VILNIAUS UNIVERSITETO

MEDICINOS FAKULTETO DISERTACIJOS

2018

VILNIUS UNIVERSITY

FACULTY OF MEDICINE DISSERTATIONS

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Pratarmė

Gebėjimas žengti kartu su skubančiu laiku, taikant mokslo pasiekimus ir tyrimų duomenis nūdienos poreikiams – viena stipriausių Vilniaus universiteto Medicinos fakulteto bendruomenės savybių. Mokslas visada reikalauja kantrybės, kruopštumo, užsispyrimo ir nuoširdaus darbo – to tikimasi iš kiekvieno pasirenkančio doktorantūros studijas. Dažnu atveju tyrimai persipina tarpdisciplininiais ryšiais, reikalauja inovacijų, plataus akiračio ir didelės erudicijos. Būtent tokiomis savybėmis pasižymi Medicinos fakulteto doktorantai.

Moderni, šiuolaikiška ir iššūkiams atvira Alma Mater kasmet parengia aukštos kompetencijos mokslininkus, teikia kokybiškas žinias, vykdo podiplominių studijų procesą bei skatina aukšto lygio mokslinių darbų publikavimą. Gerbdama savo mokytojus ir brangindama praeitį, medikų bendruomenė veržliai juda pirmyn, inovatyviai skleisdama mokslo žinias.

Ketvirtus metus iš eilės leidžiamas „Disertacijų santraukų rinkinys“ jau tapo gražia ir prasminga tradicija, jame rasite 2018 metais Medicinos fakultete apgintų disertacijų santraukas. Ši knyga skirta Medicinos fakultete vykdomų doktorantūros darbų ir tyrimų reprezentacijai. Leidiniu siekiama skatinti mokslo sklaidą, tarpdisciplininį ir tarptautinį bendradarbiavimą, raginti doktorantus rengti aukštos kokybės disertacijas, didinti jaunųjų mokslininkų motyvaciją ir populiarinti mokslą visuomenėje.

Prof. (HP) dr. Algirdas Utkus
Medicinos fakulteto dekanas

Preface

The ability to go along with rushing time by applying scientific achievements and research data to today's needs is one of the strongest features of the community of the Faculty of Medicine of Vilnius University. Science always requires patience, diligence, stubbornness, and sincere work; this is expected from every doctoral study. Research is often interdisciplinary and requires innovation, wide-ranging intelligence, and high-level erudition. These are the qualities of PhD students at the Faculty of Medicine.

Modern, up-to-date and challenging, our alma mater annually prepares high-quality scientists, provides high-quality knowledge, conducts its postgraduate study process, and promotes the publication of high-level scientific papers. Respecting its tutors and cherishing the past, the medical community is moving forward vigorously by innovatively disseminating scientific knowledge.

For the fourth year in a row, the "Summary of Dissertations" will become a beautiful and meaningful tradition, with summaries of the dissertations defended at the Faculty of Medicine in 2018. This book is dedicated to the representation of doctoral studies and research at the Faculty of Medicine. The aim of the publication is to promote the dissemination of science, further interdisciplinary and international cooperation, encourage doctoral students to prepare high-quality dissertations, boost the motivation of young scientists, and advance science in society.

Prof. Dr. Algirdas Utkus
Dean of the Faculty of Medicine

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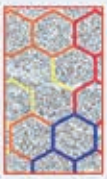
Justinas Besusparis

THE ASSESSMENT OF THE BREAST CANCER PROLIFERATION RATE AND ITS INTRATUMOR HETEROGENEITY USING DIGITAL IMMUNOHISTOCHEMISTRY METHODS

KRŪTIES VĖŽIO PROLIFERACINIO AKTYVUMO IR JO HETEROGENIŠKUMO NUSTATYMAS SKAITMENINĖS IMUNOHISTOCHEMIJOS METODAIS

Biomedical Sciences, Medicine (06B)
Biomedicinos mokslai, medicina (06 B)

Thesis at a glance

THE AIM OF THE STUDY				
To develop and test methodologies for a comprehensive Ki67 labeling index with an intra-tumor heterogeneity assessment based on digital immunohistochemistry methods.				
Study	**Objectives**	**Methods**	**Results**	**Conclusions**
I	To develop a methodology for ensuring and improving the accuracy of the approach for digital image analysis in breast cancer Ki67 IHC.	A comparison of the visual estimation of Ki67 LI, a digital image analysis result and reference values obtained with a stereology in TMA of breast cancer tissue.	We achieved a DIA misclassification rate of 5–7%, as opposed to that of 11–18% for the VE-median-based prediction.	Ki67 LI obtained by digital image analysis outperforms visual estimates, taking manual stereological counts as a reference value. An accurate Ki67 LI estimation can be achieved by DIA which is based on proper validation, calibration, and measurement error correction procedures guided by quantified bias from reference values.
 II	To develop a methodology for comprehensive Ki67 LI quantification with heterogeneity assessment and hot spot detection, perform analytical validation in a breast cancer patient cohort.	The WSI DIA-generated data were subsampled by hexagonal tiling, and spatial distribution parameters were calculated	The degree of proliferation measured by various automated Ki67 LI indicators was associated with higher histological grade/ more aggressive types of breast cancer. The manual hotspots of Ki67 LI were associated and comparable with the Pareto hotspot median Ki67 LI.	A systematic subsampling of DIA-generated data into hexagonal tiles would enable a comprehensive Ki67LI analysis that would reflect the aspects of intra-tumor heterogeneity and could serve as a methodology for improving digital immunohistochemistry.

Study	Objectives	Methods	Results	Conclusions
III	To optimize breast cancer tissue sampling requirements to represent Ki67 LI taking its intra-tumor heterogeneity into account.	The hexagons in the HexT were chosen to simulate virtual TMA cores/ fields of view in conventional microscopy, with Ki67LI established by DIA. The sampling simulations were carried out for different heterogeneity levels.	To achieve low error rates, 8 TMA cores or 4 000 nuclei are required when the heterogeneity levels are unknown. Respectively, 5–6 cores/3 000 nuclei or 11–12 cores/7 000 nuclei are required in the subgroups of homogeneous and heterogeneous tumors.	Hexagonal tiling data provide a useful model for establishing tissue sampling requirements for biomarker studies and visual estimations, which depend on intra-tissue heterogeneity and must be determined on a peruse basis.
IV	To evaluate the prognostic value of the comprehensive Ki67 LI estimation method in a breast cancer patient cohort.	The Ki67 LI indicators, extracted from WSI DIA-generated data, were subsampled using hexagonal tiles and compared with the data of overall patient survival.	All visual and DIA-generated indicators of the level of Ki67 expression provided significant cutoff values as single predictors of OS. Only the bimodality indicators (Ashman's D) were independent predictors of OS in the context of hormone receptor and HER2 status.	The spatial heterogeneity indicators (the bimodality status in particular) of proliferative tumor activity, measured by the DIA of Ki67 IHC expression and analyzed using the HexT approach, can serve as an independent, prognostic indicator of OS in breast cancer patients and outperform the prognostic power of the level of proliferative activity.

1. Įvadas

1.2 Darbo aktualumas

Krūties vėžys yra labiausiai paplitęs piktybinis susirgimas tarp moterų visame pasaulyje: 2013 metais diagnozuota 1,8 mln. naujų krūties vėžio atvejų, dėl šios priežasties per minėtą laikotarpį mirė 464 000 moterų [1, 2]. Išgyvenamumo rodikliai varijuoja nuo 80 % Šiaurės Amerikoje, Japonijoje, Švedijoje iki 40 % besivystančiose šalyse [2]. Šių rodiklių gerinimas gali būti pasiektas taikant ankstyvos diagnostikos programas arba adjuvantinę chemoterapiją, kuri derinant su hormoniniu gydymu yra labai efektyvi, tačiau pasižymi potencialiai pavojingais šalutiniais reiškiniais ir yra brangi [3]. Dėl šios priežasties gydant hormonų receptoriams pozityvius krūties navikus yra itin svarbu nuspręsti, ar papildomai yra reikalinga adjuvantinė chemoterapija, į kurios skyrimo indikacijas yra įtraukti patikimi prognostiniai faktoriai aukštai rizikai identifikuoti [3]. Tradiciniai prognostiniai faktoriai, tokie kaip morfologiniai naviko bruožai ar metastazių limfmazgiuose statusas, yra nepakankami, todėl pastaraisiais metais buvo rasta daug naujų krūties vėžio prognostinių ir predikcinių žymenų, kurių dauguma yra susiję su naviko ląstelių ciklo reguliacija ir proliferacija [4-7].

Krūties navikų gydymo taktikos pasirinkimas priklauso nuo naviko potipio, kuris yra apibrėžiamas remiantis imunohistochemine estrogenų receptorių (ER), progesterono receptorių (PgR) raiška, žmogaus epitelio augimo faktoriaus 2 (HER2) statusu ir Ki67 proliferacinio indekso (Ki67 PI) vertėmis navikiniame audinyje. Luminaliniai A navikai yra gydomi vien tik endokrinine terapija, kuri yra ir luminalinio B potipio gydymo strategijos dalis. Papildomai chemoterapija yra taikoma daugumai pacienčių, sergančių luminaliniu B, HER2 teigiamu arba trigubai neigiamu krūties vėžiu [8]. Pagrindinė skirtis tarp luminalinių A ir luminalinių B navikų yra Ki67 proliferacinis aktyvumas, kurio ribinės vertės yra žemos ir įvairiose rekomendacijose varijuoja nuo 14 % iki 30 % [8-11]. Norint taikyti minėtą klasifikaciją yra būtinas patikimas ir išsamus ER, PgR, HER2 ir Ki67 PI imunohistocheminių reakcijų vertinimas, kuris yra ypač svarbus priimant tinkamą klinikinį sprendimą ir parenkant gydymo strategiją.

Ki67 yra plačiai naudojamas imunohistocheminis žymuo, skirtas proliferuojančių ląstelių daliai tiriamajame audinyje nustatyti. Vizualus Ki67 proliferacinio indekso vertinimas yra pirmo pasirinkimo metodas kasdienėje praktikoje. Tačiau, literatūros duomenimis, vizualaus vertinimo atkartojamumas tarp skirtingų patologų yra žemas, ypač turint omeny ribines, kliniškai svarbiausias vertes nuo 10 % iki 20 % [12]. Klaidingas Ki67 proliferacinio indekso įvertinimas gali lemti netinkamai pasirinktą krūties navikų gydymo taktiką. Dėl šios priežasties vizualaus vertinimo rekomendacijose yra nurodoma vertinti skaičiuojant didelius kiekius (mažiausiai 500–1 000)

naviko ląstelių branduolių [11]. Tačiau tokie skaičiai yra per aukšti rankiniam vertinimui ir sunkiai įgyvendinami rutininiame darbe. Daugelis patologų renkasi subjektyvų vizualų vertinimą arba taiko individualius metodus, pagreitinančius proliferacinio indekso įvertinimą. Įprastinė imunohistocheminių preparatų interpretacija yra pagrįsta žmogaus vizualiu sugebėjimu identifikuoti audinių struktūras ir atlikti pusiau kiekybinius vertinimus. Šis procesas pasižymi žemu atkartojamumu ir gali reikšmingai paveikti galutinį Ki67 proliferacinio indekso vertinimą tiriant naviką [11]. Nors šis metodas yra pakankamas įprastiems diagnostiniams tikslams pasiekti, jis gali būti netinkamas priimant personalizuoto gydymo sprendimus.

Intranavikinis Ki67 žymens raiškos heterogeniškumas – labai būdingas krūties navikų bruožas [13-16], kuris nėra plačiai tyrinėtas ir potencialiai yra viena iš pagrindinių neefektyvios terapijos priežasčių [17]. Proliferacinio indekso vertinimas yra rekomenduojamas tuose naviko plotuose, kuriuose žymens raiška yra ryškiausia. Vis dėlto nėra vienos nuomonės, ar Ki67 PI turėtų būti apskaičiuojamas kaip Ki67 teigiamų naviko ląstelių procentas visame invazyvaus naviko plote, ar tik vadinamuosiuose „karštuosiuose taškuose“ [11, 18]. Pabrėžtina, kad dabartinėse gairėse „karštųjų taškų“ apibrėžimai ir imunohistocheminių žymenų intranavikinio heterogeniškumo nustatymas nėra standartizuoti. Ankstesni literatūros duomenys apie intranavikinio heterogeniškumo nustatymą ir apskaičiavimą histologiniuose preparatuose yra riboti. Ki67 proliferacinio indekso ribinės vertės kasmet žymiai varijuoja (14 %, 20 %, 20–29 %, ≤ 10 %, 20–30 %) [8-11]. 2016 metais Amerikos klinikinės onkologijos draugija (angl. ASCO) išleido klinikinės praktikos gaires [19], kuriose yra nurodoma, kad dėl vidutinio įrodymų lygio Ki67 proliferacinis indeksas neturėtų būti taikomas kaip atrankos kriterijus skiriant adjuvantinę chemoterapiją. Kadangi vizualus vertinimas yra sudėtingesnis dėl minėtų faktorių, kurie sumažina Ki67 PI prognostinę vertę, naujų, išsamių ir patikimų vertinimo metodologijų paieška yra labai aktuali.

Klinikiniuose tyrimuose skaitmeninės vaizdo analizės metodų taikymas tampa vis dažnesne alternatyva vizualiam vertinimui. Literatūros duomenimis, automatizuotas Ki67 proliferacinio aktyvumo vertinimas pasižymi aukštu atkartojamumu ir potencialiai galėtų būti pritaikytas kasdienėje praktikoje [20]. Tačiau intranavikinis Ki67 žymens raiškos heterogeniškumo nustatymas nėra standartizuotas ir tai yra pagrindinė prasto atitikimo tarp vizualaus ir automatizuoto vertinimo priežastis [21]. Ankstesniuose skaitmeninės vaizdo analizės taikymo darbuose buvo gilinamasi į optimalų naviko ląstelių segmentavimą ir automatizuotą žymenų karštųjų taškų aptikimą [22-26] išvengiant vaizdo analizės algoritmų analitinės ir klinikinės validacijos. Dėl šios priežasties automatizuoto imunohistocheminių žymenų vertinimo ir skaitmeninės vaizdo analizės pritaikomumas kasdienėje praktikoje vis dar yra ribotas.

1.3 Darbo tikslas

Sukurti metodologiją, užtikrinančią krūties navikų Ki67 proliferacinio indekso vertinimo tikslumą, ir įvertinti šio indekso intranavikinio heterogeniškumo lygį audinyje, naudojant skaitmeninę vaizdo analizę.

1.4 Darbo uždaviniai

1. Sukurti metodologiją, užtikrinančią skaitmeninės vaizdo analizės algoritmų tikslumą Ki67 proliferacinio indekso vertinimui krūties navikų imunohistocheminiuose preparatuose.
2. Sukurti metodologiją išsamiam Ki67 proliferacinio indekso skaičiavimui, jo heterogeniškumo nustatymui ir karštųjų taškų aptikimui, atlikti analitinę naujų metodų validaciją pacienčių, sergančių krūties vėžiu, imtyje.
3. Nustatyti imunohistocheminių Ki67 krūties navikų preparatų vertinimo ir audinių mikrogardelių mėginių ėmimo parametrus, atsižvelgiant į intranavikinį Ki67 proliferacinio indekso heterogeniškumo lygį audinyje.
4. Įvertinti naujo Ki67 proliferacinio indekso skaičiavimo metodo prognostinę vertę pacienčių, sergančių krūties piktybiniais navikais, imtyje.

1.5 Ginamieji teiginiai

1. Remiantis palyginimu su pamatine Ki67 PI verte, teigtina, kad kalibruotais skaitmeninės vaizdo analizės metodais nustatyto Ki67 proliferacinio indekso tikslumas yra didesnis nei patologo vizualaus vertinimo atveju.
2. Krūties navikų Ki67 proliferacinio aktyvumo intranavikinis heterogeniškumas, nustatytas segmentuojant skaitmeninės vaizdo analizės duomenis į šešiakampes gardeles, yra nepriklausomas prognostinis krūties vėžiu sergančių pacienčių bendro išgyvenamumo rodiklis ir pasižymi stipresne prognostine verte nei vien proliferacinio aktyvumo lygis.

1.6 Tyrimo naujumas

1. *Analitinė skaitmeninės vaizdo analizės validacija.* Aprašoma imunohistocheminių preparatų skaitmeninės vaizdo analizės tikslumo gerinimo metodologija pagrįsta analitinės validacijos procedūromis, kurios atliktos remiantis pamatinėmis vertėmis, apskaičiuotomis taikant stereologijos principus. Ankstesnėse panašiose studijose analitinės validacijos procedūros arba nebuvo atliekamos, arba pamatinėmis vertėmis buvo pasirenkami subjektyvaus vertinimo rezultatai.
2. *Šešiakampių gardelių principas.* Sukurta nauja imunohistocheminių žymenų vertinimo ir erdvinių parametrų nustatymo metodologija, pagrįsta šešia-

kampių gardelių principu ir išbandyta Ki67 proliferacinio indekso matavimuose. Skenuotų preparatų segmentavimo į šešiakampes gardeles principas anksčiau nebuvo taikytas patologijoje. Metodika taip pat pritaikyta imunohistocheminių žymenų karštiesiems taškams aptikti.

3. *Heterogeniškumo parametrų nustatymas.* Darbe pristatomi nauji ir standartizuoti metodai, skirti intranavikiniam Ki67 PI heterogeniškumui kiekybiškai įvertinti ir erdvinės tekstūros parametrams apskaičiuoti. Šiame darbe pirmą kartą nustatyta, kad skaitmeninės vaizdo analizės metodais apskaičiuotų išvestinių heterogeniškumo parametrų prognostinė vertė yra didesnė nei įprastinių Ki67 PI matavimo metodologijų. Ši išvada gali potencialiai sustiprinti Ki67 PI prognostinę vertę.
4. *Ki67 vizualaus vertinimo ir audinių mikrogardelių eksperimentų kriterijai.* Šešiakampių gardelių principas buvo pritaikytas Ki67 PI vizualaus vertinimo bei audinių mikrogardelių eksperimentų ėminių kriterijams nustatyti, atsižvelgiant į intranavikinį heterogeniškumą. Eksperimentai atlikti remiantis išsamiu statistiniu modeliavimu ir daugybiniais pakartojimais. Pabrėžtina, kad ankstesni panašūs tyrimai buvo paremti fiziniu audinių mėginių ėmimu, kuris buvo pagrindinis apribojimas patikimam statistiniam modeliavimui atlikti.

2. Metodai

Šiame skyrelyje trumpai aptariami tik pagrindiniai metodai, taikyti disertaciniame darbe. Detalesnė informacija yra pateikta ankstesnėse publikacijose (Etapai I–IV [27-30]). Darbe naudotos trys skirtingos krūties piktybiniais navikais sergančių pacienčių imtys. Atskiros darbo dalys buvo patvirtintos Lietuvos bioetikos komiteto ir Notingamo mokslo tiriamųjų darbų etikos komiteto. Pacienčių sutikimas dalyvauti tyrime buvo gautas. Statistinė analizė atlikta naudojant SAS 9.3, *Microsoft Excel* (*Microsoft*, Redmondas, Vašingtono valstija, JAV) ir *OpenOffice Calc* (*Oracle*, Redvud Sitis, Kalifornijos valstija, JAV) programinės įrangos paketus. Statistinio reikšmingumo lygmuo buvo naudotas ties $p < 0{,}05$ reikšmėmis.

Pacienčių imtys

1. 164 pacienčių audinių mikrogardelių histologiniai preparatai, dažyti Ki67 imunohistocheminiu žymeniu. Pacientės gydytos 2007–2009 metais Nacionaliniame vėžio institute, Vilniuje. Tyrime naudota pacienčių klinikinė ir histologinio atsakymo informacija. (Imtis naudota 1-am uždaviniui įgyvendinti.)

2. 302 pacienčių audinių viso pjūvio histologiniai preparatai, dažyti Ki67 imunohistocheminiu žymeniu. Pacientės gydytos 2013–2014 metais Nacionaliniame vėžio institute, Vilniuje. Tyrime naudota pacienčių klinikinė ir histologinio atsakymo informacija. (Imtis naudota 2-am ir 3- iam uždaviniams įgyvendinti.)
3. 152 pacienčių audinių viso pjūvio histologiniai preparatai, dažyti Ki67 imunohistocheminiu žymeniu. Pacientės gydytos 1986–1998 metais Notingame (Jungtinė Karalystė). Tyrime naudota pacienčių klinikinė ir histologinio atsakymo informacija bei išgyvenamumo duomenys. (Imtis naudota 4-am uždaviniui įgyvendinti.)

Audinių paruošimas

Formaline fiksuoti ir parafinuoti krūties navikų audinio preparatai buvo panaudoti ruošiant 3 µm storio histologinius pjūvius tolesniam imunohistocheminiam dažymui. Imunohistocheminės reakcijos atliktos naudojant monokloninius Ki67 antikūnus (klonas MIB-1; DAKO, Glostrupas, DK). Detalūs imunohistocheminio dažymo aprašymai yra pateikti ankstesnėse publikacijose [27, 30]. Histologiniai preparatai buvo skenuoti *Aperio Scan-Scope XT Slide* skeneriu (*Aperio Technologies*, Vista, Kalifornijos valstija, JAV) taikant 20x padidinimą (0,5 µm rezoliucija).

Pirmajame etape audinių mikrogardelių konstrukcijai buvo panaudoti 1 mm skersmens krūties navikinio audinio stulpeliai, kurie buvo atsitiktinai pasirinkti patologo. Iš kiekvienos pacientės buvo panaudota po vieną TMA stulpelį. Audinių mikrogardelių konstrukcijos eksperimentas yra plačiau aprašytas [31]. Kituose šio darbo etapuose (II–IV) buvo naudojami viso pjūvio histologiniai preparatai.

Skaitmeninė vaizdo analizė

Skaitmeninė vaizdo analizė buvo atlikta naudojant dvi skirtingas programinės įrangos platformas: I–III etapuose taikyti *Aperio Genie and Nuclear v9* algoritmai, IV etape skaitmenizuoti histologiniai preparatai analizuoti HALO™ audinių klasifikavimo moduliu ir *CytoNuclear v1.4* algoritmu (*Indica Labs*, Naujosios Meksikos valstija, JAV). Naudojant programinės įrangos algoritmus buvo atliktas automatizuotas naviko ploto atpažinimas ir kiekybinė naviko ląstelių branduolių profilių analizė. Audinio klasifikavimo moduliai buvo kalibruoti invazyvių navikinių kompleksų aptikimui, eliminuojant stromos, uždegimo ir kitus audinio plotus, turinčius artefaktų. Naviko ląstelių analizė atlikta automatizuotu būdu kiekybiškai klasifikuojant ląstelių branduolių profilius į turinčius neigiamą ir teigiamą Ki67 imunohistocheminę raišką. I etape atlikti keli vaizdo analizės algoritmų kalibracijos žingsniai:

1. Baziniai nustatymai, 2. Nustatymai pagal subjektyvų analizės rezultatų kokybės vertinimą, 3. Modifikuojant branduolių atpažinimo algoritmą pagal statistinį paklaidos vertinimą, gautą yginant antro algoritmo modifikacijos žingsnio duomenis su pamatinėmis Ki67 vertėmis.

I etapas. *Skaitmeninė vaizdo analizė: kalibracija, kiekybinė analizė ir validacija*

Pamatinių Ki67 proliferacinio indekso verčių generavimą rankiniu būdu atliko 3 patologai žymėdami ir skaičiuodami teigiamus ir neigiamus naviko ląstelių profilius taikant stereologinį tinklelį. Skaitmeninė skenuotų preparatų analizė atlikta taikant *Aperio Genie, Nuclear v9* algoritmus. Skaitmeninės vaizdo analizės algoritmų kalibracija atlikta trimis etapais (1. Baziniai nustatymai, 2. Intuityvi kalibracija pagal vizualų vertinimą,

3. Programinės įrangos nustatymų koregavimas pagal statistinės analizės

rezultatus, gautus antrojo kalibracijos etapo analizės rezultatus lyginant su pamatine verte). Automatizuoto Ki67 proliferacinio indekso (trijų kalibracijos etapų) vertės ir vizualaus (5 patologų) vertinimo vidurkis bei atskiros individualių vertintojų Ki67 PI vertės palygintos su pamatine Ki67

PI verte. Palyginimui naudoti koreliacijos, tiesinės regresijos ir vienfaktorinės dispersinės analizės ANOVA modeliai. Duomenų palyginimui poromis taikytas Duncano daugialypio lyginimo testas.

II etapas. *Erdvinė ir multiparametrinė analizė: heterogeniškumo matavimai, šešiakampių gardelių taikymas*

Skenuoti Ki67 imunohistocheminiai krūties navikų preparatai segmentuoti į lygius regionus taikant šešiakampių gardelių principą. Atlikta skaitmeninė skenuotų preparatų analizė *Aperio Genie, Nuclear v9* algoritmais ir Ki67 proliferacinis indeksas apskaičiuotas kiekvienoje šešiakampėje gardelėje bei visame invazyvaus naviko plote. Naudojant šiuos duomenis ir „bendrosios masės matricą“ (angl. *Co-occurrence matrix*) apskaičiuoti proliferacinio indekso heterogeniškumo parametrai. Atliktas automatizuoto proliferacinio indekso karštųjų taškų aptikimas, remiantis viršutiniu segmentavimo rezultatų kvintiliu, kuris buvo pavadintas Pareto karštuoju tašku. Automatizuoto Ki67 vertinimo rezultatai palyginti su klinikiniais duomenimis ir vizualiu patologo vertinimu. Atlikta faktorinė duomenų analizė.

III etapas. *Šešiakampių gardelių principo pritaikymas Ki67 PI vizualaus vertinimo ir audinių mikrogardelių eksperimentų ėminių kriterijų nustatymui*

Šiame etape buvo atliktas skenuotų Ki67 imunohistocheminio dažymo krūties navikų preparatų segmentavimas į šešiakampes gardeles ir pritaikytas virtualiam audinių mikrogardelių konstrukcijos eksperimentui. Remiantis skaitmeninės vaizdo analizės rezultatais Ki67 PI reikšmės įvertintos skirtinguose šešiakampių gardelių „virtualių audinių mikrogardelių" rinkiniuose (N = 1, …, 15) ir palygintos su Ki67 PI vertėmis visame konkretaus atvejo plote. Procedūra pakartota po 50 000 kartų kiekviename „virtualių audinių mikrogardelių" rinkinyje ir kiekvienu tiriamuoju atveju (N = 297). Norint palyginti rezultatus, vizualaus vertinimo kriterijų bei audinių mikrogardelių konstrukcijos parametrų nustatymui apskaičiuotos klaidos koeficiento reikšmės. Tokiu būdu įvertinama, kokia yra audinio kiekio įtaka galutiniam Ki67 PI vertinimui. Tiriamieji atvejai ir eksperimentai suskirstyti į atskiras grupes pagal žymens raiškos heterogeniškumo lygį audinyje, naudojant II etape aprašytą metodologiją.

IV etapas. *Klinikinė validacija: automatizuotų Ki67 PI parametrų palyginimas su pacienčių bendro išgyvenamumo duomenimis*

Automatizuoto krūties navikų Ki67 proliferacinio indekso ir jo heterogeniškumo nustatymo metodologija ištestuota krūties navikų imtyje (Notingamas, Jungtinė Karalystė), turinčioje ilgamečio stebėjimo rezultatus. Skaitmeninė skenuotų preparatų analizė atlikta taikant HALO™ Classifier Module/CytoNuclear v1.4 algoritmus, kurie buvo kalibruoti patikimam naviko struktūrų atpažinimui ir kiekybiniam naviko ląstelių branduolių vertinimui. Šešiakampių gardelių principas buvo pritaikytas erdvinės naviko tekstūros matavimui, heterogeniškumo parametrų nustatymui ir automatizuotam Ki67 PI karštųjų taškų aptikimui. Ki67 PI vertinimo rezultatai palyginti su pacienčių išgyvenamumo duomenimis taikant Coxo multiparametrinės regresijos analizę ir Kaplano–Meierio išgyvenamumo kreives (nuolatiniai kintamieji buvo dichotomizuoti naudojant viešos prieigos įrankį „Cutoff Finder" [32]). Atlikta faktorinė duomenų analizė. Kiekybiniams duomenims palyginti naudota multifaktorinės variacijos analizė ANOVA (palyginimui poromis – Bonferoni testas).

3. Rezultatai

I etapas. *Skaitmeninė vaizdo analizė: kalibracija, kiekybinė analizė ir validacija*

Vidutinė apskaičiuota pamatinė Ki67 proliferacinio indekso vertė – 40,2 ± 25,3 %, lyginant su vizualaus 5 patologų vertinimo mediana ir kalibruoto skaitmeninės vaizdo analizės algoritmo rezultatu, atitinkamai 37,2 ± 27,4 % ir 36,5 ± 20,2 %. Vizualaus 5 patologų vertinimo mediana ir kalibruoto skaitmeninės vaizdo analizės algoritmo rezultato koreliacijos koeficientų reikšmės, lyginant su pamatinėmis Ki67 PI vertėmis, atitinkamai 0,930 ir 0,949 ($p < 0,0001$). Tiesinės regresijos modelių rezultatai (pamatinę Ki67 PI vertę laikant nepriklausomu kintamuoju): vizualaus 5 patologų vertinimo mediana – $R^2 = 0,86$ ($p < 0,0001$), kalibruoto skaitmeninės vaizdo analizės algoritmo rezultatas – $R^2 = 0,9$ ($p < 0,0001$). Pacienčių dichotomizavimo į kliniškai svarbias grupes ties ribinėmis vertėmis > 10 %, > 15 %, > 20 % tikslumas (klaidingai suklasifikuotų atvejų dalis), lyginant su pamatinėmis Ki67 PI vertėmis: a) naudojant vizualaus 5 patologų vertinimo medianą – 11 %, 14 %, 18 %; b) naudojant kalibruoto skaitmeninės vaizdo analizės algoritmo Ki67 PI rezultatą – 7 %, 9 %, 7 %.

II etapas. *Erdvinė ir multiparametrinė analizė: heterogeniškumo matavimai, šešiakampių gardelių taikymas*

Gauta ideali koreliacija ($r = 0,9967$, $p < 0,0001$) tarp Ki67 PI, automatizuotai apskaičiuoto visame histologiniame pjūvyje, ir Ki67 PI medianos šešiakampėse gardelėse. Aukštesnis Ki67 PI asocijuotas su aukštesniu naviko diferenciacijos laipsniu ($p < 0,0001$); aukštesnės Ki67 PI entropijos reikšmės yra būdingos aukštesnio diferenciacijos laipsnio navikams ($p < 0,0001$). Faktorinės analizės metu išskirti 4 svarbūs faktoriai: 1. Heterogeniškumo faktorius, 2. Proliferacijos faktorius, 3. Bimodališkumo faktorius, 4. Ląstelingumo faktorius. Ki67 PI Pareto karštųjų taškų plote, kuris persidengia su manualiniu žymėjimu, buvo statistiškai reikšmingai didesnis nei likusiame naviko plote (*T* testas: vidutiniai skirtumai 5,8 %, 5,8 % ir 6,7 %; $p < 0,0003$, $p < 0,0002$ ir $p < 0,0001$). Trijų vertintojų Ki67 PI vidurkis karštuosiuose taškuose reikšmingai nesiskyrė ($p = 0,0675$) nuo Pareto karštojo taško įvertinimo (90-ojo procentilio).

III etapas. *Šešiakampių gardelių principo pritaikymas Ki67 PI vizualaus vertinimo ir audinių mikrogardelių eksperimentų ėminių kriterijų nustatymui*

Optimalūs audinių ėminių ir Ki67 PI vertinimo parametrai varijuoja priklausomai nuo audinio heterogeniškumo lygio. Norint pasiekti 10 % klaidos koeficiento vertę yra reikalinga naudoti 8 TMA stulpelius nežinomo heterogeniškumo navikams; homogeniškiems navikams – 5 TMA stulpelius; heterogeniškiems navikams – 11 TMA stulpelių. Norint pasiekti 10 % klaidos koeficiento vertę reikalinga įvertinti 4 000 naviko ląstelių branduolių vizualiam Ki67 PI nustatymui, kai intranavikinio heterogeniškumo lygis yra nežinomas. Vertinant atvejus, kai Ki67 PI yra žemas (< 20 %), yra reikalingas didesnis ėminių / ląstelių skaičius, norint pasiekti tą pačią paklaidos tikimybę, kaip ir aukšto Ki67 PI navikų atveju.

IV etapas. *Klinikinė validacija: automatizuotų Ki67 PI parametrų palyginimas su pacienčių bendro išgyvenamumo duomenimis*

Vidutinis pacienčių stebėjimo laikas – 143,4 ± 71,4 mėnesio, 79 iš 152 pacienčių mirė. Trijuose Coxo regresijos modeliuose vienas iš automatizuotu būdu apskaičiuotų heterogeniškumo indikatorių (bimodališkumo parametras) buvo identifikuotas kaip nepriklausomas blogos pacienčių prognozės veiksnys. Nė vienas iš Ki67 proliferacinio aktyvumo parametrų (apskaičiuotų tiek rankiniu, tiek automatizuotu būdu) nebuvo identifikuotas kaip nepriklausomas veiksnys, prognozuojantis bendro pacienčių išgyvenamumo rodiklius. Faktorinės analizės metu išskirti 5 faktoriai, iš kurių svarbiausi yra 1. Proliferacijos faktorius, 2. Heterogeniškumo faktorius ir 4.Bimodališkumo faktorius. Multifaktorinės variacijos analizės (ANOVA) duomenimis, aukštesnis 1 faktorius asocijuotas su aukštesniu diferenciacijos laipsniu (p < 0,0001). Trigubai neigiami navikai pasižymėjo aukštesnėmis 1 faktoriaus reikšmėmis nei hormonų receptoriams pozityvi grupė.

4. Rezultatų aptarimas

Doktorantūros studijų metu atliktų tyrimų rezultatai atskleidžia keletą aspektų, kurie yra svarbūs siekiant pagerinti krūties navikų gydymo taktikai pasirinkti naudojamo Ki67 proliferacinio indekso prognostinę vertę. Nustatyta, kad automatizuoto Ki67 proliferacinio indekso vertinimo tikslumas yra didesnis nei patologų vizualaus vertinimo atveju. Pabrėžtina, kad vaizdo analizės algoritmai buvo kalibruojami kaip pamatines vertes naudojant Ki67 PI įverčius, gautus atlikus nešališkus skaičia-

vimus pagal stereologijos taisykles. Antra, proliferacinio naviko aktyvumo erdvinis heterogeniškumas gali būti pamatuotas ir vizualizuotas taikant šešiakampių gardelių principą, kurį taip pat įmanoma pritaikyti biožymenų raiškos karštųjų taškų aptikimui ir jų kiekybiniam įvertinimui. Be to, šešiakampių gardelių metodas buvo pritaikytas ir audinių mikrogardelių eksperimentų reikalavimų optimizavimui, atsižvelgiant į krūties vėžio Ki67 PI heterogeniškumą. Krūties navikų proliferacinio aktyvumo erdvinio heterogeniškumo rodikliai, išmatuoti taikant skaitmeninę vaizdo analizę ir šešiakampes gardeles, gali būti nepriklausomas kintamasis, prognozuojantis pacienčių bendro išgyvenamumo rodiklius ir pranokstantis vien tik proliferacinio aktyvumo įverčius.

Vaizdo analizės metodų taikymas skaitmenizuotų imunohistocheminių preparatų vertinimui yra sparčiai besivystantis ir tobulėjantis procesas patologijos tyrimų srityje. Didžiausias skaitmeninės vaizdo analizės privalumas – dideli audinio informacijos kiekiai, kurie gali būti lengvai išgaunami analizuojant aukštos raiškos skenuotus histologinius preparatus. Automatizuotos analizės pajėgumas yra gerokai didesnis nei rankinio preparatų vertinimo. Pavyzdžiui, šiame tyrime buvo išanalizuota 36 mln. ląstelių bendrame 15 000 mm^2 naviko plote bei 13 mln. ląstelių 6 000 mm^2 naviko plote, atitinkamai eksperimentuose, aprašytuose II ir IV tyrimuose. Antrojo tyrimo metu viename histologiniame preparate vidutiniškai įvertinta 121 000 navikinių ląstelių profilių. Remiantis šiuo požiūriu vienintelio indikatoriaus (t. y. Ki67 PI) apskaičiavimas galėtų būti laikomas nepakankamu duomenų panaudojimu. Skaitmeninei vaizdų analizei yra būdingas ne vien tik didesnis tikslumas, atkartojamumas ir pajėgumas, tačiau automatizuotas imunohistocheminių preparatų vertinimas pasižymi ir aukštesne prognostine verte lyginant su vizualiu vertinimu [33]. Remiantis navikų mikroskopinės architektūros požymiais, apskaičiuotais skaitmeninės vaizdų analizės metodais, įmanoma stratifikuoti krūties navikus į turinčius skirtingą metastatinį potencialą [34]. G. Stålhammaras su bendraautoriais aprašo eksperimentą, kurio metu skaitmenizuotas Ki67 PI vertinimas pranoko rankinį mitozių skaičiavimą ir Ki67 PI nustatymą įprastiniais metodais bei turėjo papildomos prognostinės informacijos [35]. Be to, automatizuoto Ki67 PI vertinimo rezultatai reikšmingai koreliuoja su molekulinių modelių, pasižyminčių itin aukštu ligos recidyvo prognozavimo jautrumu ir specifiškumu, rezultatais [36].

Nepaisant sparčiai tobulėjančios programinės įrangos, skirtos skaitmeninei histologinių preparatų analizei, minėti algoritmai yra beveik netaikomi kasdienėje patologijos praktikoje. Validacijos procedūrų netikslumai yra didžiausias ankstesnių eksperimentų, tyrinėjusių vaizdo analizės algoritmų pritaikomumą, trūkumas. Ankstesniuose darbuose algoritmų validacija arba nebūdavo atliekama, arba pamatinėmis vertėmis buvo pasirenkami subjektyvaus vertinimo rezultatai, kurie negali būti taikomi kaip „tiesos kriterijus“. Šio darbo pirmajame etape buvo atlikta išsami

skaitmeninės vaizdo analizės algoritmų kalibracija ir sukurtas vadinamasis „aukso standartas“, paremtas stereologijos taisyklėmis ir rankiniu naviko ląstelių profilių skaičiavimu. Šiame etape nustatyta, kad 5 patologų atlikto vizualaus Ki67 PI vertinimo vidurkis nepasiekė tikslumo lygio, gauto taikant kalibruotą skaitmeninės vaizdo analizės algoritmą. Taip pat buvo apskaičiuotas vizualaus ir automatizuoto vertinimo tikslumas skirstant pacientes į kliniškai svarbias grupes pagal dažniausiai naudojamas Ki67 PI ribines vertes (10 %, 15 % ir 20 %). Eksperimento metu nustatyta, kad paklaidos tikimybė yra du kartus didesnė, jei yra naudojamas patologo vertinimas. Šis rezultatas buvo iš dalies netikėtas, tačiau tai yra papildomi įrodymai, kad vizualus pusiau kiekybinis imunohistocheminių Ki67 preparatų vertinimas negali būti naudojamas kaip patikimas matavimas, kuriuo remiantis priimami klinikiniai sprendimai arba nustatomos pamatinės vertės, atliekant skaitmeninės vaizdo analizės algoritmų validavimą. Šiame eksperimente taip pat nustatyta, kad skaitmeninės vaizdo analizės kokybė gerėjo po kiekvieno kalibracijos žingsnio, o geriausias rezultatas buvo pasiektas statistiškai išanalizavus algoritmų paklaidas. Remiantis šiais rezultatais galima teigti, kad pirmas žingsnis skaitmeninės vaizdo analizės eksperimentuose turėtų būti kruopšti algoritmų validacija, naudojant pamatines vertes, apskaičiuotas nešališkais vertinimo metodais.

Doktorantūros studijų metu buvo sukurtas naujas ir pažangus automatizuotas metodas, skirtas erdvinei biologinių žymenų tekstūrai ir heterogeniškumui histologiniuose preparatuose nustatyti ir įvertinti. Metodika yra pagrįsta sisteminiu skenuotų preparatų skaitmeninės vaizdo analizės rezultatų padalijimu į lygias šešiakampes gardeles. Kaip ir tikėtasi, taikant šį metodą buvo nustatyti naviko erdvinės tekstūros parametrai ir jiems suteiktos skaitinės reikšmės, kurios vėliau kartu su pasiskirstymo duomenimis gali būti panaudotos atvejų palyginimui ir navikų stratifikacijai į skirtingo heterogeniškumo grupes. Ankstesniuose panašiuose tyrimuose navikų erdvinio heterogeniškumo nustatymas buvo atliktas lyginant mėginius, paimtus iš to paties naviko skirtingų regionų. Dažniausiai buvo naudojami fiziniai audinių mikrogardelių (TMA) mėginiai, kuriuose lyginti tiriamojo biologinio žymens raiškos skirtumai tarp TMA stulpelių, paimtų iš to paties naviko [37-39]. Nors, šių eksperimentų duomenimis, tam tikri navikinio audinio variacijos skirtumai gali būti identifikuoti, tačiau heterogeniška ER, PgR, HER2 raiška buvo aptinkama nedažnai (< 10 % atvejų) [39]. Pagrindinis TMA metodo trūkumas nustatant biologinių žymenų heterogeniškumą yra tiesioginė rezultatų priklausomybė nuo audinių ėminių skaičiaus ir viso eksperimento struktūros. Dažnai per mažas TMA ėminių kiekis neatspindi viso navikinio audinio įvairovės ir gerokai apriboja heterogeniškumo tyrimus tokio tipo eksperimentuose.

Skaitmeninės vaizdo analizės metodų taikymas intranavikiniam heterogeniškumo lygiui įvertinti nėra naujas. S. J. Pottsas kartu su bendraautoriais pritaikė ekolo-

gijos mokslų srityje naudojamus variacijos rodiklius krūties navikų imunohistocheminės HER2 raiškos heterogeniškumo nustatymui histologiniuose preparatuose, iš anksto pasirinktuose regionuose [17]. Tyrimo metu, naudojant skaitmeninės vaizdo analizės rezultatus, buvo sukurtas virtualus žemėlapis, parodantis HER2 ekspresijos lygį skirtingose naviko vietose. Tyrimo išvadose autoriai pabrėžia, kad, norint tiksliai nustatyti erdvinę variaciją, analizėje naudotų regionų skaičius gali būti nepakankamas. Tokie tyrimai turėtų būti atlikti naudojant viso pjūvio histologinius preparatus, kuriuose būtų analizuojamas visas naviko plotas, o ne smulkūs jo fragmentai. Pabrėžtina, kad rengiant disertaciją sukurta šešiakampių gardelių metodika yra paremta sisteminiu skaitmeninės vaizdo analizės rezultatų segmentavimu ir naviko erdvinės tekstūros parametrų nustatymu visame atlikto pjūvio histologiniame preparate. Suklasifikavus šios analizės metu gautus rezultatus (Ki67 PI atskiruose šešiakampiuose) į skirtingus intervalus, gautus duomenis galima būtų panaudoti naujų, kompleksinių ir potencialiai biologiškai reikšmingesnių indikatorių kūrimui nei vien Ki67 proliferacinio indekso vertės.

Šiame darbe yra aprašomas naujas ir pranašus metodas, skirtas biožymenų imunohistocheminės raiškos karštiesiems taškams aptikti. Jis paremtas stabilios naviko dalies (90-ojo procentilio) vertinimu ir pavadintas Pareto karštuoju tašku. Literatūros duomenimis, skaitmeninės vaizdo analizės algoritmai yra plačiai taikomi biožymenų raiškos karštiesiems taškams aptikti audiniuose [22, 23, 25, 26, 36, 40, 41]. Nepaisant to, dalyje ankstesnių eksperimentų vis dar yra taikomi metodai, paremti rankiniu skaičiavimu [24], daugybiniu vizualiu vertinimu [42] arba audinių mikrogardelių konstrukcijos principų taikymu [37, 39]. Kitose mokslo srityse (ekologijoje, geografijoje) erdvinės paviršiaus tekstūros matavimams yra plačiai taikomi statistiniai metodai, paremti tiriamojo objekto segmentavimu į smulkesnius lygius fragmentus. Medicinos srities mokslo tiriamuosiuose darbuose šiam tikslui dažniausiai yra naudojamos stačiakampio formos gardelės [26, 36, 41]. Pavyzdžiui, E. Gudlaugssonas su bendraautoriais [41] aprašo metodą, skirtą Ki67 PI karštiesiems taškams aptikti krūties navikuose, selektyviai pasirenkant keturkampio formos laukus su vizualiai didžiausia Ki67 teigiamų ląstelių dalimi ir juose atliekant skaitmeninę vaizdo analizę. Tyrimo metu nustatyta, kad tokiu būdu apskaičiuotos Ki67 PI vertės pasižymi aukštu atkartojamumu ir yra prognostiškai reikšmingos. Vis dėlto toks metodas tiesiogiai priklauso nuo rankiniu būdu pasirinktų analizės plotų, o rezultatai gauti neatsižvelgus į galimą skirtingą ląstelių tankį navike. Šie eksperimentai iš dalies yra panašūs į doktorantūros studijų metu atliktus darbus, nes histologiniai preparatai buvo virtualiai segmentuojami į lygius smulkesnius regionus. Tačiau šešiakampių gardelių principas, kuris anksčiau dar niekada nebuvo pritaikytas histologinių preparatų segmentavimui, pasižymi beveik idealiu viso naviko ploto padengimu ir yra nepriklausomas nuo nereguliarios bei kompleksiškos karštojo taš-

ko formos ir dydžio. Naudojant stačiakampes gardeles, audinyje lieka nepadengtų regionų (ypač audinio kraštuose), o šešiakampių gardelių principas užtikrina, kad yra vienoda tikimybė pasirinkti bet kurį audinio regioną. Pabrėžtina, kad taikant Pareto principą yra vertinama stabili naviko ląstelių dalis (20 % aukščiausią Ki67 PI vertę turinčių šešiakampių gardelių), tačiau šios taisyklės gali būti lengvai modifikuojamos, priklausomai nuo eksperimento struktūros ir tikslų. Apibendrinant galima teigti, kad skaitmeninės vaizdo analizės taikymas karštajam taškui aptikti gali būti naudingas dėl kelių priežasčių: a) tai gali būti panaudota standartizuoto karštojo taško apibrėžimui kurti, aprašant jų formą, dydį, „temperatūrą" ir kitas savybes, b) objektyviam ir aukštu atkartojamumu pasižyminčiam karštojo taško aptikimui, c) taikant šią metodiką yra įmanoma išryškinti naviko vietas (šešiakampių gardelių grupes), kuriose Ki67 imunohistocheminė raiška yra ryškiausia, tokiu būdu palengvinant kasdienį patologo darbą identifikuojant sunkiai randamus karštuosius taškus tiriamajame audinyje, d) pacienčių atrankos individualizuotai terapijai gerinti.

Darbo metu nustatyta, kad skirtingų vertintojų sutarimas aptinkant karštuosius Ki67 PI taškus audinyje yra gana prastas. II ir IV etapuose karštieji taškai buvo apibrėžti ir įvertinti trijų patologų. Lyginant tarp skirtingų vertintojų, sutarimas aptinkant bent vieną karštąjį tašką audinyje varijavo priklausomai nuo vertintojų porų (kapa koeficientai nuo 0,2 iki 0,5 II etapo metu (n = 50) ir nuo 0,55 iki 0,85 IV etapo metu (n = 152)). Vertinant karštojo taško dydžio ir formos persidengimą tarp skirtingų patologų, erdvinė variacija buvo gauta labai ryški. Kaip ir tikėtasi, šie duomenys atitinka nurodytus literatūroje [12, 41, 43, 44] ir akcentuoja biologinių žymenų raiškos karštųjų taškų apibrėžimo standartizavimo svarbą. Šiame darbe patologų aptiktų karštųjų taškų įverčiai buvo panašūs į esančius šešiakampėse gardelėse, turinčiose aukštesnę Ki67 PI vertę, ir buvo lyginti su Pareto karštojo taško įverčio mediana.

Atlikus faktorinę šešiakampių gardelių segmentavimo duomenų (išvestinių heterogeniškumo parametrų) analizę buvo išryškinti 4 pagrindiniai faktoriai: entropijos, proliferacijos, bimodališkumo ir ląstelingumo. Šie faktoriai vėliau buvo panaudoti klasterinėje analizėje, kurioje išryškėjo heterogeniškų navikų potipiai su dominuojančia entropija, bimodališkumu ir skirtingomis proliferacinio indekso vertėmis. Tačiau siekiant tiksliau stratifikuoti navikus į homogeniškus ir heterogeniškus yra būtini įrodymais pagrįsti apibrėžimai, kurie idealiu atveju būtų paremti pacienčių išgyvenamumo duomenų analize.

Šešiakampių gardelių segmentavimo principas buvo pritaikytas Ki67 imunohistocheminio vertinimo standartizacijos pagerinimui ir reikalavimų TMA eksperimentams nustatymui, atliekant daugybinius virtualių TMA gardelių modeliavimo eksperimentus. Rezultatai suskirstyti atsižvelgiant į audinio heterogeniškumo lygį. Šio eksperimento metu buvo gauti nauji ir dalinai prieštaringi literatūros duome-

nims rezultatai. Nustatyta, kad optimalūs preparatų vertinimo parametrai priklauso nuo tiriamojo žymens raiškos heterogeniškumo lygio audinyje. Šio tyrimo metu nustatyta, kad patikimam TMA eksperimentų modeliavimui yra reikalingi didesni audinių ėminių kiekiai, nei yra aprašoma literatūroje. 5–6 audinių mikrogardelių stulpeliai yra būtini, kai žymens raiška – homogeniška, 11 stulpelių – heterogeniškiems navikams ir 8 stulpeliai, kai heterogeniškumo lygis yra nežinomas. Ankstesniuose panašiuose darbuose rekomenduojamas TMA stulpelių skaičius varijuoja nuo 1 iki 4. Tokie ryškūs nesutapimai tarp literatūros ir šio eksperimento duomenų gali būti paaiškinami labai mažu pakartojimų dažniu ankstesniuose eksperimentuose, kurie dažniausiai yra paremti vienu atsitiktinio modelio sukūrimu. Tai ir yra pagrindinė priežastis, apsunkinanti išsamų statistinį modeliavimą. Šio eksperimento metu virtualus audinių mikrogardelių modeliavimo eksperimentas buvo atliktas po 50 000 kartų kiekvienu atveju, kiekviename skirtingame virtualių TMA stulpelių rinkinyje. Pirmą kartą nustatyti vizualaus krūties navikų Ki67 imunohistocheminio žymens vertinimo parametrai, remiantis išsamaus statistinio modeliavimo rezultatais. Nustatyta, kad norint pasiekti mažą paklaidos tikimybę vizualiai vertinant Ki67 PI krūties piktybiniuose navikuose, kai heterogeniškumo lygis yra nežinomas, tikslinga įvertinti apie 4 000 naviko ląstelių branduolių. Taip pat yra būtina skaičiuoti daugiau naviko ląstelių branduolių, kai Ki67 PI yra žemas (< 20 %). Pagal dabartines rekomendacijas minimalus ląstelių skaičius, būtinas vizualiam vertinimui, yra 500–1 000 branduolių [11]. Pabrėžtina, kad šio tyrimo metu nustatytas minimalus ląstelių kiekis viršija esamas rekomendacijas 2 kartus ir turėtų būti taikomas, kai intranavikinio heterogeniškumo lygis yra nežinomas. Tokie aukšti ląstelių vertinimo reikalavimai, žinoma, yra sunkiai įmanomi kasdienėje praktikoje visais atvejais, tačiau potencialiai galėtų būti taikomi, kai yra reikalingas labai tikslus proliferacinio indekso vertinimas norint priimti klinikinius sprendimus (10–30 % Ki67 PI intervale).

Pirmą kartą nustatyta, kad krūties navikų Ki67 PI erdvinio heterogeniškumo rodikliai, apskaičiuoti taikant šešiakampių gardelių principą, pasižymi didesne prognostine verte nei konservatyvus navikų proliferacinio aktyvumo vertinimas. Ketvirtajame doktorantūros etape automatizuota heterogeniškumo matavimo metodologija buvo pritaikyta krūties navikų imčiai, turinčiai ilgamečio pacienčių stebėjimo ir išgyvenamumo duomenis. Šiame eksperimente buvo ištirta įvairių Ki67 proliferacinio indekso, audinio erdvinės tekstūros, bimodališkumo parametrų ir vizualaus vertinimo duomenų prognostinė vertė. Pabrėžtina, kad daugumai kiekybinių Ki67 automatizuoto ir vizualaus vertinimo parametrų buvo nustatytos statistiškai patikimos ribinės vertės stratifikuojant pacientes į kliniškai svarbias grupes. Tačiau tik bimodališkumo parametrai (Ashmano D koeficientas) buvo identifikuoti kaip nepriklausomi bendro pacienčių išgyvenamumo rodikliai hormonų receptoriams ir HER2 pozityviuose navikų subtipuose bei pranoko prognostinę įprastinių

Ki67 PI parametrų vertę. Šis rezultatas buvo netikėtas, tačiau gali turėti praktinę reikšmę. Dėl ryškios krūties navikų biologinės įvairovės ir audinio heterogeniškumo variacijos yra problemiška apibrėžti Ki67 PI ribines vertes ir užtikrinti tikslų kiekybinį Ki67 imunohistocheminių preparatų vertinimą. Skaitmeninės vaizdo analizės metodais apskaičiuoti išvestiniai Ki67 PI bimodališkumo parametrai, tikėtina, yra mažiau jautrūs šiems veiksniams. Šio tyrimo rezultatai įrodo, kad intranavikinio proliferacinio aktyvumo variacijos rodikliai gali būti svarbesnis ligos agresyvumo rodiklis, lemiantis individualią prognozę, nei vien Ki67 PI vertinimas.

Apibendrinant pasakytina, kad šios disertacijos rezultatai patvirtina, jog aukštas Ki67 imunohistocheminio vertinimo tikslumas yra sunkiai pasiekiamas įprastiniais vizualaus vertinimo metodais. Pabrėžtina informacinių technologijų svarba Ki6 PI vertinimo efektyvumui gerinti, apskaičiuojant standartizuotus heterogeniškumo parametrus ir automatizuotu būdu aptinkant karštuosius žymens raiškos taškus viso pjūvio histologiniuose preparatuose. Išvestiniai audinio erdvinės tekstūros parametrai, apskaičiuoti skaitmeninės vaizdo analizės metodais, gali būti potencialiai naudojami pasirenkant krūties navikų gydymo taktiką ateityje.

5. Darbo tęstinumas

Skaitmeninės vaizdo analizės įdiegimas į klinikinę praktiką

Reali skaitmeninės vaizdo analizės įrankių klinikinė nauda gali būti pasiekta įdiegus juos į patologijos laboratorijų informacines sistemas ir naudojant kaip sprendimų palaikymo įrankius patologų kasdienėje praktikoje. Tai ir būtų pagrindinis šioje disertacijoje aprašomų eksperimentų tikslas. Nepaisant to, kad vaizdo analizės algoritmų integracija nebuvo šio disertacinio darbo uždavinys, Valstybiniame patologijos centre (Vilnius, Lietuva) jau yra atlikti bandomieji eksperimentai, kurių metu validuotas vaizdo analizės algoritmas buvo įdiegtas į informacinę laboratorijos sistemą ir pritaikytas rutininiam Ki67 PI vertinimui krūties navikuose.

Heterogeniškumo apibrėžimai ir klinikinė validacija

Taikant šiame darbe aprašytą šešiakampių gardelių principą yra įmanoma modeliuoti keletą skirtingų biožymenų karštųjų taškų apibrėžimų, kurie šiame darbe nebuvo nagrinėti. Geriausias būdas validuoti šiuos skaitmeninės vaizdo analizės metodais pagrįstus apibrėžimus yra palyginti juos su pacienčių išgyvenamumo duomenimis ir nustatyti jų prognostinę reikšmę. Biožymenų raiškos erdvinio hetero-

geniškumo parametrus galima apskaičiuoti remiantis skaitmeninės analizės rezultatais, tačiau navikų stratifikavimas į homogeniškus ir heterogeniškus taip pat turi būti pagrįstas klinikiniais eksperimentais. Kituose šio darbo etapuose yra numatytas išvestinių vaizdo analizės parametrų prognostinės vertės įvertinimas plečiant tiriamųjų pacienčių grupes ir renkant išgyvenamumo duomenis.

Šešiakampių gardelių principo pritaikomumas

Šešiakampių gardelių metodas buvo išbandytas krūties navikų Ki67 imunohistocheminiuose preparatuose, tačiau jį galima lengvai pritaikyti ir kitiems tikslams ar audiniams. Automatizuotas Ki67 PI vertinimas galėtų palengvinti kruopštų proliferacinio indekso vertinimą virškinamojo trakto neuroendokrininiuose navikuose, kai ribinės vertės labai žemos (1 %, 3 % ir 20 %), o vizualus vertinimas yra labai apsunkintas. Skaitmeninė vaizdo analizė ir karštųjų taškų aptikimas galėtų palengvinti šią užduotį ir užtikrinti geresnį pacienčių skirstymą į kliniškai reikšmingas grupes.

Kita sritis, kurioje skaitmeninės vaizdo analizės algoritmai galėtų būti sėkmingai pritaikyti, yra estrogenų, progesterono ir HER2 receptorių vertinimas krūties navikuose. Literatūros duomenimis, automatizuotas heterogeniškumo nustatymas minėtuose biologiniuose žymenyse buvo labai retai taikomas. Šiame darbe aprašyti metodai galėtų būti lengvai pritaikyti minėtai užduočiai atlikti, prieš tai įgyvendinus išsamią naujai sukurtų skaitmeninės vaizdo analizės algoritmų validaciją.

6. Išvados

1. Sukurta metodologija, užtikrinanti ir pagerinanti skaitmeninės vaizdo analizės algoritmų Ki67 proliferacinio indekso vertinimo tikslumą. Krūties navikų Ki67 proliferacinio aktyvumo indeksas, nustatytas skaitmeninės vaizdo analizės metodais, pranoksta vizualų patologo vertinimą, lyginant su pamatine verte, apskaičiuota taikant stereologinius metodus. Skirstant krūties karcinomomis sergančias pacientes į terapiniu požiūriu svarbias grupes (Ki67 PI > 10, 15 ir 20 %) paklaidos tikimybė yra 2 kartus mažesnė remiantis automatizuoto Ki67 proliferacinio indekso matavimo vertėmis nei taikant vizualų 5 patologų Ki67 proliferacinio indekso vertinimo vidurkį. Vaizdo analizės algoritmai gali būti toliau sėkmingai tobulinami, matuojant jų paklaidą ir analizuojant galimas sistemines neatitikimų priežastis.
2. Sukurta tikslaus Ki67 PI vertinimo metodologija, paremta šešiakampių gardelių segmentavimo principu. Šiuo metodu yra įmanoma pamatuoti ir

vizualizuoti Ki67 proliferacinio aktyvumo indekso erdvinę tekstūrą navike bei apskaičiuoti žymens raiškos intranavikinio heterogeniškumo parametrus, skaitmeninės vaizdo analizės metodais analizuotuose Ki67 imunohistocheminiuose preparatuose. Išvestiniai heterogeniškumo parametrai gali būti pritaikyti krūties navikų dichotomizavimui į turinčius homogenišką ir heterogenišką Ki67 imunohistocheminę raišką. Šis principas buvo panaudotas automatizuotam Ki67 PI karštųjų taškų aptikimui, kuris yra kiekybiškai apskaičiuojamas remiantis viršutiniu segmentavimo rezultatų kvintiliu ir pavadintas Pareto karštuoju tašku. Šešiakampių gardelių metodas taip pat pasižymi lengvu pritaikomumu kitose patologijos srityse tiriant įvairius imunohistocheminius žymenis ar audinius, siekiant nustatyti jų raiškos heterogeniškumą arba atlikti kokybės kontrolės procedūras.

3. Optimalūs audinių mikrogardelių mėginių ėmimo ir / arba preparatų vertinimo parametrai priklauso nuo tiriamojo žymens raiškos heterogeniškumo lygio audinyje. Mokslo tiriamosiose studijose nurodoma, kad Ki67 imunohistocheminio žymens tyrimuose, atliekamuose su krūties piktybinių navikų audiniais, 5–6 audinių mikrogardelių stulpeliai yra būtini, kai žymens raiška – homogeniška, 11 stulpelių – heterogeniškiems navikams ir 8 stulpeliai, kai heterogeniškumo lygis yra nežinomas. Norint pasiekti mažą paklaidos tikimybę vizualiai vertinant Ki67 proliferacinį aktyvumą krūties piktybiniuose navikuose, kai heterogeniškumo lygis yra nežinomas, tikslinga įvertinti apie 4 000 naviko ląstelių branduolių. Vertinant atvejus, kai Ki67 PI yra žemas (< 20 %), yra reikalingas didesnis ėminių / ląstelių skaičius, norint pasiekti tą pačią paklaidos tikimybę, kaip ir aukšto Ki67 PI navikuose.
4. Erdvinio navikų proliferacinio aktyvumo heterogeniškumo parametrai (ypač bimodališkumo statusas), apskaičiuoti taikant skaitmeninę vaizdo analizę ir šešiakampių gardelių segmentavimo principą, gali būti naudojami kaip nepriklausomi prognostiniai faktoriai, apibūdinantys krūties piktybiniais navikais sergančių pacienčių bendro išgyvenamumo rodiklius ir pranokstantys prognostinę Ki67 proliferacinio aktyvumo reikšmę.

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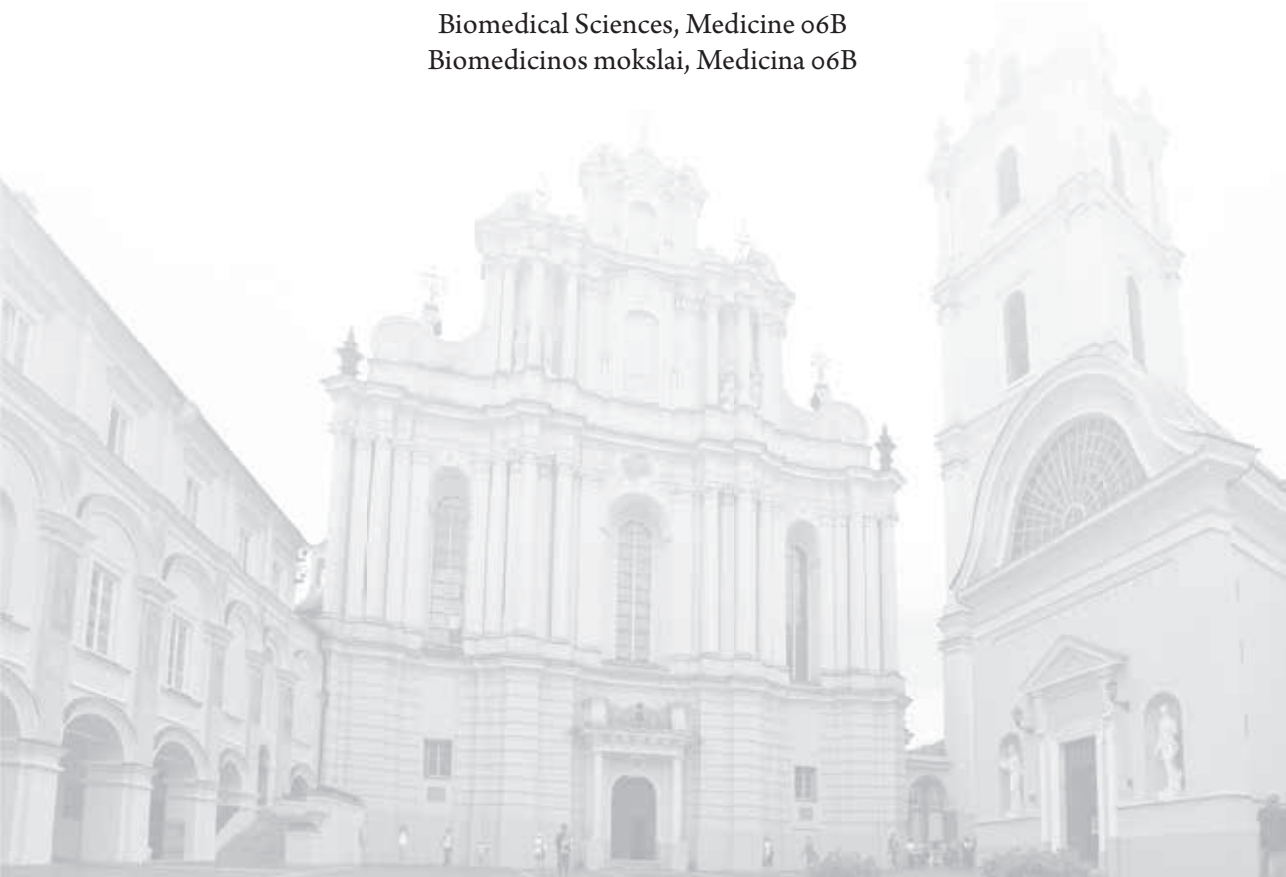
Birutė Burnytė

EVALUATION OF GENETIC AND PHENOTYPE HETEROGENEITY IN HEREDITARY NEUROPATHIES

GENOTIPO IR FENOTIPO HETEROGENIŠKUMO ĮVERTINIMAS PAVELDIMŲ NEUROPATIJŲ GRUPĖJE

Biomedical Sciences, Medicine 06B
Biomedicinos mokslai, Medicina 06B



Abbreviations

AD – autosomal dominant
AR – autosomal recessive
CMT – Charcot-Marie-Tooth disease
CMT1A – Charcot-Marie-Tooth disease type 1A
HNPP – Hereditary neuropathy with susceptibility to pressure palsies mt
DNA – mitochondrial DNA
NCV – nerve conduction velocity
NGS – next-generation sequencing
PCR – polymerase chain reaction
WES – whole exome sequencing

Introduction

Background

Hereditary neuropathies are a broad clinically and genetically heterogeneous group of neurodegenerative disorders. The clinical phenotypes of all of these diseases overlap. Combinations with the mechanisms of other diseases are also possible.

Hereditary neuropathies, based on their origin, are categorized as neuropathies in which neuropathy is the main symptom of the disease such as Charcot-Marie-Tooth Disease (CMT), hereditary sensory and autonomic neuropathies (HSAN), distal hereditary motor neuropathies (dHMN), etc. and neuropathies in which the clinical manifestation of the neuropathy is part of a widespread neurological or multisystem disorder. Currently, pathogenic variants of hereditary neuropathies and related phenotypes have been clearly described for more than 100 genes, but a large proportion of genetic causes still remain unexplained. Autosomal dominant (AD), autosomal recessive (AR), X-linked and mitochondrial inheritance forms are distinguished. Primary mitochondrial disorders can also manifest as peripheral neuropathies. The use of Next Generation Sequencing (NGS) technology in the study of hereditary neuropathies continually increases the frequency of discovery of genetic causes. Although there is in fact no etiological treatment for hereditary neuropathies, knowing the molecular diagnosis of the disorder is important for many patients as it is not necessary to continue to search for other possible causes of the disease and it is possible to determine the probability of a recurrence risk for children and other family members. An exact molecular diagnosis is important if we want to identify patients for further research, including clinical trials of new therapies.

A lot of effort is being made around the world to investigate the causes of hereditary neuropathies. There were more than 5,000 publications in the PubMed database based on the search criteria "Hereditary Neuropathy" in the period of writing this thesis. Currently, in different countries, including Lithuania, genetic testing in

stages is performed most often, based on the determination of the nerve conduction velocity (NCV) criteria, the pattern of inheritance of the disease in the family, and the prevalence of the disease in the affected population. In Norway, almost 96% of the pathogenic variants identified in patients with CMT were in only four genes (*PMP22* duplication, *GJB1*, *MPZ* and *MFN2*) associated with CMT (Østern R et al., 2013). These genetic findings are consistent with those observed in other countries. In the United Kingdom, more than 90% of the 1607 patients studied had CMT causes in the same four genes (Murphy SM et al., 2012). Meanwhile, in Denmark, where a CMT1A genetic study started in 1990, the results of 1442 genetically tested patients in 1992-2012 showed that the molecular diagnosis of only four major CMT genes confirmed the diagnosis for only 21.6% of the patients (Vaeth S et al., 2017). In Hungary, in 531 patients with CMT, the duplication of *PMP22* was for 40.5%, and the pathogenic variants for the *GJB1* gene were for 9.2% (Milley GM et al., 2018). In Russia, in 174 CMT patients, the duplication of *PMP22* was detected in 59 patients and amounted to 33.9% (Mersiyanova IV et al., 2000). In Lithuania, a CMT1A genomic region study was started in 2000, and for the second most common cause – the *GJB1* gene – only in 2010. Mitochondrial DNA analysis in patients with mitochondrial syndromes have not been previously performed in Lithuania. Usually these patients have multisystem impairment, including peripheral neuropaty. The aim of this study was to identify the most common and novel genetic factors and to evaluate genetic and phenotype relationships in a group of patients with hereditary neuropathies using Sanger sequencing and NGS methods.

The Aim of the Study

To identify the most common and novel genetic defects and to evaluate the genetic and phenotype heterogeneity in a group of patients with hereditary neuropathies.

The Objectives of the Study

1. To determine the distribution of Charcot-Marie-Tooth type 1A disease and analyse the heterogeneity of the phenotype in patients with Charcot-Marie-Tooth disease type 1A and hereditary neuropathy with liability to pressure palsies and to determine the phenotype manifestation dependence on gender in the group of patients with Charcot-Marie-Tooth disease type 1A.
2. To investigate the most common genes associated with Charcot-Marie-Tooth disease (*GJB1*, *MPZ*, *MFN2*), to evaluate the incidence and effect of identified pathogenic variants on the phenotype in patients with Charcot-Marie-Tooth disease.

3. To investigate the genetic region of *MT-ATP6/8* in patients with Charcot-Marie-Tooth type 2 and Charcot-Marie-Tooth intermediate type, to evaluate the incidence and phenotype of the identified pathogenic variants in these groups.
4. To determine and evaluate the incidence of mitochondrial DNA pathogenic variants and the clinical manifestation of neuropathy in the group of patients with classical mitochondrial syndromes.
5. To improve genetic diagnosis using next-generation sequencing technologies and to determine the diagnostic value of the targeted gene panel associated with Charcot-Marie-Tooth disease and related neuropathies in a group of patients with Charcot-Marie- Tooth disease.

Defended Statements

1. When suspecting the mitochondrial syndrome, first of all it is necessary to investigate the most common pathogenic variants of mtDNA, and afterwards, if the molecular diagnosis is not set, perform the complete mtDNA sequencing.
2. The distribution of CMT1A and the manifestation of phenotypes do not differ from the literature data.
3. The pathogenic variants of the *GJB1* gene are the second-most common molecular cause in patients with Charcot-Marie-Tooth disease.
4. Research based on next-generation sequencing technology is an effective tool for rational molecular diagnosis in hereditary neuropathies.

The Scientific Novelty of the Study and Implementation in Clinical Practice

There are no scientific studies in the field of genetic heterogeneity and prevalence of hereditary neuropathies in Lithuania.

The molecular research strategy for patients with hereditary neuropathies in Lithuania is not defined. The aim of this work was to identify the most common causes of CMT in Lithuania and improve the molecular diagnostic strategy of patients with CMT, which would be useful in providing personal health care to patients and would also be useful to their supervising physicians.

MtDNA sequencing for diagnostic purposes has never been performed in Lithuanian clinical genetics practice. The results obtained during the present study allowed to identify molecular causes of mtDNA in a group of patients with mitochondrial syndromes. The obtained results show that molecular research on mtDNA is useful to patients and is important for the differentiation of these phenotypically heterogeneous disorders.

New genetic factors identified during present study have provided more knowledge about the molecular etiology and its impact on the phenotypic spectrum of hereditary neuropathies.

Materials and Methods

Patients Studied

A total of 163 patients were enrolled in the study and composed two groups. Sixteen patients were included in a group based on diagnostic criteria of mitochondrial disorder. Others were attributed to a second group. 122 patients were considered to have Charcot-Marie-Tooth disease based on family history, neurological and neurophysiological examinations after excluding other acquired causes. These patients were classified as demyelinating (CMT1, n = 64), axonal (CMT2, n = 37) or intermediate (CMT-I, n = 21). 22 patients were also included with suspected HNPP or motor neuropathy by means of neurophysiological measurements, i.e. 17 patients with a medical history of transient pareses and/or sensory loss related to typical nerve compression points (pressure palsies), conduction blocks and mild demyelinating neuropathy were considered for a clinical phenotype of hereditary neuropathy with liability to pressure palsies and five patients with motor neuropathy. Three pediatric patients were included in the research study with phenotypicaly unspecified neuromuscular disease with prominent neuropathy features. The present study was approved by the Vilnius Regional Research Ethics Committee.

Retrospective Study

Retrospective study was conducted of 122 unrelated CMT patients who had been referred to a clinical diagnostic laboratory for *PMP22* region analysis from January 2012 to December 2017. 38 (31.14%) patients reached a molecular diagnosis of CMT1A. Clinical data of 37 patients were reviewed by analysing available medical records and compared between female and male patients. In addition, we evaluated phenotypic features of six patients who reached molecular diagnosis of HNPP.

Prospective Study

Most of the genetic studies in the present work have been performed prospectively. The genetic testing shares a common methodological workflow based on the generation of genetic data, analysis and interpretation, and a consecutive validation of the findings. The results were reported back to the physicians and were used in the clinical care of these patients.

Genetic Analysis

Blood samples were collected from all patients and their family members where appropriate. In addition, urine samples were collected from all 16 patients with mitochondrial syndrome. The whole genomic DNA was extracted from peripheral blood and urine sediment cells using the standard phenol-chloroform-isoamyl alcohol extraction method.

Mitochondrial genome analysis. Complete mitochondrial genome (mtDNA) sequencing was performed by Sanger sequencing using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), on automated genetic analyser ABI PRISM 3130xl (Applied Biosystems). The data were analysed and edited, respectively, by software program BioEdit 7.2.6.1. The obtained sequences were compared with the revised Cambridge Reference Sequence (rCRS) from the MITOMAP database (www.mitomap.org). Variants were considered rare when they occurred ≤ 10 times on the MITOMAP database with 32,059 mtDNA sequences. Long-range PCR was performed for all patients for the detection of deletions/duplications of mtDNA.

Single gene sequencing. The genetic testing of genes *GJB1*, *MFN2*, *MPZ*, *PMP22* and *MT-ATP6/8* was carried out by Sanger sequencing. It was performed by PCR and the sequencing reactions of each gene, analysing all coding exons and their intronic flanking sequences, except the *GJB1* gene, for which the part of the promoter sequence was also examined. All coding exons of the relevant genes were covered both by forward and reverse strand sequencing using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and the ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems) according to the manufacturer's protocol. The obtained sequences were compared with the reference sequences listed in the www.ensembl.org database. *MT-ATP6/8* gene sequences were analysed as mentioned above. Novel sequence variations were analysed with the most used *in silico* prediction programs to predict their functional effects.

Next-generation sequencing (NGS). A custom-designed gene panel was used for sequence detection for 34 subjects. The panel targeted the coding regions (+25 base pairs at exon-intron boundaries) of 150 genes associated with CMT and related hereditary neuropathies. The panel was designed for Ion AmpliSeq™ technology (Ion Torrent, Thermo Fisher Scientific). The DNA libraries were sequenced on an Ion PGM™ Sequencer (Life Technologies). The obtained genetic data were analysed and annotated. FASTQ sequencing files were aligned to human genome assembly GRCh37 on the Ion Torrent Suite™ Server. The average mean read depth was 168.13 ± 61.32 fold coverage in more than 99% of the targeted regions. Variants with population frequency over 1% in the dbSNP v137, Exome Variant Server (EVS;

http://evs.gs.washington.edu/EVS/) and 1000 Genome (http://www.1000genomes.org/) databases were filtered out. Only variants predicted to affect the coding regions (including non- synonymous, predicted missense, nonsense, splice acceptor and donor site, and insertions or deletions) were selected for further analysis. Several *in silico* prediction programs (MutationTaster, PolyPhen-2, SIFT) were used to predict the functional effect as well as the evolutionary conservation score, etc. Variants were correlated with patients' phenotypes and the results of clinical and neurophysiological investigations. Sanger sequencing with specific primers was conducted to confirm the selected variants.

Whole exome sequencing (WES). WES was performed on three of the affected paediatric patients and three adult patients with negative results after targeted NGS in cooperation with partners from Germany and Japan. Exome libraries were captured using hybridization with the Agilent SureSelect V2, and WES performed on an Illumina HiSeq 2000 (Illumina Inc.). High-throughput sequencer data were filtered based on inheritance models, the frequency of the variants in large datasets and the variants' effect.

Statistical analysis. Free software environment R, version 3.4.3, and Excel (Microsoft) 2016, were used to generate descriptive statistics. Continuous quantitative variables are presented as means ± Standard Deviation (SD). Categorical variables are presented as numbers and percentages. The Shapiro-Wilk test was used to determine the normal distribution of the data. If the data was not normally distributed, the non-parametric Mann-Whitney rank-sum test was performed. Group differences were analysed by t-test (2-tailed, unpaired). Probability values of $p < 0.05$ were considered statistically significant.

Results

Results of Retrospective Studies

Evalutation of CMT1A Cohort Phenotypes

A total of 38 (31.14%, 38/122) unrelated CMT1A patients were diagnosed between 2012 and 2017. Clinical data were available in 37 patients. The mean age at the time of onset was 9.08 ± 8.37 years, and the mean age at molecularly confirmed diagnosis of CMT1A was 28.10 ± 17.82 years. The mean age at the time of onset was 9.80 ± 9.27 in females, and 8.23 ± 7.36 in males. The mean age at molecularly confirmed diagnosis was 34.25 ± 19.60 in females, and 20.88 ± 12.47 in males (Fig. 1).

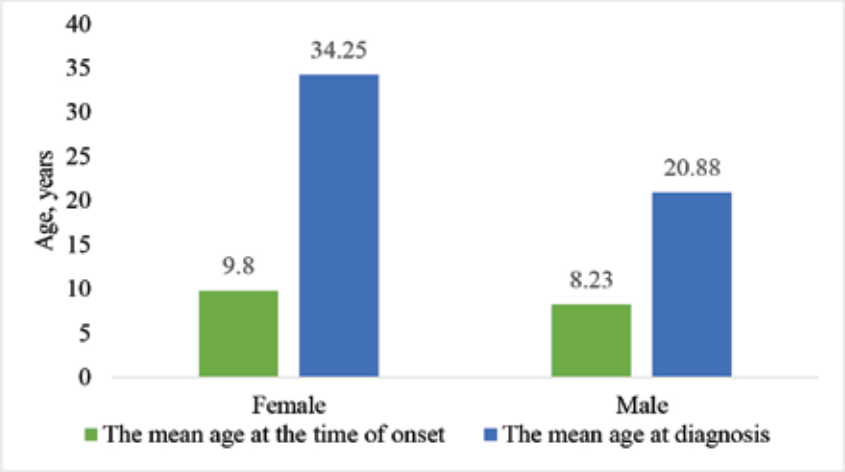


FIGURE 1. The age distribution between genders among the CMT1A patients.

The most common presented complaints were difficulties in walking, running and jumping. Atypical presentation in our cohort observed one male patient with predominantly upper limb involvement (postural hand tremor and difficulty writing). In female patients, sensory impairment in the lower limbs and absent tendon reflexes were significantly frequent, $p < 0.05$. Female patients received a rehabilitation treatment significantly more periodically, $p < 0.05$. An occasional finding was scoliosis. Auditory disturbances were not observed in our cohort. The clinical characteristics recorded in the mutation-positive patients with CMT1A are summarized in Table 1.

TABLE 1. Clinical characteristics of CMT1A patients analyzed in this study

Items	Female, n=20	Male, n=17	Total, n=37	p
Mean age at onset, years	9.80 ± 9.27	8.23 ± 7.36	–	0.713
Mean age at diagnosis, years	34.25 ± 19.60	20.88 ± 12.47	–	0.067
Initial symptoms • Lower limb, % • Upper limb, %	 20 (100 %) –	 16 (94.12 %) 1 (5.88 %)	 36 (97.30 %) 1 (2.70 %)	 0.460 0.460
Muscle weakness • Lower limb, % • Upper limb, %	 17 (85.00 %) 8 (40.00 %)	 12 (70.59 %) 3 (17.65 %)	 29 (78.38 %) 11 (29.73 %)	 0.428 0.138
Muscle atrophy	15 (75.00 %)	9 (52.94 %)	24 (64.86 %)	0.161

Items	Female, n=20	Male, n=17	Total, n=37	p
Sensory impairment • Lower limb, % • Upper limb, %	 16 (80.00 %) 7 (35.00 %)	 7 (41.18 %) 2 (11.76 %)	 23 (62.16 %) 9 (24.32 %)	 0.015* 0.137
Absent tendon reflexes • Lower limb, % • Upper limb, %	 20 (100 %) 6 (30.00 %)	 11 (64.71 %) 1 (5.88 %)	 31 (83.78 %) 7 (18.92 %)	 0.005* 0.097
Gait disturbance, %	12 (60.00 %)	7 (41.18 %)	19 (51.35 %)	0.254
Gait disturbance on heels, %	19 (95.00 %)	15 (88.24 %)	34 (91.89 %)	0.584
Foot deformation, %	19 (95.00 %)	15 (88.24 %)	34 (91.89 %)	0.584
Hand tremor, %	6 (30.00 %)	4 (23.53 %)	10 (27.03 %)	0.725
Medication for pain in legs, %	6 (30.00 %)	2 (11.76 %)	8 (21.62 %)	0.246
Surgery, %	3 (15.00 %)	4 (23.53 %)	7 (18.92 %)	0.680
Rehabilitation, %	14 (70.00 %)	4 (23.53 %)	18 (48.65 %)	0.005*

* statistically significant p value.

Phenotypic Findings of Patients with Hereditary Neuropathy with Liability to Pressure Palsie s

In the retrospective study of this cohort, we analyzed the phenotypic data of six patients with a deletion of CMT1A genetic domain that determined HNPP. The mean age of the initial symptoms was 24.83 ± 8.95 years, and the mean age at di-

TABLE 2. Demographic and phenotypic data of the HNPP patients

Gender	Age at diagnosis, y	Age at onset, y	**Classic** phenotype		**Atypic** phenotype		Positive genealogy	**NCV (*N.medianus*)**	
			Reccurent palsies	Isolated palsy	CMT-like disease	Chronic sensory neuropathy		< 38 m/s	> 38 m/s
F	56	20	-	-	+	-	+	+	-
M	38	36	-	-	-	+	+	-	+
M	31	22	+	-	-	-	-	+	-
M	17	15	-	-	+	-	-	+	-
M	34	20	+	-	-	-	+	-	+
F	36	36	+	-	-	-	-	-	+

F: female; M: male; +: yes, -: no.

agnosis was 35.33 ± 12.58 years. The genealogy data of three patients showed AD inheritance. Neuropathic pain and provocative factors have not been reported by any patient. The classic HNPP phenotype was found in 50% of patients. The phenotype of CMT-like disease was present in 33.33%, and chronic sensory neuropathy in 16.67% of patients. In all patients, the initial symptoms were leg insensitivity and numbness. Foot deformity (*pes cavus*) was observed in only one patient.

The results of the NCV study showed an increase in the rate of conduction of the middle nerve. In three patients, the results of the NCV showed a motor and sensory demyelinating type of neuropathy after detecting a slow pulse rate at extended distal latent periods. Patients' demographic and phenotypic data are summarized in Table 2.

Results of Prospective Studies

Mitochondrial Genome Findings and Phenotypes of Patients with Primary Mitochondrial Syndromes

We ascertained 16 patients with suspicion of mitochondrial syndrome (between 2015 and 2017) on the basis of clinical criteria for mitochondrial disorders (*Leigh* syndrome n = 3; Mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS) n = 4; Neuropathy, ataxia and retinitis pigmentosa (NARP) n = 1; Progressive External Ophthalmoplegia n = 4; *Kearns- Sayre* syndrome (KSS) n = 1; Leber hereditary optic neuropathy (LHON) n = 3). Objective signs of neuropathy were observed in all patients. Other clinical findings classically showed a multisystem involvement and the findings differed amongst all patients. Seven patients showed pathogenic variants in the mitochondrial genome. Maternally inherited *Leigh* syndrome was confirmed for two children (patient 1 and patient 2) with pathogenic variants NC_012920.1: m.9185T>C (p.(Leu220Pro)) and NC_012920.1:m.8993T>C (p.Leu156Pro)) in the *MT-ATP6* gene, respectively. Brain MRI abnormalities in these *Leigh* syndrome patients included symmetric alterations to the basal ganglia or brain stem. Other phenotypic findings are summarised in Table 3.

Three adult patients (patients 3, 4 and 5) were identified as harbouring the pathogenic variant NC_012920.1:m.3243A>G (p.(Ala3243Gly)) in the *MT-TL1* gene corresponding with MELAS, and in one patient (patient 6) the MERRF-like syndrome was concluded by detecting the new variant NC_012920.1:m.8353T>C *tRNA (Lys)* gene. Phenotypically, all three patients with m.3243A>G had short stature, ptosis, hearing impairment, dysarthria, dysphonia, feeding difficulties, exercise intolerance and muscle weakness as well gastrointestinal dysmotility, diarrhoea, constipation and abdominal distension (Table 4).

TABLE 3. Phenotypic findings of patients with *Leigh* syndrome

Organ systems	Pt 1	Pt 2
Age of onset	By 6 months	By 6 months
Motor delay	+	+
Ocular signs (nystagmus, strabismus)	+	+
Dysphagy	+	-
Epilepsy	-	-
Failure to thrive	+	-
Hearing impairment	-	-
Breathing disorders	-	-
Cardiologic dysfunction	-	-
Liver dysfunction	-	-
Renal dysfunction	-	-
Haematologic disorders	-	-
Neuropathy	+	+
Growth delay	-	-
Microcephaly	-	-
Intellectual dissability	-	-

Pt: patient; +: yes; -: no.

TABLE 4. Phenotypic characterization of the three female patients with m.3243A>G included in this study

Organ systems	Features	Pt 3	Pt 4	Pt5
	Onset	2rd-3rd decade	1st-2nd decade	2rd-3rd decade
	Age at last examination, years	36	30	55
	Height, cm	157	162	162
	Weight, kg	33	58	45
	BMI	13.4	22.1	17.1
CNS	Stroke/stroke-like episodes	-	-	-
	MRI findings	Brain atrophy, cerebellar atrophy	Subcortical lesions in the occipital lobes	Subcortical lesions
	Encephalopathy	-	-	-
	Cognitive dysfunction	-	-	+
	Ataxia	+	-	-
	Seizures	-	-	-
	Migraine	-	-	-

Organ systems	Features	Pt 3	Pt 4	Pt5
PNS	Neuropathy/hyporeflexia	+	+	+
Muscular	Myopathy/weakness	+	+	+
	Exercise intolerance	+	+	+
	Myopatic EMG	-	+	-
Ear	Hearing loss	+	+	+
Eye	Ptosis/ophthalmoplegia	+	+	+
	Pigmentary retinopathy	+	-	-
Gastro-intestinal	GI dysmotility	+	+	+
	Feeding difficulties	+	+	+
Heart	Cardiomyopathy	-	-	-
	ECG abnormalities	+	-	-
Liver	Hepatic dysfunction	-	-	+
Endocrine	DM type I	+	-	-
	DM type II	-	-	+
Metabolic	Lactic acidosis	-	+	-

Pt: patient; BMI: body mass index; DM: diabetes mellitus; ECG: electro- cardiography; EMG: electromyography; MRI: magnetic resonance imaging; +: yes; –: no.

One patient (patient 7) of the 16 patients studied for mtDNA deletions and duplications was identified as carrying the novel 5888 bp mtDNA deletion NC_012920.1:m.6069_11956del. This patient exhibited a classical *Kearns-Sayre* syndrome phenotype.

Results of Single Gene Analysis

GJB1 gene

We identified 5 pathogenic and/or likely pathogenic *GJB1* variants in 6 patients, and 2 of them were novel: the missense hemizygous variant NM_001097642.2:c.290A>G (p.(His97Arg)) and heterozygous frameshift deletion NM_000166.5:c.476delG (p.(Gly159Alafs)). The phenotypes and genotypes of patients with *GJB1* variants in this study are summarized in Tables 5 and 7, respectively.

TABLE 5. Phenotypic features of patients carrying *GJB1* variants

Items	Pt 1	Pt 2	Pt 3	Pt 4	Pt 5	Pt 6
Gender	F	M	M	M	F	M
Variant	c.34G>A	c.43C>T	c.290A>G	c.290A>G	c.476delG	c.547C>T

Items	Pt 1	Pt 2	Pt 3	Pt 4	Pt 5	Pt 6
CMT type*	CMT2	CMT-T	CMT-T	CMT2	CMT1	CMT-T
Age at onset, years	15	8	16	15	35	12
Age at diagnosis, years	21	28	29	19	36	12
Initial symptoms	Distal limb numbness	Steppage gait	Steppage gait	Steppage gait, distal muscle weakness	Right foot drop and numbness	Acute transient CNS manifestations
Muscle weakness (UL/LL)	- /+	++/++	+/++	++/++	-/++	-/-
Muscle atrophy	Mild	Severe	Moderate	Moderate	Mild	-
Sensory disturbance	-	V	P>V	V	-	-
Tendon reflex (knee/ ankles)	B/B	A/A	A/A	B/A	B/A	B/A
Foot deformity	+	+	+	+	+	+
Other symptoms	Tremor, cognitive impairment	-	Tremor	Scoliosis	-	-

Pt: patient; UL: Upper limb; LL: Lower limb; Muscle weakness: -: Normal; +: Distal weakness ≥4/5 on MRC; + +: Distal weakness <4/5 on MRC scale. P: Pinprick; T: Touch; V: Vibratory; Tendon reflex: B: brisk; A: absent; Foot deformity: +: *pes cavus*; -: not observed; CMT: Charcot-Marie-Tooth; * based on nerve conduction velocity findings.

MPZ gene

The sequencing of the *MPZ* gene was performed for 76 patients. The pathogenic variants of the *MPZ* gene were detected in 2 (2.63%) patients. PN occurring during infancy associated with pathogenic variants of the *MPZ* gene is characterized by delayed self-paced walking when children begin to walk from at least 15 months of age (on average 18-48 months) and symptoms up to 5 years of age. Patient 1 is a 4-year-old girl who presented a generalized hypotonia at 2 months of age with a history of gradual weakness, more prominent in the upper limbs proximally. Respiratory and bulbar difficulties led to tracheostomy tube and gastrostomy tube at 4 and 8 months, respectively. Deep tendon reflexes were absent. Needle EMG/NCVs indicated very severe sensorimotor demyelinating- axonal polyneuropathy with predominant severe demyelination. WES identified a novel *de novo* heterozygous frameshift deletion NM_000530.7:c.558delG (p.(Arg196Serfs*)) in the *MPZ* gene.

Patient 2 is a 7-year-old girl who presented generalized hypotonia and delayed gross motor skills. She was able to walk independently at the age of 2 years and 4 months. Her neurological examination revealed an inability to walk on heels or toes, proximal and distal weakness. Deep tendon reflexes were preserved. Needle EMG/NCVs demonstrated borderline CMAP amplitude with neurogenic changes on needle examination, asymmetric, length-dependent, sensory- predominant sensorimotor, primarily demyelinating polyneuropathy. She harboured a novel *de novo* heterozygous missense mutation of NM_000530.7:c.263A>G (p.(Tyr88Cys)) in the *MPZ* gene. Characterization of the novel *MPZ* variants are presented in Table 7.

MFN2 gene

We performed a *MFN2* gene screening in a clinically well- characterized group of 54 unrelated index patients with CMT2 (n=34) and CMT-I (n=18). We detected a pathogenic variant in one patient (1.85%) with CMT2. The patient carried a heterozygous mutation NM_014874.3:c.281G>A (p.(Arg94Gln)) in close proximity of the GTPase domain. At the onset of the disease at the age of 12, foot instability, *pes cavus* and painful involuntary muscle contractions occurred. These symptoms have slowly progressed over time. At the age of 32, the patient had mild sensory impairment in the hands, distal sensory loss in her legs, distal muscle weakness and atrophy. ENG revealed a reduced amplitude of the compound motor nerve action potentials of the peroneal and tibial nerves and normal nerve conduction velocities (Table 6).

TABLE 6. Motor and sensory nerve conduction velocity results

	Nerve	Amplitude (uV) (ref.)	Latency (mS) (ref.)	Conduction velocity (m/s) (ref.)
Motor nerve	Median	8.75 (3.9)	3.96 (3.9)	54.3 (48.4)
	Ulnar	7.35 (4.8)	2.74 (3.7)	50.0 (49.9)
	Right nervi tibialis	0.66 (3.0)	3.80 (3.9)	37.4 (40.3)
	Left nervi tibialis	2.97 (3.0)	4.30 (3.9)	41.1 (40.3)
	Right peroneal nerve	9.92 (3.8)	3.30 (4.3)	41.1 (40.0)
	Left peroneal nerve	9.51 (3.8)	2.90 (4.3)	37.2 (40.0)
Sensory nerve	Median	10.7 (15.1)	4.60 (3.7)	38.0 (48.1)
	Ulnar	Not elicited		
	Suralis	Not elicited		

PMP22 gene

The sequencing of the *PMP22* gene was performed for 31 patients (CMT1, n = 15, and HNPP, n = 16). Only the previously reported pathogenic variant

NM_000304.3:c.319+1G>A (p.?) was identified in intron 4 of the *PMP22* gene in a patient with CMT1. The patient developed gait abnormalities from the age of 16. Neurological examinations at age 20 demonstrated diffusely diminished deep tendon reflexes in the legs. NCVs showed generalized prolonged distal latencies and F-wave latencies of the motor or sensory nerves and markedly decreased motor and sensory nerve conduction velocities in the hands.

TABLE 7. Pathogenic/likely pathogenic and novel variants identified by the use of single gene analysis.

Variant	Genotype	Data from large databases[1]: 1000G; ESP; ExAC	*In silico* prediction[2]: GERP; SIFT; PP2; CADD
NM_000166.5(*GJB1*):c.34G>A (p.(Gly12Ser))	htz	-	5.63; 0*; 1*; 34*
NM_000166.5(*GJB1*):c.43C>T (p.(Arg15Trp))	hmi	-	4.36; 0*; 0.99*; 31*
NM_000166.5(*GJB1*):c.290A>G (p.(His97Arg))	hmi	-	4.67; 0*; 0.79*; 19.98*
NM_000166.5(*GJB1*):c.476delG (p.(Gly159Alafs))	htz	-	4.81; -; -; -; -
NM_000166.5(*GJB1*):c.547C>T (p.(Arg183Cys))	hmi	-	4.99; 0*; 1*; 29.2*
NM_000530.7(*MPZ*):c.263A>G (p.(Tyr88Cys))	htz	-	4.69; 0*; 0.99*; 25.1*
NM_000530.7(*MPZ*):c.558delG (p.(Arg186Serfs))	htz	-	4.7 ; -; 0.97*; -
NM_014874.3(*MFN2*):c.281G>A (p.(Arg94Gln))	htz	-	5.4; 0*; 0.99*; 30*
NM_000304.3(*PMP22*):c.319+1G>A (p.?)	htz	-	5.76; -; -; -

Genotype: hmi: hemizygous; htz: heterozygous;
[1] 1000G: 1000 Genome project (http://www.1000genomes.org); ESP: Exome Sequencing Project (http://evs.gs.washington.edu/EVS/); ExAC: Exome Aggregation Consortium (http://exac.broadinstitute.org/);
[2] GERP: Genomic Evolutionary Rate Profiling; PP2: PolyPhen2 (http://genetics.bwh.harvardPedu/pph2/); SIFT: (http://sift.jcvi.org/); CADD: (https://cadd.gs.washington.edu/);
* predicts pathogenicity.

MT-ATP6/8 gene

A standard PCR reaction was performed to amplify overlapping genes *MT-ATP6/8* in 49 CMT patients (CMT2, n = 34, and CMT-I, n = 15). All patients showed negative results after CMT1A genetic locus analysis and single genes, the *GJB1*, *MPZ* and *MFN2*, sequencing. Patients whose genealogy data showed a clear male-to-male inheritance were not included in the study. No pathogenic variants of *MT-ATP6/8* genes have been identified in this group. Since the mitochondrial genome is characterized by significant variability, all non-pathogenic changes identified in this study are presented in Table 8.

TABLE 8. mtDNA variants identified in this study

Variant	Number of patients	State
CO2:m.8167T>C (p.(Gly194=))	1	homoplasmic
CO2:m.8251G>A (p.(Gly222=))	2	homoplasmic
NC7:m.8270_8278delCACCCCCTC	1	homoplasmic
NC7:m.8289_8290insCCCCCTCTA	2	homoplasmic
ATP8:m.8395C>T (p.(Pro10=))	1	homoplasmic
ATP8:m.8410C>T (p.(Pro15=))	1	homoplasmic
ATP8:m.8422A>G (p.(Thr19=))	1	homoplasmic
ATP8:m.8464C>T (p.(Tyr33=))	1	homoplasmic
ATP8:m.8472C>T (p.(Pro36Leu))	1	homoplasmic
ATP8:m.8473T>C (p.(Pro36=))	1	homoplasmic
ATP8:m.8512A>G (p.(Lys49=))	1	homoplasmic
ATP6:m.8610T>C (p.(Pro28=))	2	homoplasmic
ATP6:m.8697G>A (p.(Met57=))	5	homoplasmic
ATP6:m.8715T>C (p.(Thr63=))	1	homoplasmic
ATP6:m.8818C>T (p.(Leu98=))	1	homoplasmic
ATP6:m.8825T>C (p.(Met100Thr))	1	homoplasmic
ATP6:m.8836A>G (p.(Met104Val))	1	homoplasmic
ATP6:m.8860A>G (p.(Thr112Ala))	49	homoplasmic
ATP6:m.8994G>A (p.(Leu156=))	2	homoplasmic
ATP6:m.9055G>A (p.(Ala177Thr))	2	homoplasmic
ATP6:m.9123G>A (p.(Leu199=))	1	homoplasmic
ATP6:m.9614T>C (p.(Leu30=))	2	homoplasmic

Efficacy of targe tednext-generation sequencing

In this study we analyzed 34 patients with CMT and evaluated the effectiveness of the targeted sequencing of a CMT gene panel using NGS. The studied group consisted of 34 patients with no evidence of duplication in the genetic locus comprising

the *PMP22* gene, and no pathogenic variants of the *GJB1*, *MPZ* and *MFN2* genes using the Sanger sequencing method. Patient selection was based on an individual assessment, which included genealogy data, clinical neurological findings and NCV results. In suspicion of AR inheritance, we selected all patients with early onset and severe phenotype, scoliosis, foot deformities and other accompanying symptoms. For other patients, an AD inheritance pattern was suspected.

In the NGS group, 25 (73.53%, 25/34) of the patients were selected with CMT2 type diagnosed by clinical evaluation, because after genetic testing of the most common CMT genes, the genetic defect rate was the lowest in this group. Five (14.71%, 5/34) and four (11.76%, 4/34) patients were assigned for CMT-I and CMT1 types, respectively. The ratio of women to men was 0.89. The mean age at the time of the CMT diagnosis was 26.79 ± 17.46 years. The mean age at the time of the first symptoms was 20.26 ± 17.63 years. The predominant first symptoms were gait disturbances and foot deformities. Analysis of the targeted gene panel NGS results revealed that pathogenic or likely pathogenic variants were detected at 29.41% (10/34), novel pathogenic variants were identified 17.65% (6/34), and variants of unknown clinical significance in 17.65% (6/34) of patients. Characteristics of pathogenic, novel pathogenic and variants of unknown clinical significance are presented in Table 9.

TABLE 9. Characteristics of variants identified by the targeted NGS panel.

Variant	Genotype	Data from large databases[1]: 1000G; ESP; ExAC	*In silico* prediction[2]: GERP; SIFT; PP2; CADD
Pathogenic/likely pathogenic variant			
NM_001122955.3(*BSCL2*):c. 455A>G (p.(Asn152Ser))	htz	-	5.63; 0*; 1*; 34*
NM_001005361(*DNM2*): c.1609G>A (p.(Gly537Ser))	htz	-	5.36; 0.06*; 0.99*; 27.4*
NM_018972.2(*GDAP1*): c.844C>T (p.(Arg282Cys))	hmz	-	4.99; 0*; 1*; 35*
NM_000166.5(*GJB1*): c.34G>A (p.(Gly12Ser))	htz	-	4.36; 0*; 0.99*; 25.6*
NM_005340.6(*HINT1*): c.110G>C (p.(Arg37Pro))	hmz; htz	0.0002; 0.0001; 0.0001	-0.93; 0.02*; 0.115; 23.3*
NM_000304.3(*PMP22*): c.353C>T (p.(Thr118Met))	htz	0.0008; 0.0053; 0.0029	5.15; 0*; 1*; 28.8*
NM_021625.4(*TRPV4*): c.68G>A (p.(Ser23Asn))	htz	-	3.62; 0.01*; 0.12; 17.05

Variant	Genotype	Data from large databases[1]: 1000G; ESP; ExAC	*In silico* prediction[2]: GERP; SIFT; PP2; CADD
Novel pathogenic variant			
NM_018188.3(*ATAD3A*): c.556C>T (p.(Arg186Trp))	htz	-; 0.0001; 0.00004	3.87; 0*; 1*; 34*
NM_001605.2(*AARS*): c.1643T>C (p.(Leu548Pro))	htz	-	6.15; 0*; 0.93*; 31*
NM_004082(*DCTN1*): c.1295C>G (p.(Ala432Gly))	htz	-	5.65; 0.01*; 1*; 27.9*
NM_021629(*GBN4*): c.847C>T (p.(Arg283Cys))	htz	-	4.51; 0.01*; 0,99*; 35*
NM_004990.3(*MARS*):c.1340G>A (p.(Ser447Asn))	htz	-	3.88; 0*; 0.948*; 23.7*
NM_001136472.1(*LITAF*):c.418G>A (p.(Ala140Thr))	htz	-; -; 0.00001	5.01; 0.03*; 0.64; 27.1*
Variants of unknown clinical significance			
NM_014629.3 (*ARHGEF10*):c.2197C>T (p.(His733Tyr))	htz	0.001; 0.0016; 0.001	3.95; 0.03*; 0.76*; 23.1*
NM_014629.2(*ARHGEF10*): c.2456T>C (p.(Val819Ala))	htz	0.0002; -; 0.0001	-0.02; 0.02*; 0.12; 12.9
NM_018188.4(*ATAD3A*): c.1847A>T (p.(Lys616Met))	htz	0.0017; -; 0.0034	2.75; 0.02*; 0.99*; 23.7*
NM_000902.3(*MME*): c.157G>A (p.(Asp53Asn))	htz	-; 0.0001; 0.0002	5.24; 0.43; 0.01; 18.72
NM_005340.6(*HINT1*): c.299A>G (p.(Glu100Gly))	htz	-	3.52; 0.04*; 0.19; 24.9*
NM_021625.4(*TRPV4*): c.2518G>A (p.(Glu840Lys))	htz	0.0024; 0.0095; 0.0053	5.39; 0.01*; 0.11; 26.8*

Genotype: htz: heterozygous; hmz: homozygous;
[1] 1000G: 1000 Genome project (http://www.1000genomes.org); ESP: Exome Sequencing Project (http://evs.gs.washington.edu/EVS/); ExAC: Exome Aggregation Consortium (http://exac.broadinstitute.org/);
[2] GERP: Genomic Evolutionary Rate Profiling; PP2: PolyPhen2 (http://genetics.bwh.harvardPedu/pph2/); SIFT: (http://sift.jcvi.org/); CADD: (https://cadd.gs.washington.edu/);
* predicts pathogenicity.

The diagnostic yield of the targeted 150 gene panel NGS was 44.12% (15/34) in the studied cohort of CMT patients. However, slightly more than half of the patients remained with unidentified molecular CMT diagnosis. *GJB1* gene pathogenic variant NM_001097642.2:c.34G>A (p.(Gly12Ser)) was not identified with the Sanger sequencing method. *HINT1* gene pathogenic variants were identified in three patients with neuromyotonia and axonal neuropathy. Digenic inheritance was identified in one patient.

Results of Whole Exome Sequencing

Six patients (three children with unspecified neuromuscular disease and three adults with pure CMT2) were included in this study. In the three adult patients we did not identify any pathogenic/likely pathogenic or variants of unknown significance that segregate with the CMT phenotype. Molecular genetic diagnoses were attributed to all three paediatric patients.

The heterozygous *de novo* deletion NM_000530.7:c.558delG (p.(Arg186Serfs)) in the *MPZ* gene was identified in a 7-year-old girl (described in the section *MPZ* gene). The second patient harboured a rare heterozygous missense variant NM_001376.4:c.1792C>T (p.(Arg598Cys)) in the *DYNC1H1* gene. This girl presented motor developmental delay with onset of independent walking at 17 months of age. Her gait was waddling and she was not able to crouch or climb stairs unaided. On examination at 5 years of age, a symmetric distal muscle weakness and hypotrophy of the lower limbs were observed. The muscle hypotrophy and weakness were mainly restricted to the distal lower limbs along with absent reflexes in the lower extremities. The symptoms were predominantly motor. NCVs were not performed due to their unavailability. *DYNC1H1* gene pathogenic variants are linked to two distinct phenotypes. Detailed phenotypic information is required for correlating potential disease-causing variants to the clinical phenotype of the patients. The identified pathogenic variant was *de novo* origin. A third patient was found to carry a compound heterozygous variants in the *SLC52A2* gene. This patient presented motor delay from early childhood, hypotonia, bulbar paralysis and, later, signs of involvement to the lower motor neuron. Two variants were identified by WES in the *SLC52A2* gene. The rare pathogenic missense variant NM_024531.4:c.1016T>C (p.(Leu339Pro)) was inherited from the father. The paternally inherited variant (c.1016T>C) had previously been reported for many patients. In vitro studies have shown that such an altered protein does not connect ^{3}H-riboflavin (Foley AR et al., 2014). Another novel missense variant NM_024531.4:c.377G>A (p.(Cys126Tyr)) was inherited from the mother. The maternally inherited missense variant (minor allele frequency < 0.01) had not been previously reported. The variant is evaluated as likely pathogenic based on DANN (0.99), GERP (3.65), LRT (0.84), Mutation-

Assessor (0.928), MutationTaster (1), SIFT (0), PolyPhen2 (0.99) and PROVEAN (-10.88) scores. Based on a phylogenetic point of view, the novel variant located in the moderately conservative reading frame results in a loss of the protein function and is a possible pathogenic mechanism that corresponds to previous pathogenicity observations.

Discussion

The overall aim of the present study was to elucidate novel genetic causes for hereditary neuropathies and to evaluate genotype- phenotype correlations in patients with hereditary neuropathies.

In the study of CMT1A and HNPP patients we do not observed any differences with literature data. We observed gender differences between CMT1A patients with regard of data of sensory impairment, absent tendon reflexes and rehabilitation treatment with higer deterioration in women. It could be explained by the older age at diagnosis and that female patients have finer perception of symptoms than men (Colomban C et al., 2014).

Phenotype-genotype correlations in 6 patients with the *GJB1* pathogenic variants indicate that, based on NCV criteria, these patients could be classified as CMT1, CMT-I or CMT2. The novel *GJB1* variant NM_001097642.2:c.290A>G (p.(His97Arg)) is associated with a clinically distinct phenotype characterized by classical onset, distal muscle weakness and marked sensory abnormalities. NCVs of the motor median nerve vary from marked axonal demage to intermediate values in these patients. A rare presentation was found in a patient harbouring the NM_001097642.2:c.547C>T (p.(Arg183Cys)) variant and these findings are in line with the literature data (Scherer SS and Kleopa KA, 2012). In the process of analyzing the most common CMT- related genes, two novel pathogenic variants of the *MPZ* gene were identified, one of which was identified by WES.

Mitochondrial DNA analysis in patients with mitochondrial syndromes have not previously been performed in Lithuania. In this study, seven patients were diagnosed using combined mtDNA molecular testing. Three of these patients had the most common variant of the *MT-TL1* gene m.3243A>G and two were confirmed to have maternally inherited *Leigh* syndrome. One patient harboured a novel deletion of mtDNA that is responsible for *Kearns-Sayre* syndrome.

A targeted next-generation sequencing study of 150 hereditary neuropathy-related genes was performed on 34 selected patients. We identified a diagnostic efficiency of the targeted gene panel NGS for 44.12% (15/34) of the patients. Thus, we identified pathogenic variants that confirmed the molecular CMT diagnosis in

less common CMT-associated genes, except for one patient who had not been diagnosed with the *GJB1* gene variant NM_000166.5:c.34G>A (p.Gly12Ser) by the Sanger sequencing method. Three patients were identified with pathogenic variants of the *HINT1* gene. The pathogenic variant NM_005340.6:c.110G>C (p.(Arg37Pro)) was detected in a homozygous condition for two patients, and in one heterozygote in combination with a novel variant of NM_005340.6:c.299A>G (p.(Glu100Gly)). Milovidova TB et al. tested 700 CMT patients in Russia and identified the *HINT1* gene homozygous variant NM_005340.6:c.110G>C (p.(Arg37Pro)) for 30 patients (http://www.abstractsonline.com/pp8/#!/4652/presentation/2285). In the Czech Republic, variants of the *HINT1* gene are also the most common cause of neuromyotonia and axonal neuropathy (Lassuthova P et al. 2015), but they are rare in Spain and the United Kingdom (Horga A et al., 2015). Based on these results, it is rational for patients with neuromyotonia and axonal neuropathy to first choose the *HINT1* gene NM_005340.6:c.110G>C (p.(Arg37Pro)) variant testing. The digenic inheritance CMT-I variant was identified for one patient by identifying variant NM_001005361:c.1609G>A (p.(Gly537Ser)) of the *DNM2* gene and a novel variant NM_021629:c.847C>T (p.(Arg283Cys)) of the *GNB4* gene. The digenic effect of two pathogenic variants in different genes may modulate the phenotype, since both gene products are involved in endosomal sorting and cell signaling, possibly interacting in similar pathways.

Targeted NGS efficiency in this study almost matched the expected frequency of molecular diagnosis, as phenotypes of the patients were clearly defined, and according to recent literature, molecular diagnosis identified with the NGS method is on average half of well-defined patients. WES was performed for six patients, of which three were identified with molecular disease causes. A patient who was confirmed with a type 2 riboflavin transporter deficiency has promptly obtained the results of the study and specific treatment was immediately introduced. This case highlights the clinical benefit of WES where a molecular diagnosis was established without delay and the patient started to be properly treated, avoiding alternative treatments that not only do not help but could even be harmful during intercurrent illnesses.

In conclusion, we described novel pathogenic variants that were identified by Sanger sequencing and NGS methods. NGS is an effective tool for determining molecular diagnosis in patients, particularly with rare and heterogeneous diseases, whose genetic causes of the single genes analysis are not determined by conventional molecular genetic methods. A defined molecular diagnosis is vital to guide patients' clinical care and informed genetic counselling and is often required for the inclusion of patients in clinical trials.

Conclusions

1. Charcot-Marie-Tooth disease type 1A is the most common form of CMT. It comprises two-thirds of CMT1 unrelated patients and one third of the entire CMT group during the period 2012 to 2017. No statistically significant gender differences were observed in patients with Charcot-Marie-Tooth disease type 1A ($p = 0.592$).
2. The pathogenic variants of the *GJB1* gene are the second most common genetic factors of Charcot-Marie-Tooth disease. Concurrently, the pathogenic variants of genes *MPZ* and *MFN2* are rarely determined in the analyzed CMT group.
3. The pathogenic variants of the genes *MT-ATP6/8* in this study are not identified.
4. Nearly half of the patients phenotypically diagnosed with classical mitochondrial syndrome have identified pathogenic variants of the mitochondrial DNA. These patients were observed with normal to mild appearance of axonal-type neuropathy according to the electrophysiological pattern.
5. The next-generation sequencing approach for a targeted gene setup is a valuable and an effective diagnostic tool for the molecular diagnosis of patients with comprehensively characterised Charcot-Marie-Tooth disease. However, substantial genetic and phenotype heterogeneity of CMT makes it challenging for molecular diagnosis by single gene and gene panel testing. Digenic inheritance may not be established if only single genetic studies are performed.

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Winner of a grant of the European Society of Human Genetics in 2017.

Rasa Strupaitė

EVALUATION OF CLINICAL AND GENETIC CHARACTERISTICS OF THE INHERITED RETINAL DYSTROPHIES

PAVELDIMŲ TINKLAINĖS DISTROFIJŲ KLINIKINIŲ IR GENETINIŲ CHARAKTERISTIKŲ VERTINIMAS

Biomedical Sciences, Medicine 06B
Biomedicinos mokslai, Medicina 06B

Abbreviations

1000 G	–	1000 Genomes Project
ACHM	–	achromatopsia
AD	–	autosomal dominant
AF	–	autofluorescence
Alt	–	alternative
AMD	–	age-related macular degeneration
AR	–	autosomal recessive
BCVA	–	best corrected visual acuity
BE	–	both eyes
CA	–	central atrophy
CD	–	cone dystrophy
CME	–	cystoid macular edema
CNV	–	choroidal neovascularisation
CRD	–	cone-rod dystrophy
CRT	–	central retinal thickness
SD	–	standard deviation
SCT	–	subfoveal choroidal thickness
DA 3.0 ERG	–	dark-adapted 3.0 ERG
EOG	–	electrooculography
ERG	–	electroretinography
ERM	–	epiretinal membrane
EZ	–	ellipsoid zone
FAF	–	fundus autofluorescence
FDD	–	familial dominant drusen
HWE	–	Hardy-Weinberg equation
LE	–	left eye
LITGEN	–	acronym for the project called diversity of the population of Lithuania and changes of its genetic structure related with evolution and common diseases“
mfERG	–	multifocal electroretinography
N	–	number
OND	–	optic nerve disc
RD	–	retinal dystrophy/ retinal dystrophies
RE	–	right eye
Ref	–	reference
RNFL	–	retinal nerve fiber layer
RP	–	retinitis pigmentosa
RPE	–	retinal pigment epithelium
RS1	–	retinoschisin 1
SD-OKT	–	spectral domain optical coherence tomography
SNP	–	single nucleotide polymorphism
STGD	–	Stargardt disease
VA	–	visual acuity
VMT	–	vitreomacular traction
XLRS	–	X linked retinoschisis
µm	–	mikrometer (10-6 m)
µV	–	mikrovolt (10-6 V)

Gene abbreviations

ABCA4	–	ATP-binding cassette, subfamily a, member 4
CNGB3	–	cyclic nucleotide-gated channel, beta-3
EFEMP1	–	EGF-containing fibulin-like extracellular matrix protein 1
PRPH2	–	peripherin 2, mouse, homolog of
RHO	–	rhodopsin

Introduction

Inherited retinal dystrophies (IRD) constitute a vast group of genetically determined diseases with different patterns of inheritance, vision disturbance and eye fundus presentation. Consequently, both clinical and genetic heterogeneity is characteristic to the disease. To date, changes causing IRD are identified in more than 190 genes and more than 40 genetic loci. Most of these conditions share common feature: loss or dysfunction of photoreceptors as primary or secondary cause [1]. Most of these diseases result in progressive loss of vision up to blindness. IRD is a serious problem, since these diseases are the most common causes of genetic blindness in the Western countries [2]. It should be noted that the disease is caused by genetic changes related to retinal degeneration only for some of the patients [3]. Due to complex and intricate structure and function of the retina even bigger heterogeneity of the disease is expected as well as discovery of new yet unknown genes and pathogenic variants.

Earlier retinal dystrophies were diagnosed and grouped according to clinical features (e. g., retinitis pigmentosa (RP), Stargardt disease). However, improvement of diagnostic capabilities in ophthalmology and impementation of genetic testing caused better understanding of aforementioned heterogeneity of diseases; for example, it became clear that retinitis pigmentosa is the same clinical presentation of a number of different genetic diseases. Nowadays more accurate classification of retinal diseases based on information provided by molecular genetic research is possible (e. g., *ABCA4* (ATP-binding cassette, subfamily a, member 4) retinopathy, rhodopsin RP, ciliopathies). For a long period of time genetic testing was limited due to its availability, cost and time consumption, but intense development of genetics causes sequential reduction of testing costs and increasing of tests availability.

Diagnostic of IRD frequently is intricate. Careful analysis of genealogy and comprehensive ophthalmologic examination including examination of color vision, optical coherent tomography (OCT), fundus autofluorescence (FAF) and electrophysiological testing s appropriate in these diseases.

However, even when all possible diagnostic tests are performed exact diagnosis of the disease and thus it's prognosis may remain unclear. In such case, genetic testing remains the only possibility to clarify diagnosis and provide patients with comprehensive information about his/her disease, its course and further life (work, hobby) prospects. Although these diseases, except some special cases, are incurable, patient seeks definite diagnosis in order to find out prognosis of the disease, risk of its transmission to his/her descendants, and hopes that new treatment modalities shall occur in the future.

IRD may present both as isolated and multisystemic lesions. Genetic testing of patient frequently is complicated. In some monogenic diseases such as X-linked retinoschisis which is usually caused by changes in *RS1* gene testing is rather simple. However, in the most common retinal dystrophy, RP, genetic testing becomes a real challenge as presently more than 2800 mutations in more than 100 genes are identified, and this number is constantly increasing. Therefore, diagnosis of RP is established only in 50% of patients. Furthermore, often presentation of the same genetic factor may be phenotypically differently expressed and thus diagnostic of the disease or genetic change may be complicated [4].

Multiple pre-clinical and clinical trials in various scientific centers around the world are being conducted in order to identify new modalities of the treatment of IRD. In 2007, gene therapy was applied for the first time in 3 patients with Leber's congenital amaurosis [5]. Comprehensive knowledge in the field of genetics allows to identify mutation-specific clinical features in several different phenotypes and develop pathophysiology hypothesis for experimental research [6].

To date there is no data concerning incidence of the IRD in Lithuania, and only global and European data may be used.

The following issues will be analyzed and discussed in this research study: which IRD are prevalent in Lithuania, whether their phenotype and genotype agree with literature data.

Aim of the research

To evaluate the clinical and genetic characteristics of inherited retinal dystrophies in a Lithuanian patient group.

Objectives of the research

1. To develop the monitoring system of the biomedical research in accordance with standardized patient inclusion criteria, to perform data analysis.
2. To identify known and diagnose new pathogenic variants of known genes in individuals with retinitis pigmentosa.
3. To assess phenotype and genotype-phenotype correlation in subjects with retinitis pigmentosa in the Lithuanian patient group.
4. To assess phenotype of *ABCA4* retinopathies, to characterize identified pathogenic variants.
5. To identify known and diagnose new pathogenic variants of known genes, to characterize phenotype in individual cases of rare retinal dystrophy.

Statements to be defended

1. The continuous monitoring system of the inherited retinal dystrophies helps to analyze the clinical data systematically and set up the base for the new research methods and the evaluation of their effectiveness.
2. The visual electrophysiology investigations are mandatory for the identification and progression evaluation of the inherited retinal dystrophies.
3. Inherited retinal dystrophies are characterized by a high genetic and phenotypic heterogeneity.

Novelty of the scientific research

This research is the first research performed in Lithuania to identify, analyze and classify IRD, and to perform their genetic testing and assess phenotype-genotype correlation.

According to global literature, every country seeks to identify phenotype and genotype of the IRD in their inhabitants because gene pool differs in different nations causing different type and course of the IRD.

During this research a non-invasive visual electrophysiological test was introduced into clinical practice, and one of the testing modes, multifocal electroretinography (mfERG) was applied in Lithuania for the first time. Visual electrophysiological testing is necessary for the identification and analysis of IRD because in the early stages of the disease differences between various diseases could be difficult to recognize; test is also important for the assessment of progression of the disease.

It was sought to assess character and prevalence of the IRD in the Lithuanian patients' group. For the first time in Lithuania group of patients with IRD was formed with comprehensive ophthalmological examination and genetic clinical testing of these patients was performed. For the first time genetic causes of wide spectrum of IRD were identified in the Lithuanian population. To the best of our knowledge, no research evaluating phenotype and genotype in patients with IRD were performed both in Lithuania and Baltic states.

A local monitoring systems of IRD was intended to be established in the Center of Eye Diseases, Clinic of ENT and Eyed Diseases, Institute of Clinical Medicine, Faculty of Medicine (Vilnius University) during the research.

Practical significance of the research

Identification of the causes of IRD allows to assess phenotype of the disease more accurately based on gene mutations described in the literature, and assess course of the disease. This also contributes to the rapidly emerging gene therapy

area. Multiple pre-clinical and treatments for the patients with IRD. However, to achieve this identification of precise phenotype and genotype of the disease is necessary which have been done in this study.

Scientific benefits of the research:

- Molecular genomic testing allows the identification of new candidate genes that are important for the etiopathogenesis of disturbance of vision due to retinal dystrophy.
- Analysis of medical data could allows the identification of relation between genes and disease characteristics.
- Analysis of medical data will allow the identification of etiologic structure of IRD in study population.

Benefits for patients:

- In this scientific research genetic changes causing the development of IRD in a patient may be identified, prognosis of the disease and risk for patient and for descendants of his family is assessed.

1. Scope and methodology of the research

1.1. Study design

Inclusion criteria:

- Patients with clinically diagnosed IRD.
- Patients with clinically suspected IRD.
- Age: 6-99 years.
- Completed full ophthalmological examination, specimen for molecular genetic testing was obtained.

As Lithuania has no database of patients with eye diseases including inherited eye diseases, patients were selected and invited to participate in this study according to the following procedure:

- Patients who have previously visited Consultation Polyclinic of the Vilnius University Hospital Santaros H35.5 (inherited retinal dystrophies) according to ICD (patients were selected according to the data stored in in the information database).
- Ophthalmologists and clinical geneticists working at Ophthalmology division of the Vilnius University Hospital Santaros Klinikos and Center of

Medical Genetics referred patients with the diagnostic code H35.5 according to ICD for the consultation concerning participation in the research.

- Ophthalmologists working in hospitals of Vilnius region referred patients with the diagnostic code H35.5 according to ICD for the consultation concerning participation in the research.

Upon confirmation of the diagnosis patients were included into research.

All patients were interviewed, and comprehensive ophthalmological and genetic examination was conducted.

Approval for this research No. 158200-15-770-287 of Vilnius Regional Bioethics Committee was obtained, Protocol No. 2, version 2 (February 3, 2015).

1.2. Methodology of the research

1.2.1. Counselling

The following data on clinical signs were collected: onset of the disease, course of the disease (non-progressive/progressive; rapid/slow progress), complaints and symptoms (nyctalopia, loss of visual field (tunnel vision), nystagmus, deterioration of vision, nature of deterioration of vision, photophobia), history of other eye diseases or ophthalmological drugs use, eye injuries and surgeries; patient was also asked about family history of similar symptoms or other inherited eye diseases. Demographic data were collected: place of residence, age and gender.

1.2.2. Ophthalmological examination

1. Objective refraction in both eyes was identified by auto kerato- refractometer KR-1 (Topcon, Japan) in the presence of mydriasis.
2. Assessment of the best corrected visual acuity (BCVA) according to Snellen decimal system.
3. Color vision testing (Ishihara test, 38 plates, Kanehara Trading Inc., Tokyo, Japan) at the distance of 40 cm.
4. Intraocular pressure (IOP) was measured using Goldmann applanation method (HAAG-STREIT International, tonometer AT-900, Bern Switzerland) under local anesthesia with 0.5% Proxymetacaine (Alcon-Couvreur, Belgium) when patient was sitting at slit-lamp. If not possible, non-contact tonometry was employed (Topcon CT-80A, Japan).
5. Standard automatic perimetry (SAP) was conducted using *Humphrey Field Analyzer* (Carl Zeiss MediTek, Dublin, CA, USA) in *central threshold 30-20 mode*. Only reliable results were assessed (fixations, false-negative and false-positive results were observed in d 33%). In other cases, kinetic *Goldmann spherical* visual field test was conducted.

6. Ophthalmobiomicroscopy (SL-Zoom-bon, Jen-ophthalmo, Jena, Germany) was conducted in the presence of mydriasis.
7. Images of the fundi of both eyes (BE) were taken in the presence of mydriasis (Topcon TRC retinal camera, type IA, 50DX).
8. Optical coherence tomography was conducted using Spectralis SD-OCT apparatus and Heidelberg Eye Explored operating system operating in HRA + OCT and enhanced depth imaging (EDI) mode (Heidelberg Spectralis Engineering, Dossenheim, Germany). The following characteristics were assessed:
   8.1. Thickness of the retinal nerve fiber layer (RNFL) (Pm);
   8.2. Central retinal thickness (CRS) at fovea) (Pm);
   8.3. Subfoveal choroidal thickness (SCT) (Pm) (to evaluate structure of chorioidea enhanced depth imaging (EDI) mode was employed);
   8.4. Anatomical integrity and structure of retina was assessed.
   8.5. Testing of autofluorescence of the fundi of BE was conducted in the presence of mydriasis using Spectralis HRA + OCT apparatus in AF mode (Heidelberg Engineering, Heidelberg, Germany).
9. Visual electrophysiological examination: subjects underwent full- field electroretinogram (ERG); subjects with sufficient visual acuity (VA) and ability to fixate the gaze underwent additional mfERG. Visual electrophysiological examination was conducted using Reti-port/scan 21 plus apparatus (Roland Consult Stasche & Finger GmbH, Brandenburg, Germany). Examination was conducted according to the protocols of the International Society for Clinical Electrophysiology of Vision (ISCEV).

Study was conducted in April 2015 – November 2017 in the Center of Eye Diseases of the Vilnius University Hospital Santariškių Klinikos (Since May 1, 2017 – Vilnius University Hospital Santaros Klinikos). The majority of tests were conducted by the author of the study. Tests were conducted during the daytime, from 8:00 to 16:00. Tests were conducted in accordance with principles of Helsinki Declaration, 1975 (amended in 2000).

1.3. Molecular genetic testing

Peripheral blood samples were collected for all study participants for molecular genetic testing. DNA extracted using phenol-chloroform method by the standardised protocol (no. 461) at the Laboratory for Molecular Genetics and Cytogenetics of Vilnius University Hospital Santaros Klinikos Center for Medical Genetics. DNA concentration and purity was detected using NanoDrop® spectrophotometer using NanoDrop® ND-1000 Spectrophotometer User Manual. Selected target IRD

genes (their coding and non-coding sequences) have been sequenced using *Sanger* sequencing method on 3130xl Genetic Analyzer (Applied Biosystems™, Thermo Fisher Scientific, USA) and next generation sequencing method on Ion TorrentTM Personal Genome MachineTM (PGM)“ (Ion Torrent™, Thermo Fisher Scientific, USA) according to system user manuals optimized at the Department of Human and Medical Genetics, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University and the Laboratory for Molecular Genetics and Cytogenetics of Vilnius University Hospital Santaros Klinikos Center for Medical Genetics.

In the RP group, 9 SNP were determined and were compared Lithuanian, European and global data on healthy patients, distribution of reference and alternative alleles and genotypes in the RP subjects and LITGEN groups was examined, and SNP and phenotype correlations were calculated in RP subjects.

To compareIRD SNP genotypes with data on Lithuanian self- reported healthy individuals from LITGEN project were included. LITGEN (Lithuanian “Lietuvos populiacijos genetinė įvairovė ir sandaros kitimai, susiję su evoliucija ir dažniausiai paplitusiomis ligomis” (Genetic Diversity of the Lithuanian Population and Structural Alterations Related to Evolutions and Most Common Diseases)) is a project completed in 2011-2015 intended to conduct large scale genomic testing and identify genomic areas characteristic to hypothetic Lithuanians that are significant for their health (project scientific leader prof. V.Kučinskas).

To compare SNP with European and global data on healthy subjects’ data from the 1000G project were used. 1000G project (1000 genomes) is a catalogue of the genomic variations created within the collaboration project carried out by the USA, United Kingdom, China and Germany.

1.4. Statistical data analysis

Statistical calculations were performed using free statistical software R (version 4.3.2). Shapiro-Wilks test was used to test normality of quantitative variables of phenotype. Distribution of all variables, except age, were not normally distributed, therefore descriptive statistics of these quantitative variables are presented: arithmetical mean and standard deviation (SD) as well as median and interquartile differences. Gender differences in phenotype characteristics were evaluated using non-parametric Wilcoxon test for independent samples. Differences between left and right eyes were evaluated using non-parametric Wilcoxon test for depending samples. Spearman‘s correlation coefficient was calculated to measure correlations between variables. Boxplots were presented to evaluate association between phenotypic and genotypic quantitative variables.

Chi-square criterion was used to test genotype frequency complying with Hardy-Weinberg equation (HWE). Chi-square criterion was also used to evaluate dif-

ferences in frequencies in RP and LITGEN groups. When no rare allele was identified, Yate's correction for continuity was applied. Proportion differences between LITGEN (www.litgen.mf.vu.lt) and 1000 genome project (http://www.internationalgenome.org) markers were tested using proportion criterion. In this dissertation differences were considered statistically significant when p-value was lower than 0.05.

1.5. Author's contribution

Approval of Vilnius Regional Bioethics Committee	–	100%.
Development of inclusion criteria	–	100%.
Subjects' screening and enrollment	–	95%.
Subjects ophthalmological examination	–	90%.
Assessment of the ophthalmological data	–	100%.
Subjects' genetic consultation	–	0%.
Analysis and interpretation of molecular genetic testing	–	30%.
Statistical data analysis	–	50%.
Publications, poster presentations and oral presentations	–	90%.

2. Results and discussion

2.1. Study groups

One hundred and thirty-seven subjects participated in the research, 18 of which did not meet the inclusion criteria. The remaining subjects were divided into three large groups: RP, ABCA4 retinopathies, and other rare IRD. Group of rare dystrophies was divided into 10 subgroups according to different inherited retinal or vitreoretinal diseases. Figure 1 shows study groups.

35 study subjects were related – totally 16 familial cases were included in the study.

<u>18 subjects did not meet inclusion criteria due to following reasons:</u>

- Did not match age criterion.
- Insufficient evidence of suspected IRD.
- Another (not retinal) hereditaryinherited eye disease is suspected.
- Examination is not completed.

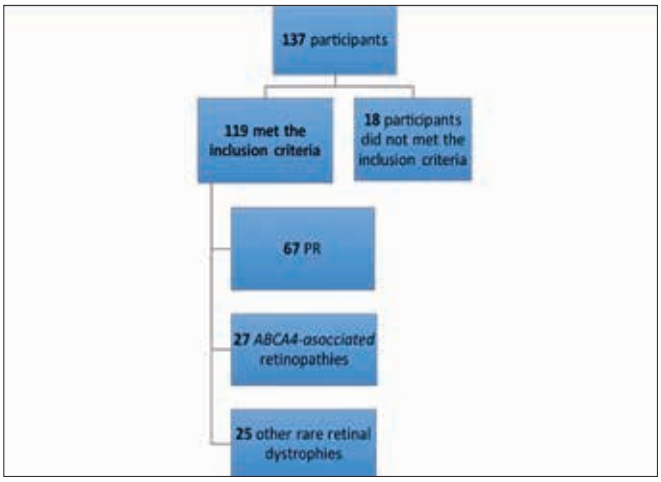

FIGURE 1. Study groups

Figure 2 shows demographic data of 119 subjects who met the inclusion criteria. Urban and rural inhabitants of 9 counties (Vilnius, Kaunas, Utena, Alytus, Panevėžys, Klaipėda, Tauragė, Telšiai and Šiauliai) participated in the study. 67 females (56.30%) and 52 males (43.70%) participated in the study. Age of subjects ranged from 6 to 77 years.

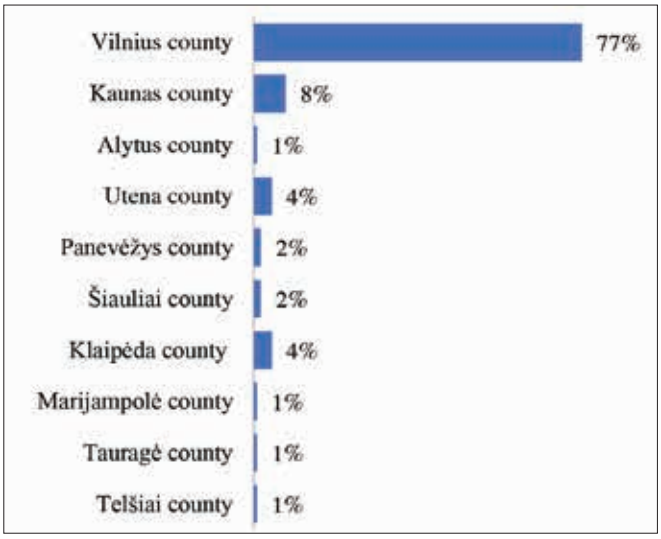

FIGURE 2. Subject distribution by county.

2.2. Subjects with retinitis pigmentosa

Sixty-seven patients were allocated in the RP group. After comprehensive ophthalmological examination, 60 subjects out of total 67 were considered as typical and 7 subjects as atypical RP. Five patients in the RP group were diagnosed with sensorineural hearing loss and syndromic RP - Usher syndrome was suspected, but were included in the typical RP group because of classical clinical RP symptoms. Atypical RP presented as unilateral, sectoral and RP sine pigmento. Figure 3 shows distribution of all subjects with RP.

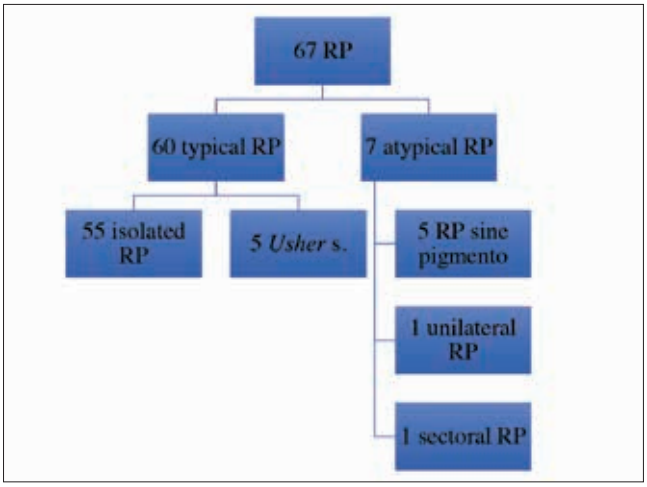

FIGURE 3. RP subject classification based on clinical signs

Demographics of RP group

Thirty-one female (57, 41%) and 23 males (42.59%) participated in the RP group. Mean age of the participants was 46.1 years (SD 16.2, median 47.5). Most of the subjects, 41 (75.93%) were inhabitants of Vilnius city of Vilnius county.

Only unrelated typical RP subjects – 54 individuals – were involved into further statistical analysis.

2.2.1. Analysis of the ophthalmological data

Subjects' BCVA ranged from 0.0 to 1.0 (Table 1). BCVA in most subjects was 0.01 and more (RE 88.9%, LE 90.74%). In these subjects mean BCVA was 0.6 (SD 0.3; median 0.55) in right eye (RE) and 0.5 (SD 0.3; median 0.5) in left eye (LE).

Assessment of refraction showed that only 1 (1.96%) subject had no refraction abnormalities. Myopic astigmatism was the most common refraction abnormality

TABLE 1. RP patients BCVA data.

		Number (%)	
		RE	LE
BCVA	Blindness (VA – 0,0)		1 (1,85 %)
	Hand movement	4 (7,41 %)	2 (3,7 %
	Finger counting	2 (3,7 %)	2 (3,7 %)
	BCVA ŧ0,01	48 (88,89 %)	49 (90,74 %)

BCVA – best corrected visual acuity, RE – right eye, LE – left eye

among subjects with RP, in 28 subjects (53.85) it was abnormality of the RE and in 29 subjects (58.86%) – in the LE.

Examination of the anterior segment using slit lamp revealed nystagmus only in 4 subjects (7.41%). Cataract was diagnosed in more than a half of subjects: 32 cataracts (53.85%) in the RE and 34 (62.96%) in the LE. During this study 18 subjects implanted IOL's were observed, 9 (12.12%) in the RE and 9 (26.74%) in the LE.

Full field ERG was carried out in the majority of subjects and and all subjects demonstrated scotopic ERG of no or significantly decreased amplitudes, as well as abnormalities of various degree of the photopic ERG.

2.2.1.1. Assessment of visual field

Visual field was assessed in 48 out of 54 subjects. In 6 subjects visual field was not assessed due to insufficient visual function. In 28 out of 48 subjects who underwent assessment of visual field Humphrey static perimetry was carried out, and in subjects with insufficient visual acuity, slow orientation and only residual visual field (20 subjects, 37.04%) Goldmann dynamic visual field was assessed. Detailed results are presented in Table 2.

TABLE 2. The results of assessment of visual field in RP patients.

		Number (%)	
		RE	LE
Humphrey visual field (N-28)	Single peripheral scotomas / other	4 (14,28%)	4 (14,28%)
	Ring scotoma	1 (3,57%)	1 (3,57%)
	Concentric constriction to 30°	5 (17,85%)	4 (14,28%)
	Concentric constriction to 20°	2 (7,14%)	2 (7,14%)
	Concentric constriction to 10°	10(50,00%)	2 (10,00%)

		Number (%)	
		RE	LE
Goldmann visual field (N-20)	Single peripheral scotomas / other	6 (30,00%)	5 (25,00%)
	Concentric constriction to 30°	1 (5,00%)	1 (5,00%)
	Concentric constriction to 20°	2 (10,00%)	1 (5,00%)
	Concentric constriction to 10°	17 (85,00%)	18 (90,00%)

RE – right eye, LE – left eye

2.2.1.2. Assessment of the central retinal thickness

Central retinal thickness and correlations

In subjects examined, CRT in the RE was 223.2 Pm (SD 110.2 Pm), and in the LE was 232.2 Pm (SD 120.8 Pm). CRT was 227.2 Pm in the RE and 237.4 Pm in the LE in male subjects, and 218.3 Pm in the RE and 226.0 Pm in the LE in female subjects (Figure 4).

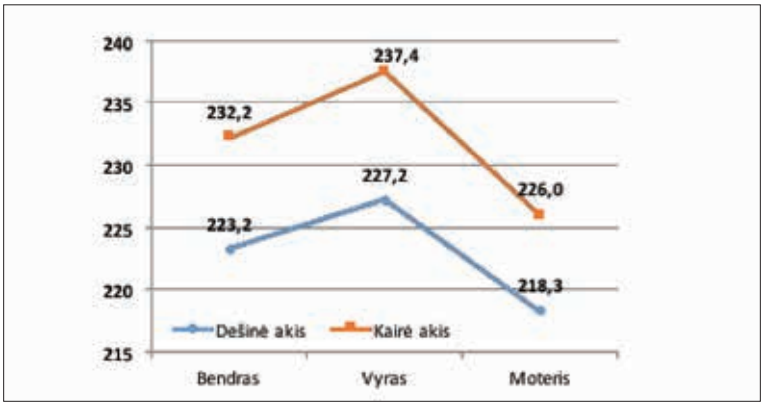

FIGURE 4. RP patients mean CRT compared by gender and between eyes.

Weak but statistically significant correlation between CRT and BCVA was found. This means that thicker central retina is associated with better BCVA. Detailed results are presented in Table 3.

TABLE 3. CRT and BCVA correlation.

	Central retinal thickness					
	RE			LE		
	Spearman's correlation coefficient	N	p	Spearman's correlation coefficient	N	p
BCVA RE	0,35	48	0,0148	0,3422	48	0,0173
BCVA LE	0,3498	49	0,0138	0,4117	49	0,0033

BCVA – best corrected visual acuity, RE – right eye, LE – left eye

Macular changes in patients with RP

Macular changes were assessed in all subjects using SD-OCT, and various changes were found (CRT was discussed previously and is not discussed in this section). Results that did not match technical requirements were not involved. No structural changes of the macula were found in 90.24% of the RE and 36% of the LE. ERM (including ERM that does not access fovea) was found in 36 RE (87.8%) and 36 LE (100%) of subjects. Combination of CGDE and ERM was the most common; it was found in 25% of right eyes and 37.84% of left eyes.

2.2.1.3. Subfoveal choroidal thickness and correlations

In subjects examined, SCT was 213.8 Pm (SD 92.0 Pm) in the RE and 220.1 Pm (SD 100.7 Pm) in the LE. CST in males (RE - 210.8 Pm, LE - 221.6 Pm) and in females (RE - 217.3 Pm, LE -218.5 Pm) showed in Figure 5.

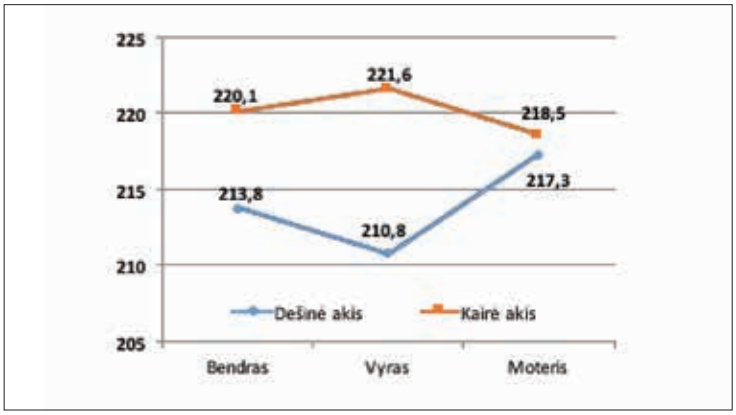

FIGURE 5. RP patients mean SCT compared by gender and between eyes.

Statistically significant association is observed between SCT and age. Spearman's correlation coefficient for RE was -0.292, and for LE it was -0.2936, showing weak association between variables (table 4). SCT decreases with age.

TABLE 4. SCT and age correlation.

	Age		
	Spearman's correlation coefficient	N	p
SCT RE	-0,292	50	0,0396
SCT LE	-0,2936	48	0,0428

SCT – subfoveal choroidal thickness, RE – right eye, LE – left eye

2.2.1.4. Assessment of the ellipsoid zone and correlations

The structure of ellipsoid zone (EZ) in the macular area was assessed using SD-OCT. First, its presense was identified and later its length was measured in the area of the macula. In 8 (15.38 percent) RE and 10 (20 percent) LE no EZ was identified (table 5).

TABLE 5. Macular EZ structure assessed by SD-OCT.

		Number (%)	
		RE	LE
Ellipsoid zone (EZ)	Absent	8 (15,38%)	10 (20%)
	Present	44 (84,62%)	40 (80%)
	Present <1mm	7 (16,28%)	5 (12,82%)
	Present >1 mm with clear borders	33 (76,74%)	32 (82,05%)
	Present through the whole scan	3 (6,98%)	2 (5,13%)

RE – right eye, LE – left eye

Correlation analysis showed statistically significant moderate correlation between length of intact EZ and BCVA and findings of Ishihara color test. BCVA and results of color test improve with the increase of length of extant EZ. This association could be observed in BE. Values of Spearman's correlation coefficient, subjects' samples and level of significance are presented in Table 6.

TABLE 6. Correlations between EZ length, BCVA and Ishihara test results.

	EZ length					
	RE			LE		
	Spearman's correlation coefficient	N	p	Spearman's correlation coefficient	N	p
BCVA RE	0,3837	41	0,0133	0,4062	37	0,0126
BCVA LE	0,4791	42	0,0013	0,5269	38	0,0007
Ishihara RE	0,5001	41	0,0009	0,4676	37	0,0035
Ishihara LE	0,4225	41	0,0059	0,4804	37	0,0026

BCVA – best corrected visual acuity, RE – right eye, LE – left eye, EZ – ellipsoid zone

2.2.1.5. Retinal nerve fiber layer

RNFL was 111.1 Pm (SD 23.0 Pm) in the RE and 114.5 Pm (SD 29.8 Pm) in the LE in the subjects examined. RNFL in males (118.1 Pm in the RE and 121.0 Pm in the LE) and females (102.8 Pm in the RE and 105.9 Pm in the LE) is presented in Table 6.

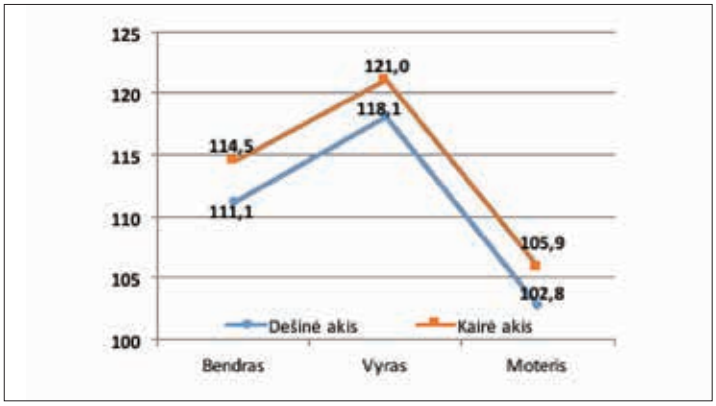

FIGURE 6. RP patients mean RNFL compared by gender and between.

2.2.1.6. Assessment of findings of fundus autofluorescence imaging

During FAF imaging the following hyperautofluorescence (hyper AF) ring characteristics were assessed: horizontal and vertical diameters of external ring boundaries (or one of them when only one could be measured). Hyper AF rings were found in 27 RE (52.94%) and 24 LE (47.06%) of subjects (detailed results are presented in Figure 7). The figure presented below (Fig. 7) shows that horizontal diameters of hyper AF rings were large in BE and in both genders.

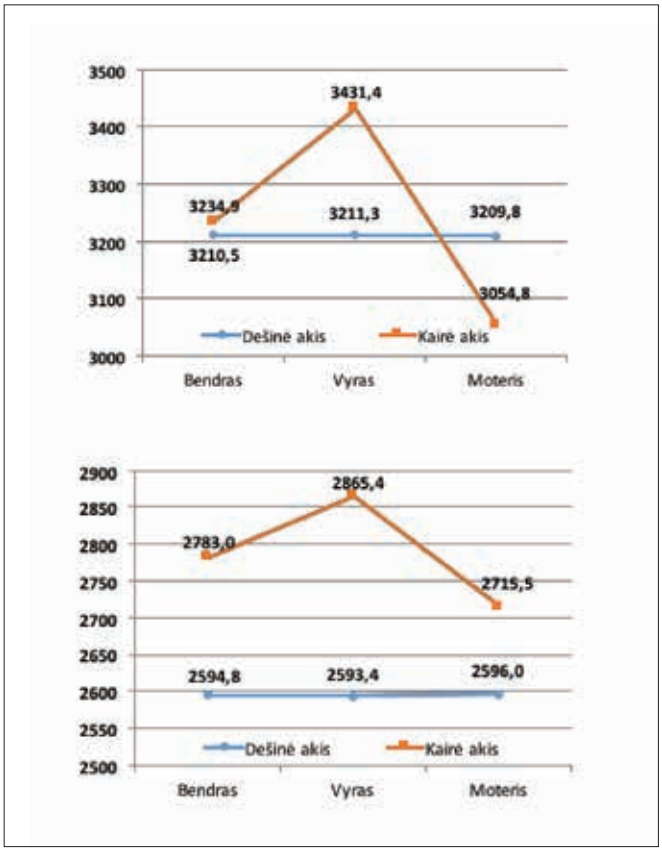

FIGURE 7. HyperAF ring horizontal (upper image) and vertical (lower image) diameters in subjects with RP compared between gender and eyes.

2.2.2. Genetically confirmed cases of typical RP TD112 typical RP case

In the TD112 (III:1) genealogy RP cases were identified in all generations, and therefore it was established that RP is inherited by AD inheritance. Genotype NM_000539.3:c.1039[C>T];[G=](NP_000530.1:p.[(Pro347Ser)];[(Pro347=)]) was identified by the analysis of coding and adjacent sequences of *RHO* gene. Heterozygote genotype of CM900200, rs29001637 variant in the *RHO* gene confirms the clinical diagnosis of AD inherited RP.

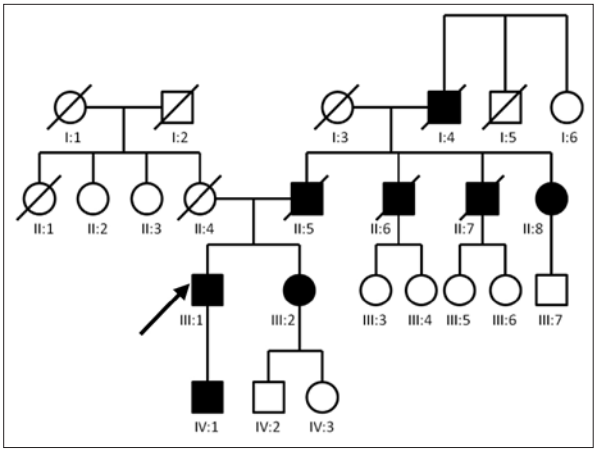

FIGURE 8. TD112 (III:1) pedigree.

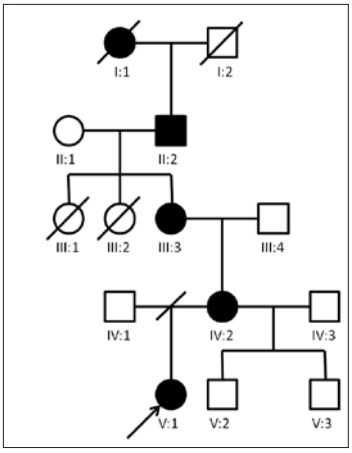

FIGURE 9. TD019 (V:1), TD030 (IV:2) ir TD127 (III:3) pedigree.

TD019 familial typical RP case

Tree female members of one family participated in our study, they underwent molecular genetic testing: TD019 (V:1), TD030 (IV:2) ir TD127 (III:3); RP cases are known in all generation of this family (Figure 9), and therefore it was determined that RP was of AD inheritance. Pathogenic variant CM930659, rs104893779 was identified in the *RHO* gene by Sanger sequencing in all tested individuals, heterozygote genotype confirming clinical diagnosis of AD inherited RP.

TD120 familial RP case

In the TD120 (III:4) genealogy RP cases are identified in most generations, and therefore AD inherited RP was determined. Analysis of coding and adjacent sequences of *RHO* gene and *PRPH2* (peripherin 2, mouse, homolog of) gene revealed heterozygote genotype NM_000322.4:c.[647C>T];[647C=] (NP_000313.2:p.[(Pro216Leu)];[(Pro216=)]). Molecular genetic testing confirmed AD inherited RP in this family.

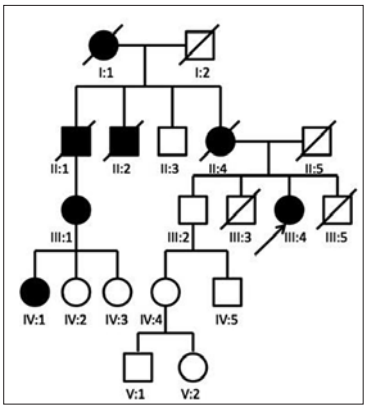

FIGURE 10. TD120 (III:4) pedigree.

TD015 case

Molecular genetic testing of subject TD015 (IV:6) identified genetic changes NM_000260:[494C>T];[596T>G;1969C>T] in the *MYO7A* gene. Familial analysis of genetic variants confirms heterozygote genotype and heterogeneity of sequence variants. Changes identified using molecular genetic testing were included in the *Deafness Variation Database* and confirm diagnosis of AD inherited RP.

2.2.3. Retinitis pigmentosa sine pigmento

Six subjects with RP sine pigmento were enrolled into subgroup of subjects with all signs characteristic to RP except retinal pigmentation - accumulation of so called „bone spicules", found during comprehensive ophthalmological examination.

Clinical data of subjects are presented in Table 7. All subjects, except TD119, had myopia. It is likely that no signs of cataract were noticed because of young age of subjects. It also could be that with ageing and progression of the disease "bone spicules" could occur in the peripheral part of retina.

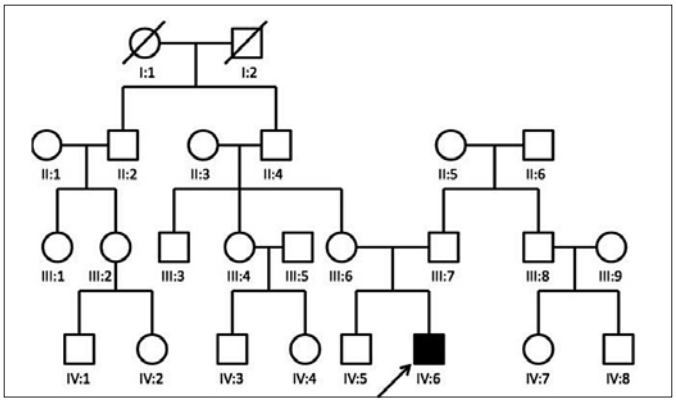

Figure 11. TD015 (IV:6) pedigree.

TABLE 7. Demographic and clinical data of subjects with RP sine pigmento.

	TD034	TD035	TD037	TD040	TD079	TD119
Age	28	29	16	19	28	20
Gender	M	M	M	M	V	M
BCVA RE/L	0,4/0,4	1,0/1,0	1,0/1,0	0,02/0,1	0,5/0,4	0,63/0,63
Ishihara test RE LE	 36/ 38 36/ 38	 38/ 38 38/ 38	 38/ 38 38/ 38	 30/ 38 33/ 38	N N	 38/ 38 38/ 38

BCVA – best corrected visual acuity, RE – right eye, LE – left eye

SD-OCT revealed diverse abnormalities: in TD040 significantly thickened retina was observed; in TD0798 significant CME was found, and in other subjects moderate decrease in CRT was observed, SCT ranged from 152 to 450 Pm (Table 8)

TABLE 8. CRT, SCT and macular EZ length results (μm) in subjects with RP sine pigmento obtained with SD-OCT.

	TD034	TD035	TD037	TD040	TD079	TD119
CRT RE LE	 179 237	 240 232	 226 226	 132 132	 870 760	 229 225
SCT RE LE	 293 288	 158 152	 301 247	 389 450	 355 293	 318 443
EZ length RE LE	 1640 1660	 3257 3049	 7860 7921	 N N	 8032 7368	 3019 2932

BCVA – best corrected visual acuity, RE – right eye, LE – left eye, CRT – central retinal thickness, SCT – subfoveal choroidal thickness, EZ – ellipsoid zone

All subjects underwent full-field ERG that showed that *a* and *b* waves were absent or of very low amplitude in the scotopic phase; waves of significantly lower amplitude were registered also in the photopic phase.

2.2.4. Unilateral retinitis pigmentosa

In subject TD062, 40 years of age, signs characteristic to RP were found only in on – left – eye. Diagnosis of unilateral RP is exceptionally rare; less than 100 cases are documented [7]. Diagnosis of unilateral RP shall meet François and Verriest

criteria [7]. TD062 noticed first signs, seeing spots, at the age of 20. Her medical history revealed that at the age of 25 years ophthalmologist have found „retinal scars". Since then subject suffers from gradually deteriorating vision of the LE. Subjects BCVA of the RE was 1.25, in the LE - 0.3.

No significant disorders of refraction were found, color vision was normal. Fluffy posterior subcapsular opacification was observed in the LE. According to subject, she had no infectious or non-infectious inflammations, traumas or systemic use of medications. Laboratory testing of possible infectious cause of the disease were negative.

Examination of visual field revealed concentric narrowing of visual field in the LE correlating with the image of fundus of eye (Figure 12)

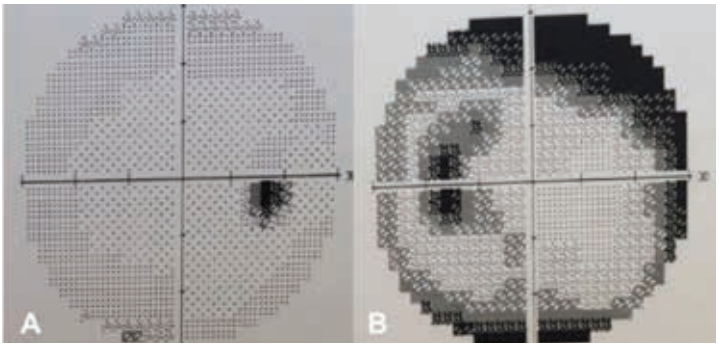

FIGURE 12. Visual field results. RE (A) – normal, LE (B) – concentric visual field constriction.

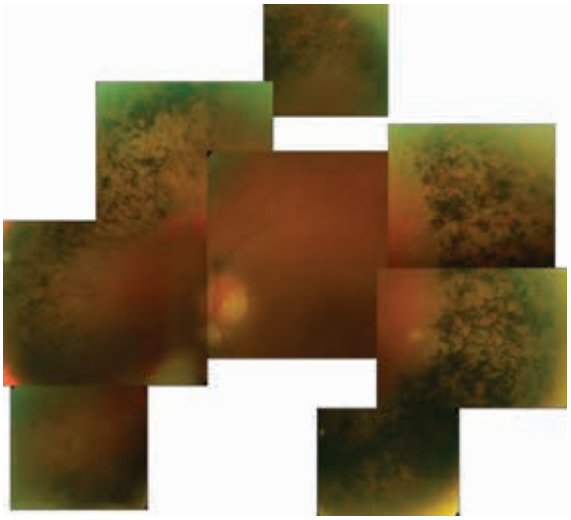

FIGURE 13. Typical RP signs in the RE of TD062.

No abnormalities were found during ophthalmobiomicroscopy if the RE while changes characteristic to the RP were observed in the LE: optic nerve disc (OND) is paler compared with the right eye, abundant „bone spicules“ are observed in the mid-peripheral portion, blood vessels are narrowed (Figure 13); furthermore, SD-OCT confirmed developing ERM et the macula slightly deforming foveal contour and mild parafoveal and cystic swelling. The latter abnormalities in the macular area and opacifications of the lens of the LE are the main courses of deterioration of VA.

Full-field ERG also revealed asymmetric responses: normal results in the right eye, and in left eye, a and b waves were absent in scotopic mode and of significantly lower amplitude in photopic mode (Figure 14).

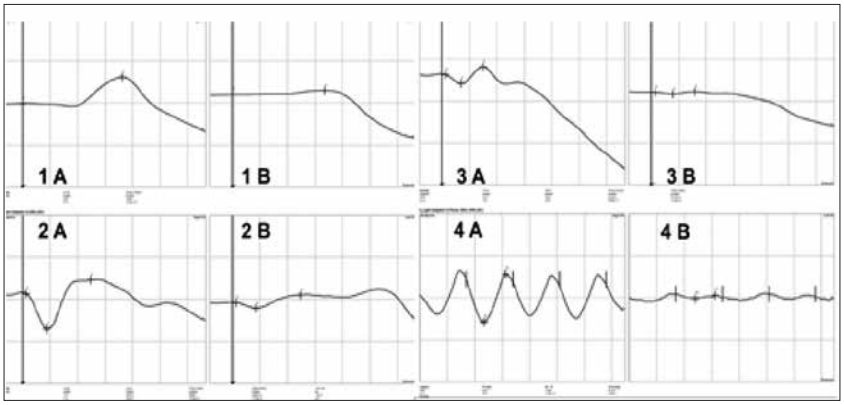

FIGURE 14. TD062 results of scotopic (1-2 A-B) and photopic (3-4 A-B) full field ERG.

Based on data collected unilateral RP was diagnosed. However, monitoring of presently intact RE for possible RP signs over time is indicated.

2.2.5. Results of the genetic testing

2.2.5.1. SNP in the RP subjects

Molecular genetic testing of coding and adjacent sequences of two genes, *RHO* and *RPRH2,* was performed in subjects with RP by Sanger sequencing. Nine different SNPs were identified: rs7984, rs56340615, rs2071092, rs2071093, rs361524, rs434102, rs425876, rs390659, rs7764439. SNPs reference and alternative alleles and genotype distribution between RP and LITGEN groups is presented in Table 9.

TABLE 9. SNPs reference and alternative alleles and genotype distribution between RP and LITGEN groups.

	Ref, n (%)	lt, n (%)	p value compared to LITGEN	Ref/Ref, n (%)	Ref/Alt, n (%)	Alt/Al, n (%)	HWE p-value	p value compared to LITGEN
rs7984								
RP	85 (85,0)	15 (15,0)	0,227	36 (72,0)	13 (26,0)	1 (2,0)	0,890	0,212
LITGEN	176 (89,8)	20 (10,2)		81 (82,7)	14 (14,3)	3 (3,1)	0,029	
rs361524								
RP	72 (72,0)	28 (28,0)	0,248	25 (50,0)	22 (44,0)	3 (6,0)	0,519	0,000
LITGEN	153 (78,1)	43 (21,9)		70 (71,4)	13 (13,3)	15 (15,3)	0,000	
rs390659								
RP	24 (24,0)	76 (76,0)	0,615	4 (8,0)	16 (32,0)	30 (60,0)	0,385	0,783
LITGEN	42 (21,4)	154 (78,6)		5 (5,1)	32 (32,7	61 (62,2)	0,764	
rs434102								
RP	24 (24,0)	76 (76,0)	0,000	4 (8,0)	16 (32,0)	30 (60,0)	0,385	0,000
LITGEN	89 (45,4)	107 (54,6)		37 (37,8)	15 (15,3)	46 (46,9)	0,000	
rs2071092								
RP	98 (98,0)	2 (2,0)	0,206	48 (96,0)	2 (4,0)	0 (0,0)	0,885	0,450
LITGEN	195 (99,5)	1 (0,5)		97 (99,0)	1 (1,0)	0 (0,0)	0,960	
rs2071093								
RP	99 (99,0)	1 (1,0)	0,057	49 (98,0)	1 (2,0)	0 (0,0)	0,943	0,294
LITGEN	185 (94,4)	11 (5,6)		90 (91,8)	5 (5,1)	3 (3,1)	0,000	

	Ref, n (%)	lt, n (%)	p value compared to LITGEN	Ref/Ref, n (%)	Ref/Alt, n (%)	Alt/Al, n (%)	HWE p-value	p value compared to LITGEN
rs7764439								
RP	52 (52,0)	48 (48,0)	0,215	15 (30,0)	22 (44,0)	13 (26,0)	0,402	0,416
LITGEN	87 (44,4)	109 (55,6)		20 (20,4)	47 (48,0)	31 (31,6)	0,777	
rs56340615								
RP	92 (92,0)	8 (8,0)	0,872	42 (84,0)	8 (16,0)	0 (0,0)	0,539	0,902
LITGEN	178 (90,8)	18 (9,2)		80 (81,6)	18 (18,4)	0 (0,0)	0,317	

Statistical analysis showed that only rs434102 results in RP group statistically significantly (p = 0.000) differ from LITGEN, Lithuanian group of healthy subjects, i. e., alternative alleles of rs434102 statistically significantly are more frequently found in subjects with RP.

Comparison between RP and LITGEN groups showed statistically significant differences between rs434102 and rs361524. Alternative genotypes were significantly more frequent in RP subjects group compared with LITGEN population (p = 0.000) (Table 9).

In the RP group, distribution of all genotypes, except rs425876 showed equilibrium when assessed according to HWE.

Data from RP subject group were compared with self-reported healthy population 1000G European and global studies.

In the Lithuanian population, compared with global population the following were statistically more frequent: rs434102 (p=0,000, p<0,05), rs425876 (p=0,000, p<0,05), rs390659 (p=0,000, p<0,05), rs7764439 (p=0,000, p<0,05). rs7984 (p=0,000, p<0,05) and rs2071093 (p=0,000, p<0,05) were significantly rarer. In Lithuanian population, compared with European population rs7984 (p=0,000, p<0,05) and rs361524 (p=0,036, p<0,05) were rarer. rs434102 (p=0,000, p<0,05), rs425876 (p=0,000, p<0,05), rs390659 (p=0,000, p<0,05), rs7764439 (p=0,000, p<0,05) were significantly more frequent. Detailed data are presented in the Figure 15.

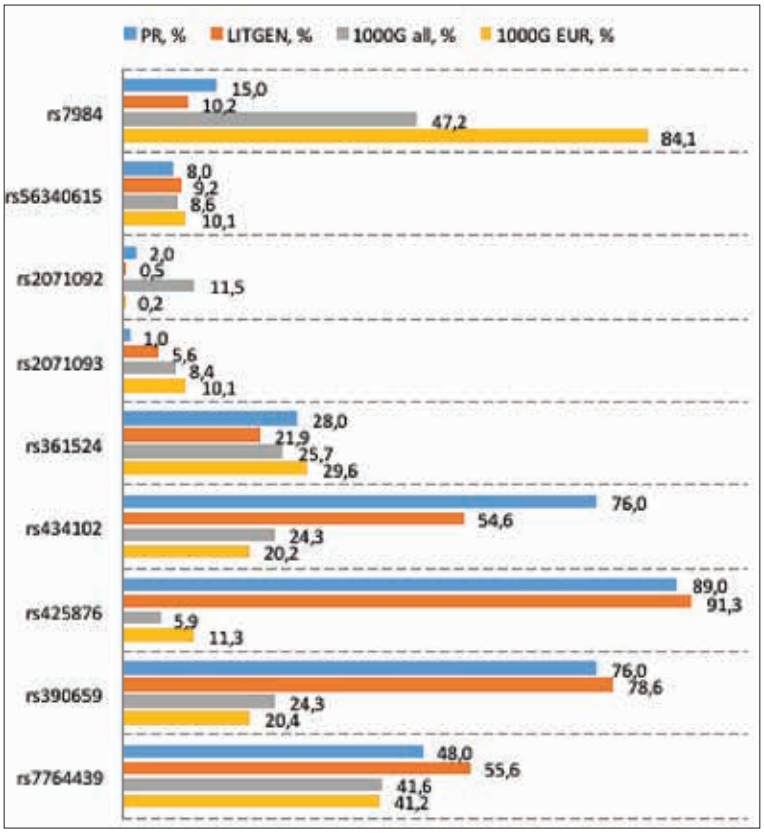

FIGURE 15. SNPs frequency distribution in Lithuanian (LITGEN), world (1000G all) and Europe (1000G Eur) populations.

2.2.5.2. SNP and phenotype correlations

Association of genetic factors with CRT

CRT association with different SNPs was analyzed. Detailed results are presented in the Figure 16 A-B. CRT median of BE in rs425876 and in RE rs361524 decreases with every rare allele, whereas in rs7984 the largest mode was observed in two rare alleles. In rs7764439 minor decrease of CRT median is observed in the LE in every rare allele. To clarify if alternative alleles of aforementioned rs425876, rs361524 and rs7764439 are associated/cause decrease of CRT comprehensive functional testing is needed.

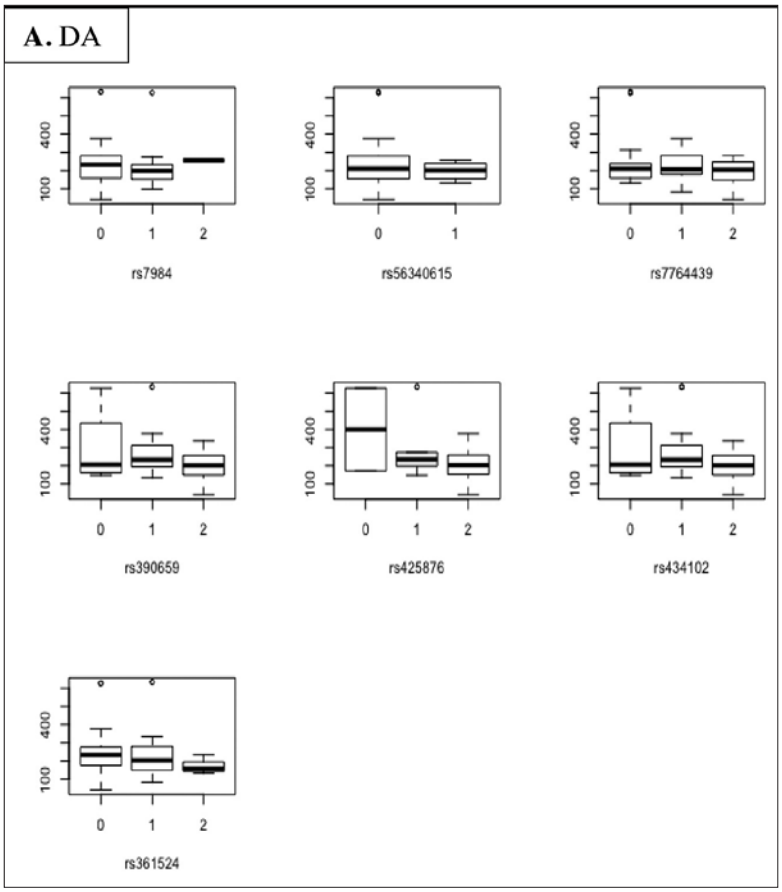

FIGURE 16. (A) The association analysis of RE and CRT with different SNPs.
0 – two reference alleles, 1 – reference and alternative alleles, 2 – two alternative alleles, y-axis – CRT (μm).

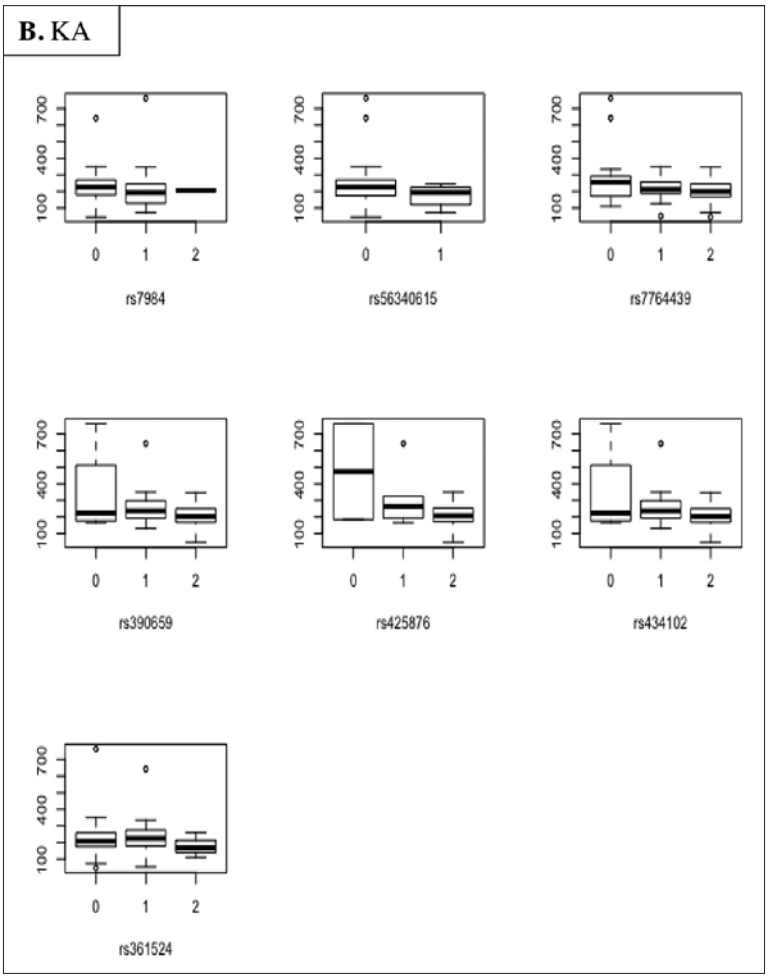

FIGURE 17. (B) The association analysis of LE and CRT with different SNPs.
0 – two reference alleles, 1 – reference and alternative alleles, 2 – two alternative alleles,
y-axis – CRT (μm).

Genetic factors association with EZ length

Association of the intact macular EZ with SNP was analyzed. Detailed results are presented in the Figure 17 A-B. Analysis of this association showed that two SNPs, rs7984 and rs361524 in BE were associated with the highest mode meaning that in cases of alternative genotypes rs7984 and rs361524 EZ layer remains intact for e longest period of time.

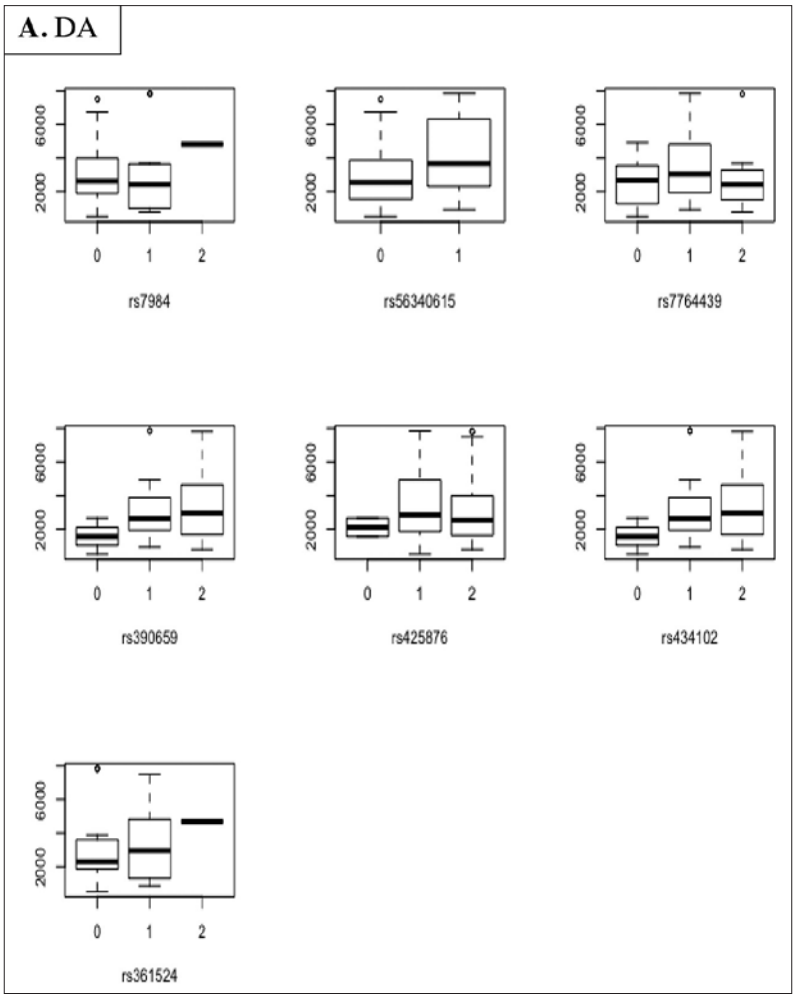

FIGURE 18. (A) The association analysis of RE and macular EZ length with different SNPs. 0 – two reference alleles, 1 – reference and alternative alleles, 2 – two alternative alleles, y-axis – macular EZ lentgh (μm).

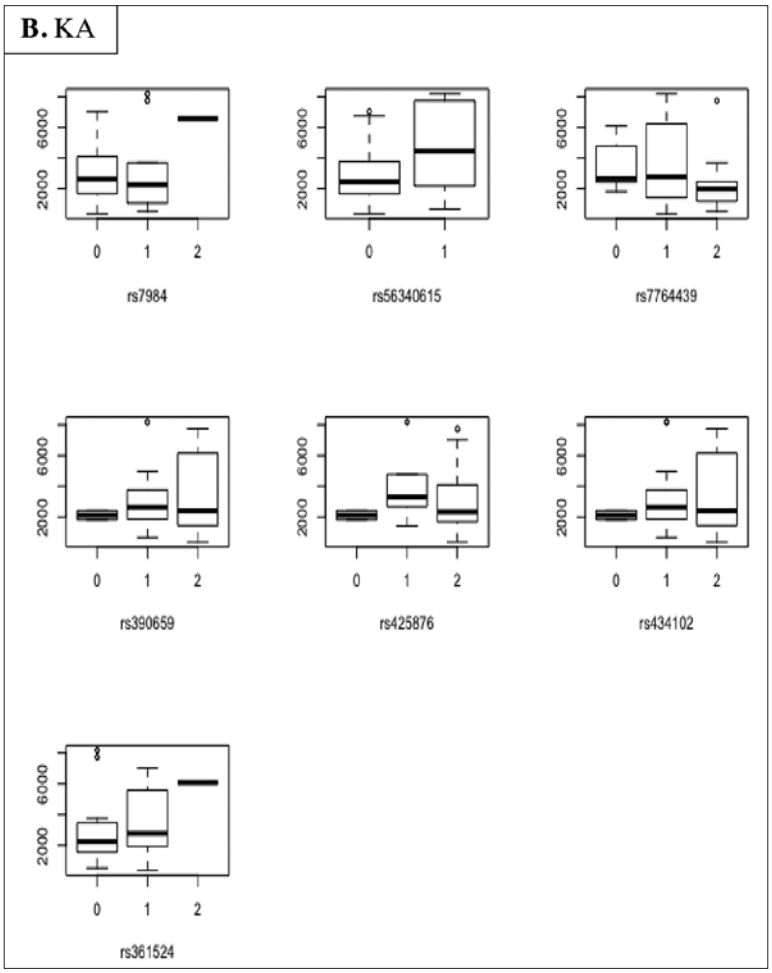

FIGURE 19. (B) The association analysis of RE and LE macular EZ length with different SNPs. 0 – two reference alleles, 1 – reference and alternative alleles, 2 – two alternative alleles, y-axis – macular EZ lentgh (μm).

Genetic factors association with SCT

BE SCT association with genotype was analyzed. In rs361524 SCT decrease in any rare allele was wound. Meanwhile, two rare alleles of SNP rs7984 were associated with the lowest mode (Figure 18 A-B). It could be summarized that alternative alleles may be associated with thinnest subfoveal choroid in cases of RP. Results are presented in the Figure 18.

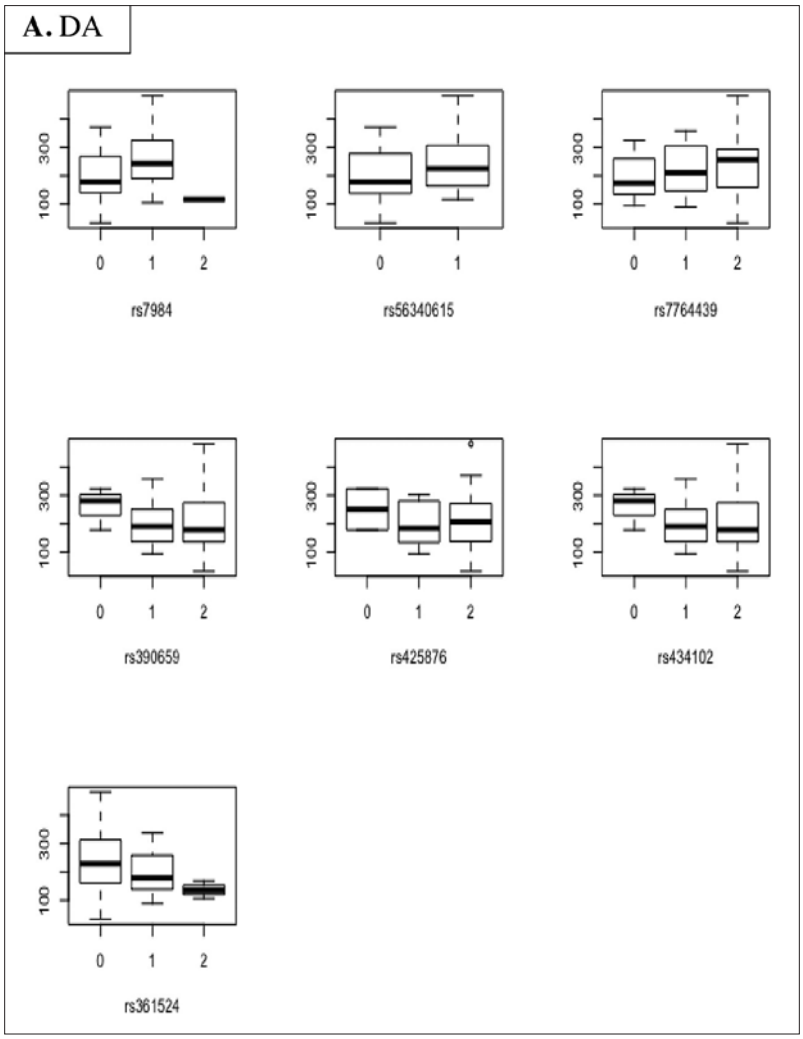

FIGURE 20. (A) The association analysis of RE and SCT with different SNPs.
0 – two reference alleles, 1 – reference and alternative alleles, 2 – two alternative alleles,
y-axis – SCT (μm).

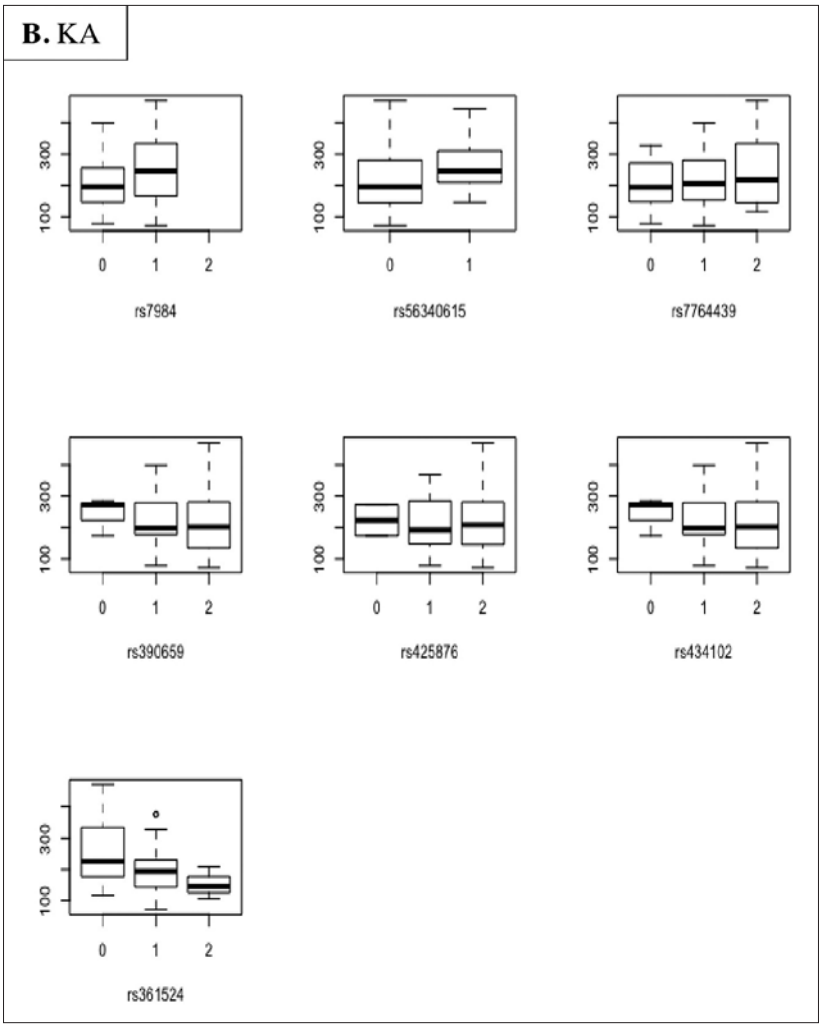

FIGURE 21. (B) The association analysis of LE and SCT with different SNPs.
0 – two reference alleles, 1 – reference and alternative alleles, 2 – two alternative alleles, y-axis – SCT (μm).

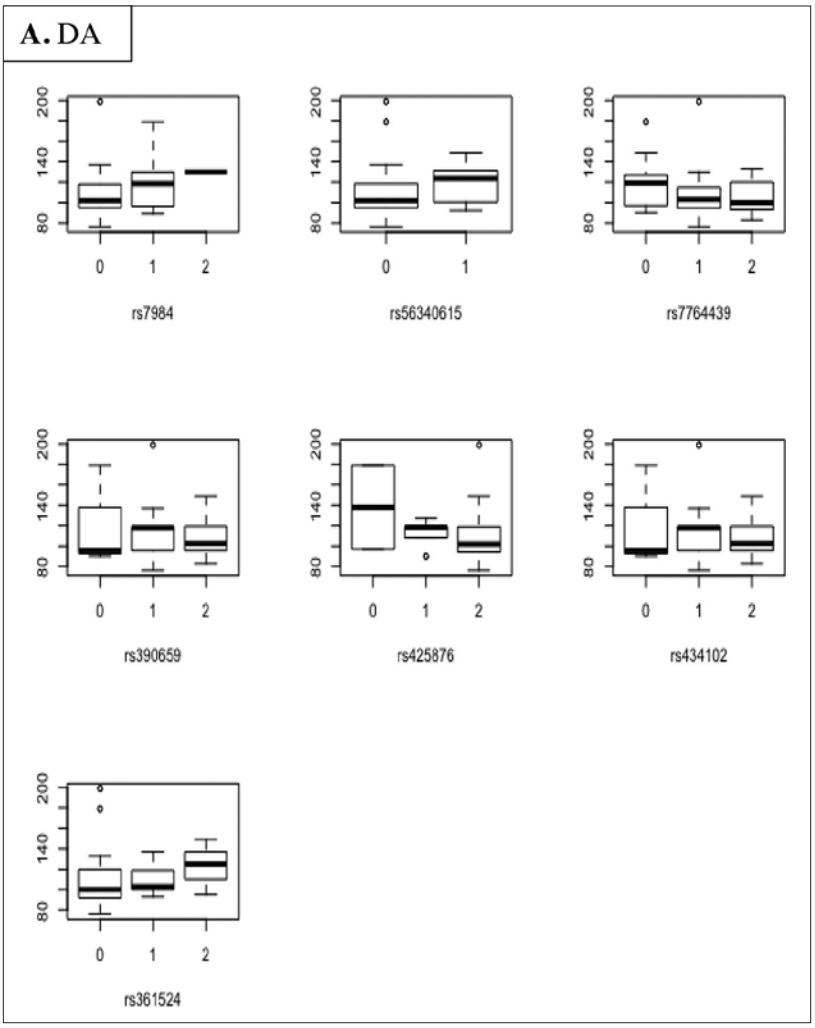

FIGURE 22. (A) The association analysis of RE and RNFL thickness with different SNPs. 0 – two reference alleles, 1 – reference and alternative alleles, 2 – two alternative alleles, y-axis – RNFL thickness (μm)

Genetic factors association with RFNL

RFNL association with SNPs in both eyes was analyzed. In SNPs rs7984 and rs361524 RFNL thickness increased in both eyes with every rare allele and in case of rs425846 RFNL in the left eye RFNL thickness decreased with every rare allele (Figure 19 A-B). It could be summarized that rs7984 and rs361524 alternative alleles may be associated with thicker RFNL, and rs425876 with thinner RFNL.

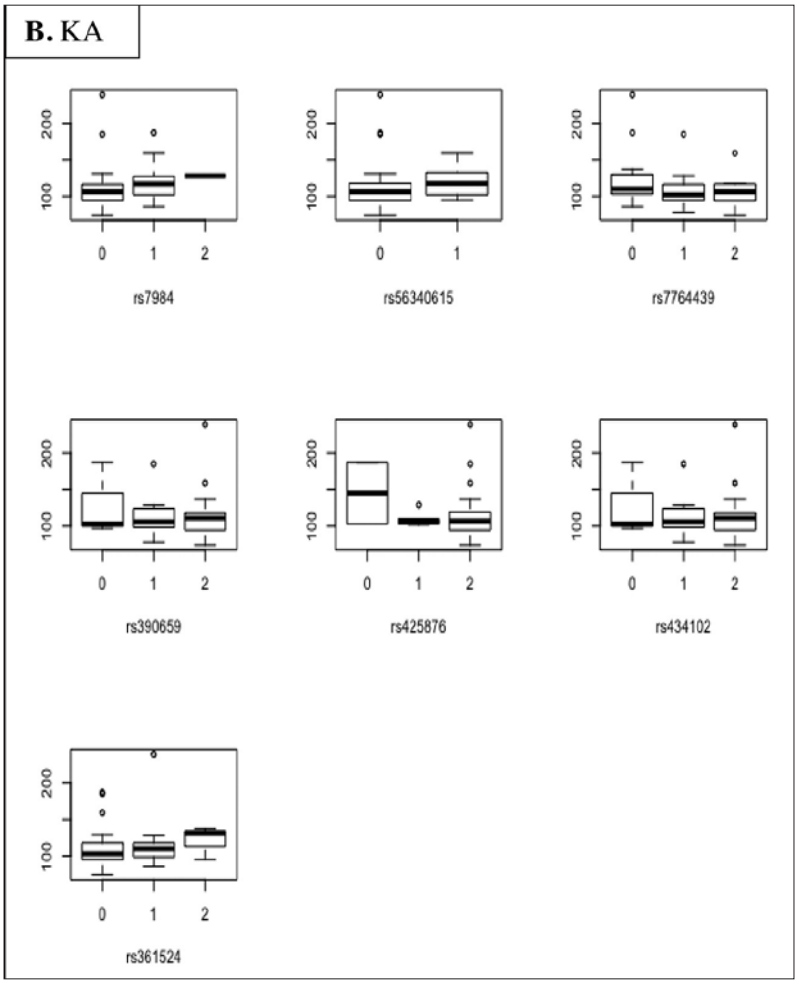

FIGURE 23. (B) The association analysis of RE and LE RNFL thickness with different SNPs. 0 – two reference alleles, 1 – reference and alternative alleles, 2 – two alternative alleles, y-axis – RNFL thickness (μm).

Genetic factors association with MD

BE visual field MD (mean deviation of visual field) association with SNP was analyzed. Different results were obtained in different SNPs. Analysis of the association of RE and rs56340615 and rs425876 showed significant differences in different alleles, and in cases of rs390659 and rs434102 analysis showed MD increase in every rare allele (Figure 20 A). In the LE, only rs361524 analysis showed MD increase in every rare allele (Figure 20 B). In summary, results are different in different SNPs, and no uniform trend is observed.

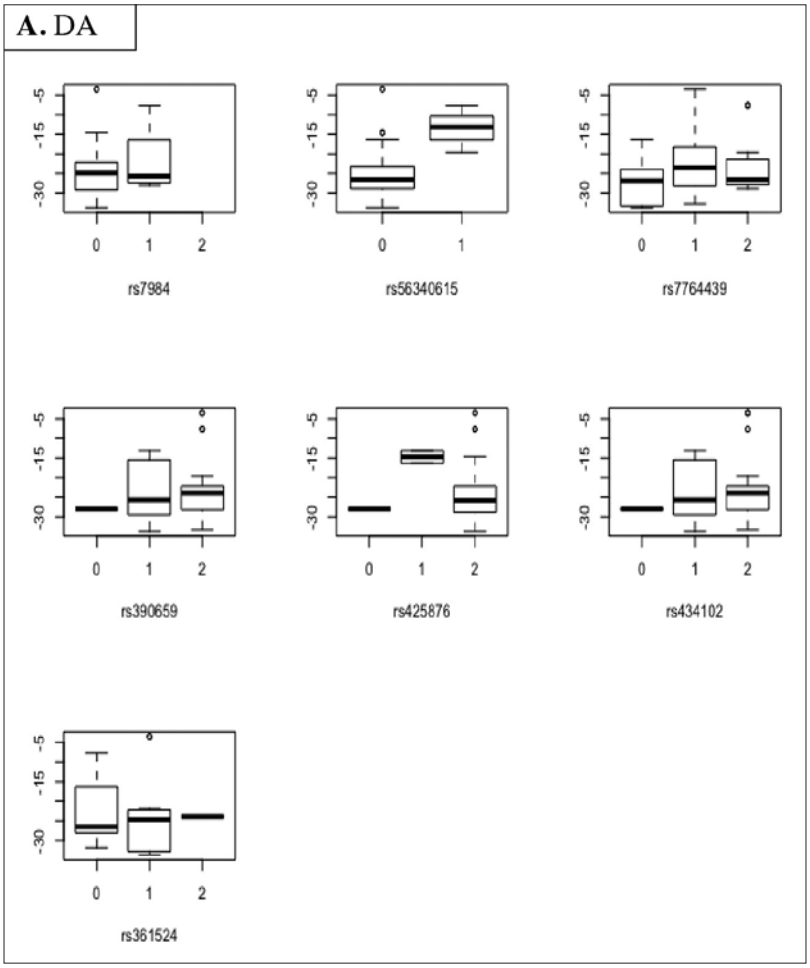

FIGURE 24. (A) The association analysis of RE and MD value with different SNPs. 0 – two reference alleles, 1 – reference and alternative alleles, 2 – two alternative alleles, y-axis – MD value (dB).

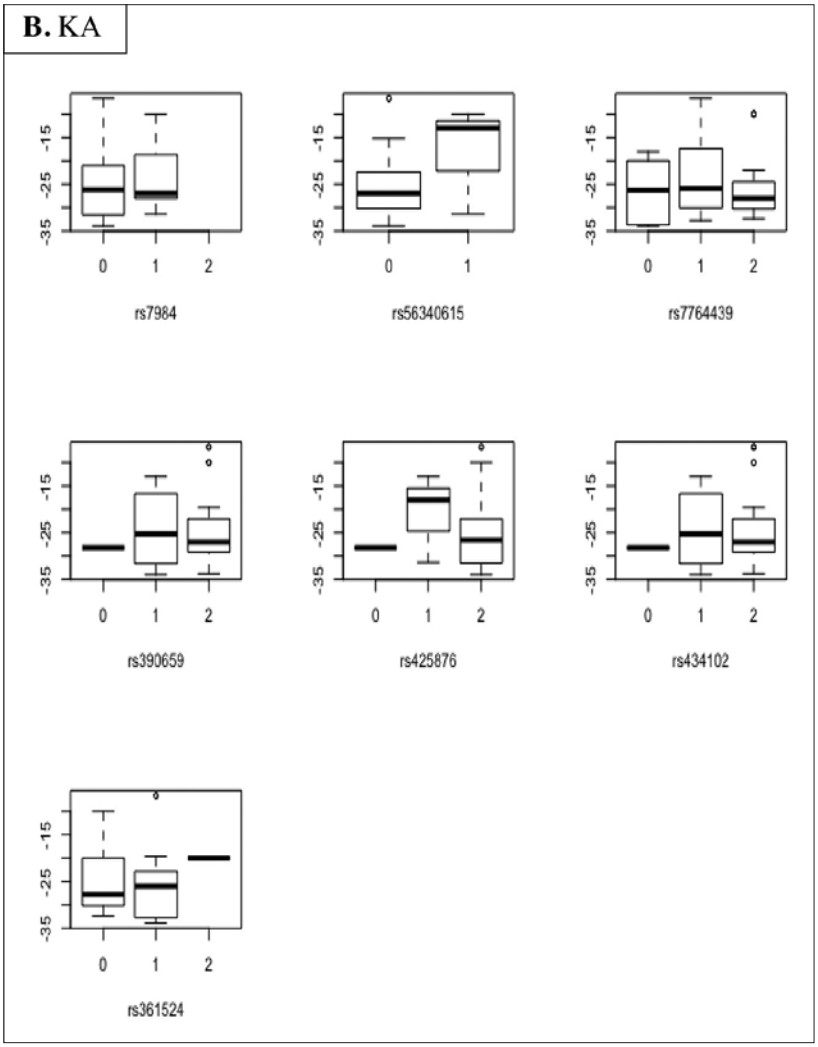

FIGURE 25. (B) The association analysis of LE and MD value with different SNPs
0 – two reference alleles, 1 – reference and alternative alleles, 2 – two alternative alleles,
y-axis – MD value (dB).

Discussion

Correlation analysis showed statistically significant moderately strong positive correlation relationship between EZ length and BCVA which is in line with findings reported by *Liu et al*. that BCVA improves with the increase of EZ length [8].

Konieczka et al. study also confirmed that shortened or lost its integrity EZ is related to poorer BCVA [9].

Sodi et al. did not find any SCT correlations with any parameters, including age, age at the onset of the disease, duration of the disease, CRT, BCVA, field of visions and ERG responses [10]. At the same time in our study we observed statistically significant association between SCT and age and we made a conclusion that SCT decreases with age. However, clinical conclusions concerning importance of choroid in the course of the RP are unclear yet, and more comprehensive studies are necessary [10].

Of the SNPs examined in our study, SNPs rs434102, rs7984 and rs56340615 were described in the literature in greater details. According to study data presented by *Shankar SP et al*. [11], SNP rs434102 (*PRPH2* gene) is found in 2% of families with AD inherited RP and in 10% of families with various clinical diagnoses such as pattern dystrophy, macular or generalised dystrophies.

Guo et al. [12] studied subjects with AD RP and established abnormal mutation of *RHO* gene as well as healthy persons belonging to one of the smallest Chinese ethnic groups, Bai family, and described two non-pathogenic SNPs, rs7984 and rs2269736. The most common rs7984 genotype among patients with RP in Bai family was found to be AA - 61.5% (n = 13), which was similar to our study, 72.0% (n = 50). In healthy persons belonging to Bai family an alternative G allele was more frequent and was found in 66.7% of subjects; however, according to LITGEN, A allele was more frequent in healthy population in Lithuania accounting for 89.8% (n = 98).

Ma Li et al. [13] studies prevalence of different sequences of *RHO* gene SNP rs56340615, and their proportion was similar to that found in our study: in *Ma Li et al*. study, in the RP sine pigmento group the proportion of genotype/heterozygous genotype/reference genotype was 0/8/12, while in our RP subject group it was 0/8/42; reference genotype was the most common in both studies.

Close cooperation of ophthalmologists, geneticists and epidemiologists in the large-scale clinical studies is necessity to analyze clinical and genetic complexity of RP [14].

2.3. *ABCA4* retinopathies

According to recent molecular studies, STGD, cone dystrophies (CD) and CRD (cone-rod dystrophies) shall be considered as inherited diseases related to *ABCA4* gene, and therefore it is more correctly to call them *ABCA4*-related retinal degenerations [15]. Since symptoms of these diseases are overlapped, establishment of their diagnosis is often possible only upon assessment of the character their progression, furthermore, the same gene abnormalities may be related to difference phenotypes and vice versa [16]. Subjects allocated to *ABCA4* group were divided into two subgroups based on clinical signs, subjects with STGD and subjects with CD/CRD.

2.3.1. Subjects with Stargardt disease

Fifteen subjects were allocated in STGD group. Nine (60%) females and 6 (40%) males participated in the study. Their age ranged from 9 to 53 years (Table 10), mean age was 35 years (SD 14, Median 31). Majority of these subjects (86.67%) were from Vilnius county. Age at the onset of the disease was considered as the beginning of the deterioration of vision, mean age was 19 years (SD 13, median 15). Mean duration of the disease was 13 years (SD 11, median 10).

In majority of subjects' refraction assessment showed myopic astigmatism (86.67%). BCVA results were the same BE, mean was 0.16 (SD 0.25, median 0.10). Disorders of color visions varied: in 3 (20%) subjects with BCVA is was too little to see test tables, 4 (26.67) subjects were able to see only test tables, and 8 subjects (53.33%) color vision still persisted or they had moderately disorders of color vision. Visual field was examined in all subjects, and central scotomas were found. Detailed information about the patients is presented in Table 10.

In all subjects ophthalmobiomicroscopy showed macular characteristic to STGS, they were especially visible during FAF test (Table 11), as well as „beaten bronze" pattern and central atrophy. Various changes were observed in subjects during FAF test (Table 11).

Macular OCT results showed that VMT was observed in 8 subjects (53.33%). EZ structure and integrity was assessed, and in 11 (73.33%) out of 15 subjects its structure was significantly damaged in EZ area, its layer could not be identified, various stages of "optical gap" were observed. In these cases, length of the defect of EZ layer was measured (Table 11). Mean CRT was 69.4 Pm (SD 35.7 Pm, median 56 Pm) in the RE and 77.8 Pm (SD 42.0 Pm, median 63 Pm) in the LE. Mean SCT was 315.4 Pm (SD 124.0 Pm, median 338.5 Pm) in the RE and 305.1 Pm (SD 126.5 Pm, median 303.5 Pm) in the LE.

OCT data, Fishmann and Lois classification FAF data are presented in Table 11. Full-field ERG results were presented according to Lois classification (Table 11).

TABLE 10. Demographic, color vision and visual field data (N/A – not performed) in STGD subjects.

Nr.	TD number	Gender	Age of VA deterioration onset (y)	Duration of the disease	Symptoms, other eye diseases	BCVA RE/ LE	Refraction RE/ LE	Ishihara test RE/ LE	Visual field
1	TD004	V	17	36	Dyschromatopsia; floaters	0,16/0,16	None/ Myopic astigmatism	2/2	Central scotomas within 10q of visual field
2	TD005	M	7	19	None	0,03/0,04	Myopic astigmatism/ Myopic astigmatism	0/0	Central scotomas within 20q of visual field
3	TD006	M	30	19	Dyschromatopsia	0,1/0,1	None/ None	5/4	Central scotomas within 20q of visual field
4	TD014	M	8	22	None	0,1/0,1	Myopic astigmatism/ Myopic astigmatism	31/31	Central scotomas within 10q of visual field
5	TD021	M	15	15	Photophobia	0,06/0,06	Mixed astigmatism/ Mixed astigmatism	0/0	Central scotomas within 20q of visual field
6	TD024	V	27	3	None	0,1/0,1	Mixed astigmatism/ Mixed astigmatism	24/33	Central scotomas within 10q of visual field
7	TD026	M	10	10	None	0,1/0,1	Myopia/ Myopic astigmatism	8/12	Central scotomas within 20q of visual field
8	TD027	V	6	36	Uveitis	0,02/0,02	Myopic astigmatism/ Myopic astigmatism	0/0	Central scotomas extend beyond the 20q range of visual field

Nr.	TD number	Gender	Age of VA deterioration onset (y)	Duration of the disease	Symptoms, other eye diseases	BCVA RE/ LE	Refraction RE/ LE	Ishihara test RE/ LE	Visual field
9	TD050	V	45	7	None	0,12/0,16	Hypermetropic astigmatism/ Hypermetropic astigmatism	38/38	Central scotomas within 10q of visual field
10	TD081	V	33	6	Floaters	0,1/0,05	Myopic astigmatism/ Myopia	31/33	Central scotomas within 20q of visual field
11	TD090	V	7	2	None	0,4/0,5	Myopic astigmatism/ Myopic astigmatism	18/12	Central scotomas within 20q of visual field
12	TD091	M	34	0,5	Uveitis	1/0,95	Mixed astigmatism/ Mixed astigmatism	38/38	Central scotomas within 10q of visual field
13	TD093	M	13	5	None	0,09/0,09	Myopic astigmatism/ Myopic astigmatism	2/2	Central scotomas within 20q of visual field
14	TD114	M	30	28	Glaucoma	0,02/0,02	Myopic astigmatism/ Myopic astigmatism	2/2	Central scotomas within 10q of visual field
15	TD123	M	10	21	None	0,005/0,005	Myopic astigmatism/ Myopic astigmatism	2/2	N/A

STGD – Stargardt disease, BCVA – best-corrected visual acuity, RE – right eye, LE – left eye

TABLE 11. Fishman classification, FAF, SD-OCT and Lois classification data in STGD subjects (NA – no data, N - normal).

Nr.	TD nr	*Fish man* classifica-tion	FAF categories	More detailed FAF evaluation	Macular appearan ce on SD-OCT RE/LE	CRT RE /LE	SCT RE/LE	Macular EZ BE	EZ defect length (µm) RE/ LE	Lois
1	TD004	1	HypoAF fovea surrounded by homogenous AF	Foveal granularity, hyperAF ring	VMT/VM T	38/ 38	446/454	N	2077/1594	I
2	TD005	3	Multiple hypoAF areas at the posterior pole on a heterogenous AF back-ground	CA, hyperAF flecks beyond the arcades	VMT/N	92/ 104	N/N	Dis-rupted		III
3	TD006	1	HypoAF fovea surrounded by homogenous AF	Foveal granularity, hyperAF flecks at the fovea	N/N	51/ 60	450/452	N	2269/2665	II
4	TD014	1	HypoAF fovea surrounded by homogenous AF	CA, hyperAF ring, hyperAF flecks at the fovea	N/VMT	40/ 174	335/340	N	2281/1525	I
5	TD021	3	HypoAF macula in the heterogeneous AF back-ground, multiple uneven AF lesions	CA, hiperAF flecks extended out of arches	N/VMT	82/ 39	226/233	Disrup ted	-	II
6	TD024	2	HypoAF fovea surrounded by homogenous AF	RE - CA, hyperAF flecks at the posterior pole/ LE - CA, hy-perAF flecks beyond the arcades	VMT/N	54/ 78	405/254	N	2868/2702	II

Nr.	TD nr	*Fish man* classifica-tion	FAF categories	More detailed FAF evaluation	Macular appearan ce on SD-OCT RE/LE	CRT RE /LE	SCT RE/LE	Macular EZ BE	EZ defect length (µm) RE/ LE	Lois
7	TD026	1	HypoAF fovea surrounded by homogenous AF	Foveal granularity, hyperAF flecks be-yond the arcades	VMT/N	56/ 58	352/298	N	2230/2099	II
8	TD027	4	Normal	Normal/ Normal	N/N	81/ 43	89/78	N		III
10	TD081	3	HypoAF macula in the het-erogeneous AF background, multiple uneven AF lesions	CA, hyperAF flecks beyond the arcades	N/VMT	44/ 44	271/243	N	5922/6957	III
11	TD090	2	HypoAF macula in the het-erogeneous AF background, multiple uneven AF lesions	Foveal granularity, hyperAF flecks be-yond the arcades	N/N	104 /118	292/309	N	2087/1764	I
12	TD091	1	HypoAF fovea surrounded by homogenous AF	Foveal granularity, hyperAF flecks ta the fovea	N/N	176 /158	381/412	N	2556/2883	I
13	TD093	2	HypoAF macula in the het-erogeneous AF background, multiple uneven AF lesions	CA, hyperAF flecks beyond the arcades	N/N	61/ 63	342/309	N	3034/3331	I
14	TD114	2	HypoAF macula in the het-erogeneous AF background, multiple uneven AF lesions	CA, hyperAF flecks beyond the arcades	N/N	39/ 49	166/189	N	3821/4101	I
15	TD123	4	Multiple hypoAF areas at the posterior pole on a heterog-enous AF background	CA, hyperAF flecks beyond the arcades	VMT/VM T	67/ 66	149/162	Dis-rupted	-	III

AF – autofluorescence, CA – central atrophy, CRS – central retinal thickness, EZ – ellipsoid zone, FAF – fundus autofluorescence, Lois – Lois classification, RE – right eye, LE – left eye, SCT – subfoveal choroidal thickness, SD-OCT – spectral – domain optical coherence tomography

MfERG was conducted in 8 subjects (53.33%): weakened responses in central zone were observed in 6 subjects (75%), and weakened responses in all mfERG zones were observed in 2 (25%) subjects.

2.3.2. Subjects with cone/cone-rod dystrophy

Subjects' ophthalmological data

Twelve subjects, 5 males (41.67%) and 7 females (58.33%) were allocated into con/cone-rod dystrophy group. Subjects' age ranged from 17 to 66 years. Majority of subjects (85.71%) were from Vilnius county, and 3 patients (27.27%) were from other counties.

Duration of the diseases ranged from 1 to 33 years, mean duration was 13.2 years (SD 9.7, median 12). As in patients with STGD, subjective assessment of the onset of the disease, beginning of the deterioration of vision was selected; median age at the beginning of the disease was 17.2 years (SD 10.8, median 15). Other complaints of subjects are presented in Table 12.

In majority of subjects refraction assessment showed myopic astigmatism (71.42%). BCVA in the RE was 0.37 (SD 0.22, median 0.45), in the LE - 0.37 (SD 0.27, median 0.36). Disorders of color visions varied: in 2 (14.28%) subjects with BCVA is was too little to see test tables, 4 (28.57%) subjects were able to see only test tables, and other subjects (57.14%) color vision still persisted or they had moderately disorders of color vision. In all patients with assessed visual field central scotomas were found. Detailed information about the patients is presented in Table 12.

Assessment of EZ layer structure showed that in 6 subjects (50%) out of 12 macular structure was significantly damaged in one or both eyes, layer could not be identified, and optical caps of various stages were observed. When optical gap was identified length of EZ defect was measured (Table 13). Mean CRT in the RE was 139.5 Pm (SD 47.9 Pm, median 142.5 Pm), in the LE - 142.3 Pm (SD 52.1 Pm, median 155 Pm). Mean CRT was 304.5 Pm (SD 140.1 Pm, median 292 Pm) in the RE and 303.3 Pm (SD 119.3 Pm, median 308 Pm) in the LE. Unlike STGD subjects, ophthalmobiomicroscopy did not showed significant macular changes; usually depigmentation or normal fundus of eye were observed. OCT data are presented in Table 13.

Findings of full-field ERG were normal in subjects with CD, and in subjects with CRD both scotopic and photopic responses were lowered or absent. MfERG was not conducted in one subject due to low BCVA, in all other subjects, abnormalities were found: flattening of the central hill or decrease of various degrees of central amplitudes.

TABLE 12. Demographic, color vision and visual field results in CD and CRD subjects (VF – visual field).

Nr.	TD nr.	Gender	Age of VA deterio ratio-nonset (y)	Disease duration (y.)	Dis-ease	Symptoms, other eye/sys-temic diseases	BCVA RE/ LE	Refraction RE/ LE	Ishihara test RE/ LE	Visual field
1	TD003	M	8	27	CD	N	0,5/0,45	Myopia/N	38/38	Ny/Ny
2	TD007	M	14	12	CD	N	0,16/0,16	Myopia/ Myopia	38/38	Central scotomas within 10q of VF
3	TD010	M	17	1	CD	Floaters	0,63/0,1	N/Hypermetro-picast	38/38	Central scotomas within 10q of VF
4	TD029	M	35	14	CD	Dyschroma-topsia	0,2/0,1	Hypermetropia/ Myopicast	2/2	Central scotomas within 10q of VF
5	TD036	M	16	9	CD	N	0,25/0,25	Myopicast/ Myo-pia	12/10	Central scotomas within 10q of VF
6	TD053	W	17	5	CD	Migraine with-out aura	0,4/0,4	Hypermetropi-cast/ Hyperme-tropicast	22/7	Central scotomas within 10q of VF
7	TD058	W	6	13	D	N	0,75/0,7	Myopic ast/Hy-permetropicast	37/38	Central scotomas extends beyond the 20q range of VF
8	TD061	W	12	10	CD	Dyschroma-topsia	0,16/0,32	Myopia/ Myopi-cast	2/2	RE – central scoto-moas within 20q of VF/ LE - within 10q
9	TD067	M	30	33	CD	N	0,5/0,95	N/ Myopicast	38/38	DA - N/KA – central scotomas within 10q VF

Nr.	TD nr.	Gender	Age of VA deterio ratio-nonset (y)	Disease duration (y.)	Dis-ease	Symptoms, other eye/sys-temic diseases	BCVA RE/ LE	Refraction RE/ LE	Ishihara test RE/ LE	Visual field
10	TD095	M	5	12	CRD	Photophobia; Renal polycys-tosis	0,5/0,45	Hypermetropi-cast/ Hyperme-tropicast	2/2	central scotomas within 10q VF
11	TD113	W	36	1	CD	N	0,5/0,5	Myopicast/ Myo-picast	17/20	RE -centrals scoto-mos within 10q of VF/ LE ->20q
12	TD123	W	10	21	CRD	N	0,005/0,005	Myopicast/ Myopic s	2/2	N

BCVA – best-corrected visual acuity, RE – right eye, LE – left eye, CD – cone dystrophy, CRD – cone-rod dystrophy, ast – astigmatism

TABLE 13. Findings of fundus ophtalmobiomicroscopy, AF, OCT and mfERG in subjects with cone/come-rod dystrophy

Nr.	TD nr	Macular appearance	More detailed FAF evaluation	Macular appearance on SD-OCT RE/ LE	CRT RE/ LE	SCT RE/ LE	EZ at the macula BE	EZ defect length (µm) RE/ LE	mfERG
1	TD003	Very mild dyspigmentation	Foveal granularity	ERM+VMT/ ERM+VMT	140/158	286/258	Normal	-	Slightly lowered central amp
2	TD007	Dyspigmentation	CA, hyperAF ring	N/N	72/59	245/277	Absent	1283/1554	Slightly lowered central amp
3	TD010	Dyspigmentation	HyperAF ring	N/N	83/70	312/310	Absent	1451/1508	Lowered central amp, eccentric fixation
4	TD029	Yellow flecks	Foveal granularity	N/N	196/200	298/327	Normal/ Absent	Normal /701	Lowered central amp
5	TD036	Very mild dyspigmentation	Foveal granularity, hyperAF ring/ CA, hyperAF ring	CME+lamellar hole/ CME+lamellar hole	145/160	106/306	Absent	662/786	Lowered central amp
6	TD053	Dull macular reflex	Foveal granularity	N/N	202/195	360/360	Normal	-	Lowered central amp
7	TD058	N	Foveal granularity	N/VMT	140/140	464/434	Disrupted	-	Flattened central *hill*
8	TD061	Dyspigmentation, hypopigmented areas at the fovea	CA, hyperAF ring	N/N	146/155	480/536	Absent	1133/1176	Lowered central amp

Nr.	TD nr	Macular appearance	More detailed FAF evaluation	Macular appearance on SD-OCT RE/ LE	CRT RE/ LE	SCT RE/ LE	EZ at the macula BE	EZ defect length (μm) RE/ LE	mfERG
9	TD067	Dyspigmentation, local RPE atrophy	Foveal granularity/ CA	VMT/ CME+lamellar hole	151/155	254/160	Disrupted/ Absent	- /2065	Flattened central *hill*
10	TD095	N	-	VMT/VMT	208/212	553/384	Normal	-	Lowered amp in all zones
11	TD113	Dyspigmentation	Foveal granularity	N/N	124/138	147/125	Normal	-	Slightly flattened central *hill*
12	TD123	Atrophy, dyspigmentations, pigmented lesions	CA, hyperAF extends beyond arcades	VMT/VMT	67/66	149/162	Normal	-	N

AF – autofluorescence, SD-OKT –spectral domain optical coherence tomography, mfERG – multifocal electroretinography, FAF – fundus autofluorescence, CRS – central retinal thickness, SCT – subfoveal choroidal thickness, EZ – ellipsoid zone, BE – both eyes, RE – right eye, LE – left eye, ERM – epiretinal membrane, VMT – vitreomacular traction, CA – central atrophy, CME – cystic macular edema, RPE – retinal pigment epithelium, amp - amplitude

2.3.3. Genetically confirmed cases of *ABCA4* retinopathy

In all subjects with *ABCA4* retinopathy molecular genetic testing of the most common pathogenic variations of *ABCA4* gene was performed. Coding and adjacent sequences of exons 12 and/or 21 and 42 were examined, fragments where most common pathogenic changes are identified in Lithuanian patients group. Molecular diagnosis of *ABCA4* retinopathy was confirmed for five patients; in all subjects compound heterozygous genotype of mutations CM990022 and CM970016 genotype was identified. In seven subjects with ABCA4 retinopathy pathogenic variation CM970016, possibly pathogenic variation HM060037 or complex disease causing allele L541P; A1038V in one allele were found.

During this study, the following sequence changes were identified in subjects with *ABCA4* retinopathy:

- CM970016 mutation frequency in the group of Lithuanian patients with *ABCA4* retinopathy is 11.84%;
- CM990022 mutation frequency in the group of Lithuanian patients with *ABCA4* retinopathy is 5.26%;
- HM060037 mutation frequency in the group of Lithuanian patients with *ABCA4* retinopathy is 1.32%;
- L541P; A1038V complex mutation allele frequency in the group of Lithuanian patients with *ABCA4* retinopathy is 3.95%. Sequence changes presented in this complex two- substituions allele is inherited in cis-configuration. Such complex is more frequently identified in population of German origin and therefore is considered as German founder effect alteration. 12.7% of patients with diagnosed *ABCA4* retinopathy are of German origin compared with 1.1% of non- German subjects.

Subject TD050

Analysis of sequences of exons 12, 21 and 42 and adjacent sequences of *ABCA4* gene identified the following genotype: c.[1622T>C];[5882G>A] (p.[(Leu541Pro)];[(Gly1961Glu)]) - rs61751392, CM990022; rs1800553, CM970016*.

Possibly compound heterozygous genotype of pathogenic mutations c.1622T>C and c.5885F>A was identified in gene *ABCA4* causing autosomal recessive STGD.

Subject TD114

Analysis of sequences of exons 12, 21 and 42 and adjacent sequences of *ABCA4* (MIM#601691) gene identified the following genotype: c.[(1622T>C(;)3200C>T)];[(5882G>A)] (p.(Leu541Pro)(;)(Ala1038Val);(Gly1961Gln)) - rs61751392, CM990022; rs61751374, CM970006; rs1800553, CM970016*.

Molecular genetic testing indentified possibly compound pathogenic heterozygous mutation genotype causing STGD (OMIM#248200, ORPHAN:827). Changes possibly identified in one allele (rs61751392+rs61751374) are reported being inherited together (complex allele).

Discussion

Results of clinical study of two subgroups are rather similar, except the ocular fundus. The most common refraction abnormality in both groups is myopic astigmatism (86.67% ir STGD subgroup and 71.42% in CD/CRD subgroup). *Ishihara* test did not show any or showed only insignificant disorders of color vision in 53.33% of subjects with STGD and 57.14% of subjects with CD/CRD. In both groups central scotomas were found in subjects during the assessment of visual field.

Using SD-OCT technique different stages of „optical gaps" were identified in 73.33% of subjects with STGD and 50% of subjects with CD/CRD. However, optical gap has been described also in other diseases such as solar retinopathy, rod monochromatism and maculopathies related to *RP1L1* mutations [17]–[20] and therefore this sign is not pathognomonic to RD related to *ABCA4*.

The importance of electrophysiological testing, ERG and EOG is still disputable. No EOG was conducted in study participants.

According to study conducted by *K. G. Noble and R. E. Carr*, full-field ERG shows decrease of the amplitude only in advanced disease (16%, 50 subjects examined) [21]. Because subjects of various age and duration of the disease underwent full-field ERG in the STGD group, assessment of changes according to *Lois* classification is presented for all three groups: 7 were assigned to group I and by 4 subjects were assigned to groups II and III. In subjects with CD full- field ERG did not identify any changes, and in both subjects from CRD group lowered responses were observed both in photopic and scotopic phases that could be expected in subjects with CRD. Due to small samples both in STGD and CD/CRD groups statistical analysis considered to be inappropriate and was not conducted, therefore comparison is impossible.

U. Kretschmann et all. examined mfERG changes in Stargardt disease. They Identified changes even is subjects with good VA: in 25 eyes out of examined 28 (89.3%) regional cone dysfunction was found even when VA was higher than 0.6 [22]. We conducted mfERG in 8 subjects (53.33%) with STGD and in all subjects (100%) changes in central or all zones were found, weakened or disappeared potentials. Also, in all 11 subjects with CD/CRD who underwent mfERG weakened or disappeared potentials were observed. These findings confirm importance of mfERG, especially in subjects with early stages of the disease when there may be no changes of fundus of eye or disorders of color.

2.4. Other inherited retinal dystrophies

Ten different IRD were identified in the group of other rare IRD (Figure 21). Diseases in which molecular genetic testing was conducted are presented below.

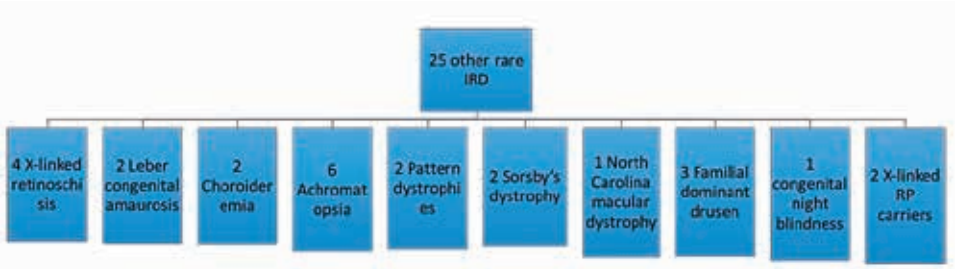

FIGURE 26. The groups of other rare retinal dystrophies.

2.4.1. Subjects with achromatopsia

Six subjects were allocated in the group of achromatopsia (ACHM).

In all subjects, symptoms of ACHM were present since birth. However, only during the study ACHM diagnosis was clinically suspected and molecular genetic testing showed abnormalities causing ACHM.

Subjects' BCVA was very similar and ranged from 0.07 to 0.16; all subjects except TD089 were hypermetropic. Detailed data are presented in Table 14.

TABLE 14. ACHM patients age, BCVA, subjective complaints and color vision data (CV – color vision, HM – hypermetropia, Ny – nystagmus, V – vision, Phb - photophobia).

	TD011	TD012	TD013	TD089	TD121	TD122
Age (y)	28	27	29	13	14	5
Complaints	Phb Low V Low CV	Phb Low V Ny No CV HM	Phb Low V Ny No CV HM	Phb Low V Ny	Phb Low V Ny	Phb Low V Ny
BCVA RA/LA	0,12/0,12	0,1/0,16	0,1/0,1	0,12/0,12	0,12/0,12	0,08/0,07
Ishihara test RA LA	8/ 38 7/38	2/38 2/38	2/38 2/38	5/ 38 3/ 38	6/ 38 7/ 38	2/ 38 2/ 38

Absence of glaze fixation and horizontal nystagmus, symptoms characteristic to ACHM, were observed in all subjects. Due to these symptoms many tests were challenging or impossible to conduct.

No specific changes of eye anterior segment were found at ophthalmobiomicroscopy. As usual in ACHM, no changes or minimum changes were found in the fundi of the eyes: lack of macular reflex (TD011, TD012, TD013) and mild depigmentation (TD011 and TD012) (Figure 22). Due to significant photophobia and age imaging of fundi of the eye was not made in TD012 and TD122.

Fluorescein angiography tests showed no significant changes, only in TD013 mild hyperAF was observed.

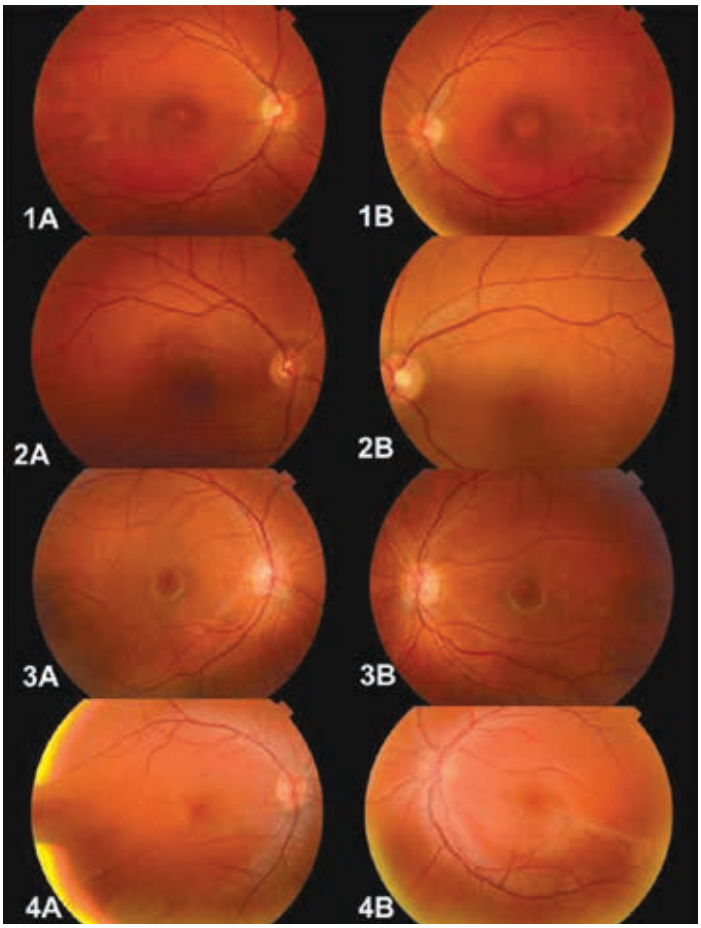

FIGURE 27. Images of fundus in ACHM subjects.

TD011: BE mild dispigmentation and dull macular reflex are found (1A-B). TD013: dull macular reflex (2A-B). TD089 ir TD121: without obvious (3A-B ir 4A-B).

SD-OCT testing revealed a variety of macular changes (Figure 23). Foveal hypoplasia of different degree was observed in all patients.

In TD122 SD-OCT testing was not conducted due to subject's young age. EZ defects in the macula were observed in 3 subjects: EZ integrity derangement with defect length in the RE 1269 Pm and in the LE 1345 Pm was observed in TD011; total loss of EZ in subfoveal area called optical gap with defect length of 715 Pm in the RE and 641 Pm in the LE was observed in TD012; and small integrity defects were observed in TD089.

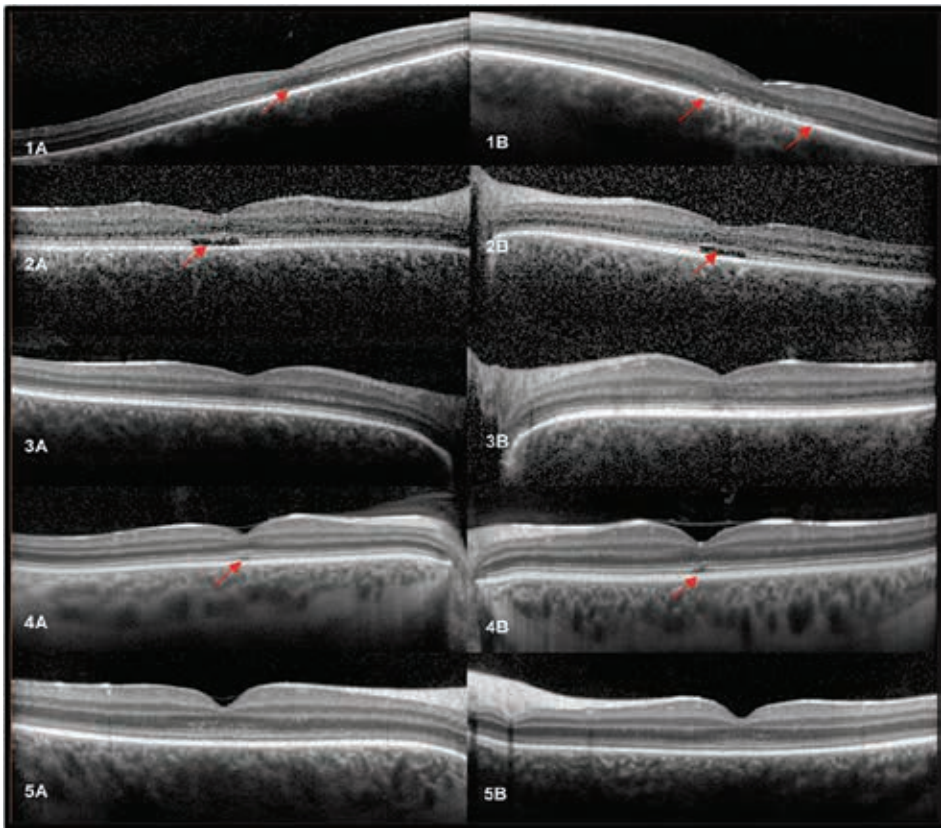

FIGURE 28. ACHM patients macular SD-OCT scans.

TD011: RE EZ loss was found (1A), LE – EZ structure is lost (1B) (red arrows). TD012: BE optical gap is observed (2A-B) (red arrows). TD013 and TD121 very mild EZ and external limiting membrane structure changes are observed (3A-B ir 5A-B). TD089: very mild EZ integrity loss (4A-B) (red arrows).

In all subjects, CRT was thinned compared with normal ranges (Table 15). This thinning could be related to the pathogenesis of the disease, lack of cone photoreceptors that are most abundant in the macula.

TABLE 15. CRT results (μm) in ACHM subjects.

CRT (μm) Subject	RE	LE
TD011	192	181
TD012	206	221
TD013	222	216
TD089	185	177
TD121	208	216

It was difficult to conduct SD-OCT due to significant photophobia, and in 2 subjects SCT was measured using the EDI mode. In TD089 it was 280 Pm in the RE and 257 Pm in LE eye. In TD121 subfoveal choroid was significantly thicker: 418 Pm in the RE and 428 Pm in the LE.

Full-field ERG was conducted in all subjects; normal, slightly lowered or halved responses were observed in scotopic phase, and in photopic phase lowered amplitudes of waves including *flicker* ERG showing isolated response of cones were observed almost no responses were seen.

Results of genetic testing

In all subjects with ACHM molecular genetic testing was performed, and pathogenic changes in *CNGB3* (cyclic nucleotide- gated channel, beta-3) gene were identified for 5 patients (Table 16). In the 16th table the references are listed, where the mutations were described for the first time.

TABLE 16. ACHM patients pathogenic CNGB3 mutations.

Patient	Gene	Genotype	Identified mutation	Literature
TD011	*CNGB3*	compound heterozygous	c.31insC (p.Val11fs*9) c.1148delC (p.Thr383Ilefs*13)	*Kohl et al.*, EJHG; 2005 *Sundin et al.*, Nature Genet; 2000
TD012	*CNGB3*	homozygous	c.819_826del (p.Arg274Valfs*13)	*Sundin et al.*, Nature Genet; 2000

Patient	Gene	Genotype	Identified mutation	Literature
TD013	*CNGB3*	homozygous	c.819_826del (p.Arg274Valfs*13)	*Sundin et al.*, Nature Genet; 2000
TD121	*CNGB3*	homozygous	c.1148delC:p.Thr383IlefsX13	*Sundin et al.*, Nature Genet; 2000
TD122	*CNGB3*	homozygous	c.1148delC:p.Thr383IlefsX13	Sundin et al., Nature Genet; 2000

Discussion

Specific instrumental ophthalmologic testing is of extreme importance in the diagnosis of ACHM because ophthalmobiomicroscopy shows no or only very mild non-specific changes. Lack of macular reflex or mild depigmentation was observed in only 4 out of 6 subjects (66.67%). *Genead et al.* found changes in the yellow-spot area in 58.3% of subjects [23], similar results were reported in other studies [24], [25].

OCT also showed a variety of macular changes: hypoplasia of *fovea* of various degrees, loss of EZ structural integrity or optical gap, thinning of CRT. Only in one subject, TD012, optical gap was observed. Prevalence of optical gap on ACHM patients is unknown; it was found in the presence of various genotypes [20], [26], [27]. Different morphological changes are observed even in sibs (TD12 and TD13) with identified pathogenic deletion c.819_826del p.Arg274Valfs*13 in *CNGB3* gene. These results are in line with the literature data arguing that the same abnormal change may cause different phenotype, ant presently the cause of this is unknown [23]. Due to small sample size we are unable to confirm or dispel hypothesis set up by *Thiadens et al.* that different phenotypes of the same genotype could be observed due possible progression of ACHM [20].

FAF images of the subjects showed characteristic hyperAF which is considered to be early sign of ACHM and could be useful to confirm diagnosis [18].

In subjects, pathogenic changes in *CNGB3* genes were identified which combined are responsible for 75% of ACHM cases [27]–[29]. Prevalence of gene mutations causing ACHNM in Lithuanian population is not known yet, and molecular genetic testing in larger samples is necessary.

Identified in subjects TD012 and TD013 8b deletion c.819_826del p.Arg274Valfs*13 in *CNGB3* gene causing frame shift was reported for the first time by *Sundinir et al.* in 2000 [30]. This mutation causes premature termination of translation of *CNGB3* protein, and therefore necessary areas of protein channel composing pair, S6 transmembrane part and cGMP connecting domain are eliminated [31].

Identified in subjects TD121 and TD122 deletion c.1148delC:p.Thr383IlefsX13 in *CNGB3* gene causing frame shift was reported for the first time by *Sundin et al*. in 2000 [30]. This mutation also causes premature termination of translation of *CNGB3* protein [32].

2.4.2. Subjects with X-linked inherited juvenile retinoschisis

Four subjects with clinically suspected XLRS participated in the study; molecular genetic testing revealed pathogenic mutation of *RS1* gene in these study patients.

All subjects were males, their main complaints were difficulties reading, tiredness of eyes looking from short distances and text flickering while reading.

Subjects' age and data on VA are presented in Table 17. Hypermetropic astigmatisms presented refraction abnormalities in subjects. Anterior segment was unremarkable.

TABLE 10. XLRS patients' age and BCVA data.

	TD002	TD017	TD022	TD137
Age	12	9	17	6
BCVA RE/LE	0,32/ 0,32	0,6/ 0,5	0,6/ 0,3	0,3/ 0,3
Near BCVA RE/ LE	0,6/ 0,6	0,6/ 0,6	0,6/ 0,6	0,4/ 0,4

Images of fundi of the eyes of subjects are presented in Figure 23; ophthalmoscopy shows no changes of OND, lack of macular reflex is observed as well as macular cystic changes and presentation or „wheel spokes" which is especially characteristic to XLRS. In 2 subjects out of 4 (50%) changes in the peripheral part of retina were observed: peripheral schisis in the lower and nasal part, mild traction and lamellar gap of internal layers and obliterated blood vessels were observed in TD002 and demarcation lines in the nasal part in the RE and in the paramacular temporal part in the LE were observed in TD017.

SD-OCT revealed retinoschisis in the macula, predominantly in the internal nuclear layer and less prominent in the external retinal, external nuclear and retinal ganglion cells layers; formation of intraretinal gaps of various sizes was also observed (Figure 25). CRT ranged from 373 Pm to 777 Pm; mean CRT of all subjects was 498.5 Pm in the RE, and 498.25 Pm in the LE. SCT was evenly distributed among subjects and ranged from 304 Pm to 375 Pm; mean SCT of all subjects was 323.75 Pm in the RE and 340 Pm in the LE.

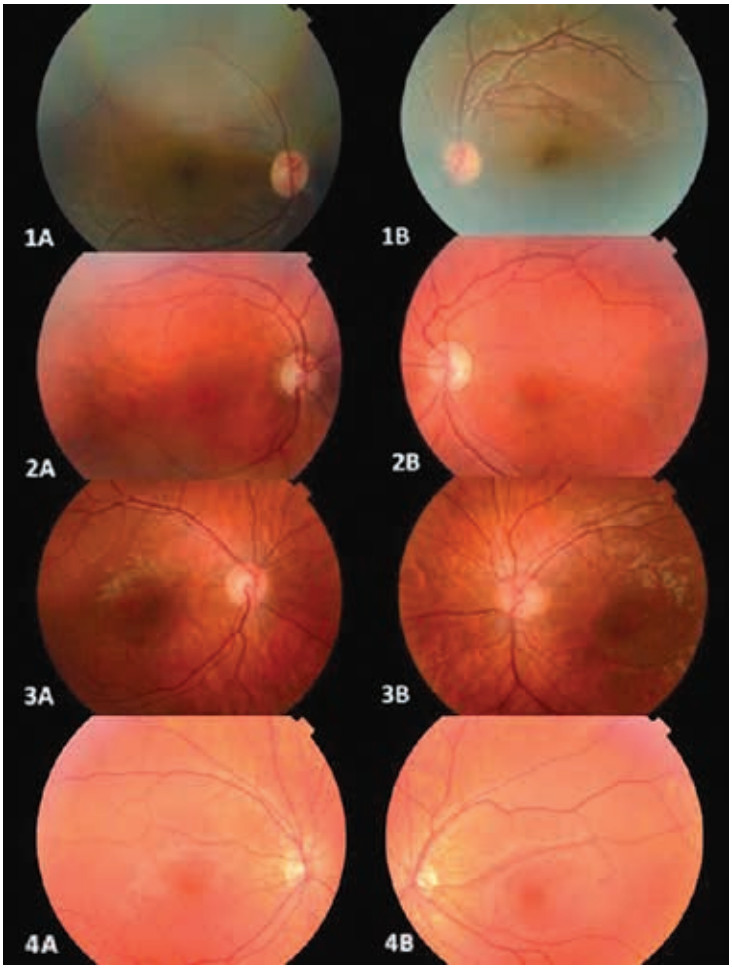

FIGURE 29. Images of fundus in XLRS subjects, macular appearance. TD002: dystrophic changes at the macula, dyspigmentation, CME (1A-B); TD017:"wheel spokes" like CME, foveoschisis, "star"-like (3A-B); TD137 – CME without dyspigmentation.

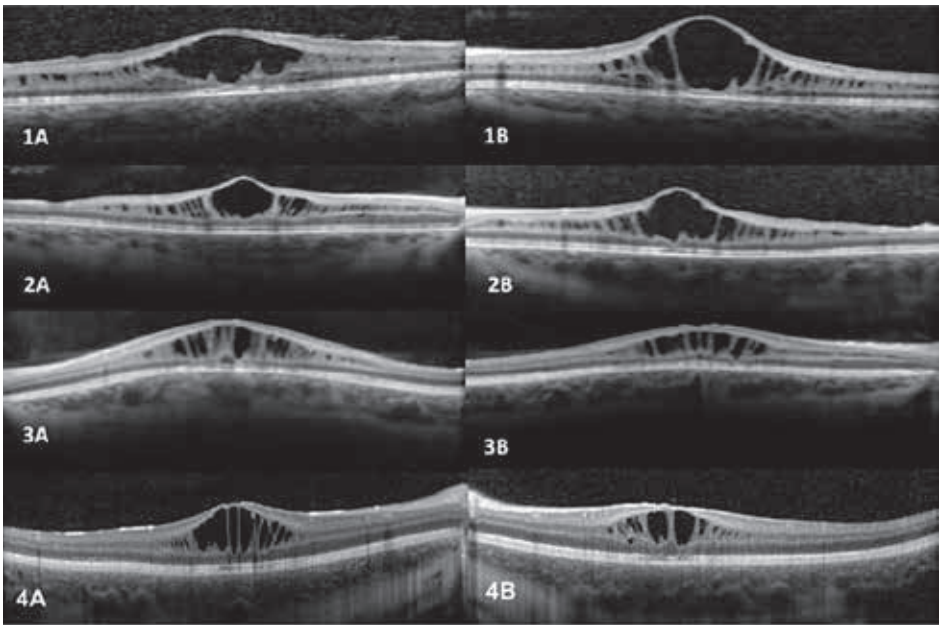

FIGURE 30. Macular SD-OCT retinal schisis with large i/r cavities are observed for all patients: TD002 CTS RE – 600 µm, LE – 661 µm (1A- B); TD017 CRT RE – 570 µm, LE – 777 µm (2A-B); TD022 CRT RE – 373 µm, LE – 434 µm (3A-B), TD137 CRT RE – 450 µm, LE - 379 µm (4A-B).

Ishihara color test was normal in all subjects, 38 out of 38 pictures were assessed correctly. pictures were assessed correctly.

Full-field ERG was conducted in 3 out of 4 subjects (Figure 26), end 3.0 ERG mode showed negative ERG, pathognomonic sign of XLRS. Mild lowering of amplitude of b-wave was observed in subjects under conditions of photopia. mfERG was additionally conducted in TD017 and TD022, and significant lowering of amplitudes of central responses were observed. In TD137 full-field ERG was unreliable due to young age of the subject; it is not involved into discussion.

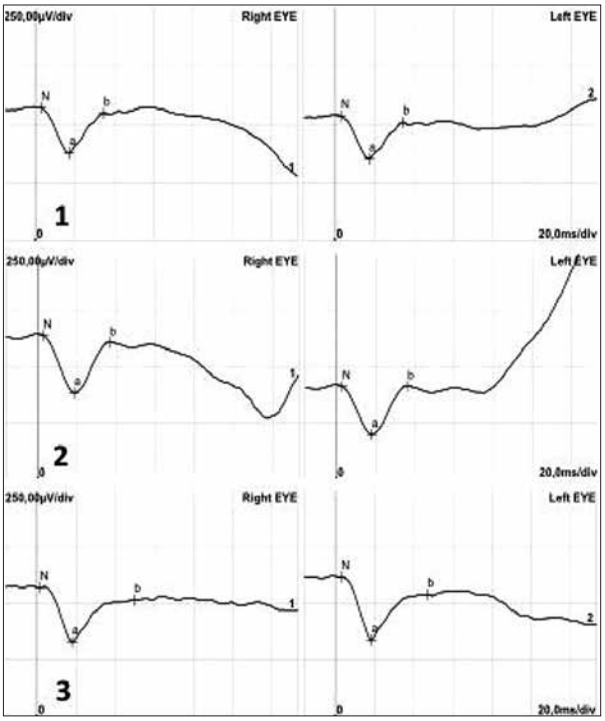

FIGURE 31. Full-field ERG combined response shows a negative waveform the plot showed an electronegative ERG with b/a wave ratio of: RE-0,876 and LE-0,848 for TD002 (1), RE-0,875 and LE-1,0 for TD017 (2) and RE-0,769 and LE-0,723 for TD022 (3).

Results of genetic testing

Genealogy of subjects is inconclusive.

Mutation NM_000330.3: c.599G>T (p.R200L) was indentified in subjects TD022 and TD137, *in silico* analysis confirmed pathogenicity (disease-causing mutation (mutationtaster.org); PROVEAN score -6.883 – dexterous; probably damaging). Three other different changes of the same aminoacid (CM095237 [33], CM981767 [34], CM981768 [34]) supporting pathogenicity of identified variations are published in the Human Genome Mutation Database (HGMD). NM_000330.3:c.(92_97)insC (p.W33fs) insertion creating frame shift is identified in subject TD002, and *in silico* analysis, again, shows pathogenicity of this variant (mutationtaster.com). Previously reported [34] pathogenic mutation NM_000330.3:c.422G>A (p.R141H), HGMD CM981753 was identified in third subject, TD017. Sequenograms of *Sanger* sequencing results are presented in Picture 27.

Mothers of all patients were examined; they had no clinical ophthalmological signs of XLRS. *Sanger* sequencing confirmed that mothers of all subjects were heterozygous carriers of the identified pathogenic mutations.

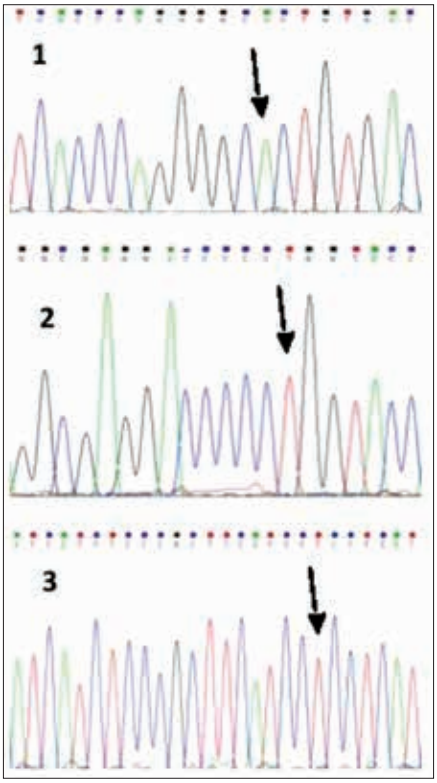

FIGURE 32. The Sanger sequencing sequenograms indicating the identified variants: 1 – TD017 (NM_000330.3:c.422[G>A];[0] (NP_000321.1:p.[(Arg141His)];[(0)]), 2 - TD002 (NM_000330.3:c.599[G>T];[0] (NP_000321.1:p.[(Arg200Leu)];[(0)])), 3 – TD022 ir TD137 the same variant was identified (NM_000330.3:c.96[insC];[0] (NP_000321.1:p.[(Trp33Leu)];[(0)]).

Discussion

According to the literature, foveal retinoschisis was observed in 68% to 100% patients with XLRS [35], and detachment of peripheral retina was observed in 43% to 60% of patients. In our study significant foveal retinoschisis was observed in all four subjects with XLRS, and peripheral retinoschisis was observed in 2 subjects (50%). These results are in line with the literature data. Various other possible abnormalities of peripheral retina are also reported [35], [36] with prevalence ranging from 93% to 38%; peripheral abnormalities were observed in 2 of our subjects.

OCT testing revealed retinoschisis mostly in the internal nuclear layer, and less frequently it was observed in the external retinal, external nuclear and retinal ganglion cells layer, i/r formations of various sizes were also observed in places; these findings were in line with data previously reported by *Padrón-Pérez at al.* and *Yu at al.* [37], [38].

Sign which is reported as pathognomonic, electronegative dark adaptations 3.0 cd*s/m^2 (DA 3.0) ERG, were found in all our subjects; however, these signs is not always seen in subjects with XLRS [39].

At the moment, alterations of single *RS1* gene are responsible for XLRS [40]. Pathogenic alterations of *RS1* gene were identified in all subjects, pathogenic changes that are undocumented yet were identified in 2 subjects [33], [34]. According to *Fahim et al.*, patients with identifies pathogenic *RS1* gene mutation and peripheral retinal abnormalities has higher risk of complications of retinoschisis [36].

2.4.3. Subjects with familial dominant drusen

Three subjects are included into this group and all of them are female relatives (sisters) TD100, TD104 and TD110.

It is important to emphasize that subjects had no ophthalmological complaints.

Subjects' age ranged from 38 to 41 years, VA was 1.0-1.5, color vision was normal.

In all subjects, anterior segment was normal, ophthalmobiomicroscopy showed normal OND. Images of the fundi are presented in Figure 28, yellowish-whitish drusen of various abundance was observed in the macula of all subjects.

Fundus AF showed sporadic hyperfluosescence spots corresponding drusen at fundus.

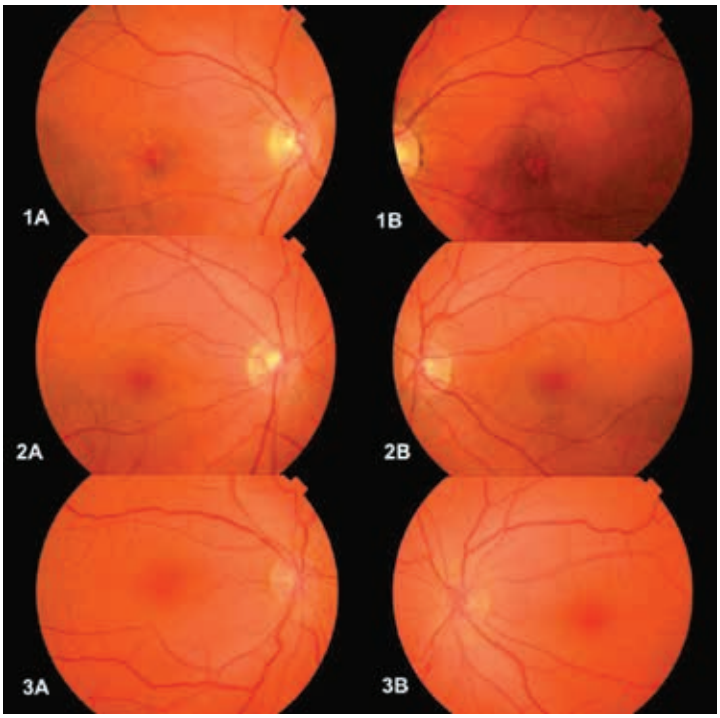

FIGURE 33. For all patients yellowish-white macular flecks were observed.

TD100 – 1A-B; TD104 – 2A-B; TD110 – 3A-B.

SD-OCT testing was conducted in all subjects (Figure 29). Most significant changes of SD-OCT were observed in subject TD100 who had the most abundant drusen.

KP 30-2 testing did not show any central scotoma in subjects.

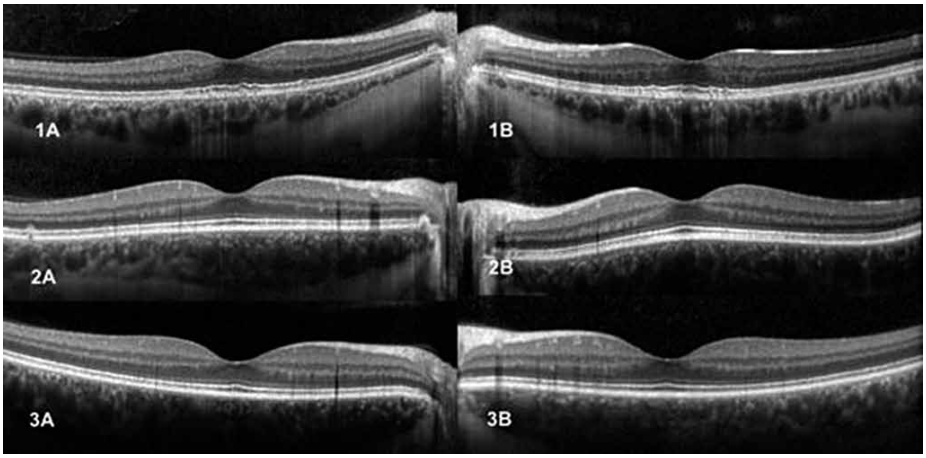

FIGURE 34. On macular SD-OCT sub-retinal pigment epithelium deposits are observed for TD100 (1A-B), the OCTs of TD104 (2A-B) and TD110 (3A-B) were without RPE irregularities.

In all subjects full-field ERG was conducted, results were normal, no data indicating disorder of function of photoreceptors were obtained.

Whereas abnormalities were characteristic to familial dominant drusen disease, targeted molecular genetic testing was conducted.

Results of genetic testing

Molecular genetic testing of *EFEMP1* (EGF-containing fibulin-like extracellular matrix protein 1) gene causing AD inherited FDD disease was performed in all patients. Most common mutation c.1033C>T, p.Arg345Trp of *EFEMP1* gene was analysed and not identified in any of the tested research patients. Promotors, introns and other sequences were not included into analysis, and therefore probability of possible changes of these parts of gene could not be denied. *EFEMP1* :c.1033C>T, p.Arg345Trp is the most common molecular cause of AD inherited FDD [41].

Discussion

The only known mutation of *EFEMP1* gene causing FDD - non-conservative R345W (arg345trp) - was identified in 1999 [42]. Therefore, sequencing and target

analysis of parts of 9th and 10th exons and adjacent sequences of gene *EFEMP1* were conducted in our subjects. Aforementioned tests did not reveal pathogenic mutation .1033C>T, p.Arg345Trp in our subjects. Possibility of FDD could not be denied because cases when FDD diagnosis is based on clinical data are reported (*Tarttelin et al.*) [43], although molecular genetic testing does not reveal pathogenic changes in *EFEMP1* gene. In cases of AMD no changes of *EFEMP1* gene are found despite significant similarity of phenotype; this correlation between inherited and age- related diseases is characteristic to many age-related diseases [44].

Clinical symptoms of subjects are in line with reported in the literature. No one of subjects (their age range from 38 to 41 years) presently has no ophthalmologic complaints; however, according to the literature, symptoms usually occur at the age of 40-50 years [45].

2.4.4. Subjects with North Carolina macular dystrophy

Subject TD071, 50 years of age, several months ago noticed deteriorating and distorted vision with her RE. She has poor VA of her LE for 30 years.

FIGURE 35. In the fundus photo of the RE confluent yellow macular deposits are observed (1A), in FAF hypoAF area at the macula surrounded by hyperAF (2A), on SD-OCT SRF is observed (3A).
In fundus photo of the coloboma-like atrophicmacular lesion is observed (1B), at the FAF large macular hypoAF lesion (2B), on SD- OCT atrophy of the neurosensorial retinal and RPE is observed (3B).

Based on history and clinical findings, North Carolina macular dystrophy was suspected (Figure 30).

Results of genetic testing

Changes in copy number variation in the genomic region involving *PRDM13* gene are related to North Carolina macular dystrophy [46]. SNP comparative genome hybridization was performed with analysis of 220000 probes tag SNPs in the SNP chip located in the clinically significant genome sites (including subtelomeric and pericentromeric regions and sex chromosome) and in ~400 disease-related gene areas, no pathogenic changes of copy number variantion related to reported diseases and syndromes were identified.

Discussion

North Carolina macular dystrophy penetration is full and it is considered as non-progressive RD with various phenotypic presentation and usually bilateral lesion [47], [48]. Although disease is non-progressive, complications such as CNV may cause deterioration of VA [49].

After intense genetic testing in 1999, abnormalities causing North Carolina macular dystrophy were identified in 6q16 chromosome (MCDR1, MIM:136550) in several families of different ethnic origin [50]–[53]. Similar phenotype was identified in the second locus 5p15-p13 (*MCDR3*, MIM:608850) [47] [54]. Recently, 3 new single nucleotide variations were identified in 11 families, in *MCDR1* locus, DNase hypersensitivity site (DHS), non-coding interval between PRDM13 and adjacent overlapping genes *CCNC/TSTD3* [46].

Conclusions

1. Registry and monitoring system of the inherited retinal diseases was established and developed in the Center of Eye Diseases, Clinic of Ear, Nose, Throat and Eye Diseases, Institute of Clinical Medicine of the VU Faculty of Medicine.
2. Pathogenic changes confirming molecular diagnosis of inherited retinal dystrophies were identified in 16,81% of subjects. Four pathogenic variants alterations of *RHO*, *PRHP2* and *MYO7A* genes were identified in the largest group of 67 subjects with retinitis pigmentosa.
3. Biostatistical analysis showed statistically significant correlations between central retinal thickness, subfoveal choroidal thickness, remained ellipsoid zone, diameters of hyperautofluorescent ring, visual field and SNPs of the retinitis pigmentosa genes.

4. The group of *ABCA4*-linked retinopathies was divided in two subgroups according the phenotype. In 6 subjects with *ABCA4*- linked retinopathies variants of *ABCA4* gene were identified and the prevalence of these variants were determined: CM970016 (11,84%), CM990022 (5,26%), HM060037 (1,32%), L541P;A1038V (3,95%).
5. In the group of other rare inherited retinal dystrophies pathogenic variants were identified:
   5.1. Variants of *CNGB3* gene were identified in 5 subjects with achromatopsia.
   5.2. In the group of subjects with X-linked hereditary juvenile retinoschisis 3 variants of *RS1* gene were identified, two of them were novel.

Practical recommendations

1. Considerable clinical and genetic heterogeneity of IRD requires close cooperation between ophthalmologists and geneticists in order to establish precise diagnosis. Consultations by other specialists are advisable in syndromic cases.
2. Definite diagnosis of IRD enables to inform patient about course of the disease, collect data on the disease and its pathogenesis in order to develop possible treatment strategies and assess likelihood of inheritance of the disease.
3. Genetical testing of patients using Next generation sequencing may help establish definite clinical diagnosis and conduct more precise clinical examination and therefore it should be recommended for patients.

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The approbation of the results

Publications

1. **Strupaitė R**, Tumienė B, Kohl S, Utkus A. Achromatopsija, nulemta *CNGB3* geno homozigotiniai pokyčiai: šeiminio atvejo pristatymas. Laboratorinė medicina. 2017, t. 19, Nr. 1, p. 42 – 48.

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Fellowships and observerships attended:

- **2017 (1 month)** – Observership at Eye Clinic of the University Hospital Erlangen (Erlangen, Germany)
- **2017 (1 week)** – Salzburg seminars of Ophthalmology, (Salzburg, Austria)
- **2016 (1 week)** – Clinical Electrophysiology of Vision courses at Moorfields Eye Hospital (London, UK)
- **2015 (1 week)** – Venice Summer Ophthalmology School (Venice, Italy)
- **2014 (1 month)** – Observership at University Eye Hospital (Ljubljana, Slovenia)
- **2013 (3 months)** – Fellowship at Heidelberg University Clinics Eye Department (including Fellowship at David J Apple Laboratory) (Heidelberg, Germany)
- **2008 – 2009** Fellowship at Leipzig University Hospital Department of Ophthalmology (duration – 2 weeks); fellowship at Institut of Virology, Leipzig (duration - 2 weeks) (Heidelber, Germany)
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Alina Urnikytė

AN EVALUATION OF THE GENETIC STRUCTURE AND EVOLUTIONARY FORCES OF THE LITHUANIAN POPULATION ACCORDING TO HIGH-DENSITY GENOTYPING DATA: PAST AND FUTURE

LIETUVOS POPULIACIJOS GENETINĖS STRUKTŪROS IR EVOLIUCINIŲ VEIKSNIŲ ANALIZĖ, REMIANTIS PLATAUS MASTO GENOTIPAVIMO DUOMENIMIS: PRAEITIS IR DABARTIS

Biomedicinos mokslai, medicina (06 B)
Biomedical Sciences, Medicine (06 B)

1. Introduction

The contemporary Lithuanian population is composed of a complex mixture of the former Baltic tribes. Thus, the roots of the present Lithuanian population are deep, and it is highly probable that the inhabitants of present-day Lithuania have preserved this ancient genetic composition. The availability of high-throughput genotyping platforms and next-generation sequencing techniques and the development of new statistical and computational methods in the field of evolutionary genomics allow us to infer evolutionary forces and more finely scale the genetic structure of a population. The analysis of geographically specific regions and the characterization of fine-scale patterns of genetic diversity may facilitate a much better understanding of the microevolutionary processes affecting local human populations.

Aim of the Study

An evaluation of the the local patterns of population structure, the signatures of adaptive positive selection and evolutionary demographic parameters from high-density SNP genotyping data generated in the Lithuanian population.

Tasks of the Research

1. To infer and evaluate the genetic structure of the Lithuanian population using high-density SNP genotyping data.
2. To investigate the signatures of positive selection in the Lithuanian population using high-density SNP genotyping data.
3. To infer the demographic parameters of the Lithuanian population and the changes in long-term effective population size including the date of the Lithuanian split in comparison with other populations.
4. To reconstruct past events between the two main ethnolinguistic groups (the Aukštaičiai and Žemaičiai) of Lithuania using effective population size and estimated divergence time.
5. To infer the recent effective population size using inferred long segments of identity by descent and to estimate the effective/census size ratio in the Lithuanian population.

Statements to be Defended

1. The Lithuanian population is homogeneous and genetically differentiated from its neighboring populations but only within the expected general European context.

2. Specific signals of positive selection do exist in the Lithuanian population.
3. The long-term effective population size is small compared to other European populations.
4. A statistically significant difference in effective population size may suggest a potential genetic difference between the Aukstaičiai and Žemaičiai groups.
5. The population of Lithuania is small and has historically suffered the effects of population bottlenecks and expansions, which might produce very small *Ne*/*N* values.

This research contributes to the progress of scientific knowledge and is of special importance in clarifying the relationship between natural selection and disease as well as improving our understanding of the evolutionary mechanisms observed at the individual and population levels.

2. Materials and methods

2.1. Samples

The data set consisted of 425 samples from unrelated Lithuanian individuals who indicated at least three generations of Lithuanian nationality. The samples were collected randomly from six regions of Lithuania: three groups from Aukštaitija (the Western (n = 79), Southern (n = 67) and Eastern (n = 79) regions) and three groups from Žemaitija (the Northern (n = 79), Western (n = 43) and Southern (n = 78) regions) (Fig. 2.1). In accordance with the Declaration of Helsinki, forms of written informed consent were received from all of the study participants.

FIGURE 2.1. Map of Lithuanian ethno-linguistic groups.

Genomic DNA was extracted from whole venous blood using either the phenol-chloroform extraction method or the automated DNA extraction platform TECAN Freedom EVO (TECAN Group Ltd., Männedorf, Switzerland) based on the paramagnetic particle method. DNA concentration and quality were measured with a NanoDropR ND-1000 spectrophotometer (NanoDrop Technologies Inc., US).

This study is part of the LITGEN project, which was approved by the Vilnius Regional Research Ethics Committee 235 No. 158200-05-329-79 on May 3, 2011.

2.2. Genotyping

Genotyping was performed at the Department of Human and Medical Genetics of the Biomedical Science Institute, Faculty of Medicine, Vilnius University, Lithuania using the Illumina HumanOmniExpress-12v1.1 (296 samples) and the Infinium OmniExpress-24 (129 samples) arrays (Illumina, San Diego, CA, US), which include an overlap of 707 138 SNPs that were distributed genome-wide. Genotyping data quality control was performed according to the standard recommendations of the manufacturer. Individuals and SNPs with > 10% missing data and SNPs with minor allele frequency (MAF) < 0.01 were excluded from the analysis. SNPs with deviations from the Hardy-Weinberg equilibrium ($P < 10^{-4}$) were eliminated from the study. After quality control, 1 individual was excluded with more than 10% missing genotypes (MIND > 0.1), and 532 836 autosomal SNPs remained out of 589 752.

2.3. Data Analysis

2.3.1. Population Genetic Structure Analysis

To characterize the Lithuanian population in a broader genetic context, we merged these SNP genotyping data to those downloaded from the 1 000 Genomes Project Phase3 dataset [1], generating a pooled dataset of 264 950 genome-wide distributed autosomal SNPs in a total of 2 928 individuals from 20 populations and 4 main geographical regions: African populations, including the Yoruba in Ibadan, Nigeria (YRI), the Luhya in Webuye, Kenya (LWK), the Gambian in the Western Divisions in the Gambia (GWD), the Mende in Sierra Leone (MSL) and the Esan in Nigeria (ESN); European populations, including Utah residents with ancestries from Northern and Western Europe (CEU), the Toscani in Italy (TSI), the Finnish in Finland (FIN), the British in England and Scotland (GBR) and the Lithuanians (LT); East Asian populations, including the Han Chinese in Bejing, China (CHB), the Japanese in Tokyo, Japan (JPT), the Southern Han Chinese, China (CHS), the Chinese Dai in Xishuangbanna, China (CDX) and the Kinh in Ho Chi Minh City, Vietnam (KHV); South Asian populations, including the Gujarati Indians in Hous-

ton, Texas (GIH), the Punjabi from Lahore, Pakistan (PJL), the Bengali from Bangladesh (BEB), the Sri Lankan Tamil from the UK (STU) and the Indian Telugu from the UK (ITU).

A principal component analysis (PCA) was carried out with independently pruned SNPs using SmartPCA from EIGENSOFT 7.2.1 [2]. SNPs in linkage disequilibrium were removed with the indep-pairwise option of PLINK v1.07 using a window size of 50 SNPs, a step size of 5 and an r^2 threshold of 0.5 [3]. The PCA was performed for the six ethnolinguistic groups of the Lithuanian sample set alone as well as on the merged Lithuanian 1 000 Genomes Project Phase3 dataset.

Ancestry analysis was performed with ADMIXTURE v.1.3.0 varying the number of ancestral populations K between 2 and 9 [4]. The best K was identified using the cross-error estimation implemented in ADMIXTURE.

2.3.2. The Detection of Signals of Positive Selection

Previous to the analysis of selection, genetic relationships and consanguinity were inferred through the kinship and the inbreeding coefficients, which were estimated with KING v.2.1 [5] and PLINK v1.07 [3], respectively. Negative F values were converted to zero, as they probably represent sampling errors [6]. Outlier samples on the PCA plots and individuals with inbreeding coefficients higher than those expected for offspring from second cousin mating (F values ≥ 0.0156) were removed from the further study.

Genotyping data were phased with SHAPEIT2 [7]. Any signatures of recent or ongoing positive selection were investigated using the locus fixation index (F_{ST}) [8] and the XP-EHH statistic [9], which were computed between the following pairs of populations: LT-CEU, LT-FIN, LT-YRI, CEU-FIN, CEU-YRI and FIN-YRI. The F_{ST} values were calculated using VCFtools v.0.1.13 [10]. XP-EHH was run using Selscan v1.2.0a [11]. For each comparison, a score of XP-EHH per SNP was obtained, and any XP- EHH scores > 2 were considered as indicative of positive selection. We selected as candidates for positive selection any genomic region with two or more SNPs located at the 0.1% top extreme of the XP-EHH genome-wide empirical distribution and with at least one SNP presenting an F_{ST} p-value < 0.01.

Older signals of selection were investigated through the Tajima's D neutrality statistic, which was calculated with the PopGenome package implemented in R [12] considering 100 kb sliding windows across all autosomal regions with a step size of 10 kb. Windows containing missing variants were ignored. As for F_{ST}, the extreme negatives of Tajima's D values were identified considering the rank of the score in the genomic distribution. In particular, the windows were sorted in an ascending order based on Tajima's D values; we considered for further analysis those with empirical p-values less than 0.01.

Variant annotation in the candidate regions for selection was performed with ANNOVAR [13] using GRCh37 (hg19), RefSeqGene, dbSNP147 [14] and CADD version 1.3 [15].

2.3.3. Long-Term *Ne* and Divergence Time Analysis

Long-term effective population size (*Ne*) in the Lithuanian population and their ethnolinguistic groups was estimated using the R package NeON v1.0 based on LD patterns [16]. NeON v1.0 uses binary PLINK files as inputs and updates the genetic map information of the markers to calculate the *Ne* over time, exploiting the relationship between *Ne* and the average squared correlation coefficient of LD (r^2) within any predefined recombination distance categories.

The estimates of *Ne* for the comparison were obtained from a study by M. Mezzavilla and S. Ghirrotto (2015, University of Trieste, Italy), estimated in the HGDP-CEPH panel populations [17] with the R package NeON v1.0 [16].

We merged the Lithuanian population SNP genotyping data to those downloaded from the HGDP- CEPH dataset [17], generating a pooled dataset of 239 325 genome-wide distributed autosomal SNPs in a total of 1 234 individuals from 22 populations and 6 geographical regions: Africa, the Middle East, Central South Asia, Europe, East Asia and Maya. Having the estimates of *Ne* and knowing the population's differentiation, measured by F_{ST} with the software 4P [18], we have also estimated the time of divergence in generations between the Lithuanian and HGDP-CEPH populations and between the six ethnolinguistic groups of the Lithuanian population using the NeON R package [16].

To visualize the evolutionary relationships among the studied populations, a neighbor-joining (NJ) phylogenetic tree was calculated from the divergence time matrix using the R package Phangorn [19].

2.3.3. Recent *Ne* Estimation

To infer the history of the recent effective population size in the Lithuanian population and its ethnolinguistic groups, we used a non- parametric method based on the Wright-Fisher discrete-generation model, implemented in the open-source IBDNe v.04Sep15.e78 software package published by Browning and Browning (2015) [20]. This method is based on the identical by descent (IBD) segments that provide information about the *Ne* around 50 generations from the present one using SNP array data. The length filter used to detect IBD segments with the IBDseq v. r1206 software package was 7 cM.

3. Results

3.1. Analysis of the Lithuanian Population's Genetic Structure

To investigate the genetic similarity between each pair of individuals in the Lithuanian population, we estimated the kinship [3] and inbreeding coefficients [5] using the generated genome-wide SNP data, which were, respectively to the coefficients, 0.00075 and 0.0022. Out of 424 individuals, four had F values higher than expected for offspring from second cousin mating (0.0156). Next, the genetic relationships of the six ethnolinguistic groups in Lithuania were explored at the regional level by performing a PCA using the 232 752 genome-wide-pruned SNPs successfully genotyped in the 424 Lithuanian samples. At that scale, the first two principal components (PC), explaining 22.36% of the variance, showed that the six ethnolinguistic groups formed a single cluster and displayed 8 outliers (Fig. 3.1).

To obtain a European context for the genetic diversity of the Lithuanian population, we then performed a PCA using 158 633 SNPs on a merged dataset with four European populations (CEU, FIN, GBR and TSI) from the 1 000 Genomes Project Phase3 dataset [1] (Fig. 3.2). The first PC, explaining 27.11% of the genetic variance,

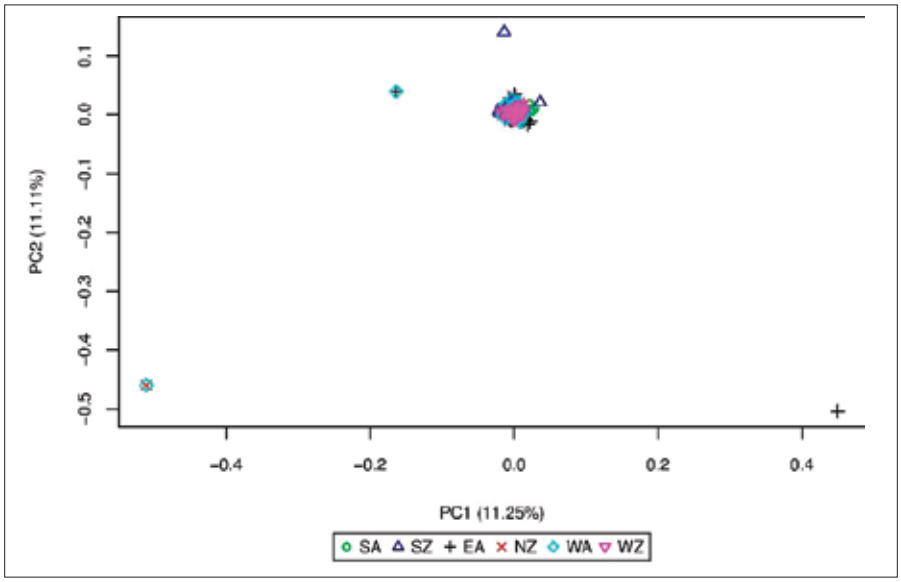


FIGURE 3.1. A principal component analysis of the six ethnolinguistic groups of the Lithuanian population. EA – Eastern Aukštaičiai, SA – Southern Aukštaičiai, WA – Western Aukštaičiai, NZ – Northern Žemaičiai, SZ – Southern Žemaičiai (SZ), WZ – Western Žemaičiai.

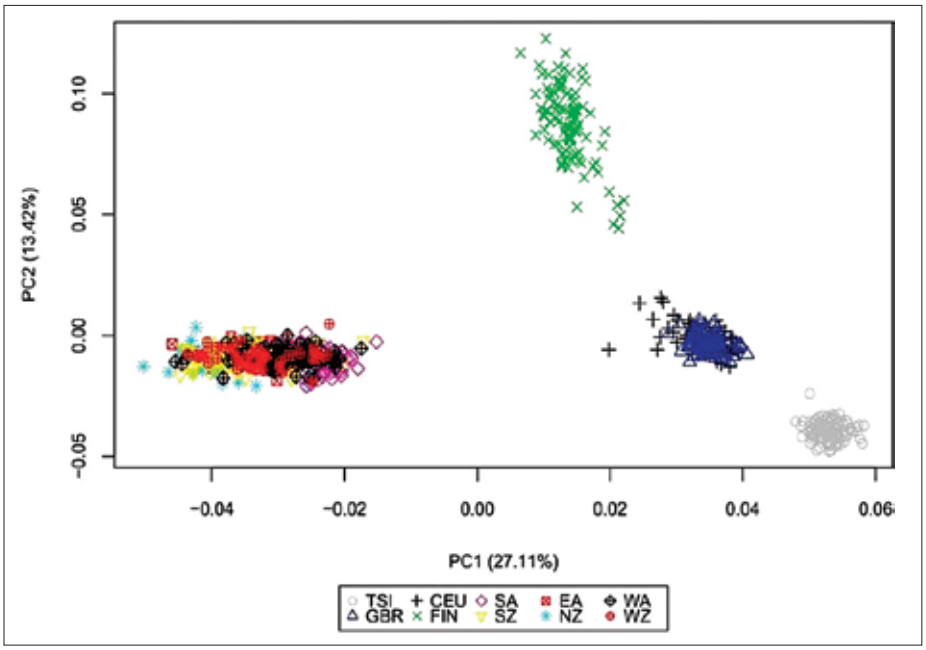


FIGURE 3.2. Figure 1. A principal component analysis of the six ethnolinguistic groups in Lithuania and the neighboring European populations. EA – Eastern Aukštaičiai, SA – Southern Aukštaičiai, WA – Western Aukštaičiai, NZ – Northern Žemaičiai, SZ – Southern Žemaičiai, WZ – Western Žemaičiai. CEU – Utah residents with ancestry from Northern and Western Europe, FIN – the Finnish in Finland, GBR – the British in Italy and Scotland and TSI – the Toscani in Italy.

separates Lithuanians from the four European populations included in the analysis, whereas the second PC, explaining 13.42% of the genetic variance, separates the FIN population, which appears to be more widely dispersed in the plot from the remaining populations. Notably, GBR clusters are grouped together with CEU, whereas the TSI is more closely related with CEU and GBR than with LT and FIN. Finally, Lithuanians lay closer to CEU and GBR than to FIN and TSY.

Population differentiation was also assessed through a calculation of pairwise F_{ST}. The F_{ST} values between LT-CEU and LT-GBR were similar (0.006 and 0.007, respectively) and LT was most differentiated from TSI ($F_{ST} = 0.011$). These results correlate with the population substructure observed in the PCA plot of PC1 vs. PC2.

To verify the European context of the genetic diversity of the Lithuanian population, a PCA was then performed including 19 worldwide populations from the 1 000 Genomes Project Phase3 dataset (excluding those of the American origin)

[1]. The first two PCs explained 52.7% and 32.3% of the variance, respectively, and showed a clear clustering of all populations according their continent. As expected, the Lithuanian population appeared within the European cluster.

To assess any potential genetic components and population structure within the Lithuanian population, a model-based ancestry analysis was subsequently performed with the ADMIXTURE software [4]. When analyzing the Lithuanian population with four European populations (CEU, FIN, GBR and TSI) from the 1 000 Genomes Project Phase3 dataset [1], the lowest cross-validation error was achieved with 3 ancestry components (Fig. 3.3). At K = 3, one main ancestry component (yellow) is distinguished in the Lithuanian population, which is found at similar proportions along the six ethnolinguistic groups. Notably, CEU, GBR and especially TSI exhibited high proportions of the second largest ancestry component (green), which has only a very low presence in Lithuanians. The FIN population presented the highest proportion of the third genetic component (brown), observed at K = 3, which was also found in considerable proportions in other European populations, such as the GBR and CEU, but at very low frequencies in Lithuanians.

Global ancestry profiles in Lithuanians were next inferred including additional worldwide populations from the 1 000 Genomes Project Phase3 dataset [1] (Fig. 3.4). A total of 2 317 individuals from 20 populations and 187 447 SNPs were used in this global ADMIXTURE analysis. The lowest cross-validation error was achieved with eight ancestry components. At K = 2, all African populations (yellow) were distinguished from populations in East Asia, Europe and South Asia (brown). At K = 3, a new ancestry component (green) distinguished Europe and South Asia from East Asia (brown) and Africa (yellow). At K = 4, a new component (yellow) distinguished all the European populations (including LT) from South Asians, and

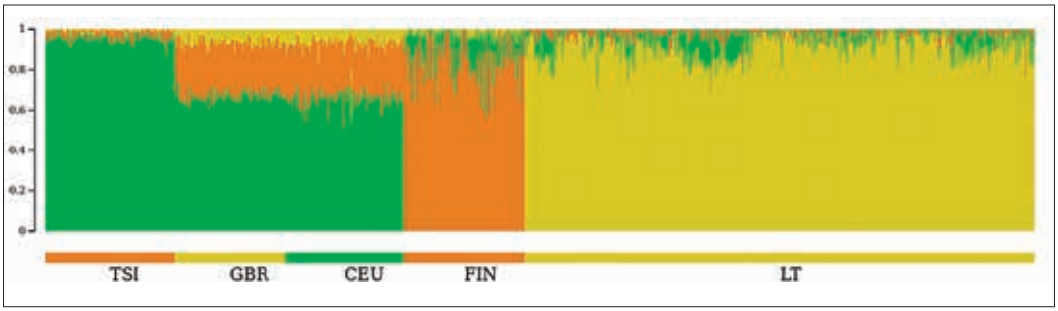


FIGURE 3.3. The ADMIXTURE plot at K = 3 of the individuals from the LT, CEU, FIN, GBR and TSI populations. Each individual is represented by a vertical colored bar, in which each segment of different color represents the proportion of an individual's ancestry derived from one of the K populations.

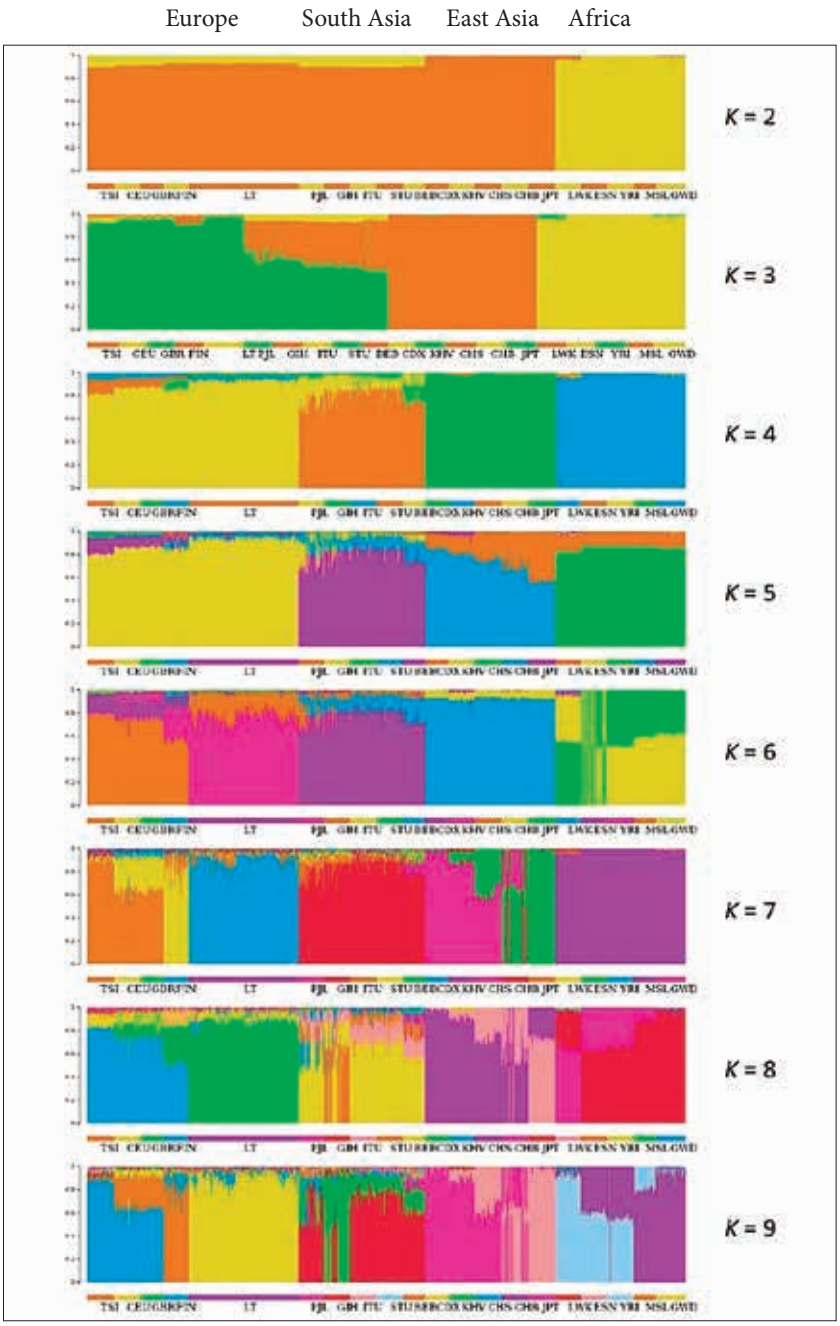


FIGURE 3.4. An ADMIXTURE analysis of Lithuanians and 19 external populations from the 1 000 Genomes Project Phase3 dataset [1]. ADMIXTURE plots from K = 2 to K = 9 are shown. Individuals are represented as vertical colored bars, in which each segment of different color represents the proportion of an individual's ancestry derived from one of the K populations.

it is not until K = 6 that an ancestry component appears specifically in high proportion in the Lithuanian population (fuchsia). At K = 8 (the lowest cross-validation error), the Lithuanians were characterized by a predominant ancestral genetic component (green) shared in low proportions with other neighboring Europeans (CEU, GBR and FIN) even if they also displayed small ancestry components belonging to South Asia and Africa.

3.2. Signatures of Positive Selection in the Lithuanian Population

We carried out genome-wide scans for different signatures of positive selection. To detect local recent selective events, we calculated F_{ST} and XP–EHH between different pairs of populations (LT-CEU, LT-FIN, LT-YRI, CEU-FIN, CEU-YRI and FIN-YRI) and selected those specific in the Lithuanian population. Per each population comparison, the genome-wide distribution of signals detected with XP-EHH and F_{ST} is shown in Figures 3.5 and 3.6, respectively. We considered as top candidates for recent selection those genomic regions that had presented at least 2 SNPs over the top 0.1% of XP-EHH empirical values and a minimum of 1 SNP with an F_{ST} rank score p-value < 0.01. Out of the 32 signals of recent selection detected in the Lithuanian population, four were shared with other European populations: three between LT and CEU and one between LT and FIN.

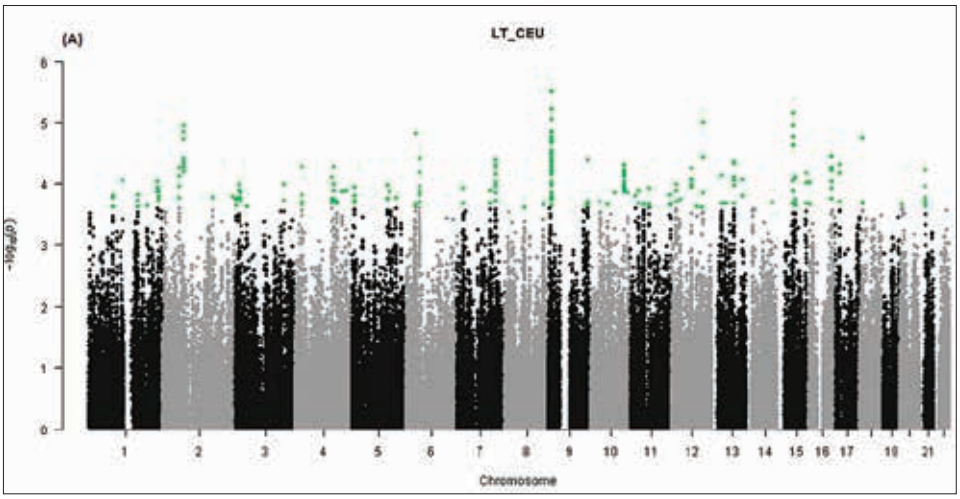
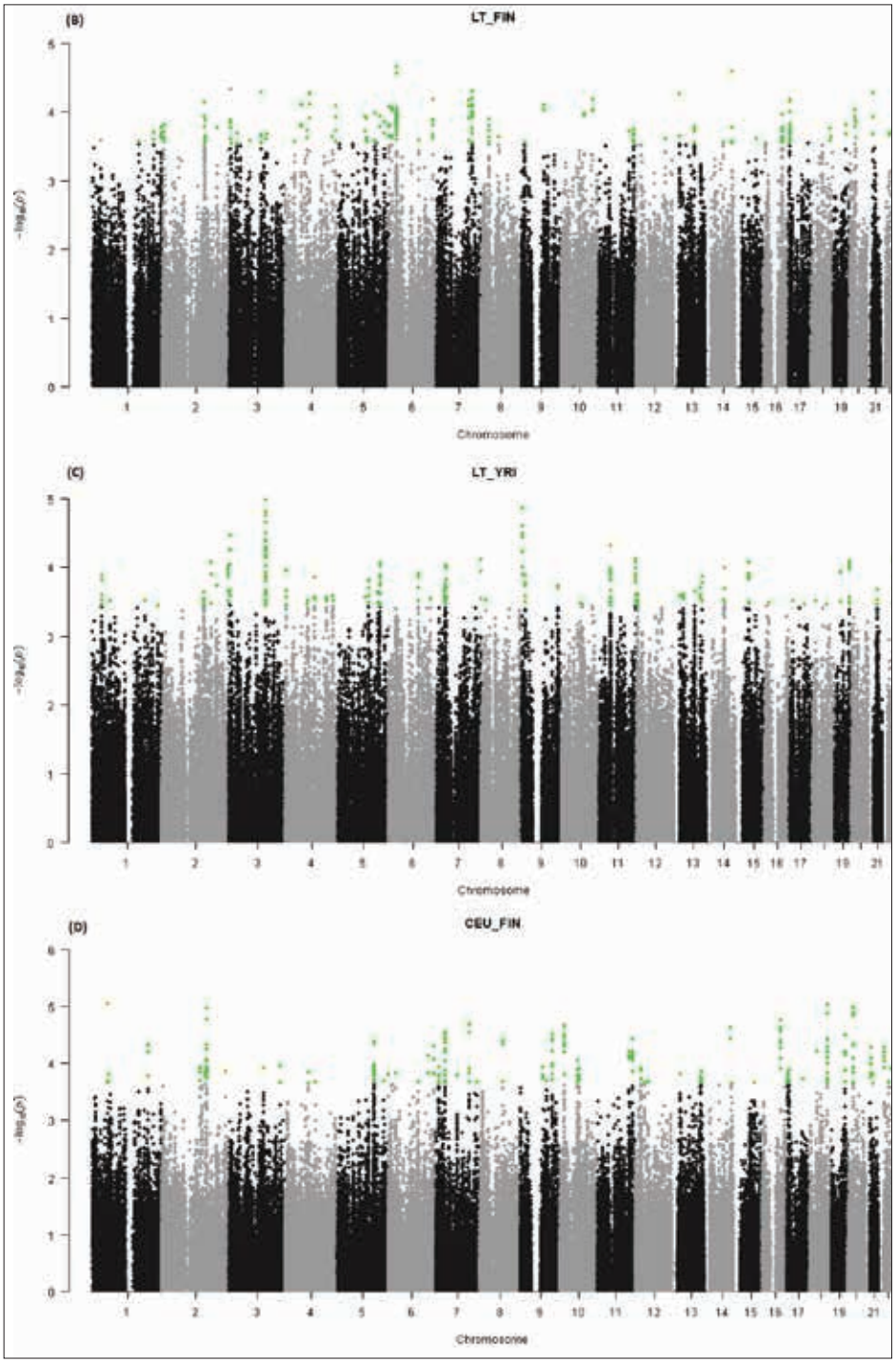
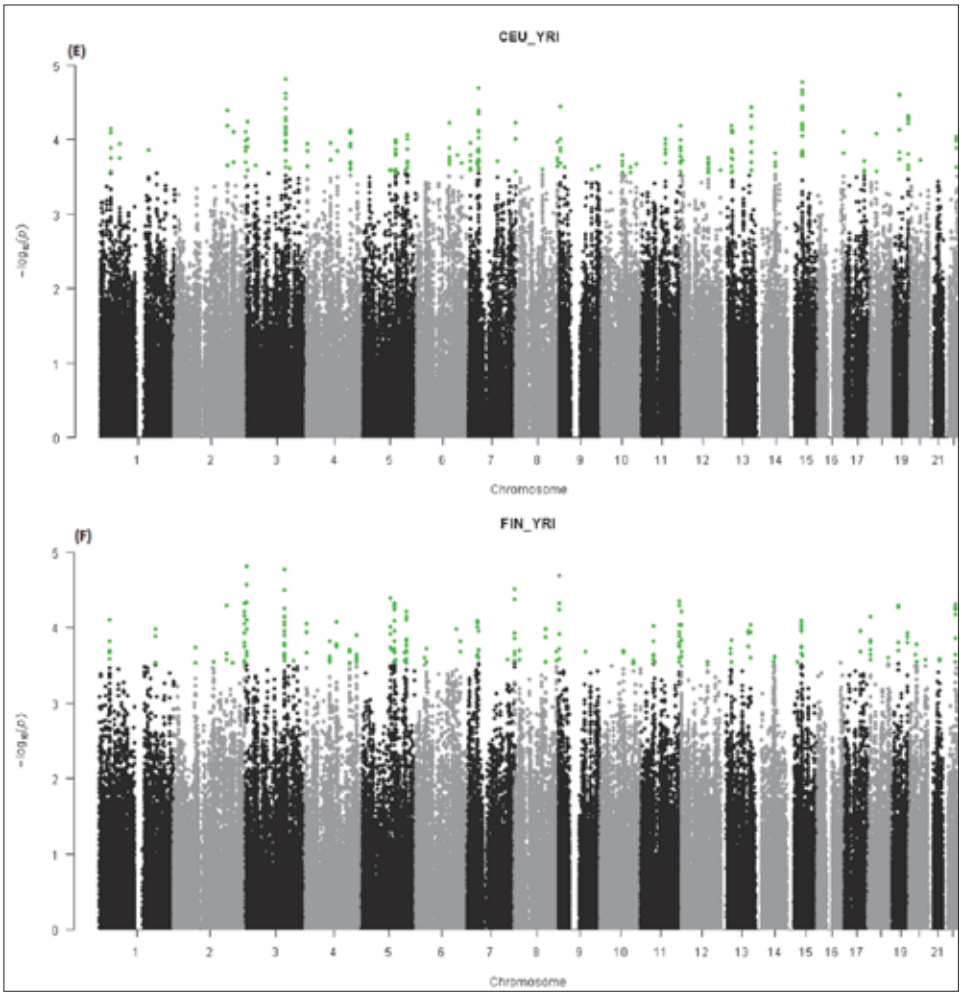


FIGURE 3.5. A Manhattan plot of XP-EHH signals across the autosomes. (a) XP-EHH in LT-CEU, (b) XP-EHH in LT-FIN, (c) XP-EHH in LT-YRI, (d) XP-EHH in CEU-FIN, (e) XP-EHH in CEU-YRI, (f) XP-EHH in FIN-YRI. In each plot, the green dots indicate 0.1% outlier regions.


(B)
LT_FIN
(C)
LT_YRI
(D)
CEU_FIN
Chromosome



One of the strongest signals detected with XP-EHH and F_{ST} was found at an ~284 kb region in chr6:27811815–28096280, which comprises several members of the histone and olfactory receptor gene families. Functional variant annotation along the region revealed two non-synonymous SNPs in the *OR2B6* gene: rs7767176 (exon1:c.G349A) and rs9380030 (exon1:c.A809G). Another strong signal of recent positive selection for the LT-CEU comparison was found at a ~184 kb region in chromosome 9 comprising *PTPRD-AS2* and *TYRP1* genes. The *TYRP1* gene encodes a melanosomal enzyme that participates in the melanin biosynthetic pathway, is involved in lighter skin pigmentation and has been described as a candidate for adaptive selection in European populations only [21]. Notably, the *SLC24A5* gene,

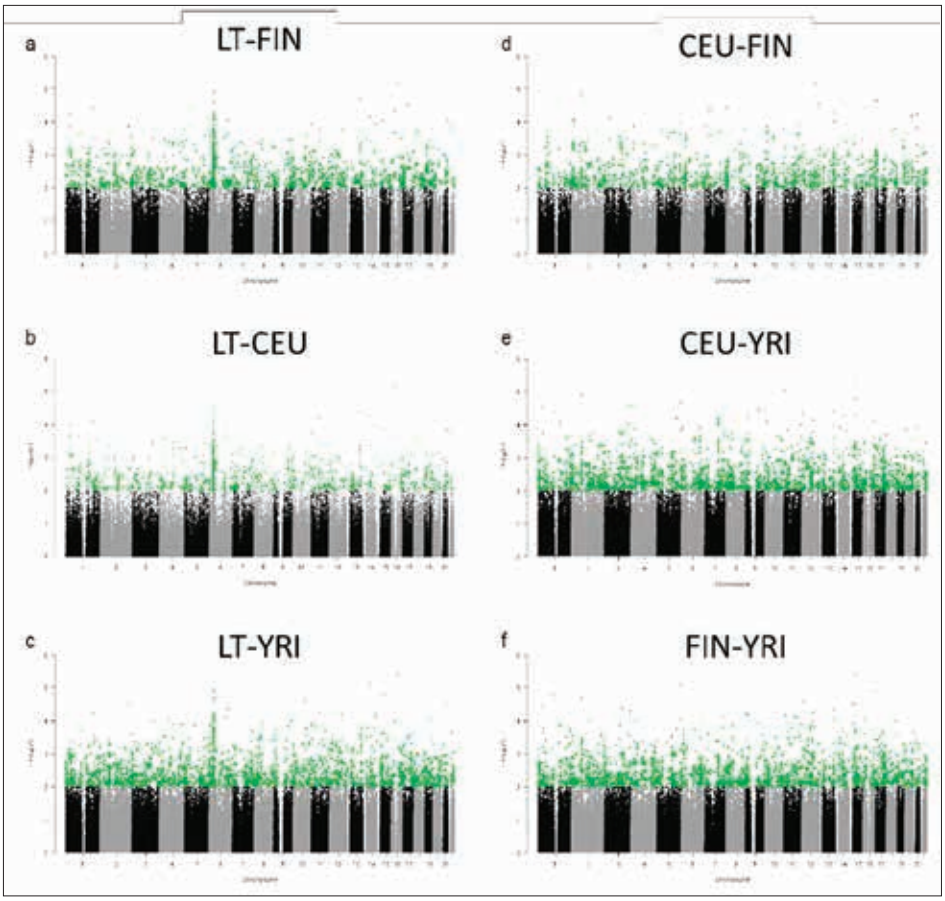


FIGURE 3.6. A Manhattan plot of F_{ST} p-values. The results are plotted as negative log-transformed empirical p-values of F_{ST} between (a) LT-CEU, (b) LT-FIN, (c) LT-YRI, (d) CEU-FIN, (e) CEU-YRI, (f) FIN-YRI. In each plot, the green dots indicate F_{ST} values with $p < 0.01$.

which is a known target of recent positive selection related to light pigmentation in non-African populations, was detected as a shared signature in CEU and LT when compared to the Yoruba in our analysis [22]. Another strong signal detected in the LT-YRI comparison was found in a ~225 kb region in chromosome 3, which comprises the *COL6A5* and *COL6A6* genes encoding for the collagen type VI alpha 5 and alpha 6 chains, respectively. Interestingly, a non-synonymous variant in *COL6A5* (rs12488457) with a CADD value of 23.2 was found among the top XP-EHH and F_{ST} outliers along the region. Even if the signal was only significant in the LT-YRI comparison, all European populations present high frequencies (above 0.748)

for the derived Pro allele at rs12488457, which is found at very low frequencies in YRI (~0.013). An additional signal in the LT-YRI comparison mapping on chromosome 1 includes two extreme XP-EHH and F_{ST} outliers (rs274750 and rs274752) at the 3' UTR of the *COL8A2* gene, which encodes for the collagen type VIII alpha 2 chain. Again, the signal was only significant in the LT-YRI comparison but the derived alleles at rs274750 and at rs274752, C and A respectively, are nearly fixed in the three European populations and found at intermediate frequencies in YRI. Interestingly, polymorphisms in *COL6A5* have been associated to body mass index [23] and dermal phenotypes, such as eczema and atopic dermatitis [24], while mutations at *COL8A2* have been linked to corneal endothelial dystrophies[25]. Moreover, two signals probably related to immunity have been identified, one in chromosome 6 comprising the *IL26* and *IL22* interleukine genes, and the other in chromosome 12 containing the *BRD2* and *HLA* genes. In particular, one variant downstream *IL22* (rs1182844) was identified as the most differentiated variant with significant XP-EHH values along the region.

By considering the empirical rank p-values that are less than 0.01, we detected up to 36 genomic regions with extreme negative Tajima's D values as potential candidates for older signals of positive selection in the Lithuanian population (Table 3.1). Notably, seven of the detected signals in the Lithuanians where shared with the two external European populations used (CEU and FIN), 5 were shared only with FIN and eight additional signals shared with CEU but not with FIN. The strongest signal specific for the Lithuanian population was identified in chr1:35818960-5948959 with 22 windows in the region and a p-value of 0.0008. Genes found in the region are *KIAA0319l* (Dyslexia-associated protein KIAA0319-like protein) and *ZMYM4* (Zinc finger MYM-type protein 4). Another strong signal with 20 windows in total was detected on chr8:48621077-8801076 with *CEBPD* (CCAAT/enhancer-binding protein delta), *PRKDC* (DNA-dependent protein kinase catalytic subunit) and *SPIDR* (DNA repair-scaffolding protein) genes in the region. Furthermore, a quite strong signal, specific to the Lithuanian population, was detected in chr7:30280729-30470728 with *NOD1* (Nucleotide-binding oligomerization domain-containing protein 1) and *ZNRF2* (E3 ubiquitin-protein ligase ZNRF2) genes.

TABLE 3.1. Candidate regions under positive selection in the Lithuanian population as detected with Tajima's D statistic.

Genome coordinates	Windows*	P-value	Region	Genes	Shared signal
chr1:35818960–35948959	4 (22)	0.0008	exonic	*KIAA0319L;* ZMYM4	LT

Genome coordinates	Windows*	P-value	Region	Genes	Shared signal
chr1:49988960–50728959	6 (10)	0.0003	exonic	*AGBL4;* ELAVL4	LT
chr1:188788960–188968959	9 (17)	0.0003	intergenic	*LINC01037;* BRINP3	LT; CEU
chr2:21728675–21888674	7 (7)	0.0004	intergenic	*TDRD15;* LINC01822	LT; FIN
chr2:179468675–179648674	9 (10)	0.0003	exonic	*TTN*	LT; CEU
chr3:50343412–51893411	4 (17)	0.0006	exonic	*C3orf18;CACN* A2D2;CISH; CYB561D2;	LT; CEU
				DCAF1; DOCK3;GRM2; HEMK1;HYAL2; IQCF3;IQCF6; MANF; MAPKAPK3; NPRL2; RAD54L2; RASSF1; RBM15B; TEX264; TMEM115; TUSC2; ZMYND10	
chr3:128763412–128903411	5 (13)	0.0006	exonic	*CNBP; GP9; ISY1;* ISY1-RAB43; RAB43	LT;CEU; FIN
chr3:143543412–143683411	5 (8)	0.0001	exonic	*SLC9A9*	LT;CEU; FIN
chr4:171930684–172360683	4 (9)	0.0002	ncRNA_exonic	*LINC02431;* MIR6082	LT
chr4:176190684–176400683	11 (15)	0.0005	intergenic	*ADAM29;* GPM6A	LT; FIN
chr5:50531164–50691163	6 (7)	0.0003	exonic	*ISL1*	LT
chr5:126311164–126441163	4 (7)	0.0005	exonic	*C5orf63*	LT
chr6:35265879–35395878	4 (7)	0.0005	exonic	*DEF6;* PPARD	LT; FIN
chr6:97855879–98005878	6 (6)	0.0007	ncRNA_exonic	*MIR548H3*	LT
chr7:30280729–30470728	8 (16)	0.0006	exonic	*NOD1;* ZNRF2	LT

Genome coordinates	Windows*	P-value	Region	Genes	Shared signal
chr7:151730729–151870728	5 (7)	0.0002	exonic	*GALNT11;* KMT2C	LT; CEU
chr8:48621077–48801076	9 (20)	0.0007	exonic	*CEBPD;* PRKDC;SPIDR	LT
chr8:93731077–93901076	8 (9)	0.0002	exonic	*TRIQK*	LT; CEU
chr9:38474202–38614201	5 (6)	0.0001	exonic	*ANKRD18A*	LT; FIN
chr9:125434202–125574201	5 (8)	0.0006	exonic	*OR1K1;OR1L3;* OR1L4;OR1L6; OR5C1	LT
chr10:66065709–66215708	6 (7)	0.0002	intergenic	*REEP3; ANXA2P3*	LT; FIN
chr10:118195709–118325708	4 (11)	0.0008	exonic	PNLIP; PNLIP-RP3	LT
chr11:71554229–71734228	9 (11)	0.0004	exonic	DEFB131B; IL18BP; NUMA1; RNF121	LT
chr12:1296235–1426234	4 (6)	0.0009	exonic	*ERC1*	LT; CEU; FIN
chr12:15736235–15886234	6 (11)	0.0003	exonic	*EPS8;PTPRO*	LT; CEU; FIN
chr13:34108565–34278564	8 (11)	0.0005	UTR5	*STARD13*	LT
chr14:64046743–64236742	10 (13)	0.0005	exonic	SGPP1; WDR89	LT
chr15:48357093–48477092	3 (4)	0.0005	exonic	MYEF2; SL-C24A5	LT; CEU; FIN
chr15:69617093–69737092	3 (6)	0.0002	exonic	KIF23; PAQR5	LT; CEU
chr16:67321264–67501263	7 (20)	0.0005	exonic	ATP6V0D1; HS-D11B2; KCTD19; LRRC36; PLE-KHG4; TPPP3; ZDHHC1	LT; CEU

Genome coordinates	Windows*	P-value	Region	Genes	Shared signal
chr17:29252345–29392344	5 (5)	0.0002	exonic	ADAP2; LOC107984974; RNF135	LT; CEU; FIN
chr18:30389383–30559382	8 (11)	0.0001	exonic	*CCDC178*	LT; CEU
chr19:50580913–50700912	3 (4)	0.0005	exonic	*IZUMO2*	LT
chr20:58399095–58539094	3 (8)	0.0006	exonic	CDH26; FAM217B; PHACTR3; PPP1R3D; SYCP2	LT; FIN
chr21:44859932–44989931	3 (8)	0.0005	exonic	*HSF2BP*	LT

*Number of SNPs significant at the top 0.1% of the distribution. The total number of SNPs in the region is shown in brackets.

Further data analysis and studies on data sets are required to confirm such selection signatures.

3.3. Long-Term *Ne* and Divergence Time Analysis

To infer evolutionary relationships between Lithuania and other populations contained in the HGDP-CEPH panel, we reconstructed two human evolutionary forces – effective population size and the divergence time between populations by analyzing LD patterns in the SNP array data with the R package NeON [16]. We analyzed a total of 295 samples from unrelated Lithuanian individuals. The estimates of *Ne* for the populations contained in the HGDP-CEPH panel were obtained from a study by Mezzavilla and Ghirrotto (2015, University of Trieste, Italy), estimated with the R package NeON [16].

The *Ne* values for the Lithuanian population were obtained from 6 000 to 200 generations ago, assuming a generation time of 25 years. The estimated long-term *Ne,* calculated as the harmonic mean [26], is 5 404 for the Lithuanian population with a confidence interval (CI) [4 910; 5 643]. There is variation in *Ne* estimates through time for the Lithuanians (Fig. 3.7). Over the 150 000–25 000 YBP (years before present) period, the *Ne* of Lithuanians was in continuous reduction. The expansion is observed around 25 000 YBP.

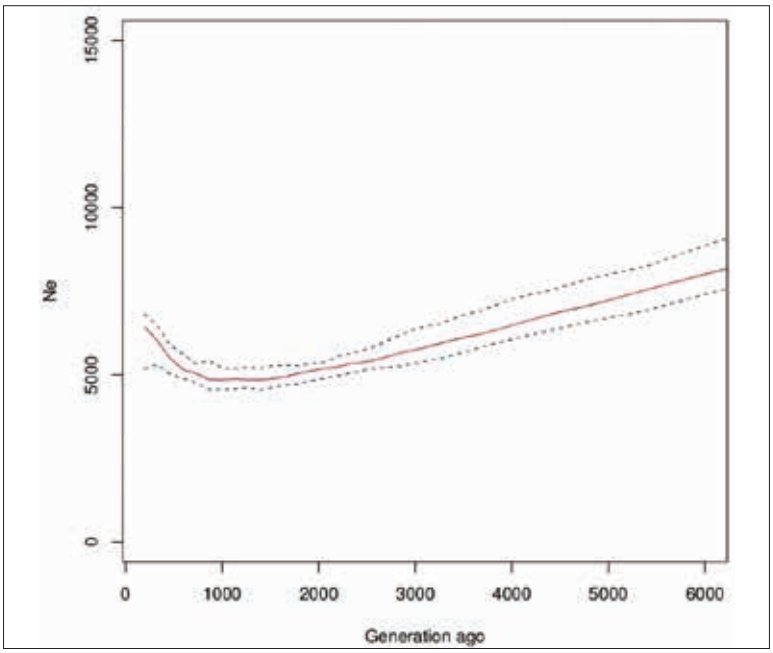


FIGURE 3.7. Lithuanian effective population size through time estimated from the LD analysis. The x-axis represents the time measured in generations; the y-axis represents the *Ne* values with the confidence interval (the 5th and 95th percentile) values in dashed lines.

The *Ne* estimates and the matrix of inter-population *Fst* values of 23 studied populations were used to reconstruct the divergence times summarized in a neighbor-joining phylogenetic tree (Fig. 3.8). In concordance with other authors, we observed three major groupings: Africans, East Asians and Europeans with Central South and Middle East Asians. The phylogenetic tree provides us with a clear picture saying that the most recent separations of populations and the geographical areas are related.

The oldest split is observed between African (Yoruba, Mandenka, Biaka Pygmy) and East Asian (Han, Yakut, Japanese) populations, 70 368 YBP, CI [64 036; 76 700]; another separation can be observed in 67 821 YBP, CI [50 989; 84 653], for Africans and Maya comparisons. The average divergence time between African and European populations occurred around 56 536 YBP, CI [54 000; 59 071] as well as between European and East Asian populations in 31 261 YBP, CI [27 089; 35 433]. The most recent separation occurred between European and Middle Eastern ancestors in 7 074 YBP, CI [5 973; 8 176], as well as between the European and Central South Asian ancestors in 8 970 YBP, CI [8 191; 9 750].

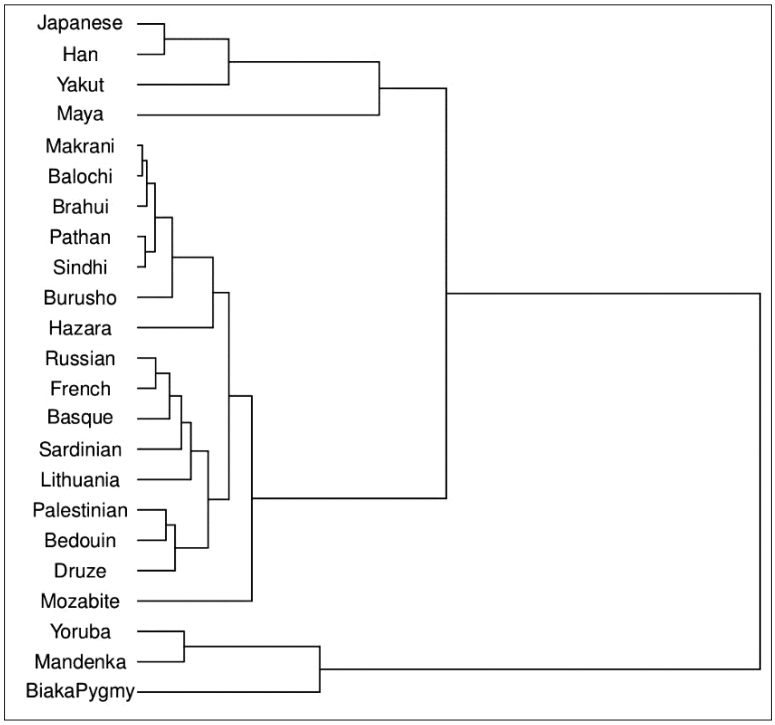


FIGURE 3.8. A neighbor-joining (NJ) clustering tree, based on the *Ne* and *Fst* estimates, shows the divergence between populations.

Considering the Lithuanian population, we observed that Lithuania first split from Africans in 52 160 YBP, CI [44 169; 60 151], and much later from East Asians – in 26 201 YBP, CI [12 272; 40 129]. The split from Central South and Middle East Asians happened around the same time – in 8 082, CI [7 198; 8 965], and 8 880 YBP, CI [6 179; 11 581], respectively. The most recent genetic separation happened with the Russians in 2 814 YBP and the French in 3 790

YBP. The results also showed that Lithuania was the first population, when compared with other studied European populations (French, Basque, Sardinian), to split from the Middle East Asian population.

In aiming to reconstruct past events between the ethnolinguistic groups of Lithuania, we analyzed the same 295 samples. The estimated long-term *Ne* for each ethnolinguistic group ranged from 4 940 [4 674; 5 304] in the West Žemaičiai (WZ) group to 5 314 [4 829; 5 490] in the West Aukštaičiai (WA) group (Figure 3.9). The difference in the estimated long-term *Ne* values between the two main ethnolinguistic groups (Žemaičiai and Aukštaičiai) of Lithuania was statistically significant ($p < \alpha$, $\alpha = 0.001$, Wilcoxon-Mann-Whitney-Test).

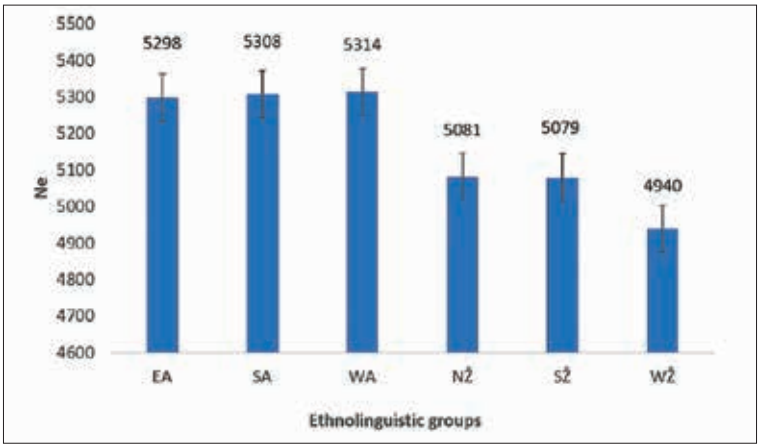


FIGURE 3.9. Long-term effective population size estimated in the Lithuanian population ethnolinguistic groups with 95% confidence intervals. EA – Eastern Aukštaičiai, SA – Southern Aukštaičiai, WA – Western Aukštaičiai, NŽ – Northern Žemaičiai, SŽ – Southern Žemaičiai, WŽ – Western Žemaičiai.

Considering the *Ne* values through the observed duration, we can conclude that all ethnolinguistic groups of the Lithuanian population suffered similar fluctuations in *Ne*. The *Ne* estimates between the two main ethnolinguistic groups (the Aukštaičiai and Žemaičiai) of Lithuania over the 150 000–37 500 YBP (6 000–1 500 generations ago) period show a constant reduction in *Ne*, possibly as a result of a series of founder effects of the migration of modern humans out of Africa. During the Neolithic Era, all ethnolinguistic groups started to expand; it is known that an intense cultural development occurred in Lithuania during this period as ceramics and farming had first appeared [27]. Distinct growth trajectories observed in WA show the strongest expansion in population size, and WŽ show the strongest reduction in *Ne* during the Neolithic period. Considering the recent *Ne* values, we observed that there were more recent reductions in *Ne* in the Žemaičiai group compared to the Aukštaičiai group.

The estimated times of divergence between the ethnolinguistic groups of Lithuania showed that WŽ is the oldest diverged group, 9 975 YBP (Table 3.2). As expected, the separations happened more recently for ethnolinguistic groups from the same geographical area. The most recent separation occurred between the Northern Žemaičiai (NŽ) and the Southern Žemaičiai (SŽ) groups (4 775 YBP).

TABLE 3.2. Calculated divergence times, in years, between six Lithuanian ethnolinguistic groups. EA – Eastern Aukštaičiai, SA – Southern Aukštaičiai, WA – Western Aukštaičiai, NŽ – Northern Žemaičiai, SŽ – Southern Žemaičiai, WŽ – Western Žemaičiai.

	SA	SŽ	EA	NŽ	WA	WŽ
SA	0					
SŽ	6 725	0				
EA	7 325	6 450	0			
NŽ	7 125	4 775	5 800	0		
WA	5 850	5 650	5 275	5 225	0	
WŽ	9 975	8 350	9 650	8 250	9 350	0

3.4. Recent *Ne* Analysis

We estimated the recent effective population size for 50 generations (*g*), or 1 250 years from the present, from IBD segments using the newest non-parametric approach [20] on the Lithuanian population (Figure 3.10). One generation is considered to be 25 years. Fifty generations ago, the effective population size in Lithuania was 11 900, whereas from 2015 to 1991, corresponding to generation 0, it was

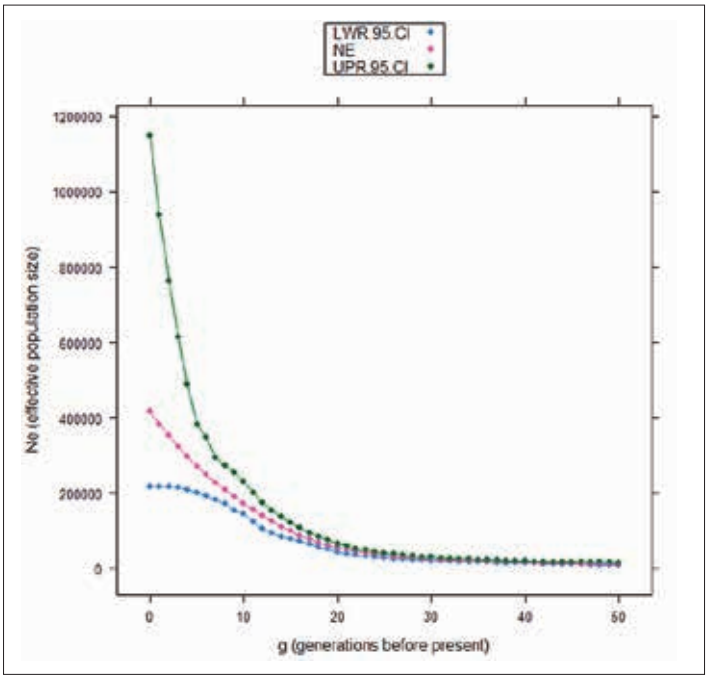


FIGURE 3.10. Recent effective population size estimated in the Lithuanian population for 50 generations with 95% confidence intervals.

41 7 000 (95% confidence interval, CI [218 000; 1 150 000]), and the mean census size was 3 373 154. The average estimated *Ne* for generations 30–50 was 16 228. An increased exponential growth is observed from generation 25.

We evaluated the ratio of effective population size to census size, *Ne/N*, in the Lithuanian population at selected time points. We obtained reliable census size data for only three generations of the Lithuanian population from Eurostat, the statistical yearbook of Lithuania (Official Statistics Portal: http://osp.stat.gov.lt/en/statistiko sleidiniu- katalogas?%20publication=1673) and from the e-book of the first Population Census of pre-war Lithuania (Stulginskis and Galvanauskas, 1923) [28]. For generation 1 (1990–1966), the *Ne* was 383 000 (95% confidence interval, CI [217 000; 938 000]), and the mean census size was 3 351 015. Furthermore, for generation 2 (1965–1941), the *Ne* was 352 000 (95% confidence interval, CI [217 000; 763 000]), and the mean census size was 2 816 800. The estimated ratio was 0.125 (95% CI [0.077; 0.271]) for $g = 2$ (corresponding to 1941), 0.114 (95% CI [0.065; 0.280]) for $g = 1$ (corresponding to 1966), and 0.124 (95% CI [0.065; 0.341]) for $g = 0$ (corresponding to 1991) (Figure 3.11). The estimates of *Ne* were approximately at one-tenth of the Lithuanian population size based on the census.

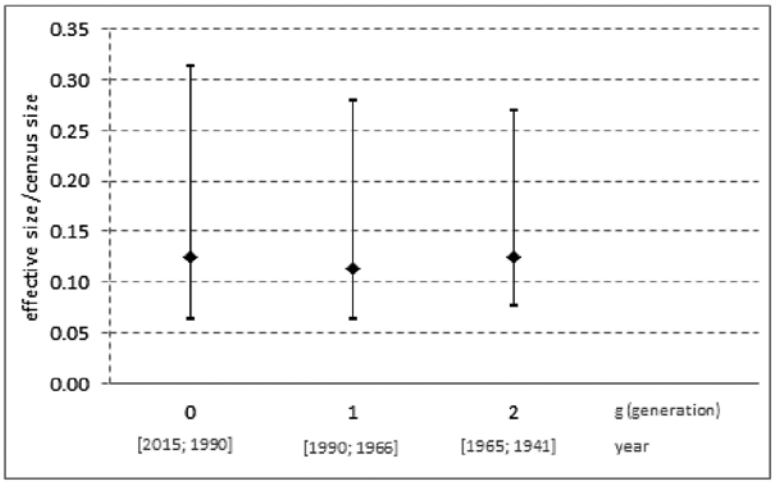


FIGURE 3.11. The ratio between *Ne/N* in the Lithuanian population for three generations with 95% confidence intervals.

4. Discussion

In this study, we analyzed the local patterns of population structure, the signatures of adaptive positive selection and evolutionary demographic parameters from high-density SNP genotyping data generated in the Lithuanian population. After exploring the genetic relationships among the six ethnolinguistic groups present in Lithuania based on PCA, we found a clear homogeneous genetic landscape across them. The estimated small F_{ST} values among the six geographical regions confirmed their close genetic proximity. In turn, Lithuanians as a whole displayed high genetic similarity to CEU ($F_{ST} = 0.006$), and their genetic diversity was found in agreement with their European context. The global ancestry profiles obtained with ADMIXTURE when using several external worldwide populations from the 1 000 Genomes Project Phase3 dataset [1] revealed that at K = 8 (the lowest cross-validation error), the Lithuanians have a predominant ancestral genetic component shared at low proportions with other neighboring Europeans (CEU, GBR and FIN), even if they also displayed small ancestral components form South Asia and Africa. Any potential bias on the structure analysis due to the higher sample size of the Lithuanian population was rejected by twice subsampling 120 Lithuanian samples. Overall, these results indicated that Lithuanians are a homogenous population, genetically differentiated from their neighboring populations but within the expected general European context.

We next investigated whether the specific signals of positive selection could be identified in the Lithuanian population. A strong selection signal was identified in the intergenic region of chr9:2352971–12537279 comprising *PTPRD-AS2* and *TYRP1* genes. However, no obvious functional variant have been identified among the SNPs displaying the top outlier XP-EHH and F_{ST} values in that region. Interestingly, polymorphisms in the *TYRP1* gene have been associated with hair and iris colors in European populations [29].

Strong selection signals detected in chromosome 3, which comprises the *COL6A5* and *COL6A6* genes, and in chromosome 1 with *COL8A2* gene. Interestingly, polymorphisms in *COL6A5* have been associated to body mass index [23] and dermal phenotypes, such as eczema and atopic dermatitis [24], while mutations at *COL8A2* have been linked to corneal endothelial dystrophies[25]. Moreover, selection signals related to immunity have been identified (*IL26*, *IL22*, *BRD2* and *HLA* genes).

The results of the long-term *Ne* showed that the *Ne* of the Lithuanian population is quite low – 5 404 – likely the consequence of bottlenecks associated with the Last Glacial period of 25 000–12 000 YBP in Europe [27, 30]. The obtained divergence time estimates between study populations are in agreement with recent studies [16, 31, 32]. Our results support an initial migration from Africa to East Asia in 70 000

YBP and a later dispersal into Europe in around 56 000 YBP and another into the Middle East, Central South and North Asia in around 52 000 YBP. The divergence analyses showed that Lithuania was the first population, if compared with other studied European populations (the French, Basque, Sardinian, and Russian), to have split from the Middle East Asia in around 8 800 YBP. The reconstructed *Ne* between the two main ethnolinguistic groups (the Aukštaičiai and Žemaičiai) of Lithuania showed significant differences between the groups. Indo-Europeans, which had arrived in the Lithuanian territory during the Neolithic period, contributed to the formation of different Baltic tribes and may have had an important influence in the genetic variation and the differences of Lithuanians.

The obtained values of *Ne/N* ratios are small (0.1) compared with other genetics-based estimates of between 0.21 and 0.65 [33]. According to Nunney and Campbell (1994) and Nunney (1996) [34, 35], the *Ne/N* ratio is usually close to 0.5 and only rarely outside the range of 0.25–0.75. However, very low estimates of *Ne/N* (<0.1) raise the possibility that other factors acting to reduce *Ne* have been underestimated, e.g., the variation in female fecundity [34].

Furthermore, natural levels of fluctuations in population size, in the hypothetical absence of any other influence, are often sufficient to depress the *Ne/N* to small values [36]. A single factor is sufficient to produce very small *Ne/N* values, and additional factors tend to depress these values even further [36]. We conclude that natural levels of fluctuations, such as the variance in size, reproduction, sex ratio, as well as the degree to which generations overlap, have probably caused the small values of *Ne/N* in the Lithuanian population. The population of Lithuania is small and has historically suffered the effects of population bottlenecks (a rapid decrease in population size in generation 2) and expansions (a rapid increase in population size in generation 0), which might have produced very small *Ne/N* values.

Furthermore, the small sample size of this study and the Lithuanian population structure could introduce oscillations for the most recent generations. However, considering our results, we think that the true effective size is contained within the bootstrap confidence interval.

Further data analysis and studies on data sets are required to confirm the findings, as well as the selection signatures, of this study.

5. Conclusions

1. After exploring the genetic relationships among the six ethnolinguistic groups present in Lithuania based on PCA, we found a clear homogeneous genetic landscape across them. Lithuanians are a homogenous population,

genetically differentiated from their neighboring populations but within the expected general European context.

2. Signatures of positive selection in the Lithuanian population were investigated over different time frames using three statistics: XP-EHH, F_{ST} and Tajima's D:
   2.1. Candidate regions for positive selection were identified in the Lithuanian population that are related with pigmentation (*SLC24A5*, *TYRP1*) immunity (*IL26, IL22, HLA, BRD2)* and other traits (*COL6A5*, *COL8A2)*.
3. According to long-term effective population size and divergence time estimates:
   3.1. Lithuania was the first population, if compared with other studied European populations (the French, Basque, Sardinian, and Russian), to have split from the Middle East Asia in around 8 800 YBP.
   3.2. According to long-term *Ne* estimates between the two main ethnolinguistic groups of Lithuania (the Aukštaičiai and Žemaičiai), a statistically significant difference was determined.
   3.3. Indo-Europeans, which had arrived in the Lithuanian territory during the Neolithic period, contributed to the formation of different Baltic tribes and may have had an important influence on the genetic variation and differences of Lithuanians.
4. According to the estimated recent effective population size and to the evaluated effective/census size ratio in the Lithuanian population, a small *Ne*/*N* ratio was obtained (0.1).
   4.1 The population of Lithuania is small and has historically suffered the effects of population bottlenecks (a rapid decrease in population size in generation 2) and expansions (a rapid increase in population size in generation 0), which might have produced very small *Ne*/*N* values.

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Traineeship:

Evolutionary Population Genetics Lab of the Department of Experimental and Health Sciences at the Universitat Pompeu Fabra in Barcelona, Spain, from 11-02-2017 to 11-05-2018.

Andrė Amšiejienė

MULTIVARIATE ANALYSIS OF LITHUANIAN INFERTILE WOMEN'S BIOLOGICAL FACTORS

LIETUVOS NEVAISINGŲ MOTERŲ POPULIACIJOS BIOLOGINIŲ VEIKSNIŲ DAUGIAMATĖ ANALIZĖ

Biomedical sciences, Medicine 06 B
Biomedicinos mokslai, medicina 06 B

Abbreviations

NICE - National Institute for Health and Care Excellence
IUI - intrauterine insemination
IVF - in vitro fertilization
ICSI - intracytoplasmic sperm injection
IMSI - intracytoplasmic morphologically selected sperm injection
ESHRE – European Society of Human Reproduction and Embryology
ASRM - American Society of Reproductive medicine
WHO – World Health organization
BMI – body mass index
WHR – waist to hip ratio
AMH – Anti-Müllerian hormone
AFC – antral follicle count
FSH – follicle-stimulating hormone
LH – luteinizing hormone
FAI – free androgen index
ART – assisted reproductive technologies
TFR – total fertility rate

Introduction

Infertility is a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse (Zegers-Hochschild, Adamson et al. 2009). Most (about 80-90 percent) couples get pregnant during the first year of planning and from 5 to 15 percent get pregnant during the second year (Kuohung 2015). One out of six couples experience fertility problems at least once during their fertile life (2016). About 186 millions of people have fertility problems (Inhorn and Patrizio 2015). Infertility is a chronic disease generating disability, affecting both physical and mental health (Schmidt, Christensen et al. 2005). According to WHO, infertility in women was ranked the 5th highest serious global disability (2011).

Family infertility can be analyzed through social, medical and demographical aspects. One of the most commonly used indicators is total fertility rate, which defines the total number of children born or likely to be born to a woman in her childbearing (reproductive) years if she were subject to the prevailing rate of age-specific fertility in the population. This rate should be more than 2,1 to sustain population levels. The term "lowest-low fertility" is defined as TFR at or below 1.3. In the European Union TFR is about 1,58 (2017). In modern societies there is a trend for families to have fewer children and for women to delay childbearing (Stankūnienė, Baublytė et al. 2016, Stankūnienė 2017). Fertility decreases with increasing age, so delaying childbearing means bigger need for medical help such as ART (Baird, Collins et al. 2005, 2009, Daniluk and Koert 2012). Advanced maternal age is associated with lower fertility and various comorbidities, but most women rely on modern medical technologies and think that with ART women can get pregnant until meno-

pause (Daniluk, Koert et al. 2012). The ability to bear children is just one of many factors influencing fertility. Sexual behavior, family size limitation, social situation, advanced maternal age, usage of contraceptive methods, ART possibilities and other factors can be influencing women's reproductive health (Bonde and Olsen 2008).

In Lithuania TFR started declining steadily after 1990, reaching as low as 1,23 in 2002. From 2006 it started going up slowly, but is still low – 1,69 in 2016 (Stankūnienė, Jasilionis et al. 2014). Natural increase rate is negative for many years. From 1992 to 2018, Lithuania's population decreased by 23% from 3.7 million to 2.81 million (Drąsutienė 2007, Stankūnienė 2017). Maternal age increased significantly in Lithuania: in 2001, the average age was 27, and in 2016, - 29.1 years (Medical data of Birth Register from 2000 through 2016). The same trend is seen all over the Europe (Ledger 2009, Beaujouan and Sobotka 2017).

Lithuania's demographical situation and family infertility problems can partially be solved using modern medical technologies such as ART. However, it is not always clear what factors influence the success of ART. This study can reveal part of factors influencing infertility in Lithuanian society.

1. Aim of dissertation

To evaluate age, anthropometrical measures and ovarian reserve of Lithuanian infertile women and to perform multivariate analysis of factors influencing success of ART.

2. Objectives of the dissertation

1. To evaluate the influence of woman's age on pregnancy, live birth and miscarriage rates after ART.
2. To evaluate the association of body size and composition with pregnancy, live birth and miscarriage rates after ART.
3. To evaluate the influence of ovarian reserve on pregnancy, live birth and miscarriage rates after ART.
4. To evaluate the differences of analyzed factors in different women and men infertility groups.
5. To perform multivariate analysis of all analyzed factors.

3. Relevance and scientific novelty of the research

This research is one of few scientific studies in Lithuania that analyses causes of infertility and biological factors influencing ART success. The demographic situation in our country has reached a critical point. Decreasing birth rates raise concern for society and in particular for the medical community. The search for a link between the morphological characteristics of the woman's health and her physical condition is the subject of research in many countries. Various factors affecting pregnancy and delivery after ART are discussed in scholarly literature. The links between bodies' morphological and biochemical markers and their relationship with female health are being studied by Vilnius University anthropologists and clinicians in the last decades. The specifics of each country's life, social factors are associated with public health. Changing conditions of living, specific diet, ethnical and climatic factors affect these relationships. Therefore, it is no coincidence that the topic of scientific work done at the Vilnius University Clinic of Obstetrics and Gynecology is intended to explain the associations mentioned above. This research, aiming to identify infertile women's anthropometric and biochemical markers, extends the research of Vilnius University scientists on the links between female anthropometric and metabolic indexes and complements the research of other countries.

The findings of this research can be useful for doctors consulting infertile families, predicting probability of pregnancy and delivery after ART and choosing the best examination and treatment plan.

4. Materials and methods

Patients undergoing IVF in Vaisingumo klinika between 2013 and 2015 were included in this research.

General anthropometric and metabolic biochemical (hormone) indicators were obtained. The study used standard anthropometric methods. The following body size anthropometric indicators were measured: height, body weight, waist circumference, hip circumference. The circumferences of the body (cm) in women were measured in standing position: the waist circumference (in the middle between the lower rib cage and the pelvic crests) and the hip circumference (measuring the widest site of gluteal region). Digit length was measured on the palmar surface of the hand from the basal crease of the digit to the tip using Vernier calipers.

Hormones (FSH, LH, estradiol, testosterone, SHBG, AMH) were measured on cycle day 3-5 using Roche Cobas e411 analyzer. AFC was measured on cycle day 2-3 using transvaginal ultrasound.

Daily gonadotrophin dose was started on cycle day 2-3. When dominant follicle reached a maximum diameter of 14 mm, daily GnRh antagonist injections were given. When at least 3 follicles reached 18 mm in diameter, human chorionic gonadotropin was administered. Transvaginal oocyte retrieval was performed 36 hours after hCG injection. Oocytes were fertilized conventionally or via ICSI. Embryo transfer was performed using Cook catheter on day 2-3. Surplus embryos were cultured to the blastocyst stage. Cryopreservation was performed for surplus embryos. Only clinical pregnancies were evaluated. A single serum βhCG measurement was performed 12 days after embryo transfer procedure. If serum hCG was >40 IU/l, indicating pregnancy, the luteal support (Crinone 8 % one applicator per day vaginally until the 8th week of gestation) was continued until the ultrasound scans. Clinical pregnancy was confirmed when an intrauterine gestational sac with fetal heartbeat was detected during transvaginal ultrasound examination.

Women were divided to 3 age groups:

- 35 years old and younger
- 36-40 years old
- Older than 40 years

Women were divided to 4 groups according to BMI:

- < 18,5 - underweight
- 18,5 - 24,9 - normal range
- 25 - 29,9 - overweight
- ≥ 30 - obese

Women were divided to 4 groups according to waist to hip ratio (Kidy FF, Dhalwani N et al. 2017):

- < 0,75 - excellent
- 0,75 - 0,79 - good
- 0,80 - 0,86 – average
- > 0,86 – at risk

Women were divided to 3 groups according to AFC:

- ≤ 4 – likely low ovarian response to stimulation
- 5-16 – likely normal ovarian response to stimulation
- >16 – likely high ovarian response to stimulation

Women were divided to 3 groups according to AMH:

- ≤ 0,76 ng/ml - likely low ovarian response to stimulation
- 0,77-3,49 ng/ml - likely normal ovarian response to stimulation
- ≥ 3,5 ng/ml - likely high ovarian response to stimulation

Women were divided to 3 groups according to FSH:

- FSH > 8,9 IU/L- likely low ovarian response to stimulation
- FSH 4–8,9 IU/L - likely normal ovarian response to stimulation
- FSH < 4,0 IU/L - likely high ovarian response to stimulation

The statistical analysis of the data was performed using "IBM SPSS". The calculation of anthropometric and biochemical indicators mean values, standard deviations, minimum and maximum values was performed. The data of different groups were compared using Student's t test, chi-square and ANOVA analysis. Logistic regression and multivariate factor analysis were performed. The selected level of statistical significance – $p<0.05$.

5. Results

5.1. Physical status of participants

5.1.1. Characteristics of women

863 women aged from 21 to 47 (M = 33,4, SN = 4,48) participated in this research. 1166 controlled ovarian hyperstimulation cycles and oocyte retrievals were performed. Women with female factor infertility were statistically significantly (p=0,007) older (N=749, M=33,67, SN=4,33) than women with male factor infertility (N=417, M=32,93, SN=4,71). Embryo transfer was performed in 1051 case (90,14 %). Embryo transfer was not performed in 115 cases (table 1).

TABLE 1. Reasons for canceled embryo transfer

Reasons	N	%
No oocytes retrieved	18	15,65
No spermatozoon retrieved	2	1,74
No embryos formed	55	47,83
No good quality embryos formed	14	12,17
Freeze all	26	22,61
Total	115	100

On average 9,46 oocytes were retrieved (SD=6,11, min-max 0-45 oocytes) and 5,08 embryos were formed (SD=3,84, min-max 0-29 embryos) during one cycle. Statistically significantly (p=0,000) less oocytes were retrieved for women with female factor infertility (N=749, M=8,87, SD=6) compared to women with male factor infertility (N=416, M=10,52, SD=6,17). The mean embryo number did not differ

significantly (N=748, M=5,03, SD=3,88 vs N=416, M=5,17, SD=3,77). Pregnancy rate per oocyte retrieval was 40,1 %, live birth rate - 25,6 % (N=298). Multiple pregnancy rate - 17,2 % (N=79). Pregnancy rate per transfer - 44,4 % (N=467), live birth rate - 28,4 % (N=298). Pregnancy and live birth rate did not differ in women with female factor infertility compared to women with male factor infertility.

Indications for ART are listed in table 2.

TABLE 2. Indications for ART

Indications	N	%
Tubal infertility	280	24
Endometriosis	155	13,3
Ovulatory dysfunction	47	4
Male factor	417	35,8
Unexplained infertility	247	21,2
Other reasons	20	1,7
Total	1166	100

TABLE 3. Pregnancy and delivery rates

Indications	Pregnancy		No pregnancy		Delivery	
	N	%	N	%	N	%
Tubal infertility	109	41,4	154	58,6	69	26,2
Endometriosis	66	48,9	69	51,1	52	38,5
Ovulatory dysfunction	14	35,9	25	64,1	8	20,5
Male factor	169	45,2	205	54,8	103	27,5
Unexplained infertility	104	46,6	119	53,4	62	27,8
Other reasons	5	29,4	12	70,6	4	23,5
Total	467	44,4	584	55,6	298	28,4

Pregnancy and delivery rates presented in table 3 were not significantly different in various indication groups (p=0,384 and p=0,110).

5.1.2. Women's body size

TABLE 4. Women's anthropometrical indicators

Measure	N	Min	Max	M	SD
Height (m)	1166	1,48	1,89	1,69	0,05
Weight (kg)	1166	43	130	66,18	11,69
BMI	1166	16,05	44,98	23,19	3,96
Waist circumference (cm)	640	54	125	76,38	12,31
Hip circumference (cm)	638	68	138	96,06	10,78
WHR	638	0,62	1,34	0,79	0,08

Table 4 shows women's anthropometrical indicators. Mean BMI is within normal range (18,5-24,99). Waist to hip ratio is associated with woman's reproductive health (ability to get pregnant and deliver). Mean waist to hip ratio was 0,79.

TABLE 5. Women's anthropometrical indicators in various indication groups

Indicator	Cause of infertility						P
	Female factor			Male factor			
	N	M	SD	N	M	SD	
Height (m)	749	1,69	0,05	417	1,69	0,05	0,044
Weight (kg)	749	65,84	11,72	417	66,8	11,64	0,180
BMI	749	23,15	4,07	417	23,27	3,75	0,616
Waist circumference (cm)	410	76,49	12,64	230	76,2	11,72	0,772
Hip circumference (cm)	409	95,69	10,77	229	96,7	10,78	0,257
WHR	409	0,8	0,09	229	0,79	0,07	0,072

Anthropometrical indicators were similar in women with female factor infertility compared to women with male factor infertility (table 5).

TABLE 6. Digit length

Indicator	N	Min	Max	M	SD
II digit (cm)	648	5,5	9	6,67	0,33
IV digit (cm)	648	5	10	6,75	0,39
2D:4Dratio	648	0,7	1,12	0,99	0,27

We measured the length of 2nd and 4th digit of 648 women. 2D:4D ratio is sexually dimorphic and shows the exposure to androgens and estrogens in prenatal period. Mean 2D:4D ratio was 0,99 (table 6).

TABLE 7. Digit length in various indication groups

Indicator	Cause of infertility						P
	Female factor			Male factor			
	N	M	SD	N	M	SD	
II digit (cm)	412	6,67	0,32	236	6,68	0,35	0,543
IV digit (cm)	412	6,73	0,36	236	6,79	0,42	**0,036**
2D:4Dratio	412	0,99	0,03	236	0,98	0,03	**0,001**

Mean 2D:4D ratio was statistically significantly higher in women with female factor infertility compared to women with male factor infertility (table 7).

5.1.3. Biochemical markers

Table 8 shows biochemical markers that are important in infertility treatment. AMH plays an important role in predicting response to ovarian stimulation. Mean AMH level was within normal range (M=3.54 μg/L).

TABLE 8. Biochemical markers

Indicator	N	Min	Max	M	SD
FSH (mIU/ml)	577	1,93	17,4	7,28	2,23
LH (mIU/ml)	577	1,1	21	6,15	2,75
Testosterone (nmol/l)	366	0,08	34,4	1,75	3,91
SHBG (nmol/l)	346	19,77	200	85,15	37,93
FAI	337	0,1	10,95	1,51	1,28
AMH (μg/L)	325	0,03	18,92	3,54	3,17

Mean AMH was statistically significantly higher in women with male factor infertility compared to women with female factor infertility (table 9).

TABLE 9. Biochemical markers in various indication groups

Indicator	Cause of infertility						P
	Female factor			Male factor			
	N	M	SD	N	M	SD	
FSH (mIU/ml)	375	7,36	2,24	202	7,13	2,2	0,233
LH (mIU/ml)	358	6,1	2,63	199	6,24	2,95	0,563
Estrodial (pmol/l)	237	1,55	3,55	129	2,11	4,51	0,198
Testosterone (nmol/l)	226	85,68	38	118	84,12	37,94	0,718
SHBG (nmol/l)	221	1,5	1,31	116	1,52	1,22	0,912
FAI	214	3,23	3,15	111	4,14	3,14	**0,014**
AMH (µg/L)	409	0,8	0,09	229	0,79	0,07	0,072

5.1.4. Ultrasound testing of ovarian reserve

Female genitalia, ovarian reserve and ovarian response to gonadotropin stimulation can be evaluated by ultrasound testing. We measured antral follicle count of 325 women. Mean AFC was 11,91 (min-max 4-33). Mean AFC was statistically significantly (p=0,024) higher in women with male factor infertility (N=111, M=12,94, SD=5,67) compared to women with female factor infertility (N=214, M=11,38, SN=5,99).

5.2. Differences between women who conceived and who did not

Women who conceived were statistically significantly (p=0,000) younger (M=32,72 years, SD=4,08, min-max 21-47 years) than those who did not conceive (M=33,8 years, SD=4,68, min-max 22-47 years) after ART.

Women who got pregnant after ART were statistically significantly taller, leaner and had lower BMI and WHR than women who did not conceive (table 10).

TABLE 10. Anthropometrical indicators

Indicator	Pregnancy			No pregnancy			P
	N	M	SD	N	M	SD	
Height (m)	467	1,7	0,05	584	1,68	0,52	**0,002**
Weight (kg)	467	64,09	9,71	584	67,48	12,63	**0,000**
BMI	467	22,31	3,25	584	23,76	4,24	**0,000**
Waist circumference (cm)	254	75,33	11,05	327	77,47	13,24	**0,034**
Hip circumference (cm)	252	95,67	10,26	327	96,67	11,28	0,268
WHR	252	0,79	0,07	327	0,8	0,09	**0,033**

Mean 2D:4D ratio was not different in women who got pregnant after ART compared to those who did not get pregnant. Mean AMH was statistically significantly higher in women with male factor infertility compared to women with female factor infertility (table 11).

TABLE 11. Digit length

Indicator	Pregnancy			No pregnancy			P
	N	M	SD	N	M	SD	
II digit (cm)	255	6,69	0,37	332	6,67	0,3	0,546
IV digit (cm)	255	6,77	0,43	332	6,75	0,36	0,608
2D:4Dratio	255	0,99	0,03	332	0,99	0,03	0,979

TABLE 12. Biochemical markers

Indicator	Pregnancy			No pregnancy			P
	N	M	SD	N	M	SD	
FSH (mIU/ml)	239	7,07	2,07	286	7,36	2,22	0,115
LH (mIU/ml)	232	6,1	2,51	275	6,16	2,74	0,799
Testosterone (nmol/l)	161	1,87	4,64	176	1,7	3,44	0,692

Indicator	Pregnancy			No pregnancy			P
	N	M	SD	N	M	SD	
SHBG (nmol/l)	151	87,83	37,4	168	83,9	38,65	0,358
FAI	148	1,33	0,92	162	1,66	1,54	0,017
AMH (µg/L)	130	3,72	3,11	165	3,58	3,05	0,684

Only FAI was statistically significantly higher in women who did not conceive compared to whose who did (table 12).

Mean AFC was statistically significantly (p=0,025) higher in women who got pregnant (N=130, M=13,05, SD=5,71) after ART compared to those who did not get pregnant (N=165, M=11,5, SD=5,61). Significantly (p=0,001) higher dose of gonadotropins was used for women who did not get pregnant (N=556, M=1563,4 IU, SD=560,96) after ART compared to those who got pregnant (N=452, M=1540,64 IU, SD=464,74). The retrieved number of oocytes was statistically significantly (p=0,007 higher in women who got pregnant (N=467, M=10,18 SD=5,69) after ART compared to those who did not get pregnant (N=584, M=9,05, SN=5,44). Statistically significantly (p=0,000) higher number of embryos formed in women who got pregnant (N=467, M=5,89, SD=3,59), after ART compared to those who did not get pregnant (N=584, M=4,88, SD=3,4).

5.3. Differences between women who gave birth and who miscarried

467 women got pregnant after ART, 298 (63,8 %) of them gave birth. Mean age of women who delivered after ART was 32,2 years: SD 3,7, min-max 21-47 years. Mean age of women who suffered a miscarriage after ART was 33,63 years: SD 4,54, min-max 23-44 years. The difference was statistically significant, p=0,000.

We found no significant differences between women who gave birth and miscarried after ART in terms of height and weight, but women who miscarried had significantly higher BMI compared with those who gave birth after ART (table 13).

TABLE 13. Body size differences between women who delivered and miscarried

Indicator	Delivery			Miscarriage			P
	N	M	SD	N	M	SD	
Height (m)	298	1,7	0,05	169	1,69	0,06	0,063
Weight (kg)	298	63,63	9,0	169	64,91	10,82	0,170
BMI	298	22,06	2,98	169	22,77	3,64	**0,025**
Waist circumfer-ence (cm)	159	74,4	10,09	95	76,89	12,4	0,081
Hip circumfer-ence (cm)	157	95,2	9,21	95	96,45	11,8	0,350
WHR	157	0,78	0,07	95	0,8	0,07	0,082

TABLE 14. 2D:4D ratio differences

Indicator	Delivery			Miscarriage			P
	N	M	SD	N	M	SD	
II digit (cm)	160	6,71	0,41	95	6,65	0,3	0,244
IV digit (cm)	160	6,79	0,5	95	6,74	0,3	0,395
2D:4Dratio	160	0,99	0,03	95	0,99	0,02	0,499

We found no differences in 2D:4D ratio between women who gave birth after ART and those who suffered a miscarriage after ART (table 14).

TABLE 15. Differences in biochemical markers

Indicator	Delivery			Miscarriage			P
	N	M	SD	N	M	SD	
FSH (mIU/ml)	159	7,08	2,02	80	7,04	2,19	0,909
LH (mIU/ml)	159	5,97	2,46	73	6,39	2,62	0,235
Testosterone (nmol/l)	106	1,94	4,97	55	1,74	3,97	0,792

Indicator	Delivery			Miscarriage			P
	N	M	SD	N	M	SD	
SHBG (nmol/l)	102	88,19	35,81	49	87,06	40,89	0,863
FAI	99	1,29	0,92	49	1,40	0,94	0,478
AMH (µg/L)	87	3,98	3,34	43	3,21	2,53	0,187

We found no differences in biochemical markers between women who gave birth after ART and those who suffered a miscarriage after (table 15).

The mean number of oocytes retrieved was higher in women who suffered a miscarriage after ART compared to women who delivered, but the mean number of embryos formed was the same in both groups (table 16).

TABLE 16. Differences between women who delivered and miscarried

Indicator	Delivery			Miscarriage			P
	N	M	SD	N	M	SD	
AFC	87	13,06	5,48	43	13,02	6,24	0,975
Dose of gonadotropins (IU)	292	1418,73	452,87	160	1508,88	481,65	**0,048**
Number of oocytes retrieved	298	9,96	5,66	169	10,57	5,73	0,264
Number of embryos formed	298	5,93	3,65	169	5,82	3,49	0,757

Significantly higher dose of gonadotropins was used for ovarian stimulation in women who later miscarried after ART compared with women who delivered. The mean number of antral follicles was similar in both groups (table 16).

5.4. The influence of age on ART success

Female age is one of the most important factors influencing fertility. Increasing age, especially if woman is older than 35 years, is associated with decreasing fertility. Most of women (N=727, 69,2 %) who participated in this research were younger than 36 years (fig.1).

The pregnancy rate was statistically significantly higher (p=0,000) in younger women, reaching 48,8 % (N=355) in women who were younger than 36 years. Delivery rates were also statistically significantly (p=0,000) different in different age groups (table 17).

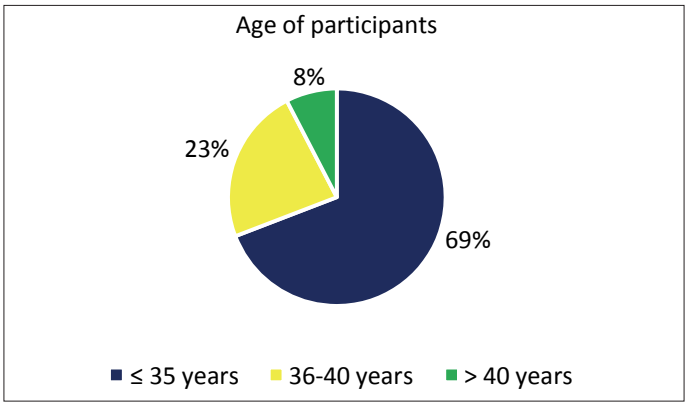


FIG. 1. Age of participants

TABLE 17. Pregnancy and delivery rates in different age groups

Female age	Pregnancy		No pregnancy		Delivery	
	N	%	N	%	N	%
≤ 35 years	355	48,8	372	51,2	245	33,7
36-40 years	96	39,3	148	60,7	51	20,9
>40 years	16	20	64	80	2	2,5
Total	467	44,4	584	55,6	298	28,4

Advanced maternal age is associated with higher risk of miscarriage. Miscarriage rate was statistically significantly (p=0,000) higher in older women, reaching 87,5 % in women older than 40 years (table 18).

TABLE 18. Delivery and miscarriage rates of women who got pregnant after ART

Female age	Delivery		Miscarriage	
	N	%	N	%
≤ 35 years	245	69	110	31
36-40 years	51	53,1	45	46,9
>40 years	2	12,5	14	87,5
Total	298	63,8	169	36,2

The odds of pregnancy after ART was 1,8 times higher in women younger than 36 years old compared with older women (OR-1,8; 95% CI 1,4-2,4). The odds of delivery after ART was 2,6 times higher in women younger than 36 years old com-

pared with older women (OR – 2,6; 95% CI 1,9-3,6). The odds of miscarriage after ART was 2,5 times higher in women older than 35 years old compared with younger women (OR 2,5; 95% CI 1,6-3,8).

5.5. The influence of obesity on ART success

Obesity has a negative impact on reproductive health. Obese women tend to have menstrual cycle and ovulatory dysfunctions and perinatal complications occur more often during pregnancy.

Fig. 2 shows the distribution of participants according to the BMI. Most of participants were in the normal BMI range (N=760, 72%).

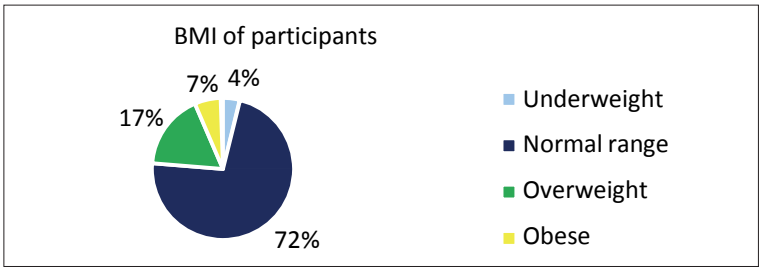


FIG. 2. BMI of participants

TABLE 19. Pregnancy and delivery rates in different BMI groups

BMI	Pregnancy		No pregnancy		Delivery	
	N	%	N	%	N	%
Underweight	20	48,8	21	51.2	13	31.7
Normal range	375	49.3	385	50.7	246	32.4
Overweight	31.5	31.5	124	68.5	30	16.6
Obese	14	20.6	54	79.4	8	11.8
Total	466	44.4	584	55.6	297	28.3

The biggest pregnancy rate was observed in normal BMI range group (N=375, 49,3 %). Pregnancy rate in obese women group was more than two times lower (N=14, 20,6 %). The difference was statistically significant (p=0,000). Delivery rates were also statistically significantly (p=0,000) different in different BMI groups. The biggest delivery rate was observed in normal BMI range group (N=246, 32,4 %) while delivery rate in obese women only reached 11,8 % (N=8) (table 19).

TABLE 20. Delivery and miscarriage rates of women who got pregnant after ART

BMI	Delivery		Miscarriage	
	N	%	N	%
Underweight	13	65	7	35
Normal range	246	65.6	129	34.4
Overweight	30	52.6	27	47.4
Obese	8	57.1	6	42.9
Total	297	63.7	169	36.3

The trend towards higher miscarriage rate in overweight and obese women was noticed, but the differences were not statistically significant (p=0.275) (table 20).

The odds of pregnancy after ART was 2,5 times higher in women who had no overweight (BMI <25) compared with obese women (OR-2,5; 95% CI 1,8-3,3). The odds of delivery after ART was 2,7 times higher in women who had no overweight (BMI <25) compared with obese women (OR – 2,7; 95% CI 1,8-3,9). The odds of miscarriage after ART was 1,7 times higher in obese women (BMI≥25) compared with women who had no overweight (OR 1,7; 95% CI1-2,8).

TABLE 21. Pregnancy rate in different BMI and age groups

BMI	≤35 years	36-40 years	>40 years	P
Underweight	48.3 %	44.4 %	66.7 %	0.797
Normal range	53.0 %	43.4 %	23.9 %	0.000
Overweight	33.0 %	36.1 %	10.0 %	0.083
Obese	25.7 %	18.2 %	9.1 %	0.465
P	**0.000**	0.135	0.086	

We noticed the trend that in all age groups the pregnancy rate was going down when women's BMI was higher (table 21).

TABLE 22. Delivery rates in different BMI and age groups

BMI	≤35 years	36-40 years	>40 years	P
Underweight	37.9 %	11.1 %	33.3 %	0.319
Normal range	37.2 %	24.3 %	0.0 %	0.000
Overweight	19.0 %	16.4 %	5.0 %	0.307
Obese	14.3 %	13.6 %	0.0 %	0.416
P	**0.000**	0.382	0.003	

The delivery rate was also going down when women's BMI was higher. Statistically significant difference was only observed in young (≤35 years) and older (>40 years) women's group (table 22).

TABLE 23. Miscarriage rate of women who got pregnant after ART

	≤35 years	36-40 years	>40 years	P
Underweight	21.4 %	75.0 %	50.0 %	0.126
Normal range	29.9 %	43.9 %	100.0 %	0.000
Overweight	42.4 %	54.6 %	50.0 %	0.676
Obese	44.4 %	25.0 %	100.0 %	0.394
P	0.315	0.426	0.077	

Table 23 shows the miscarriage rate of women who got pregnant after ART which was as high as 100 % in older women (>40 years) who were in the normal BMI age and obese. Miscarriage rate was statistically significantly influenced by age only in normal BMI range group.

5.6. The influence of WHR on ART success

WHR correlates with fertility. Circulating estrogen preferentially stores lipid deposits in the gluteofemoral region, including the buttocks and thighs. Body fat deposition can be evaluated by measuring waist to hip ratio. Fig. 3 shows the distribution of participants according to WHR. Most of participants (N=319, 55 %) had WHR lower than 0,8.

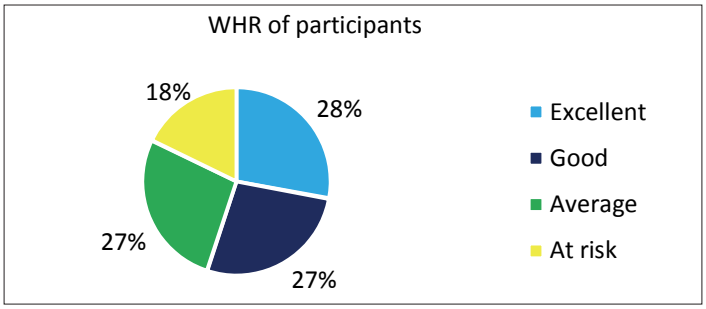


FIG. 3. Waist to hip ratio of participants

Table 24 shows pregnancy and delivery rates in different WHR groups. Lower pregnancy and delivery rates were observed in women who had high WHR, but the differences were not significant (p=0,185; p=0,068).

TABLE 24. Pregnancy and delivery rates in different WHR groups

WHR	Pregnancy		No pregnancy		Delivery	
	N	%	N	%	N	%
Excellent	76	46,9	86	53,1	48	29,6
Good	71	45,2	86	54,8	51	32,5
Average	70	44,6	87	55.4	39	24,8
At risk	35	34,0	68	66,0	19	18,4
Total	252	43,5	324	56,5	157	27,1

We noticed that the miscarriage rate is higher in women who have higher WHR, but the differences were not significant (p=0.170) (table 25).

TABLE 25. Delivery and miscarriage rates of women who got pregnant after ART

WHR	Delivery		Miscarriage	
	N	%	N	%
Excellent	48	62,3	28	36,8
Good	51	71,8	20	28,2
Average	39	55,7	31	44,3
At risk	19	54,3	16	45,7
Total	157	62,3	95	37,7

The odds of pregnancy after ART was 1,4 times higher in women who had WHR≤0,8 compared with women who had WHR>0,8 (OR-1,4; 95% CI 1-1,9). The odds of delivery after ART was 1,8 times higher in women who had WHR≤0,8 compared with women who had WHR>0,8 (OR – 1,8; 95% CI 1,2-2,7). The odds of miscarriage after ART was 1,9 times higher in women who had WHR>0,8 compared with women who had WHR≤0,8 (OR 1,9; 95% CI 1,1-3,3).

TABLE 26. Pregnancy rates in different WHR and age groups

WHR	≤35 years	36-40 years	>40 years	P
Excellent	51,3 %	31,4 %	50 %	0,116
Good	48,2 %	45,9 %	10 %	0,067
Average	53,3 %	30,8 %	9,1 %	0,003

WHR	≤35 years	36-40 years	>40 years	P
At risk	35,9 %	35,5 %	12,5 %	0,409
P	0,146	0,498	0,091	

When female age and WHR is increasing, the pregnancy rate is decreasing, but statistically significant differences were only observed in average WHR group. The same tendencies are seen in delivery rates (tables 26 and 27).

TABLE 27. Delivery rates in different WHR and age groups

WHR	≤35 years	36-40 years	>40 years	P
Excellent	32,8 %	22,9 %	12,5 %	0,292
Good	37,3 %	24,3 %	10 %	0,101
Average	30,8 %	15,4 %	0 %	0,023
At risk	23,4 %	12,9 %	0 %	0,174
P	0,301	0,552	0,515	

We noticed the trend that when the age and WHR was increasing, miscarriage rate was increasing as well. However, the differences were not statistically significant (table 28).

TABLE 28. Miscarriage rate of women who got pregnant after ART

WHR	≤35 years	36-40 years	>40 years	P
Excellent	36,1 %	27,3 %	75 %	0,229
Good	22,6 %	47,1 %	0 %	0,123
Average	42,1 %	50,0 %	100 %	0,466
At risk	34,8 %	63,6 %	100 %	0,156
P	0,184	0,394	0,344	

5.7. The influence of ovarian reserve on ART success

Success of ART depends on the ovarian response to gonadotropin stimulation. Factors, influencing ovarian response, may have prognostic value in predicting pregnancy and delivery rates after ART. Antral follicle count shows ovarian reserve. Fig.4 shows distribution of participants according to AFC.

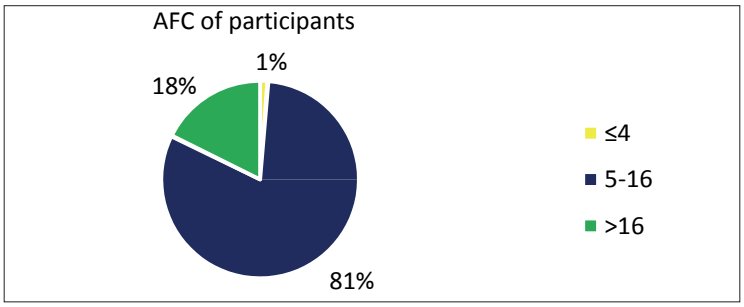


FIG. 4. AFC of participants

TABLE 29. Pregnancy and delivery rates in different AFC groups

AF C	Pregnancy		No pregnancy		Delivery	
	N	%	N	%	N	%
≤4	0	0	4	100	0	0
5-16	105	43,9	134	56,1	72	30,1
>16	25	48,1	27	51,9	15	28,8
Total	130	44,1	165	55,9	87	29,5

Table 29 shows pregnancy and delivery rates in different AFC groups. No woman got pregnant in the low AFC group (AFC ≤4), but we found no significant differences in pregnancy and delivery rates between different AFC groups (p=0,174; p=0,421).

TABLE 30. Delivery and miscarriage rates of women who got pregnant after ART

AFC	Delivery		Miscarriage	
	N	%	N	%
≤4	0	0	0	0
5-16	72	68,6	33	31,4
>16	15	60,0	10	40,0
Total	87	66,9	43	33,1

Delivery and miscarriage rates of women who got pregnant after ART were not significantly different in different AFC groups (p=0,413) (table 30).

TABLE 31. Pregnancy rates in different AFC and age groups

AFC	≤35 years	36-40 years	>40 years	P
≤4	-	-	0 %	-
5-16	53,8 %	34,7 %	12,5 %	0,000
>16	52,2 %	16,7 %	-	0,102
P	0,843	0,367	0,454	

Table 31 shows pregnancy rates in different AFC and age groups. We found statistically significant differences only in likely normal ovarian response group (AFC 5-16). When the age is increasing, the probability of pregnancy decreases even when ovarian reserve is normal. We see the same trend when analyzing delivery rates in different AFC and age groups (table 32). When ovarian reserve is normal, delivery rate (41,3%) in women younger than 36 years old is more than 2 times higher compared with older women (18,1 %).

TABLE 32. Delivery rates in different AFC and age groups

AFC	≤35 years	36-40 years	>40 years	P
≤4	-	-	0 %	-
5-16	41,3 %	18,1 %	0 %	0,000
>16	32,6 %	0 %	-	0,097
P	0,296	0,254	-	

Miscarriage rate of women who got pregnant after ART was significantly increasing with age in normal ovarian reserve group (Table 33).

TABLE 33. Miscarriage rate of women who got pregnant after ART

AFC	≤35 years	36-40 years	>40 years	P
≤4	-	-	-	-
5-16	23,4 %	48 %	100 %	0,002
>16	37,5 %	100 %	-	0,211
P	0,172	0,308	-	

Level of AMH shows ovarian reserve and likely ovarian response to gonadotropin stimulation. Fig. 5 shows distribution of research participants according to AMH level. AMH level was 0,77-3,49 ng/ml in 55 % (N=162) of participants, meaning that the likely ovarian response to stimulation was normal.

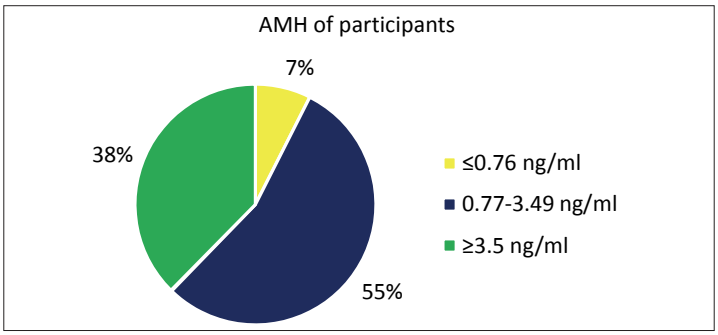


FIG 5. AMH of participants

TABLE 34. Pregnancy and delivery rates in different AMH groups

AMH	Pregnancy		No pregnancy		Delivery	
	N	%	N	%	N	%
≤0,76 ng/ml	9	40,9	13	59,1	5	22,7
0,77-3,49 ng/ml	72	44,4	90	55,6	46	28,4
≥3,5 ng/ml	49	44,1	62	55,9	36	32,4
Total	130	44,1	165	55,9	87	29,5

Pregnancy and delivery rates were similar in different AMH groups, no significant differences were found (p=0,952; p=0,595) (table 34).

TABLE 35. Delivery and miscarriage rates of women who got pregnant after ART

AMH	Delivery		Miscarriage	
	N	%	N	%
≤0,76 ng/ml	5	55,6	4	44,4
0,77-3,49 ng/ml	46	63,9	26	36,1
≥3,5 ng/ml	36	73,5	13	26,5
Total	87	66,9	43	33,1

We noticed the trend that when AMH is increasing, miscarriage rate is decreasing in women who got pregnant after ART, but there were no statistically significant differences (p=0,412) (table 35).

TABLE 36. Pregnancy rate in different AMH and age groups

AMH	≤35 years	36-40 years	>40 years	P
≤0,76 ng/ml	66,7 %	37,5 %	0 %	0,051
0,77-3,49 ng/ml	55,1 %	39,2 %	13,6 %	0,001
≥3,5 ng/ml	50,5 %	15,8 %	0 %	0,014
P	0,597	0,175	0,632	

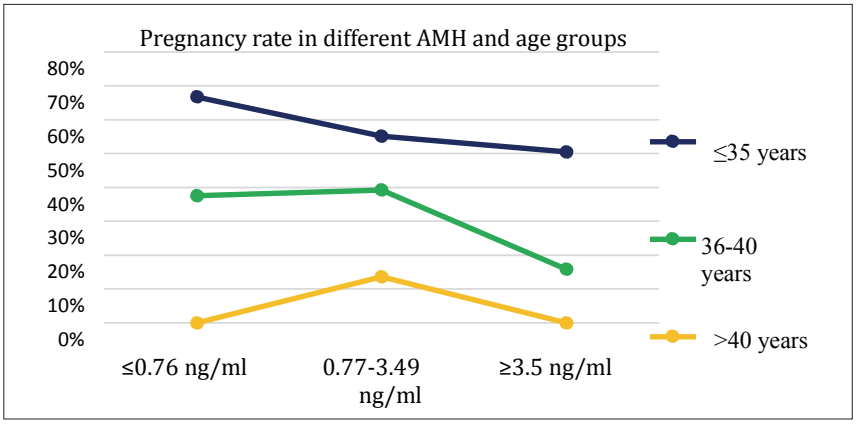


FIG. 6. Pregnancy rates in different AMH and age groups

Table 36 and fig. 6 shows pregnancy rates in different AMH and age groups. Pregnancy rate is high even when AMH is low in women younger than 36 years old. Pregnancy rate is low even when AMH is normal (likely ovarian response to gonadotropin stimulation is normal) in women older than 40 years.

Delivery rates are also significantly decreases with increasing age in all AMH groups (table 37).

TABLE 37. Delivery rates in different AMH and age groups

AMH	≤35 years	36-40 years	>40 years	P
≤0,76 ng/ml	55,6 %	0 %	0 %	0,009
0,77-3,49 ng/ml	38,2 %	23,5 %	0 %	0,001
≥3,5 ng/ml	38,5 %	5,3 %	0 %	0,015
P	0,586	0,078		

Table 38. Miscarriage rate of women who got pregnant after ART

AMH	≤35 years	36-40 years	>40 years	P
≤0,76 ng/ml	16,7 %	100 %	-	0,018
0,77-3,49 ng/ml	30,6 %	40 %	100%	0,048
≥3,5 ng/ml	23,9 %	66,7 %	-	0,104
P	0,646	0,127		

Level of AMH had no significant influence on miscarriage rates in women who got pregnant after ART, but we noticed that in women aged 36-40 years old miscarriage rate was higher in low and high AMH groups than in normal AMH group. Miscarriage rate was increasing significantly with increasing age in low and normal AMH groups (table 38).

Ovarian reserve and likely ovarian response to gonadotropin stimulation can also be measured by level of basal FSH. Fig. 7 shows participants distribution according to FSH. FSH level was 4 - 8,9 IU/L in 80 % (N=461) of women, so the likely ovarian response to stimulation was normal.

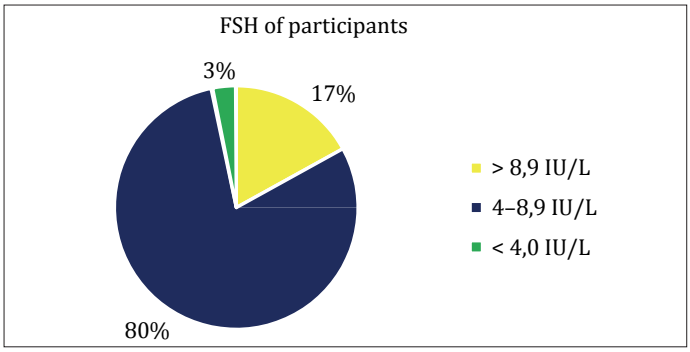


FIG. 7. FSH of participants

TABLE 39. Pregnancy and delivery rates in different FSH groups

FSH	Pregnancy		No pregnancy		Delivery	
	N	%	N	%	N	%
> 8,9 IU/L	35	35,7	63	64,3	25	25,5
4-8,9 IU/L	195	42,3	266	57,7	130	28,2
< 4 IU/L	9	50,0	9	50	4	22,2
Total	239	41,4	338	58,6	159	27,6

Table 39 shows pregnancy and delivery rates in different FSH groups. Pregnancy rate is increasing when FSH level is decreasing (likely ovarian response to stimulation is increasing). Highest delivery rate is observed in normal (4-8,9 IU/L) FSH group, but the differences are not statistically significant (p=0,366; p=0,757).

TABLE 40. Delivery and miscarriage rates in women who got pregnant after ART

FSH	Delivery		Miscarriage	
	N	%	N	%
> 8,9 IU/L	25	71,4	10	28,6
4-8,9 IU/L	130	66,7	65	33,3
< 4 IU/L	4	44,4	5	55,6
Total	159	66,5	80	33,5

Higher miscarriage rate of women who got pregnant after ART was observed in low FSH group, but the differences were not statistically significant (p=0,309) (table 40).

TABLE 41. Pregnancy rates in different FSH and age groups

FSH	≤35 years	36-40 years	>40 years	P
> 8,9 IU/L	49,1 %	32 %	0 %	0,014
4-8,9 IU/L	51,4 %	39,6 %	15,4%	0,001
< 4 IU/L	69,2 %	0 %	0 %	0,177
P	0,414	0,575	0,421	

Table 41 shows pregnancy rates in different FSH and age groups. Highest pregnancy rate (69,2 %) was observed in women who were young and had low FSH. Increasing age significantly decreases pregnancy rates in normal FSH group.

TABLE 42. Delivery rates in different FSH and age groups

FSH	≤35 years	36-40 years	>40 years	P
> 8,9 IU/L	38,2 %	16 %	0 %	0,017
4-8,9 IU/L	36,4 %	22,8 %	0 %	0,000
< 4 IU/L	30,8 %	0 %	0 %	0,657
P	0,881	0,663		

Age had significant influence on delivery rates in high and normal FSH groups. Highest delivery rate was observed in women who where young and had high FSH (table 42).

TABLE 43. Miscarriage rate of women who got pregnant after ART

FSH	≤35 years	36-40 years	>40 years	P
> 8,9 IU/L	22,2 %	50 %	-	0,127
4-8,9 IU/L	29,1 %	42,5 %	100 %	0,005
< 4 IU/L	55,6 %	-	-	
P	0,162	0,696		

High miscarriage rate was (55,6 %) observed in women who were young and had low FSH, but differences were not statistically significant. Increasing age significantly increases miscarriage rate in normal FSH group (table 43).

5.8. Multivariate analysis

Logistic regression analysis showed that increasing female age, weight, BMI, waist and hip circumference, WHR, FSH, LH and FAI decreases pregnancy and delivery rates after ART. Increasing female height, AMH, number of oocytes and embryos, AFC, increases pregnancy and delivery rates after ART (tables 44 and 45).

TABLE 44. Logistic regression for pregnancy rate after ART

Indicator	Pregnancy	No pregnancy	P
	OR (95% CI)	OR (95% CI)	
Age (years)		1.057	0.000
Height (cm)	1.037		0.002
Weight (kg)		1.028	0.000
BMI		1.112	0.000
Waist circumference (cm)		1.014	0.040
Hip circumference (cm)		1.009	0.274
WHR		9.410	0.040
FSH		1.067	0.118
LH		1.009	0.800

Indicator	Pregnancy	No pregnancy	P
	OR (95% CI)	OR (95% CI)	
E2	1.000	1.000	0.247
Prolactin	1.000	1.000	0.523
Testosterone	1.011		0.688
SHBG	1.003		0.358
FAI		1.255	0.023
AMH	1.016		0.683
2D:4D ratio	1.083		0.979
Number of oocytes	1.037		0.001
Number of embryos	1.087		0.000
Dose of gonadotropins	1.000	1.000	0.001
AFC	1.048		0.026

TABLE 45. Logistic regression for delivery rate after ART

Indicator	Delivery	No delivery	P
	OR (95% CI)	OR (95% CI)	
Age (years)		1.087	0.000
Height (cm)	1.107		0.000
Weight (kg)		1.028	0.000
BMI		1.125	0.000
Waist circumference (cm)		1.021	0.011
Hip circumference (cm)		1.012	0.163
WHR		24.920	0.011
FSH		1.049	0.293
LH		1.036	0.341
E2	1.000	1.000	0.625
Prolactin	1.000	1.000	0.895
Testosterone	1.014		0.619
SHBG	1.002		0.434
FAI		1.261	0.046

Indicator	Delivery	No delivery	P
	OR (95% CI)	OR (95% CI)	
AMH	1.050		0.227
2D:4D ratio	5.343		0.622
Number of oocytes	1.018		0.140
Number of embryos	1.067		0.001
Dose of gonadotropins		1.001	0.000
AFC		1.037	0.099

Principle components factor analysis for women who got pregnant and for women who did not get pregnant after ART revealed that all factors (reproductive and body size) are intertwined with each other: 8-9 factors can be distinguished, but they intertwine with each other and unrelated indicators groups cannot be singled out.

So we can make a conclusion that factors influencing body size and proportion, reproductive possibilities and hormonal balance usually are the same and inseparable (tables 46 and 47).

TABLE 46. Factor analysis for participants who got pregnant after ART

Rotated Component Matrix								
Indicator	Component							
	1	2	3	4	5	6	7	8
Number of oocytes	0.908			-0.171				
AFC	0.863	-0.116				-0.136		-0.111
LH	0.777	-0.184	-0.113		-0.352	0.139	-0.206	
WHR	0.722	0.145	0.158	0.263	0.16	0.255	0.188	
Number of embryos	0.697	-0.107	-0.228		-0.409	0.135	0.288	
Number of embryos (IVF)	0.595		-0.102		-0.562	-0.391	0.265	
Female weight	-0.197	0.925				-0.139		-0.159
Hip circumference		0.914		-0.101		-0.189		
Waist circumference	0.417	0.834	0.157		0.159			

Rotated Component Matrix								
Indicator	Component							
	1	2	3	4	5	6	7	8
BMI	-0.303	0.829			-0.165			0.33
FAI	-0.193	0.623	-0.2	-0.23	0.144	0.257	0.211	-0.165
Testosterone			0.891	-0.181				
Prolactin	-0.132		0.86				-0.175	0.262
AMH	0.23	-0.174	0.606	-0.2	-0.177	-0.115	0.495	
FSH		-0.184	-0.151	0.795		0.276	0.226	
Dose of gonadotropins	-0.165	-0.258		0.75				0.211
Progesterone	-0.277	-0.153	0.131	-0.669	0.336	0.176	0.249	0.16
Indication for ART	-0.153			-0.136	0.787		0.245	
Number of embryos (IMSI)			-0.233		0.59	-0.248		0.405
Number of embryos (ICSI)	-0.143	-0.18				0.869	0.101	
2D:4D ratio	0.468			0.288		0.722	-0.127	0.118
Estradiol				-0.224			-0.855	0.149
SHBG	0.437	-0.263	0.224	0.241	-0.256		-0.617	-0.246
Female height	0.108	0.323			0.15	-0.268	0.122	-0.81
Female weight		0.148	0.315	0.183	0.216	-0.122		0.775
% of Variance	18.141	15.795	9.361	8.691	7.948	7.849	7.639	7.475
Cumulative %	18.141	33.935	43.296	51.987	59.935	67.784	75.424	82.898

TABLE 47. Factor analysis for participants who did not get pregnant after ART

Rotated Component Matrix									
Indicator	Component								
	1	2	3	4	5	6	7	8	9
BMI	0.931			0.139					
Female weight	0.92	-0.115							0.243
Waist circumference	0.886			0.124		0.352			0.112

Rotated Component Matrix									
Indicator	Component								
	1	2	3	4	5	6	7	8	9
Hip circumference	0.881	-0.195	0.101	0.196		-0.115	-0.145	-0.109	0.2
SHBG	- 0.671	0.177	- 0.211	0.187	0.109	0.115		- 0.293	-0.12
FAI	0.656								-0.285
Number of oocytes	-0.266	0.896		-0.117				-0.205	
Number of embryos	-0.128	0.856	0.275			0.163	0.259		
AFC	-0.272	0.794			-0.203	-0.147	-0.207	-0.118	-0.15
Number of embryos (ICSI)		0.742	-0.406		0.221	0.113		0.233	
Number of embryos (IVF)		0.169	0.92			0.109		0.12	0.137
Indication			- 0.727	- 0.319	0.1	0.359		0.11	0.13
Testosterone	0.112		0.59	-0.409		0.296			-0.392
Female age		-0.273		0.818					
Dose of gonadotropins	0.454	0.161	0.218	0.69		0.112	0.211		-0.117
Prolactin				-0.126	0.909	-0.164			
LH	-0.271	0.357	-0.136	0.111	0.672	0.245	-0.156	-0.186	0.247
AMH	-0.13	0.267		-0.27	- 0.408	- 0.353	- 0.174	0.254	- 0.226
WHR	0.207	0.247	-0.11			0.829	0.255		-0.112
FSH	-0.408	-0.111	0.442	0.272		0.637	-0.255		
Number of embryos (IMSI)	-0.103			-0.241	-0.32		0.765	-0.237	0.216
Estradiol	-0.185	-0.127		0.211	0.31		0.714		
Progesterone	-0.172	-0.23			-0.114		-0.202	0.88	
2D:4D ratio	0.118	0.468	0.105	0.183		0.159	0.422	0.582	
Female height	0.273	-0.164		-0.186			0.135		0.873
% of Variance	20.045	14.161	9.347	7.653	7.228	7.2	6.895	6.137	5.573
Cumulative %	20.045	34.206	43.553	51.206	58.434	65.634	72.528	78.665	84.238

6. Discussion

Female age is one of the most important factors influencing ART success. Fewer oocytes are retrieved, fewer embryos transferred and significantly lower pregnancy rate is observed in older women (≥ 40 years) after ART treatment (Gomes, Canha Ados et al. 2009, Hourvitz, Machtinger et al. 2009). If older women do get pregnant, the risk of miscarriage is much higher. Ongoing pregnancy rate is inversely proportional to maternal age – increasing maternal age decreases clinical pregnancy rate (McCoy, Nakajima et al. 2009). Mean age (M=33,4 years) of participants of this research was higher than the mean age (M=29,1 years) of delivering women in Lithuania in 2016 (2016). Women who got pregnant and delivered after ART were significantly younger than women who did not conceive after ART. Female age had significant influence on pregnancy, delivery and miscarriage rates after ART. Pregnancy rate (48,8 %) in women younger than 36 years old was more than 2 times higher compared to women older than 40 years old (pregnancy rate 20 %); delivery rate was more than 10 times higher in women younger than 36 years old (33,7 %) compared to women older than 40 years old (2,5 %). Timing is very important, but complex side of infertility treatment. Time has influence on diagnosis, prognosis and success of treatment. Prolonged infertile couple's treatment duration can sometimes force doctors to make wrong and potentially harmful decisions. Female age, time to pregnancy and duration of possible treatment must be taken into consideration, but it is very important not to over treat. Scientific research based dynamic models evaluating probability of natural conception could be helpful in decision making and would allow to avoid unnecessary and expensive treatment (Group 2017).

Body mass index. Obesity lowers the probability of pregnancy after ART. In 2010 study aimed at assessing the impact of female BMI on the success of ART (quality of the embryos received and pregnancy rate) was published in Spain. 6500 ART cycles were analyzed in this study. No significant differences were found in treatment, oocyte fertilization rate, embryo transfer day, mean number of embryos transferred, mean number of surplus embryos, blastocyst transfer rate and cleavage stage embryo quality between obese and normal BMI range women. However, embryo implantation rate, pregnancy and delivery rate was lower in obese women compared to normal BMI range women. Increasing BMI gradually decreases pregnancy and delivery rates (Bellver, Ayllon et al. 2010). Our research showed that women who got pregnant and delivered after ART had significantly lower BMI compared to women who did not conceive after ART. Highest pregnancy (49,3 %) and delivery (32,4 %) rate was observed in normal BMI range group. Pregnancy (20,6 %) and delivery (11,8 %) rates were more than twice lower in obese women group. Losing weight before ART increases chances of live birth (Espinos, Polo et al.

2017). However, not all studies find significant correlation between BMI and clinical pregnancy rate, recommending that BMI should not be a basis for IVF treatment denial (Friedler, Cohen et al. 2017).

Morphological characteristics of female body. WHR >0,80 is associated with lower pregnancy rate after ART, even when adjusted for factors like age, BMI smoking and number of embryos transferred. Central type (android) body fat distribution can have negative effect on pregnancy rate because of a different endocrinological and biochemical environment for the oocyte in the growing follicle, poor quality oocytes and endometrial changes due to hormonal imbalance (Wass, Waldenstrom et al. 1997). We did not find statistically significant differences in pregnancy and delivery rates between different WHR groups, but we noticed the trend that increasing WHR decreases pregnancy and delivery rates and increases miscarriage rate. Thus, the morphological features of the female body undoubtedly play a role in the treatment of infertility.

Ovarian reserve. AFC, AMH and FSH levels correlate with the number of oocytes retrieved and pregnancy rates after ART (Nelson, Klein et al. 2015, Keane, Cruzat et al. 2017). However, not all studies support the prognostic value of these markers (Ashrafi, Hemat et al. 2017). In our research, we found out that mean AFC was significantly higher in women who got pregnant compared to women who did not get pregnant after ART. Mean AMH and FSH was similar in both groups. It is well known that ovarian reserve diminishes with age. As age increases, the number of primordial follicles and AMH level in blood serum decrease. AMH decreases before FSH increases. Therefore, AMH can be seen as an early indicator of ovarian reserve loss (Gomez, Schorsch et al. 2016). According to our research, pregnancy and delivery rates decrease with increasing age even when ovarian reserve is normal. Pregnancy and delivery rates in young women (≤35 years old) was high even when ovarian reserve was low. These findings from our study coincide with data from other studies. Study in Germany (1287 participating women) showed that AMH level has no influence on pregnancy rate after ART in young women (<36 years old) (Gomez, Schorsch et al. 2016). Study in Taiwan revealed that probability of live birth after ART was influenced only by the number of good quality embryos in young women (<35 years old) and in older women results were influenced by age and AMH level (Lee, Liu et al. 2009). It is interesting, that pregnancy rate in low AMH group was higher than pregnancy rate in normal and high AMH groups in young women (≤35 years old). The same trend is seen in study done in Germany. These differences might be due to different indications for ART. In the low AMH groups, ART was usually performed due to decreased ovarian reserve; in the high AMH groups, the most common indication for ART was polycystic ovary syndrome. In the normal AMH group, ART was most often performed due to male infertility, endometriosis, adenomyosis, implantation failure, and other causes (Gomez, Schorsch et al. 2016).

7. Conclusions

1. Female age is the main and most important predictor of IVF outcome. Increasing female age decreases pregnancy and delivery rates after ART. The best results are achieved in women under 36 years of age.
2. Obesity, especially central type, has a negative impact on pregnancy and delivery rates after ART. Increasing BMI and WHR increases miscarriage rate after ART. Increasing female height increases pregnancy and delivery rates after ART.
3. AMH has no influence on pregnancy and delivery rates in young women (≤35 years old) suggesting AMH level monitoring should not be mandatory in this age group. AMH level can be used as a prognostic factor in determining the probability of pregnancy after ART in women over 35 years old.
4. Pregnancy, delivery and miscarriage rates were similar in the different groups of infertility. Mean AMH was significantly higher in women with male factor infertility than in women with female factor infertility.
5. Factors influencing body size and proportion, reproductive possibilities and hormonal balance usually are the same and inseparable.

References

A list of references is provided in the manuscript of the Dissertation.

SANTRAUKA

Išvados

1. Amžius yra pagrindinis veiksnys, turintis įtakos pastojimo, gimdymo ir persileidimo dažniui po pagalbinio apvaisinimo. Didėjant moters amžiui tikimybė pastoti ir pagimdyti gyvą naujagimį po pagalbinio apvaisinimo procedūros mažėja. Geriausi rezultatai pasiekiami moterims iki 36 metų amžiaus.

2. Nutukimas, ypač centrinio tipo, neigiamai siejasi su tikimybe pastoti ir pagimdyti gyvą naujagimį po pagalbinio apvaisinimo procedūros. Esant didesniam KMI ir JKI didėja po pagalbinio apvaisinimo procedūros pastojusių moterų persileidimų dažnis. Esant didesniam moters ūgiui, didėja pastojimo ir gimdymo tikimybė po pagalbinio apvaisinimo.

3. AMH neturi įtakos jaunų (≤35 metų) moterų pastojimo ir gimdymo dažniui, todėl AMH nustatymas neturėtų būti privalomas tokio amžiaus moterims. Vyresnio amžiaus moterims (>35 metų) AMH gali būti naudojamas kaip prognostinis pagalbinio apvaisinimo procedūros sėkmės veiksnys.

4. Pastojimo, gimdymo ir persileidimo dažnis skirtingose nevaisingumo priežasčių grupėse buvo panašus. Moterų, kurioms pagalbinis apvaisinimas buvo atliktas dėl priežasčių vyro organizme, vidutinis AMH buvo reikšmingai didesnis, nei moterų, kurioms pagalbinis apvaisinimas buvo atliktas dėl priežasčių moters organizme.

5. Veiksniai, lemiantys kūno dydį ir proporcijas, reprodukcines galimybes, hormonų pusiausvyrą yra tarpusavyje labai persipynę ir jų negalima griežtai vieno nuo kito atskirti.

Publications

Articles published on the present research findings:

1. Zivile Cerkiene, Audrone Usoniene, Andre Amsiejiene, Paulius Sakalauskas, Grazina Drasutiene, Diana Ramasauskaite (2015) Impact of quantitative parameters, such as number of retrieved oocytes, number of transferred embryos and availability of surplus embryos for cryopreservation, on clinical pregnancy rates in ART, Gynecological Endocrinology, 31:sup1, 46-50, DOI: 10.3109/09513590.2015.1086507.

2. Andre Amsiejiene, Grazina Drasutiene, Audrone Usoniene, Janina Tutkuviene, Saule Vilsinskaite, Liucija Barskutyte (2017) The influence of age, body mass index, waist-to-hip ratio and anti- Mullerian hormone level on clinical pregnancy rates in ART, Gynecological Endocrinology, 33:sup1, 41-43, DOI: 10.1080/09513590.2017.1399692.

3. Donata Saulė Vilšinskaitė, Liucija Barškutytė, Andrė Amšiejienė, Grąžina Drąsutienė. Pagalbinio apvaisinimo procedūrų sėkmės priklausomybė nuo moters amžiaus, kūno masės indekso ir antimiulerinio hormono koncentracijos prieš procedūrą. Laboratorinė medicina. - T. 19, Nr. 4 (2017), p. 224–227.

Scientific presentations on the theme of the dissertation:

1. A. Amšiejienė. THE INFLUENCE OF AMH, BMI, WAIST TO HIP RATIO AND 2D/4D RATIO ON IVF SUCCESS. 3rd Annual meeting of Baltic Fertility Society. 20-23 May, 2016. Riga, Latvia.

2. A. Amšiejienė, G. Drąsutienė. THE INFLUENCE OF AGE, AMH, BMI AND WAIST TO HIP RATIO ON IVF SUCCESS. The 3rd International Conference Evolutionary medicine: Pre- existing Mechanisms and Patterns of Current Health Issues; The 14th–19th of June, 2016, Organized by the Faculty of Medicine, Vilnius University and Lithuanian Academy of Sciences.

Virginija Paliulytė

ULTRASOUND EVALUATION OF POSTPARTUM UTERUS MORPHOLOGICAL PARAMETERS IN THEIR RELATIONSHIP WITH MATERNAL AND NEWBORN HEALTH INDICATORS

GIMDOS MORFOLOGINIŲ PARAMETRŲ ULTRAGARSINIS VERTINIMAS LAIKOTARPIU PO GIMDYMO IR JŲ SĄSAJOS SU MOTERS IR NAUJAGIMIO SVEIKATOS RODIKLIAIS

Biomedical Sciences, Medicine (06B)
Biomedicinos mokslai, medicina (06 B)

Abbrevations

2D – 2 dimensional
3D – 3 dimensional
AP – anteroposterior diameter
BHCG – beta-human chorionic gonadotropin
BMI – body mass index
C/S – caesarean section
CT – computed tomography scan
GBS - Group B streptococcus
M – mean
MRI – magnetic resonance imaging
N – case number
PI – pulsatility index
PPH – primary postpartum hemorrhage
RI – resistance index
RPOC – retained products of conception
S/D – systole and diastole ratio
SD – standard deviation
SPH – secondary postpartum hemorrhage
UF – uterine fundus
UFH – uterine fundal height
VBAC – vaginal birth after caesarean

Introduction

The pathology of the period after childbirth is often impacted by the condition observed in a woman during her pregnancy (hypertension, premature childbirth, preterm rupture of membranes, multiple pregnancy, common female diseases, etc.), however, complications can develop even without any risk factors, so an obstetrician gynecologist must always be knowledgeable to recognise and manage them. The most common cause of complications after childbirth is uterine pathology. Endometritis is found in approximately 1.5% of all postpartum women and is even more frequent after cesarean section - approximately 2-2.5% [*Axelsson et al., 2017; Yang et al., 2017*]. In developed countries, during the perinatal period, postpartum bleeding or infection still cause 12-13% of deaths in all the women, while late diagnosis or unnecessary interventions increase the morbidity of women and slow down the normal process of postpartum involution [*Axelsson et al., 2017; Yang et al., 2017; Karsnitz et al. 2013*]. In developing countries, postpartum pathology is one of the main causes of young women's deaths [*Ahnfeldt-Mollerup et al., 2012; Cunningham et al., 2018; Paliulytė et al., 2011*]. The structure of the causes of maternal mortality in Lithuania shows that postpartum infections account for approximately 18% of deaths and obstetrical haemorrhage - about 14% [*Hygiene Institute, 2017*]. Therefore, one of the most important goals of an obstetrician gynecologist is timely recognition of these complications by using the safest and most accurate methods of diagnosis and treatment, more frequent ultrasound examinations in the early postpartum period, correct interpretation, recognition of normal and pathological uter-

ine involution, linking this knowledge with clinical symptoms and providing timely treatment or avoiding it.

Understanding of the normal uterine involution and the timely recognition of complications after delivery would improve the care of women. Historically, the perception of postpartum uterine pathology was based only on clinical and histological studies, while in the current period, safe visual investigation techniques can be deployed. Modern obstetrics cannot be imagined without ultrasound examination which is used to achieve optimum perinatal care: to evaluate the condition of the fetus, to diagnose and monitor pregnancy complications, to plan the delivery method and time, even to determine the fetal head position in the pelvis during labour and evaluate the prognosis of childbirth via the natural paths [*Ki Hoon et al., 2014; Molina et al., 2010; Dupuis et al., 2005; Youssef et al., 2013; Vintzileos et al., 2010; Mihu et al., 2011*]. An ultrasound examination is especially safe and very informative in postpartum examination. Today, the ultrasound machine is not only easily transportable and available in every in-patient clinic, but also allows obtaining the highest quality of images and their expression. Ultrasound can be performed in any hospital room at any time of the day, even in the delivery room at the earliest period after childbirth. It is especially important to be equipped with knowledge of the uterine echoscopy at the presence of a life-threatening hemorrhage after childbirth. According to global statistics, in the cases of primary postpartum hemorrhage (PPH), the incidence of uterine perforation after urgently performed uterine abrasion reaches up to 3 per cent, while the rate of hysterectomies for these complications is about 1 per cent [*Cunningham et al., 2018; Mulic-Lutvica et al., 2009*]. If at the moment of the occurrence of PPH, we could immediately perform uterine ultrasound diagnosis to confirm the diagnosis more precisely, unnecessary interventions would be avoided and a lot safer conservative treatment would be sufficient. Undoubtedly, in each case, the accuracy of the examination and the conclusions are determined by the qualification and experience of the researcher [*Shipp et al., 2014*].

There are still no universally accepted recommendations for postpartum uterus ultrasound screening [*Ucyigit et al., 2016*]. A number of studies, carried out on this topic, deal with the physiological involutional changes in the size of the uterus and its vascular tissues, as well as pathological findings. Most of the published studies were conducted 10-15 years ago, the range of their samples is most commonly narrow, the selection criteria are not uniform, non-standardized, the morphological characteristics under analysis are different, the data on ultrasound images of the uterus in the early postpartum period (within the first two hours) are missing, the best uterus involution monitoring indicators are still being searched for [*Ucyigit et al., 2016; Fuller et al., 2015; Fukuda et al., 2016*]. The published scholarly work does not provide sufficient data either on the normal uterine involution, or on the differ-

ences observed in the uterine involution of the primiparous or multiparous women. This topic has been studied more closely in only a few studies [*Mulic-Lutvica et al., 2006; 2007; 2009; 2012; Watagana et al., 2015; Deans et al., 2006; Guedes-Martins et al. 2015; Olayemi et al., 2002*]. Most published studies examine only either the size of the uterus, or the circulatory system and rarely assess the integrity of all the parameters of the uterine involution consistently, from day one to two months after delivery (*Olayemi et al., 2002; Mulic-Lutvica et al., 2001*]. Most published studies end with the period of six postpartum weeks. The data on morphological changes in the postpartum uterus after instrumental manipulation procedures carried out in the uterus during the early postpartum period are insufficient [*Axelsson et al., 2017; Mulic-Lutvica et al., 2007; 2009*]. An obvious problem in obstetrics is the increasing number of women after a cesarean section. A never-ending topic is an ultrasound screening of the scarred uterus as the proper criteria for determining the risk of uterine rupture have not been found yet [*Shipp et al., 2014; Sohn et al., 1988; Sokol et al., 2004; Van Schoubroeck et al., 2004; Reles et al., 1992*]. It is very important to recognise that ultrasound screening can not be the main method since the application of it may also involve diagnostic and interpretive errors, therefore, it is always necessary to associate the results with clinical symptoms [*Van den Hof et al., 2001*].

In Lithuania, the gynecologic and obstetric ultrasound examination is carried out by obstetricians gynecologists themselves as this competence is defined in the medical norm of the obstetrician gynecologist, which is why these physicians should be equipped with the knowledge of both the physiological and pathologal uterine involution criteria in order to evaluate postpartum women by ultrasound [*Medical Norm of Obstetritian-Gynecologist*].

Aim of the dissertation

To evaluate the uterine morphologic parameters obtained by ultrasound evaluation of postpartum women and to determine the relationship of these indicators with the maternal and newborn health indicators. To provide practical recommendations for obstetricians-gynecologists, to include new ultrasound uterine involution criteria when observing women after delivery in their daily practice.

Objectives of the dissertation

1. To use ultrasound examination aiming to determine the physiological changes in the morphologic parameters of the uterus size in women during the postpartum period:
   1.1. To analyse the relationship between the criteria for evaluation of uterine involution and female health indicators;
   1.2. To analyse the differences between the morphologic parameters of the uterine involution between primiparous and multiparous women;
   1.3. To identify which postpartum period is most critical in terms of making an error in evaluating the uterine involution.
2. To evaluate the relationship between the morphological markers of the uterus and the birth weight of newborns.
3. To evaluate the state of newborns in the examined women on the basis of the V. Apgar scale and to determine the umbilical arterial blood pH and lactate levels.
4. To analyse the differences between the morphologic parameters of the uterus size after normal and complicated deliveries.
5. To analyse the features of the morphologic indicators of the uterus size and the uterine scar(s) after the cesarean section during postpartum period.

Relevance and scientific novelty of the research

So far the scholarly publications available nationwide or worldwide have not provided any universally accepted methods of ultrasound monitoring of the uterine involution. The guidelines based on such methods would be relevant to all doctors in their daily practice in terms of facilitating identification of the normal ultrasound image of the uterus, the differences of the uterine image during different stages of involution in primiparous and multiparous women, pathology, introduction or avoidance of uterine intervention procedures. However, there are still many uncertainties in recognising some pathological postpartum conditions: the retained products of conception (RPOC), causes of postpartum bleeding, endometritis after delivery (*Cunningham et al., 2018; Paliulytė et al., 2011; Mulic-Lutvica et al., 2007*]. In the absence of a precise diagnosis, there is an increased risk of excess primary postpartum hemorrhage, the frequency of transfusion of blood components and the amount of associated complications. According to the literature, one percent of women after

natural childbirth need transfusion of blood components, while the rate of blood transfusion after the cesarean section (C/S) or after instrumental uterine procedures is 5-6% [*Shakur et al., 2010*]. Unnecessary medical interventions in the uterus (curettage) and possible complications after them is still a major problem in obstetrics and gynecology. The rate of complications of 7% involves to uterine perforation, endometritis, Asherman's syndrome in the future, infertility, hysterectomy [*Ki Hoon et al., 2014*]. In the developed countries, half of women who return to hospital due to secondary postpartum hemorrhage (SPH) undergo uterine abrasion. Understanding of the normal uterine involution and the causes of bleeding (RPOC, endometritis or idiopathic causes due to slowed placental site involution) and the correct interpretation of ultrasound images can help prevent such complications.

The analysis of scholarly studies published worldwide and in Lithuania revealed no ultrasound examination data on the earliest postpartum period - two hours after childbirth. Other authors have published data on uterine ultrasound examinations carried out during the period from 12 to 48 hours after delivery [*Watagana et al., 2015; Mulic-Lutvica et al., 2006; 2007; Deans et al., 2006; Guedes-Martins et al. 2015; Olayemi et al., 2002*]. Our study will provide the data about the first hours postpartum, which are important for the diagnosis of both physiological conditions and severe complications: bleeding due to the uterine atony, RPOC in the uterus, uterine evaluation after intervention procedures. Most of the authors observed women 1-4 times after delivery and most commonly only up to 6 weeks postpartum, while our subjects were consistently monitored 6 times from the first two hours to 8 weeks postpartum. In most of the studies, the researchers had studied individual parameters of the uterine involution (only the length, width and thickness of the uterus, others - only the anterior and posterior dimensions of the uterus, or only the width of the uterus cavity, still others - only the blood flow). Our study linked all the possible indicators into the whole, and we observed these indicators in our subjects from the beginning to the end of the study [*Mulic-Lutvica et al., 2006; 2007; 2009; Shipp et al., 2014; Fuller et al., 2015; Wataganara et al., 2015; Deans et al., 2006*]. For each subject, a consecutive repeated uterine arterial blood flow research (an extremely rare form of research carried out in the world), was performed in this study by deploying a high quality equipment which allowed to monitor the changes in the uterine blood circulation in all the subjects from the first hours after childbirth to the end of the involution period [*Paliulyte et al., 2017*].

The scholarly literature provides little data about the correlation between the health indicators of mature newborns in the early postpartum period and the indicators of maternal uterine involution. In examining postpartum women, we evaluated the health indicators of these newborns and the newborn weight impact on the size and involution of the uterus postpartum. Perinatal hypoxia can be better diagnosed

after determining the pH and the amount of lactate in the umbilical artery blood. The analysis of the umbilical arterial blood gas includes the following parameters: pH, pO2, pCO2 and the amount of lactate, and the base excess calculated by various algorithms [*Suidan et al., 1984; Tuuli et al., 2014*]. Generally, as soon as a newborn is delivered, the determination of alkaline and acid levels in the umbilical cord arterial blood is carried out, but there is evidence that cord arterial blood lactate levels better reflect alkaline and acid levels in the newborn body than pH. The increased levels of cord blood lactate occur earlier and persist for longer than a decrease in pH, so lactate has a very similar or even higher predictive value than pH [*Tuuli et al., 2014; Wiberg et al., 2010; Heinis et al., 2011*]. This study attempts to support this theory.

The uniqueness of this study is reinforced by the fact that ultrasound measurements and qualitative assessments of the study were performed by one researcher. Therefore, the assessment error was low. Contrary to other published studies, the researcher of this study was also performing as the obstetrician- gynecologist who supervised the women in labour, as a result of it, the author is aware of all the peculiarities of the course and end of childbirth. During the study, a large amount of good quality and useful digital ultrasound images of the uterus were compiled.

Materials and methods

In 2013-2016, a prospective observational study was conducted at the Clinic of Obstetrics and Gynecology of the Medical Faculty of Vilnius University. In all, the study involved 66 patients who had given childbirth at the Obstetrics department of Vilnius University Hospital Santaros Clinics (VULSK), and who voluntarily agreed to participate in the study and signed an informed consent (approved by Regional Bioethics Committee of the Faculty of Medicine of Vilnius University, No. 158200-13-605-183.

The women who delivered in the Obstetrics department were invited to the study. The inclusion criteria: single pregnancy, pregnancy of 37-42 weeks, a woman no younger than 18, conscious, communicating, giving consent to participate in the study, mentally healthy (absence of a mental disease that would interfere with participation in the study), with absence of confirmed severe organic pathology, which would influence the course and findings of the study (malignant tumors in the uterus, cervix, ovaries, other severe diseases which interfere with a woman's movement ability to arrive for repeated investigations), living in Vilnius (the place of residence would help to ensure the frequency and continuity of investigations).

The exclusion criteria: refusal to participate, preterm delivery (up to 36 weeks + 6 days), multiple pregnancies or fetal demise, severe postpartum hemorrhage (after

resuscitation, compressed uterine sutures or devascularisation of the uterus), congenital uterine anomalies (uterus didelphys, bicornuate uterus, unicornuate uterus), uterine myomas, untreated oncological diseases, non-residents of Vilnius.

No treatment was used during the entire study.

The primary purpose of the study was to investigate the uterine involution after uncomplicated delivery. However, during the course of the study, naturally, there were women with labour complications. Over the course of the study, depending on the course of labour and the period after delivery, the subjects were divided into several groups (Figure 1):

1) Normal labour (without instrumental or surgical intervention) and postpartum period (physiological group):
   - primiparous;
   - multiparous.
2) Complicated labour (pathological group):
   - vaginal delivery through the birth canal followed by:
     - revision of the uterine cavity for the removal of retained products of conception (RPOC);
     - a scar in the uterus as a result of the past C/S;
     - postpartum endometritis;
   - current delivery via C/S.
3) All women with uterine scars after C/S were evaluated separately:
   - with previous C/S and current vaginal birth after caesarean (VBAC);
   - with current C/S.

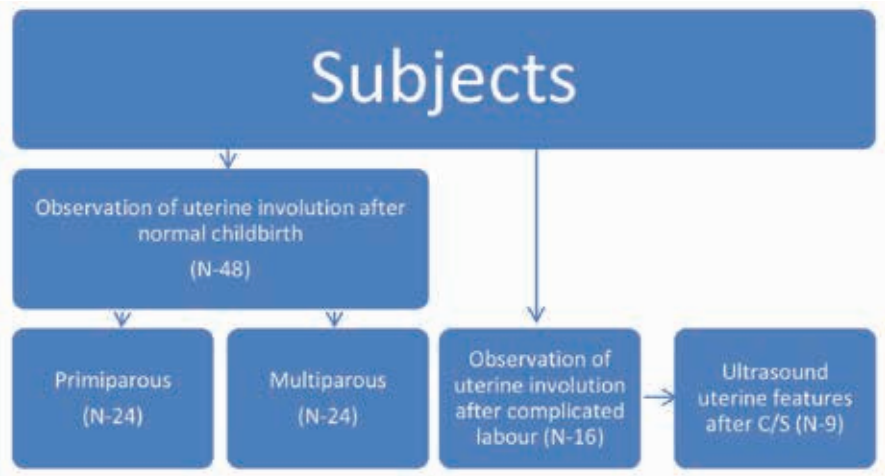


FIG. 1. Distribution scheme of subjects by groups

The subjects were examined by ultrasound 6 times: 1st, 3rd, 10th, 30th, 42nd and 60th day. On the 1st day, the ultrasound examination was performed during the first two hours after delivery (in the delivery room) or after the C/S (in the postoperative ward). The ultrasound examination involved measurement of the quantitative and qualitative indicators as follows:

- longitudinal diameter of the uterus;
- sagittal diameter of the uterus;
- transverse diameter (width) of the uterus;
- thickness of the anterior uterine wall;
- thickness of the posterior uterine wall;
- anteroposterior diameter of the uterus – (AP);
- inclination of the uterus from the longitudinal body of a woman (angle in degrees);
- width of the uterine cavity;
- echogenicity of the uterine cavity (inclusions);
- pulse index of uterine arteries (PI);
- resistance index of uterine arteries (RI);
- systolic-diastolic ratio (S/D) of uterine arteries;
- diastolic peak presence in the uterine arteries.

Measurements in women with a scar after previous C/S:

- length of the uterine scar in the longitudinal projection;
- thickness of the uterine scar in the longitudinal projection;
- echogenicity of the uterine scar;
- changes in the uterine scar localisation during the uterine involution.

Thus, over the course of this study, there were 360 meetings with women, over 5,000 different measurements of ultrasound parameters of the uterus and uterine arteries were performed, over 3,000 quality ultrasound images were compiled.

In our study all the measurements were performed by *GE Healthcare Voluson S6* (transported in delivery rooms and postoperative wards) and *Voluson S8* ultrasound apparatuses using two-dimensional, black&white transducers C1-5 and RS, and using colour and pulsed doppler to monitor and measure the blood flow uterine arteries. The abdominal ultrasound transducer was used for the 1st, 3rd and 10th days, while the vaginal transducer was used for the 30th, 42nd and 60th days. The measurements of the uterus and its blood flow were conducted in accordance with the globally adopted recommendations for the measurement of uterine parameters [*Swiss Gynaecologic Ultrasound Guideline, 2013*; *AIUM Practice Parameter for the Performance of Ultrasound of the Female Pelvis, 2014; Shipp et al., 2016; Benacerraf et al., 2000; Nicolaides et al., 2002; Park et al., 2012; Maulik et al., 2005*]. All the ultrasound images presented in this scientific work were created during this research and are the

author's copyright. All of the following schematic figures of the measurements of the morphological parameters of the uterus are created by the author.

The *longtitudinal and anteroposterior uterine diameter*, the *thickness of the anterior and posterior uterine walls*, the width of the *uterine cavity* and its *echogenicity* were measured in the longitudinal uterine projection by holding an ultrasound sensor parallel to the axis of the woman's body, with the entire uterine body and cervix displayed on the screen of the ultrasound apparatus.

The anteroposterior diameter (AP) and the *thickness of the uterine wall* in our study were measured in two places and the results of both places were compared by taking into account the controversial recommendations provided in the literature: in the widest area of the uterus in the longitudinal projection (Max AP) and 5 cm below the uterine fundus (UF) (AP 5 cm below UF).

The *width* of the uterus was measured in the transverse uterine projection by holding the ultrasound transducer across the longitudinal axis of the woman's body in the direction from the umbilicus/navel towards the pelvis.

The *sagittal diameter* of the uterus was measured only on the 1st day. This projection of the uterus is most suitable for the diagnosis of congenital uterine abnormalities and pathological inclusions. Later evaluation of this dimension loses its meaning because during the involution the uterine body moves forward or backward and angulation occurs. The *inclination* of the uterus is measured in degrees assessing the angle of the uterus body in relation to the longitudinal axis of the woman's body.

The *indexes of the uterine arteries* (pulse index (PI), resistance index (RI) and systolic-diastolic ratio (S/D)) and uterine arterial blood flow wave/peak measurements were performed by directing an ultrasound transducer to the pelvic area where the left and the right uterine arteries cross with the left and the right outer pelvic arteries and veins, while the power doppler marker was inclined by 30 degrees to the blood vessel [*Berens et al., 2016; Park et al., 2012*]. The mean values of indices for both sides of the arteries were also evaluated and assessments were made whether it is sufficient to measure only one-sided uterine arterial blood flow after childbirth, or whether it is necessary to measure the two sides, and which side is more important [*Mečėjus, 2001; Tarasevičienė et al., 2012*].

The blood flow of the uterine artery was evaluated according to the type of circulation characteristic of this artery. The blood flow of the uterine artery immediately after childbirth is characterized by a wave form, typical of pregnancy - low blood flow velocity during systole and absence of diastolic wave/peak [*Osol et al., 2014; Mečėjus, 2002*]. Later, with the advancement of the involution and narrowing of all the uterine vascular diameter, with the increasing resistance in them, the rate of blood flow during systole increases, a diastolic peak appears which, according to literature, marks the end of the uterine involution [*Mulic-Lutvica et al., 2009;*

Guedes- Martins et al., 2015; Olayemi et al., 2002; Thaler et al., 1990; Tekay et al., 1993], and a slow blood flow is observed at the end of the diastole [*Mečėjus, 2002*]. It is precisely the appearance and the time of the occurrence of a diastolic peak that were evaluated in this study.

In women with previous C/S, the *length and thickness of the scar* and the *changes in the location of the scar* in the longitudinal uterine projection during the involution were measured.

For each study study subject, an original anonymous questionnaire was completed and the parameters that could affect the uterine involution were evaluated: woman's age, BMI, smoking, number of previous miscarriages, pregnancy terminations, anemia, gestational diabetes, group B streptococcus (GBS) colonisation, duration of labour, the period of membrane rupture, colour of amniotic fluid placenta localisation, blood leukocyte count, CRP value.

The impact of newborn weight on uterine size indicators and uterine involution was evaluated. It was calculated whether the parameters of the uterine involution differ between women with newborns of less than 4,000 g and between women with newborns weighing 4,000 g or more. Also, the condition of all newborns was evaluated on the scale of V.Apgar after 1 and 5 minutes after birth. The newborns' testing involved cord blood arterial blood pH, blood gas and lactate testing according to the methodology adopted by the Vilnius University Hospital Santaros Klinikos. The indicators were determined by the auto analyser ABL90 FLEX Radiometer used in delivery rooms. Neonatal health indicators were analysed according to the group of women under investigation: the neonatal health indicators after birth between primiparous and multiparous women and between normal and high risk childbirth were compared. The value of the umbilical cord artery pH and lactate determination was compared in order to predict neonatal outcomes in different groups of women after delivery. The findings of our study on newborn conditions were included in a larger study entitled "The comparison of umbilical cord arterial blood lactate and pH values for predicting short-term neonatal outcomes", published in the Taiwanese Journal of Obstetrics and Gynecology [*Einikyte, Snieckuviene, Ramasauskaite, Paliulyte, Opolskiene, Kazenaite, 2017*].

The statistical analysis of the data was performed using MS Excel and SPSS 21. Regular variables were summed up using descriptive statistics. The study estimated the number/quantity of variables (N), mean (M), mode, median, standard deviation (SD) with minimum (min) and maximum (max) values. The cross-correlation analysis between the various indices was carried out, the Kruskal-Wallis test was used between the regular uterine variables in different groups of women (in primiparous and multiparous, between normal and pathological delivery). The chi square test was used to investigate the relationship between other qualitative variables with dif-

ferent groups of women (in normal or pathological delivery). The Mann-Whitney test was used to compare newborn umbilical cord blood pH and lactate indicators in different groups of subjects. The selected statistical significance level is $p < 0.05$.

The data were collected for scientific purposes only. The research was not influenced by either private or public interests. The investigation did not infringe the rights of the study participants or the protection of their data.

Results

1. Ultrasound examination of the uterus after normal delivery

1.1. Relationship between uterine involution and maternal health indicators

The group of women after normal labour comprised 48 women: 24 primiparous and 24 multiparous women. There were 18 women with labour complications, of whom there were 11 after giving natural childbirth via birth canal (of which 2 were after previous C/S) and 7 cases who underwent S/C during the investigation period. Two participants were excluded from the pathologic group of women after natural childbirth. There were 9 women with uterine scars after C/S who underwent separate investigation (7 of them after the current C/S and 2 after VBAC).

In 48 women with uncomplicated childbirth, no instrumental or surgical interventions were performed during labour or postpartum period and no infectious complications were diagnosed. This group of 48 women consisted of 24 primiparous (Group I) and 24 multiparous subjects (Group II). Each female underwent ultrasound examination six times. The main characteristics of women are presented in Table 1. Multiparous women were significantly older by age and a significantly shorter period of rupture of membranes was found in this group of women.

TABLE 1. Comparison of baseline characteristics between primiparous and multiparous women after normal labour

	Primiparous N=24	**Multiparous N=24**	**p**
Mean age (year) (M±SN)	28.46±2.87	32.36±3,72	**<0.001**
Mean BMI (M±SN)	21.04±2.26	22.45±3,66	0.119
Mean duration of delivery (hours) (M±SN)	8.35±2.88	6.68±2.77	0.052
Mean period of membrane rupture (hours) (M ±SN)	6.46±3.91	4.14±2.04	**0.017**

When collecting data for questionnaires, the placenta localisation in the uterus was determined according to women's medical records. Until delivery, we did not perform the ultrasound examination of the subjects. In the larger part of the subjects of both groups (I and II), the placenta was localized in the posterior uterine wall (83.3% in primiparous and 66.7% in multiparous) (Table 2). There was no statistically significant difference between these groups of subjects (p> 0.05).

TABLE 2. Questionnaire data after normal labour

Values		Primiparous		Multiparous		p
		N	%	N	%	
Smoking	No	24	100.0	23	95.8	
	yes	0	0.0	1	4.2	0.291
Abortions and miscarriages	No	23	95.8	20	83.3	
	Yes	1	4.2	4	16.7	0.127
Pregnancy or postpartum anemia	No	18	75.0	16	66.7	
	Yes	6	25.0	8	33.3	0.403
Gestational diabetes	No	21	87.5	21	87.5	
	Yes	3	12.5	3	12.5	0.909
GBS colonisation	No	23	95.8	21	87.5	
	Yes	1	4.2	3	12.5	0.255
Labour induction	No	13	54.2	16	66.7	
	Yes	11	45.8	8	33.3	0.515
Labour augmentation	No	10	41.7	15	62.5	
	Yes	14	58.3	9	37.5	0.238
Colour of amniotic fluid	Clear	21	87.5	23	95.8	
	Meconium	3	12.5	1	4.2	0.339
Placental side	Posterior	20	83.3	16	66.7	
	Anterior	4	16.7	8	33.3	0.129

1.2. Changes in the postpartum uterine diameters in primiparous and multiparous women

The reduction in the uterus size after delivery is characterised by changes in the main parameters: uterine length, width, AP (max), thickness of the anterior and posterior uterine walls. The mean values of these indicators *for all 48 women after normal labour* are presented in Table 3. In all the below given results, the 1st day indicators mean the indicators measured during the first two hours postpartum.

When assessing the differences between primiparous (Group I) and multiparous (Group II) women, the above-mentioned indicators were investigated separately. The changes in almost all of these indices during the involution period depend on the number of deliveries in the past. During the two-hour period after childbirth the uterus is larger in the group of multiparous subjects and remains larger until the end of the involution period (Table 4).

TABLE 3. Involution of mean uterus size parameters after normal labour (N=48)

Uterus size parameters (M±SN; N=48)	Examination time (postpartum days)					
	1st	3rd	10th	30th	42nd	60th
Uterus length (mm)	165.00±16.05	154.50±15.58	110.50±15.03	82.00±7.65	72.00±9.68	66.00±10.65
Uterus width (mm)	117.50±15.26	114.50±16.20	93.50±9.59	63.5±7.30	58.00±7.93	52.00±9.72
AP max (mm)	89.50±12.40	83.00±9.59	71.00±10.07	46.00±6.47	39.00±7.96	37.00±6.41
Anterior wall thickness (mm)	40.50±6.59	38.00±6.73	31.00±6.71	22.00±3.62	18.00±3.81	16.00±3.16
Posterior wall thickness (mm)	40.00±7.19	37.00±7.32	28.00±5.36	22.00±3.99	19.00±4.13	18.00±3.41

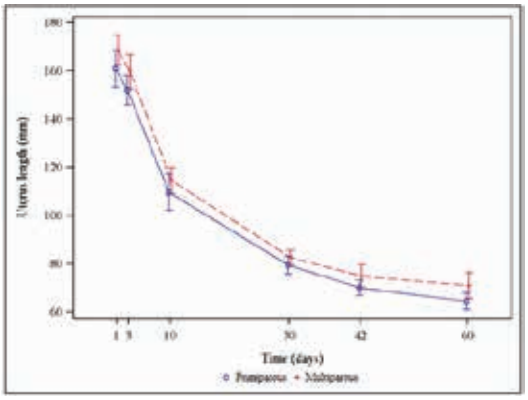


FIG 2. Longtitudinal diameter of the downward trend

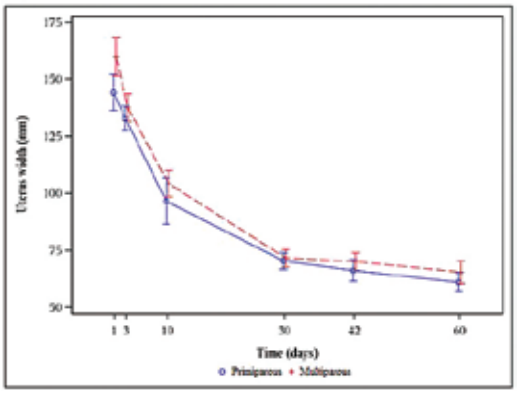


FIG 3. Transverse diameter of the uterus: uterus: downward trend

The most intense uterus decrease is observed in the first month after delivery, and later in the second month, the involution becomes slower. The downward trend of a similar pattern is observed in all the uterine diameters in groups I and II, only the diamaters are different. The changes in the length and width of the uterus (transverse dimension) during involution are given in Figures 2 and 3.

The anteroposterior (AP) diameter is larger in the group of multiparous women, when measured both at the widest area of the uterus (traditionally) and measured at a distance of 5 cm below the uterine fundus (UF) during the entire period of involution. The statistically significant AP max was higher in Group II on the 1st (p=0.01), 10th (p=0.019) and 30th (p=0.017) days after childbirth. When measured at 5 cm below

TABLE 4. Differences in uterus size parameters in primiparous and multiparous women

Uterus size parameters (M±SN)	Subjects by groups	Examination time (postpartum days)											
		1st	p	3rd	p	10th	p	30th	p	42nd	p	60th	P
Uterus length (mm)	Group I (N=24)	162.0±17.5		148.5±14.5		109.0±17.2		81.0±8.4		68.0±7.5		63.0±8.2	
	Group II (N=24)	174.0±13.5	0.148	156.5±16.1	0.113	112.5±12.43	0.28	83.0±6.7	0.284	74.0±11.2	0.08	71.5±12.1	0.069
Uterus width (mm)	Group I (N=24)	119.0±8.7		108.5±15.2		93.5±11.4		59.0±6.9		55.0±7.7		51.0±8.7	
	Group II (N=24)	116.0±12.9	0.805	122.5±16.3	**0.029**	94.0±7.4	0.417	67.0±6.6	**0.005**	60.0±8.2	0.26	53.5±10.7	0.394
AP max (mm)	Group I (N=24)	82.0±10.1		81.0±9.0		69.5±10.6		44.0±5.6		39.0±6.4		36.0±3.9	
	Group II (N=24)	94.0±13.1	**0.01**	85.5±10.2	0.391	74.5±8.0	**0.013**	48.0±6.7	**0.017**	40.5±8.9	0.103	39.0±8.0	0.059
Anterior wall width (mm)	Group I (N=24)	39.0±5.7		38.0±6.8		29.0±5.7		20.5±2.9		18.0±3.1		16.0±2.5	
	Group II (N=24)	44.0±7.0	0.095	38.5±6.8	0.525	34.0±6.9	**0.024**	23.0±3.9	**0.014**	19.5±4.4	0.182	17.0±3.7	0.529
Posterior wall width (mm)	Group I (N=24)	38.0±6.7		37.0±6.1		30.0±6.4		20.5±3.7		18.0±3.4		17.0±2.4	
	Group II (N=24)	42.0±7.7	0.357	36.5±8.5	0.48	27.5±3.9	0.295	23.0±3.9	0.062	20.0±4.4	**0.046**	19.0±3.7	**0.002**

the uterine fundus, a statistically significant difference of AP was found between the primiparous and multiparous women on the 10th (p = 0.005), 42nd (p=0.032) and 60th (p=0.015) days. The decreasing trend of in AP max and AP measured 5 cm below the uterine fundus and changes on the first day of the involution period are the same (Table 5; Fig. 4; Fig. 5). Therefore, it is debatable which AP measurement is more accurate, because when it is measured within the distance of 5 cm below the uterine fundus, this diameter due to the shrinking uterus is eventually found in the part of the cervix. On the first day after childbirth, AP max always coincides with AP at a distance of 5 cm below the uterine fundus, but it never coincides at the end of the involution.

TABLE 5. AP in the widest uterine area (AP max) and 5 cm below the uterine

AP diameter (M±SN)	Subjects by groups	Examination time (postpartum days)											
		1st	p	3rd	P	10th	p	30th	P	42nd	p	60th	p
AP max (mm)	Group I (N=24)	82.0±10.1		81.0±9.0		69.5±10.6		44.0±5.6		39.0±6.4		36.0±3.9	
	Group II (N=24)	94.0±13.1	**0.01**	85.5±10.2	0.39	74.5±8.0	**0.01**	48.0±6.7	**0.02**	40.5±8.9	0.1	39.0±8.0	0.06
AP 5 cm below UF (mm)	Group I (N=24)	79.0±10.9		71.0±10.3		62.5±10.6		41.0±8.0		28.0±5.6		22.0±5.2	
	Group II (N=24)	85.0±13.9	0.07	73.0±9.95	0.93	71.5±8.0	**0.01**	40.0±8.5	0.35	32.0±8.2	**0.03**	27.5±9.1	**0.02**

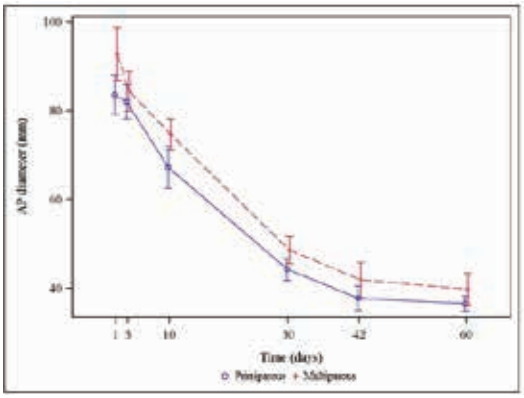


FIG 4. AP max: downward trend (mm)

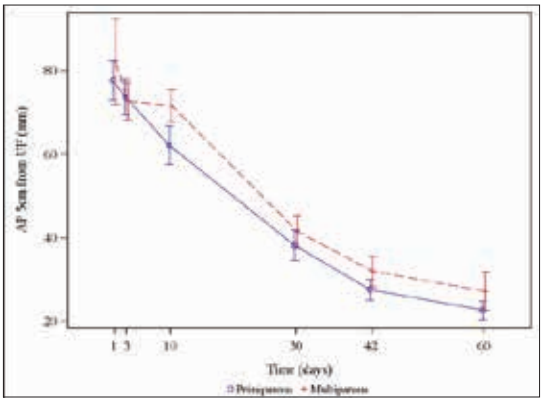


FIG 5. AP 5 cm below uterine fundus: downward trend (mm)

Thus, during the first two hours, the size of the uterus in primiparous women is 162.0 x 119.0 x 82.0 mm (length x width x thickness), and the uterus of multiparous women after uncomplicated childbirth is 174.0 x 116.0 x 94.0 mm in size. Two months after labour, the uterine size of primiparous was 63.0 x 51.0 x 36.0 mm while in multiparous it was 71.5 x 53.5 x 39.0 mm.

The sagittal uterine diameter, although measured and observed only on the 1st day (within 2 hours postpartum), was significantly higher in the group of multiparous women: 160.00±18.54 mm in group II and 144.26±18.40 mm in group I, p = 0.004. The evaluation of the shape of the uterus of the subjects in this projection revealed no congenital uterine abnormalities. Figure 6 shows some images of the sagittal uterine projection after the normal primiparous and multiparous labour.

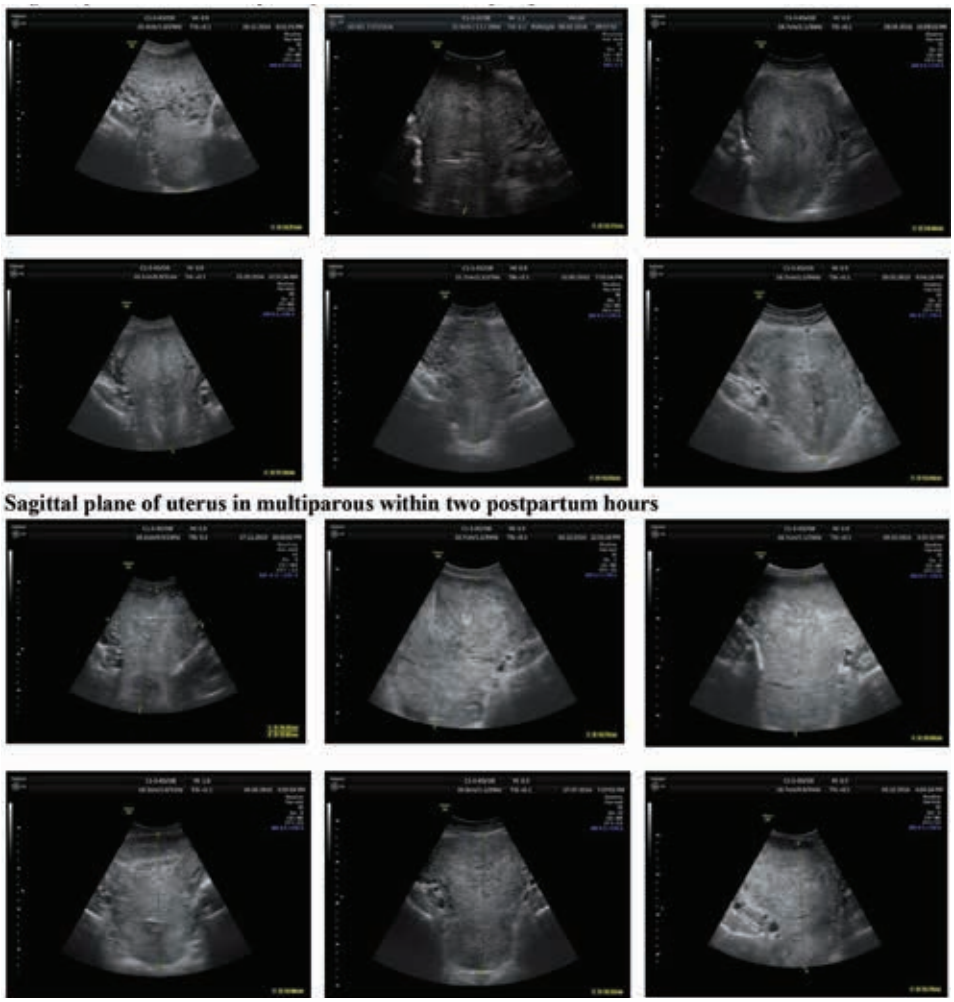


FIG 6. Sagittal uterine projection and sagittal diameter

The thickness of the anterior uterine wall in multiparous subjects was significantly higher on the 10th and 30th days of the examination (Table 6), during the so called midpuerperium period, and the posterior uterine wall was significantly thicker in multiparous women on the 42nd and 60th days (Table 7), i.e. at the end of the involution period.

TABLE 6. Differences in the anterior uterine wall thickness during the postpartum period

		Time (postpartum days)					
		1st	3rd	10th	30th	42nd	60th
Anterior uterine wall (mm) (M±SN)	Primiparous (N=24)	39.0±5.7	38.0±6.8	29.0±5.7	20.5±2.9	18.0±3.1	16.0±2.5
	Multiparous (N=24)	44.0±7.0	38.5±6.8	34.0±6.9	23.0±3.9	19.5±4.4	17.0±3.7
	P	0.095	0.599	**0.024**	**0.014**	0.182	0.529

TABLE 7. Differences in the posterior uterine wall thickness during the postpartum period

		Time (postpartum days)					
		1st	3rd	10th	30th	42nd	60th
Posterior uterine wall (mm) (M±SN)	Primiparous (N=24)	38.0±6.7	37.0±6.1	30.0±6.4	20.5±3.7	18.0±3.4	17.0±2.4
	Multiparous (N=24)	42.0±7.7	36.5±8.5	27.5±3.9	23.0±3.9	20.0±4.4	19.0±3.7
	P	0.357	0.48	0.295	0.062	**0.046**	**0.002**

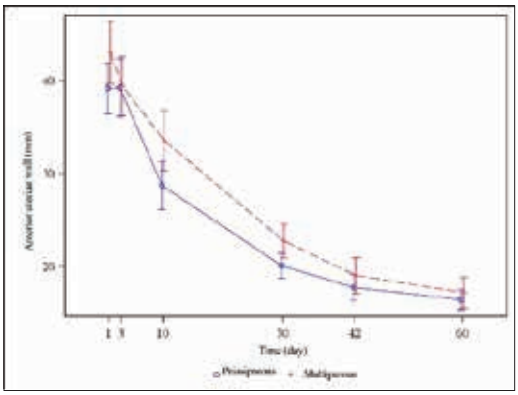


FIG 7. Decreasing anterior uterine wall

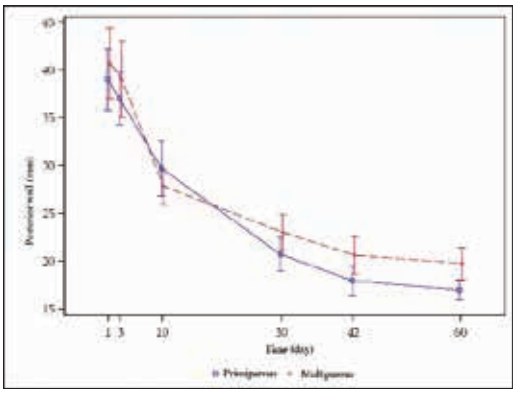


FIG 8. Decreasing posterior uterine wall

The downward trend and the changes in the thickness of the anterior and posterior uterine walls observed from the first day in women of the two groups under analysis, remained the same in both groups for the entire period of involution (Figure 7, Figure 8).

The uterine axis during the involution period was changing from retroversion toward anteversion. On the 1st day postpartum, the uterus body, due to its own weight, was inclined toward the sacrum in all subjects, then the uterus body straightened and, one month after childbirth, was almost parallel to the longitudinal axis of the female body, from the 30th day it was inclining toward the pubic bone (Figure 9).

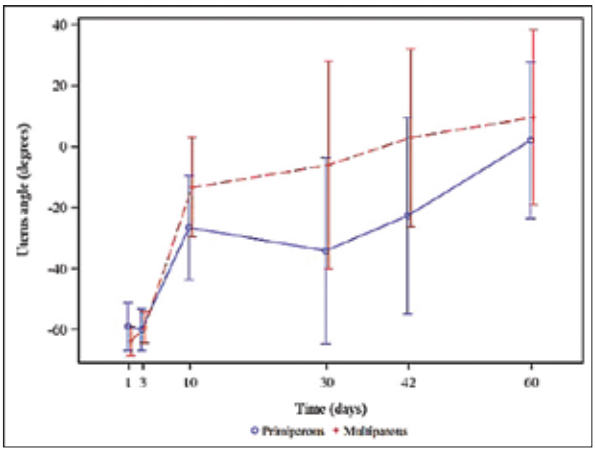


FIG 9. Changes in the uterine angle in the postpartum period

The largest changes in the uterine axis during the two months after childbirth, if compared with the uterine position during the first two hours after delivery, occurred in the group of multiparous women: their uterine axis changed by 94.0±64.1 degrees from the 1st to the 60th day after childbirth, and the median uterine axis in primiparous subjects varied only by 21.5±66.7 degrees (p=0.0005). The uterus of the multiparous women after childbirth compared to that of primiparous subjects, remained more inclined towards the pubic bone (anteversion). It was observed that

TABLE 8. Differences in the uterine angle during postpartum period

		Time (postpartum days)					
		1st	3rd	10th	30th	42nd	60th
Uterine angle(in degrees) (M±SN)	Primiparous (N=24)	-60 ±19	-60 ±16	-39 ±39	-49 ±67	-44 ±74	-20 ±59
	Multiparous (N=24)	-63 ±11	-58 ±12	-27 ±36	26 ±74	28 ±65	29 ±64
	P	0.384	0.852	0.159	0.208	0.286	0.742

the boundaries for standard deviations of the uterine angle were very broad in both groups of the subjects (Table 8), therefore, no statistically significant difference was found between the two groups.

1.3. Changes in the uterine cavity after normal childbirth

The image of the uterine cavity is very dynamic during its involution. Immediately after delivery, the width of the uterus cavity is about 1 cm in the longitudinal projection, later the uterine cavity slightly narrows, but around the 10th day the uterine cavity is wide again. On the 10th postpartum day, the width of the uterine cavity of primiparous women is again approaching the value of 1 cm, while in multiparous women it may even exceed the width of 2 cm (Table 9). One month after childbirth and until the 8th week, the uterus cavity remains narrow and reaches 3-4 mm.

TABLE 9. Width of the uterine cavity during the normal involution period

		Examination time (postpartum days)					
		1st	3rd	10th	30th	42nd	60th
Width of uterine cavity (mm) (M±SN)	Primiparous (N=24)	10.0±7.8	7.5±4.3	**9.5±9.9**	4.0±1.9	4.0±2.7	4.0±3.9
	Multiparous (N=24)	13.0±7.4	7.0±4.1	**18.5±7.2**	3.0±1.3	3.0±1.9	3.0±3.3
	P	0.384	0.231	**0.001**	0.199	0.299	0.541

The changing trend of the broadest area of the uterine cavity is similar in both groups of subjects, only on the 10th, it is clearly broader in multiparous women, and then, compared to primiparous subjects, narrows more intensively and remains narrower throughout the entire second month of involution (Figure 10).

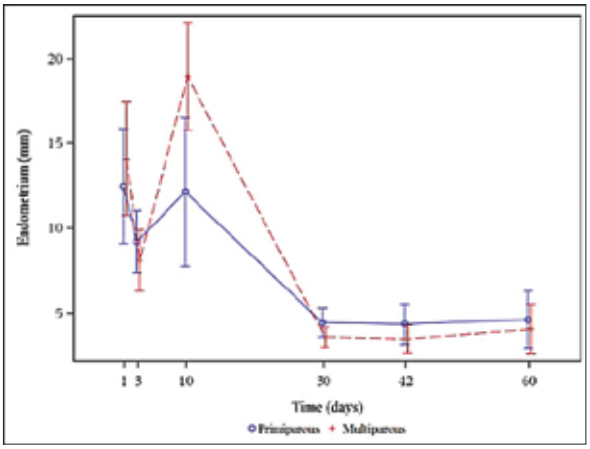


FIGURE 10. Trends in the uterine cavity changes

The uterine cavity inclusions in this study are called hyperechoic (whitish or white inclusions visible on an ultrasound screen), hypoechoic (dark band, commonly referred to as fluid in the cavity) and mixed (both of the types of inclusions are observed in the cavity). Taking into account that in the first two hours postpartum there is the highest risk of primary postpartum hemorrhage (PPH), hyperechoic band or mixed insertions predominate in longitudinal and sagittal uterine projections in subjects of both the groups, I and II. In the cases of the norm, with a Doppler regime around these inclusions, we will not see an increase in blood flow. A much wider uterine cavity, filled with fluid or mixed inclusions even gas, can be seen in the uterine isthmus rather than in the fundus (Figure 11). All the mentioned versions of ultrasound images should be considered as the norm immediately after delivery.

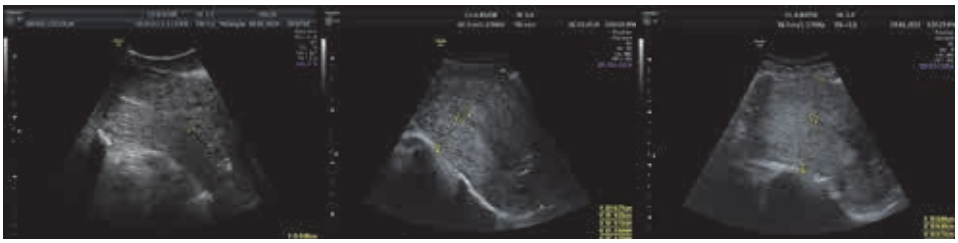


FIGURE 11. Uterine cavity view within two hours after normal childbirth

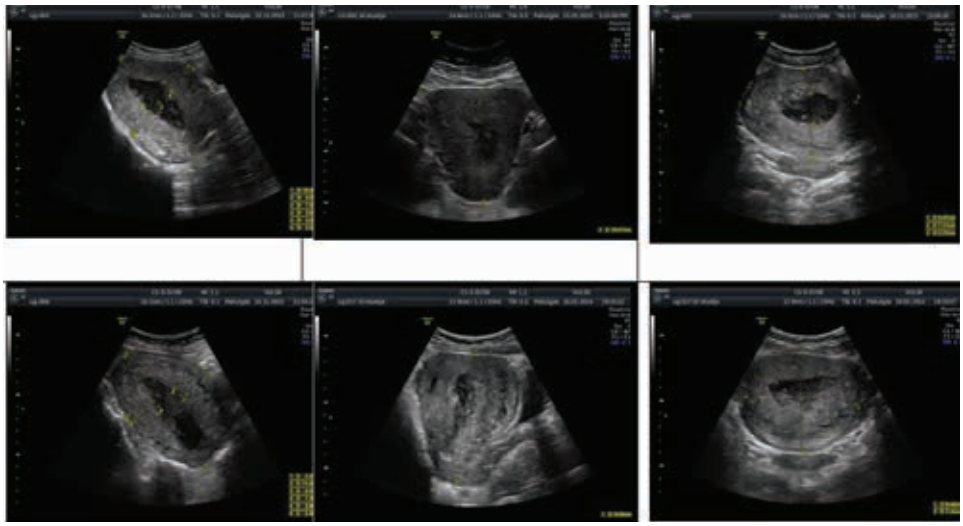


FIGURE 12. 10th day uterine cavity view in all uterine planes in primiparous and multiparous

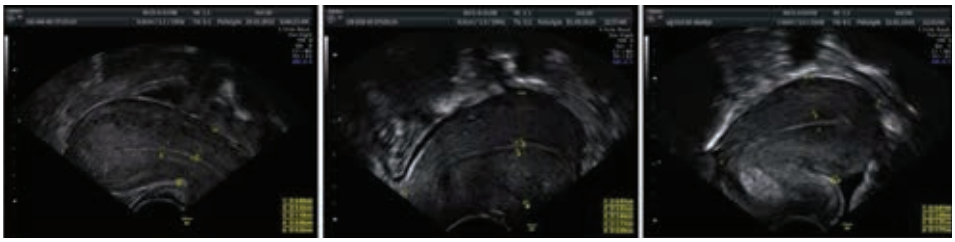


FIGURE 13. 60th day uterine cavity view

TABLE 10. Diversity in uterine cavity view from two postpartum hours to 60th day

	Hyperechoic	Mixed	Hypoechoic
	Two postpartum hours		
Primiparous	43%	40%	17%
Multiparous	33%	57%	10%
	3rd day		
Primiparous	71%	25%	4%
Multiparous	82%	18%	0%
	10th day		
Primiparous	14%	22%	64%
Multiparous	0	36%	64%
	30th day		
Primiparous	29%	0	71%
Multiparous	29%	5%	66%
	42nd day		
Primiparous	70%	4%	26%
Multiparous	86%	0	14%
	60th day		
Primiparous	70%	4%	26%
Multiparous	91%	0	9%

On the 10th day, the uterine cavity in the great majority of women in both groups is filled with fluid (hypoechoic) in all projections (Figure 12). One month after childbirth, a hypoechoic band is still observed in most women, only a much narrower one, and on the 42nd and 60th days, a narrow hyperechoic band is observed again (Figure 13, Table 10).

Therefore, when evaluating the uterine cavity in primiparous and multiparous women, it is important to emphasize that a statistically significant difference was found on the 10th day postpartum (p=0.001) (Table 9).

1.4. Changes in the blood flow of the uterine artery after normal delivery

When evaluating the uterine artery blood flow during the period after labour, a few questions emerged: if it is impacted by the localisation of the placenta before delivery and where to measure the indicators of uterine arteries in women after childbirth: on the left or the right side. The calculation of statistical reliability showed that the side for taking the measurements (left or right) was not important after either normal or complicated childbirth nor it was important on which uterine wall (front or rear) the placenta was attached, since all the measurements found no statistically significant difference ($p>0.05$). Therefore, in discussing the results both after normal and after complicated childbirth, the mean values of the average of the indicators obtained from the two sides were calculated.

Monitoring of the blood flow in the uterine arteries further highlighted the differences between the primiparous and multiparous women. The most striking differences in the uterine arterial blood flow indices were observed on the 10th day after childbirth, while the desrease in the differences was found from the 30th day.

On the 1st and 3rd postpartum days, the uterine blood flow remains the same as during pregnancy, that is why the values of the uterine artery indices are low (Table 11), although multiparous women have a higher blood flow resistance immediately after childbirth compared with primiparous. Later, the narrowing of the blood vessels results in the increased resistance and the difference between the uterine artery systole and diastole increases.

The trends in the changing blood flow indexes are shown in Figures 14, 15, 16. The most pronounced changes in the blood flow occur in the first month, later they change consistently.

TABLE 11. Indices of the uterine artery in primiparous and multiparous (RI, PI, S/D)

		Time (postpartum days)					
Indices of uterine artery (M±SN)	**Groups**	**1st**	**3rd**	**10th**	**30th**	**42nd**	**60th**
RI	**Primiparous (N=24)**	$0{,}51^{\pm 0{,}14}$	$0{,}59^{\pm 0{,}10}$	$0{,}59^{\pm 0{,}09}$	$0{,}70^{\pm 0{,}70}$	$0{,}74^{\pm 0{,}09}$	$0{,}76^{\pm 0{,}08}$
	Multiparous (N=24)	$0{,}61^{\pm 0{,}09}$	$0{,}67^{\pm 0{,}08}$	$0{,}69^{\pm 0{,}13}$	$0{,}74^{\pm 0{,}09}$	$0{,}74^{\pm 0{,}07}$	$0{,}76^{\pm 0{,}09}$
	p	**0.006**	**0.001**	**0.004**	0.182	0.049	1
PI	**Primiparous (N=24)**	$0{,}85^{\pm 0{,}20}$	$1{,}02^{\pm 0{,}35}$	$1{,}02^{\pm 0{,}29}$	$1{,}44^{\pm 0{,}37}$	$1{,}65^{\pm 0{,}45}$	$1{,}67^{\pm 0{,}40}$
	Multiparous (N=24)	$1{,}12^{\pm 0{,}37}$	$1{,}26^{\pm 0{,}31}$	$1{,}24^{\pm 0{,}29}$	$1{,}65^{\pm 0{,}45}$	$1{,}89^{\pm 0{,}40}$	$1{,}87^{\pm 0{,}65}$
	p	**0.007**	**0.002**	**0.028**	0.144	0.043	0.313
S/D	**Primiparous (N=24)**	$2{,}28^{\pm 0{,}50}$	$2{,}64^{\pm 0{,}86}$	$2{,}63^{\pm 0{,}68}$	$3{,}69^{\pm 1{,}06}$	$4{,}52^{\pm 1{,}72}$	$4{,}67^{\pm 1{,}90}$
	Multiparous (N=24)	$2{,}65^{\pm 0{,}61}$	$3{,}27^{\pm 0{,}93}$	$3{,}12^{\pm 0{,}93}$	$4{,}25^{\pm 1{,}36}$	$5{,}29^{\pm 1{,}93}$	$4{,}99^{\pm 3{,}01}$
	p	**0.031**	**0.001**	**0.035**	0.174	0.059	0.856

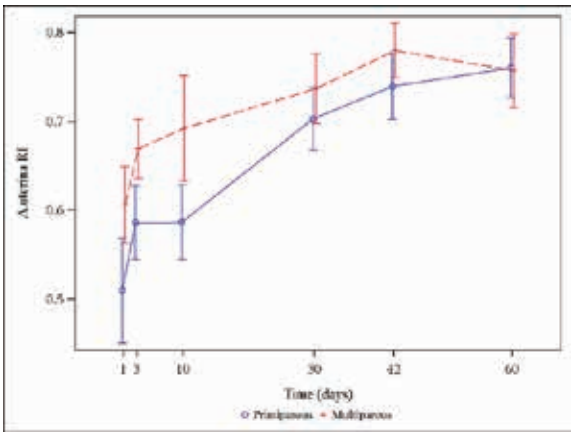


FIG 14. *A.uterina* RI changes in primiparous and multiparous

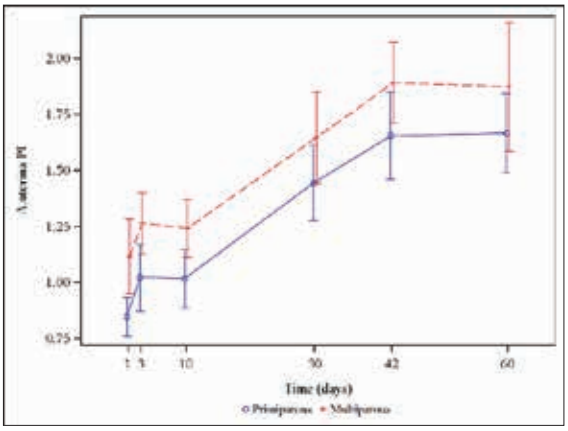


FIGURE 15. *A.uterina* PI changes in primiparous and multiparous

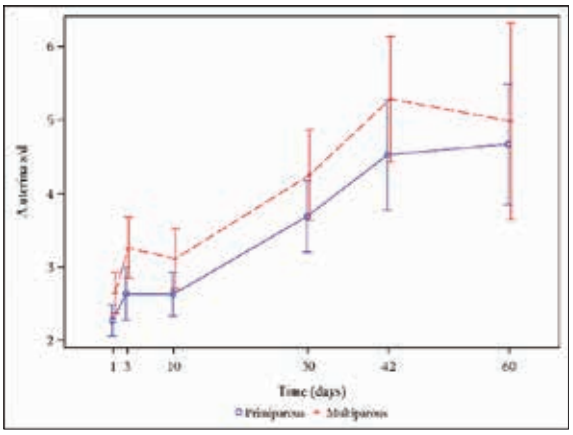


FIGURE 16. *A.uterina* S/D changes in primiparous and multiparous

The shape of the uterine artery wave in the period of involution also varies in both the groups of the subjects. In the course of involution, the diastolic peak of the uterine artery is getting more intensive (Figure 17), which is not present within two hours postpartum in all subjects belonging to the normal childbirth group, however, even two months after normal delivery, the diastolic peak does not occur in all women (Figure 18).

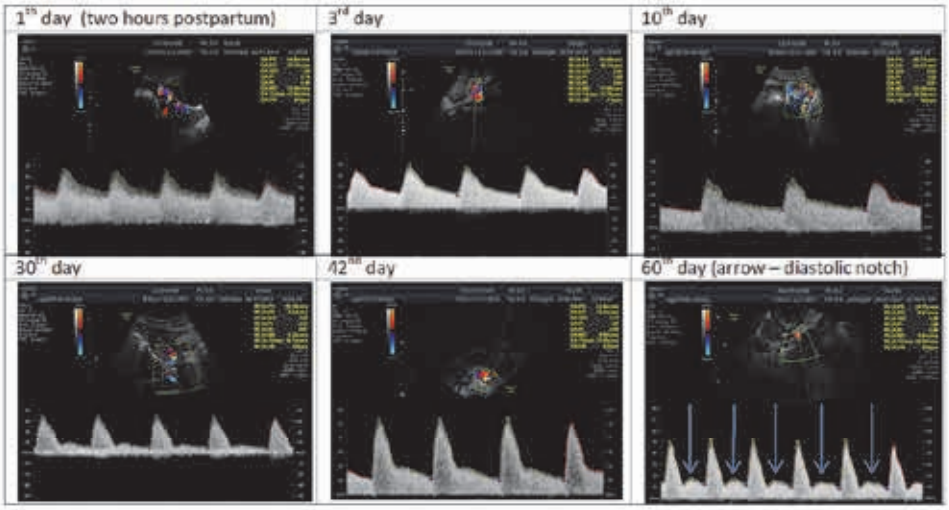


FIGURE 17. Changes in *A. Uterina* wave shape

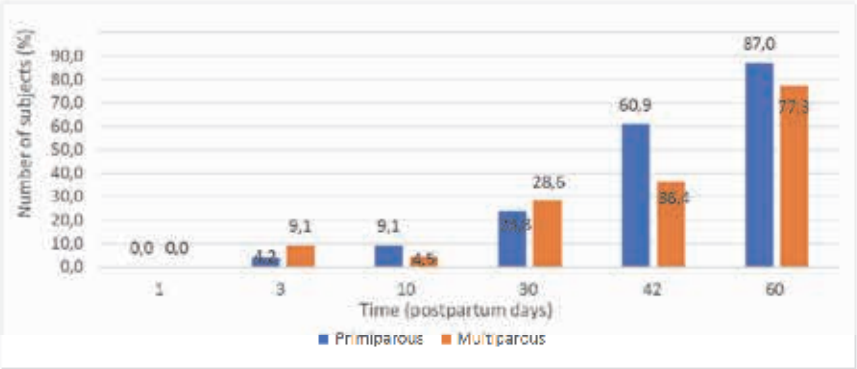


FIGURE 18. The occurrence time of the *uterine artery* diastolic notch

Also, this study investigated the influence of other factors on the normal involution of the uterus and its parameters: female age, BMI, smoking, the past history of the number of miscarriages and pregnancy interruptions, anemia, gestational diabetes, GBS colonisation, duration of labour, period of membrane rupture, amniotic fluid colour, placenta localisation, blood leukocyte count, CRB value. Unfortunately, most of these factors had no effect on the uterine involution. Only some random correlations between uterine dimensions, blood parameters and investigated factors were observed at separate stages of involution, but there are no constant trends.

2. Relationship between uterine involution and newborn birth weight after normal delivery

There was no statistically significant difference in the mean weights of newborns of primiparous and multiparous subjects: the mean weight of the 1st group's newborns (N=24) was 3,560±398 g, the mean weight of the 2nd group's newborns (N=24) was 3,588±421 g, p>0.05. We calculated how a normal and larger weight of a newborn could impact the size of the uterus after childbirth and its involution. Our female subjects were additionally divided into two groups: women with newborns weighing less than 4,000 g and those with newborns weighing 4,000 g and over. In the 1st group, 22 women gave birth to less than 4,000 g, and 2 women delivered newborns of more than 4,000 g. The influence of the weight of these women's newborns on the uterine size parameters during the involution period is presented in Table 12. In the 2nd group, 19 multiparous subjects gave birth to newborns lighter than 4,000 g and 5 women's newborns weighed 4,000 g or more. The influence of the weight of newborns delivered by the multiparous subjects on the size of the uterus is given in Table 13.

TABLE 12. Correlation of the uterine size and birth weight in primiparous subjects

Uterine size parameters (mm) (Primiparous)		Time (postpartum days)					
		1st	3rd	10th	30th	42nd	60th
Uterine length (mm)	Birth weight<4,000g (N=22)	162.00	148.50	110.00	81.00	68.00	62.50
	Birth weight ≥4,000g (N=2)	167.00	154.00	107.00	91.00	87.00	66.00
	P	0.623	0.794	0.813	0.098	0.097	0.597
Uterine width (mm)	Birth weight<4,000g (N=22)	119.00	110.50	94.00	59.50	55.00	50.50
	Birth weight ≥4,000g (N=2)	135.00	107.00	78.00	55.00	59.00	56.00
	P	0.229	0.464	0.236	0.456	0.597	0.365
AP (mm)	Birth weight<4,000g (N=22)	82.00	81.00	69.00	44.00	39.00	36.00
	Birth weight ≥4,000g (N=2)	95.50	93.00	72.00	54.00	45.00	42.00
	P	0.325	0.117	0.528	0.097	0.173	0.1978
Uterine cavity width (mm)	Birth weight<4,000g (N=22)	11.00	8.00	9.00	4.00	4.00	3.50
	Birth weight ≥4,000g (N=2)	10.00	5.50	5.00	4.00	3.00	1.80
	P	0.742	0.071	0.096	1	0.648	0.094

TABLE 13. Correlation of the uterine size and birth weight in multiparous subjects

Uterine size parameters (mm) (Multiparous)		Time (postpartum days)					
		1st	3rd	10th	30th	42nd	60th
Uterine lenght (mm)	Birth weight<4,000g (N=19)	172.50	156.00	108.00	82.00	73.00	69.00
	Birth weight ≥4,000g (N=5)	175.00	161.00	118.00	85.00	77.00	73.00
	P	0.508	0.531	0.256	0.419	0.609	0.256
Uterine width (mm)	Birth weight<4,000g (N=19)	114.50	119.00	95.00	67.00	59.00	54.00
	Birth weight ≥4,000g (N=5)	122.00	124.00	91.00	67.00	63.00	50.00
	P	0.283	0.224	0.456	0.964	0.61	0.969
AP (mm)	Birth weight<4,000g (N=19)	94.50	82.00	74.00	48.00	40.00	39.00
	Birth weight ≥4,000g (N=5)	94.00	87.00	76.00	48.00	44.00	40.00
	P	0.562	0.556	1	0.822	0.387	0.665
Uterine cavity width (mm)	Birth weight<4,000g (N=19)	13.00	7.00	20.00	3.00	3.00	2.00
	Birth weight ≥4,000g (N=5)	8.00	14.00	17.00	4.00	4.00	4.00
	P	0.215	0.285	0.969	0.406	0.779	0.354

Although the uteruses of women who gave birth to newborns heavier in weight are slightly larger, the difference is not statistically significant. Also, we did not establish a statistically reliable relationship between the changes in the size of the uterus during the involution period and the larger or smaller weight of the newborns in the groups of primiparous and multiparous women.

3. Newborn health indicators in subjects after normal childbirth

The condition of all the newborns, delivered by primiparous and multiparous women, was evaluated according to the V.Apgar scale, 1 and 5 minutes after childbirth. The study singled out the number of newborns of both groups who scored 7 or 6 or less during the 1st minute of their life. Almost all newborns in these groups were born in a satisfactory condition, while resuscitation was performed on only one newborn of a primiparous woman. There was no statistically significant difference in the evaluation of the newborn status in primiparous and multiparous women after normal childbirth (Table 14).

TABLE 14. Newborns with Apgar score 7 and less and resuscitation actions

Neonatal condition	N	Primiparous (N = 24)	Multiparous (N = 24)
Apgar score 7 after 1 min	2	2	0
Apgar score ≤6after 1 min	1	1	0
Resuscitation actions	1	1	0
CPAP	0	0	0
PPV, intubation	1	1	0

TABLE 15. Average pH and lactate values in newborns of primiparous and multiparous women

Value	Primiparous (N=24)	Multiparous (N=24)	P
pH	7.289 ± 0.061	7.324 ± 0.065	0.001
Lactate	4.28± 1.55	3.27 ± 1.604	0.043

When assessing the pH and lactate content of the newborn umbilical cord blood in primiparous and multiparous women, we found that newborns of primiparous subjects showed a significantly lower pH and higher lactate levels (Table 15).

Although the pH and lactate levels in newborns of primiparous and multiparous women were normal, the inner medium was slightly more acidic in newborns delivered by primiparous women. Unfortunately, we did not succeed in comparing how pH and lactate levels predict hypoxic neonatal conditions and how valuable they are, since these groups of subjects involved only one newborn rated 6 on the Apgar scale with the only resuscitation applied to this newborn.

4. Uterine involution after complicated labour

4.1. Changes in uterine parameters after complicated labour

As already mentioned, the group with childbirth complications consisted of 16 women:

- 9 of them with delivery through birth canal:
  - 5 women with the uterine hemorrhage after delivery were examined manually (2 patients) or instrumentally (curette, 3 patients);
  - 2 women were diagnosed with early endometritis after labour (confirmed clinically and by laboratory findings);
  - 2 women with VBAC.
- 7 women had C/S performed during this research.

TABLE 16. Comparison of the baseline characteristics of the subjects after normal and complicated labour

	Normal labour (N=48)	Complicated labour (N=16)	p
Mean age (year) (M±SN)	30.33±3.82	30.33±5.28	0.995
Mean BMI±SN	21.72±3.06	21.11±3.66	0.503
Mean duration of labour (hours) (M±SN)	7.55±2.92	9.42±5.07	0.071
Mean period of membrane rupture (hours) (M ±SN)	5.35±3.33	8.86±9.19	**0.027**
Mean infant birth weight (grams) (M ±SN)	3635.98±409.86	3723.06±320.85	0.422

TABLE 17. Comparison of the uterine size parameters after normal and complicated labour

Uterine size parameters (M±SN)	Groups of subjects	Time (postpartum days)					
		1st	3rd	10th	30th	42nd	60th
Uterine length (mm)	Normal (n=48)	165.00±16.05	154.50±15.58	110.50±15.03	82.00 ±7.65	72.00 ±9.68	66.00 ±10.65
	Complicated (n=16)	159.50±17.72	149.00±32.21	116.00±11.41	76.50 ±6.42	71.00 ±6.22	68.00 ±5.40
	P	0.284	0.299	0.486	0.057	0.858	0.831
Uterine width (mm)	Normal (n=48)	117.50±15.26	114.50±16.20	93.50 ±9.59	63.50 ±7.30	58.00 ±7.93	52.00 ±9.72
	Complicated (n=16)	118.50±14.38	118.00±15.37	93.00 ±7.29	63.50 ±9.20	61.00 ±7.72	50.00 ±9.51
	P	0.628	0.769	0.682	0.865	0.864	0.824
Anterior wall (mm)	Normal (n=48)	40.50 ±6.59	38.00 ±6.73	31.00 ±6.71	22.00 ±3.62	18.00 ±3.81	16.00 ±3.16
	Complicated (n=16)	38.00 ±7.25	38.00 ±7.22	27.00 ±4.40	20.50 ±3.66	17.00 ±3.38	18.00 ±3.44
	P	0.200	0.710	0.055	0.599	0.112	0.757
Posterior wall (mm)	Normal (n=48)	40.00 ±7.19	37.00 ±7.32	28.00 ±5.36	22.00 ±3.99	19.00 ±4.13	18.00 ±3.41
	Complicated (n=16)	36.50 ±7.24	37.00 ±6.03	26.00 ±3.75	22.50 ±3.64	19.00 ±3.20	16.00 ±2.77
	P	0.137	0.540	0.378	0.662	0.681	**0.030**

The morphological parameters of the uterus in the complicated childbirth group were compared with those found in 48 women after normal childbirth. The baseline characteristics of women after normal and complicated childbirth are presented in Table 16.

The comparison of the uterine size involution parameters observed after normal delivery and with childbirth complications, no statistically significant difference was found between the groups. In most cases, the uterine size indicators coincided both in the physiological and pathological groups (Table 17). The only statistically significant difference was determined at the end of the involution by measuring the thickness of the posterior uterine wall: two months after childbirth, the posterior uterine wall remains thicker in the normal childbirth group.

The uniform downward trend is observed in the decreasing size of the uterus during the involution period after both normal and complicated childbirth (Figure 19).

The AP diameter did not show statistically significant difference between the normal and complicated childbirth groups either (Table 18), its similar decreasing trend was observed in these groups (Figure 20), except for the normal childbirth

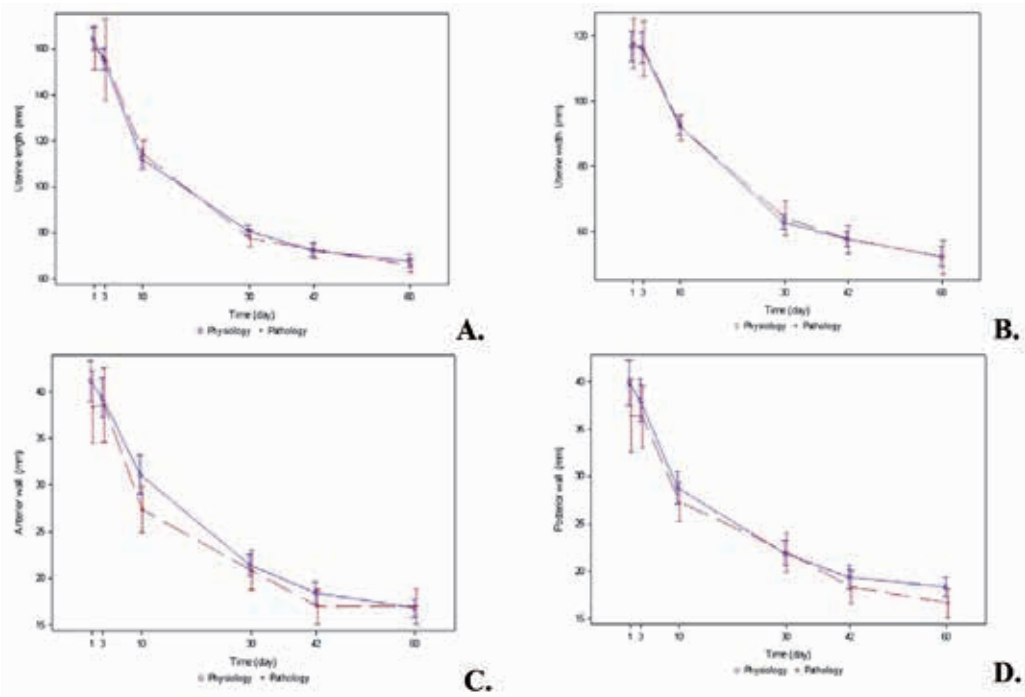


FIG 19. Uterine length (A), width (B), anterior (C) and posterior (D) wall (mm) regression after normal and complicated labour

TABLE 18. AP regression after normal and complicated labour

AP diameter (M±SN)	Time (postpartum days)					
	1st	3rd	10th	30th	42nd	60th
Normal labour (mm) (N=48)	89.50±12.4	83.00±9.59	71.00±10.07	46.00±6.47	39.00±7.90	37.00±6.41
Complicated labour (mm) (N=16)	82.00±9.46	82.00±9.92	69.00±10.33	47.50±6.22	39.00±8.87	37.00±5.58
P	0.128	0.466	0.239	0.690	0.918	0.373
p (change from the 1st day)		0.261	0.284	**0.023**	0.072	0.168

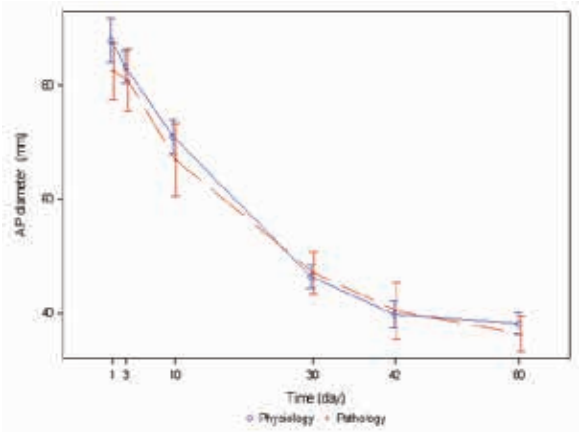


FIG. 20. The AP diameter regression trend after normal and complicated labour

group where during the first month, the AP dimension decreased significantly more than after complicated childbirth, later the AP changes were identical to both groups (Table 18).

The uterine position with respect to the longitudinal female body, both after normal and after complicated childbirth, was changing from retroversion to anteversion. Statistically significant uterus was inclining more towards the women's sacrum in the pathologic group only on the very first day, i.e. in the early postpartum period: after normal delivery the uterine angle is –61.50±15.06° and after complicated childbirth –72.50±15.77° (p=0.039). In the later period, a similar inclination and a tendency for change are observed in both groups (Figure 21).

Thus, we did not find a statistically significant difference between the dimensions of the uterus and the position of the uterus during the period of involution between the women after normal delivery and after complicated childbirth.

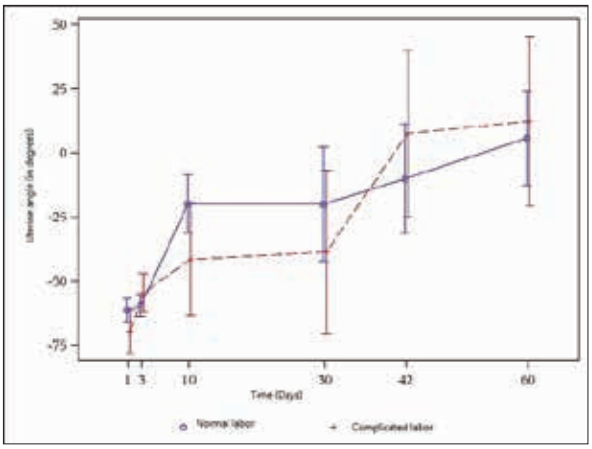


FIG. 21. Uterine angulation after normal and complicated labour

4.2. Uterine cavity changes after complicated labour

As with normal, after complicated childbirth, special attention should be paid to changes in the uterine cavity. However, on the 10th day after complicated childbirth, the width of the uterine cavity (11±7.05 mm) was not significantly different from that

TABLE 19.UTERINE cavity width after normal and complicated childbirth

Uterine cavity width (M±SN)	Time (postpartum days)					
	1st	3rd	10th	30th	42nd	60th
Normal labour [mm] (n=48)	11.0±7.6	7.0±4.2	12.0±9.2	4.0±1.7	3.0±2.4	3.0±3.6
Complicated labour [mm] (n=16)	11.0±6.8	9.0±4.7	11.0±7.1	5.0±2.8	5.0±2.0	3.0±1.5
P	0.663	0.324	0.369	0.103	**0.033**	0.696

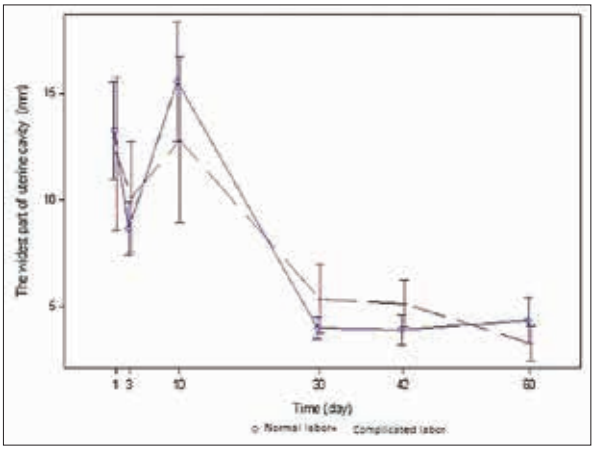


FIG. 22. Uterine cavity width regression after normal and complicated labour

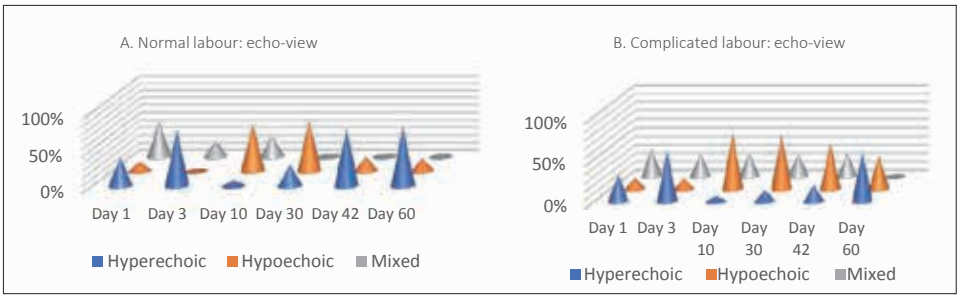


FIG. 23. Uterine cavity view after normal and complicated labour

of normal delivery (12±9.23 mm) (p=0.369). The uterine cavity was markedly wider only on the 42nd day after the childbirth complications (5±2.00 mm) compared with normal delivery (3±2.38 mm) (p=0.033) (Table 19). The narrowing of the abdominal cavity was the same in both groups (Figure 22).

On the tenth day after childbirth, the hypoechoic content of the uterine cavity prevailed after normal (62%) and after complicated childbirth (66%). In evaluating the inclusions of the uterine cavity during any period of involution, it was difficult to observe general trends, but after the complicated delivery, in the middle of the involution, hypoechoic (fluid) inclusions are more frequent than after normal delivery (Figure 23).

Specific inclusions are visible for two hours after the manual or instrumental (curette) examination of the uterus. Immediately after the intervention in the uterus, a bright hyperechoic band remains, or less commonly, mixed inclusions (Figure 24), regardless of the method of revision (both manual and instrumental) are visible. These very pronounced hyperechoic inclusions appear due to the air flow into the uterus through manipulation and are called gas inclusions. The air strongly blocks the ultrasound signal, which makes it possible to see such specific findings.

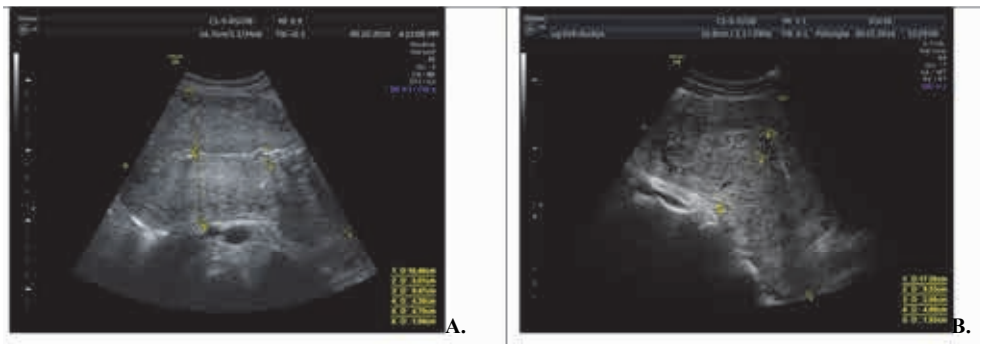


FIG. 24. Hyperechoic (A) or mixed (B) view of the uterine cavity right after RPOC removal

On the third day after the revision of the uterine cavity, still a few hyperechoic inclusions are observed in the uterine cavity for all women, but these inclusions disappear on the 10th day and only the fluid remains in the uterus.

This study estimated the differences between normal and complicated childbirth, the incidence of gas inclusions in the uterus in both groups. Gas inclusions were found in 5 out of 48 women after normal delivery (10%) and even 7 out of 16 women after complicated childbirth (44%) (Figure 25). In the group of five women after normal delivery, the gas inclusions were visible in the uterus only at the earliest postpartum period (within the first two hours), later they disappeared in all women. After complicated deliveries, these inclusions were also visible on the third day, later they disappeared in this group of subjects, too. In all women after complicated childbirth, unlike after normal delivery, gas inclusions were in the *central area of the uterus.* In subjects with endometritis, we did not detect any gas inclusions during the entire period of uterine involution.

FIG. 25. Gas (arrows - foci of higher echogenicity) after normal childbirth (isthmic part) (A), after manual placental removal (B) and after S/C (C)

4.3. Changes in the blood flow of the uterine artery after complicated labour

The evaluation of the uterine arterial blood flow indexes (RI, PI, S/D) and blood flow curve characteristics after normal and complicated delivery ($p>0.05$) showed no statistically significant difference (Table 20). In both groups of women, low levels of resistance (RI) and pulsatility indexes (PIs) were determined, low S/D ratio, while during the involution, they were consistently increasing after normal childbirth and after complicated childbirth (Figure 26).

TABLE 20. RI, PI and S/D values after normal and complicated labour

Uterine artery indices (M±SN)	Groups of subjects	Time (postpartum days), p				
		1st	P	Changes	60th	p
RI	Normal labour (N=48)	0.56±0.13	0.91	→	0.76±0.09	0.912
	Complicated labour (N=16)	0.58±0.08			0.77±0.74	
PI	Normal labour (N=48)	0.98±0.32	0.747	→	1.77±0.54	0.924
	Complicated labour (N=16)	1.00±0.28			1.78±0.47	
S/D	Normal labour (N=48)	2.46±0.58	0.882	→	4.83±2.48	0.641
	Complicated labour (N=16)	2.45±0.56			4.82±1.70	

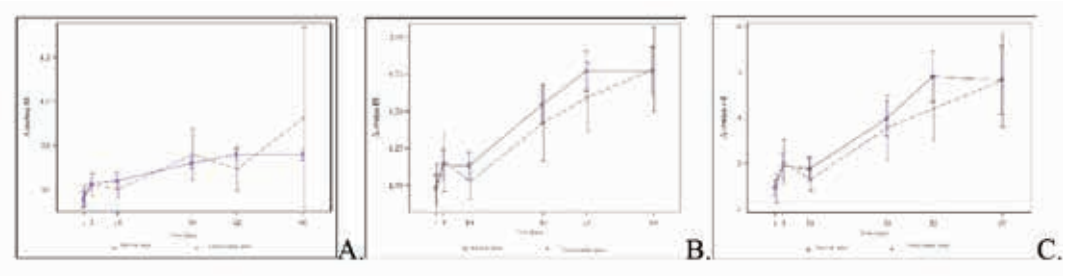


FIG. 26. Changes in RI (A), PI (B) and S/D(C) after normal and complicated labour

The time of the onset of the diastolic peak in the uterine artery was measured in all women of the complicated childbirth group and compared with the onset of the diastolic peak after normal delivery. During the first two hours after complicated childbirth, the diastolic peak was recorded in two women following an instrumental uterine cavity revision for RPOC in the uterus. There was no diastolic peak observed in the early postpartum period in women after a newly-performed caesarean section either, or with VBAC. No woman was diagnosed with the diastolic peak on the 10th day after complicated childbirth. In the subsequent period of involution, either after normal or complicated childbirth, the occurrence of the diastolic peak increases consistently. However, at the end of the involution, the diastolic peak does not appear in all women after both normal and complicated childbirth (Figure 27).

The diastolic peak was seen less frequently after childbirth complications and after 60 days postpartum than after normal delivery.

No specific ultrasound evidence of early endometritis was found during this study (only isolated cases of endometritis were investigated). The study examined the correlation between uterine involution after the pathological delivery and various parameters: presence of anemia, colonisation of GBS, meconium in the amniotic fluids, location of the placenta (anterior or posterior uterine wall), cervical rip-

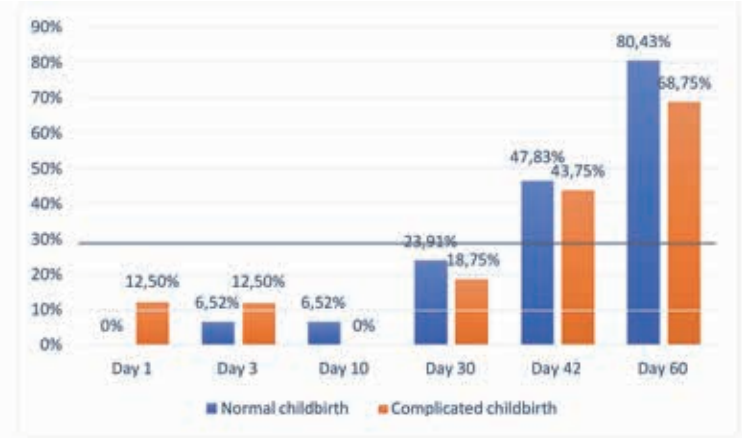


FIG. 27. Notching of the uterine artery after normal and complicated labour

ening, stimulation/ induction of labour by oxytocin, breast feeding, smoking, there were no statistically reliable correlations of these factors with the uterine involution in subjects after high risk childbirth.

4.4. Relationship between uterine involution and neonatal birth weight after complicated labour

In the complicated delivery group, the mean weight of newborns was 3,723±321 g, and in the normal childbirth group – 3,635.98±410 g. We did not find a statistically significant difference between the weight of newborns in the pathological group and normal delivery group of subjects ($p>0.05$). In the complicated childbirth group, 13 women gave birth to newborns of less than 4,000 g and 3 women delivered infants of 4,000 g and larger. The uterine dimensions were slightly larger in the second month after childbirth with the weight of newborns of 4,000 g or more, but we did not determine the statistically reliable influence of the newborn weight on the uterine involution in the group of women with complicated childbirth (Table 21). Only a few random correlations were found between the weight of newborns and the parameters of the uterine size during the involution period (Table 21). The overall declining trend in the uterus size of women after complicated childbirth followed the same pattern in both the groups - with newborns of up to 4,000 g, and those of 4,000 g and more.

Thus, we did not establish a statistically reliable relationship between the newborn birth weight and the extent of the uterine involution during the involution period.

TABLE 21. Uterine size and birth weight correlation after complicated labour

Uterine size parameters (mm)		Time (postpartum days)					
		1st	3rd	10th	30th	42nd	60th
Uterine length (mm)	Birth weight<4,000g (N=13)	161.00	152.50	115.00	76.00	71.00	67.00
	Birth weight ≥4,000g (N=3)	158.00	129.00	116.00	78.00	78.00	69.00
	P	0.736	0.07	0.885	0.309	0.275	0.217
Uterine width (mm)	Birth weight<4,000g (N=13)	123.00	118.50	92.50	61.00	58.50	49.50
	Birth weight ≥4,000g (N=3)	111.00	113.00	97.00	74.00	67.00	56.00
	P	0.253	0.885	0.717	**0.016**	0.192	0.562
AP (mm)	Birth weight<4,000g (N=13)	82.00	80.50	68.00	46.00	39.00	36.50
	Birth weight ≥4,000g (N=3)	80.00	88.00	69.00	55.00	47.00	38.00
	P	0.684	0.563	0.942	0.073	**0.029**	0.663
Uterine cavity width (mm)	Birth weight<4,000g (N=13)	12.00	8.00	10.50	5.00	4.50	3.50
	Birth weight ≥4,000g (N=3)	9.00	11.00	13.00	6.00	6.00	2.00
	P	0.544	0.885	0.612	0.813	0.609	**0.026**

4.5. Newborn health indices in subjects after complicated labour

The comparison of the neonatal health indicators of women after normal delivery and women with childbirth complications showed that there were more newborns in the normal delivery group who, after 1 minute of life, were assessed by 7 or less acccording to the V.Apgar scale (2 vs. 1). The difference between normal and complicated delivery in the neonatal condition on the basis of the V.Apgar scale was not statistically significant due to the small sample (Table 22).

TABLE 22. Newborns with Apgar score of 7 and less and resuscitation actions after normal and complicated childbirth

Neonatal condition	N	Normal childbirth (N = 48)	Complicated childbirth (N = 16)
Apgar score 7 after 1 min	3	2	1
Apgar score ≤6 after 1 min	2	1	1
Resuscitation actions	2	1	1
CPAP	1	0	1
PPV, intubation	2	1	1

We did not find a statistically significant difference after comparing the pH of the umbilical artery of newborns and the lactate levels (Table 23).

Thus, the early health indices of newborn infants did not differ significantly after normal and complicated childbirth. Due to the fact that each of our study groups comprised only one newborn, evaluated by 6 points on the V.Apgar scale, we could not compare how the pH and lactate predict the hypoxic neonatal conditions

TABLE 23. Average pH and lactate values in neonates of women after normal and complicated childbirth

	Normal childbirth (N=48)	Complicated childbirth (N=16)	P
pH	7.305 ± 0.064	7.292 ± 0.114	0.694
Lactate	3.80 ± 1.64	4.19 ± 2.603	0.920

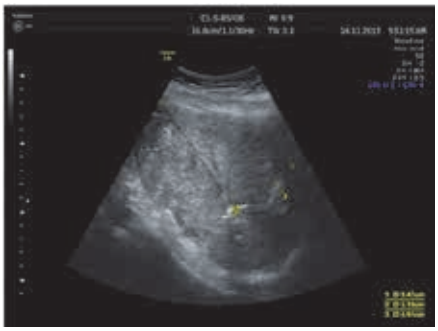
and were unable to calculate the sensitivity and specificity of these indicators in our study groups.

5. Uterine ultrasound examination with uterine scar after C/S

In total, this study involved nine women with a scar after the C/S: 7 women had C/S performed for the first time during the study period, while two women had a scar in the uterus after VBAC. All the nine subjects were included in the complicated childbirth group and were separately monitored for the changes in the uterine

1 Example. Uterine scar within two hours after C/S

1 Example. Uterine scar on the 3rd day after C/S



2 Example. Uterine scar within two hours after C/S

2 Example. Uterine scar on the 3rd day after C/S

FIG. 28. Uterine scar in two women after C/S

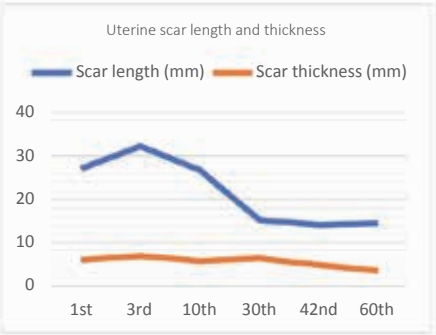


FIG. 29. Changes in the scar size

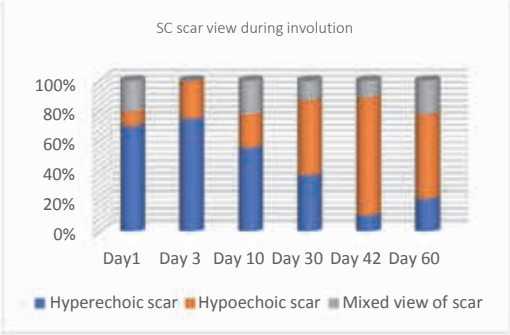


FIG. 30. Changes in the uterine scar echogenicity

scar during the involution period. None of these women experienced serious complications after childbirth: there was no hemorrhage or fever. The length, width and echogenicity of the uterus in the longitudinal uterine projection were studied in these women. These parameters change during all eight weeks after childbirth. Immediately after the cesarean section surgery (within two hours), the uterine scar is shorter and narrower than on the third day, as the uterus immediately contracts after the C/S, and 24 hours after surgery, (like after the normal postpartum) it slightly relaxes and increases (Figure 28). For the first two hours after the C/S, the average length and thickness of the scar is 27×6 mm, and 8 weeks later – 15×4 mm.

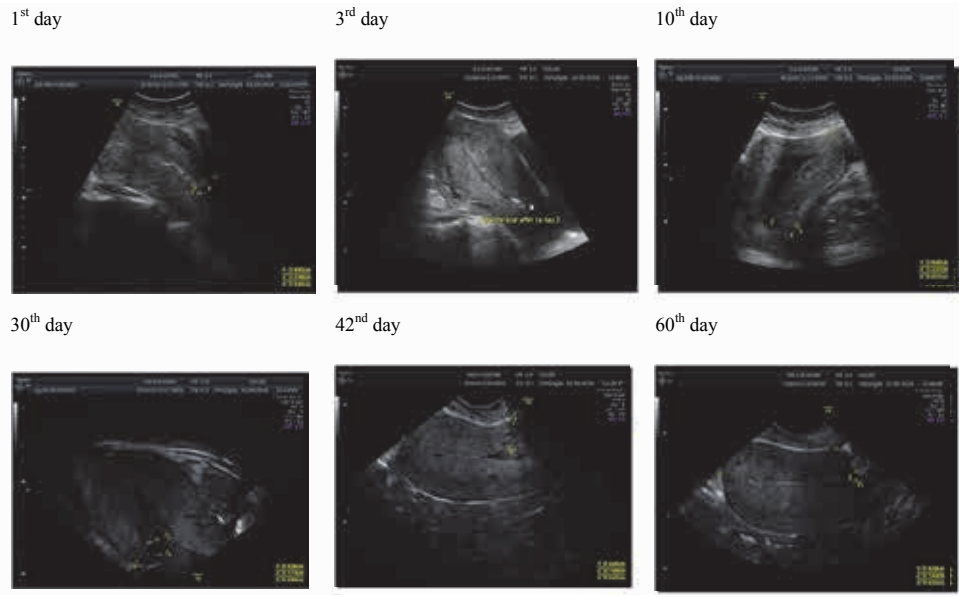


FIG. 31. Uterine scar view from 1st to 60th postpartum day

Later, the quantitative parameters of the uterine scar are decreasing consistently, and in one month after C/S, the parameters are almost unchanged until the end of the involution (Figure 29). The echogenicity of the scar varies from the hyperechoic line to the hypoechoic band, a hypoechoic niche occurs a cyst or a band occurs on the inner surface of the scar on the 30-42 postpartum days, echogenicity is rarely mixed (Figure 30, Figure 31).

There was no statistically significant difference between the quantitative parameters (uterine length, width, AP diameter) of the postsurgical uterus (N=9) and the same parameters of the unoperated uterus (48 women in the normal group).

Discussion

The acquisition of the knowledge of the normal postpartum period and understanding of the normal ultrasound imaging of the uterus help to distinguish the causes of primary postpartum hemorrhage from the uterus: atonic uterus and retained placenta or membranes [*Mulic-Lutvica et al., 2012; Maulik et al., 2005*]. In the normal period after delivery, the uterine involution is defined by changes in the size of the uterus parameters (uterine length, uterus width and anterior-posterior (AP) dimension), uterine cavity inclusions and uterine arterial blood flow changes [*Deans et al., 2006; Olayemi et al., 2002; Mulic-Lutvica et al., 2001; Berens et al., 2016; Park et al., 2012*]. Most of the scientific publications in the world involve a single researcher, while the obstetrician supervising labour is a completely different person; in our study, the same individual supervised the women in labour and performed all the ultrasound examinations from the beginning to the end of the uterine involution. The presence of the researcher from the time of the arrival of pregnant women to the delivery room to the end of the childbirth ensures the most accurate collection of anamnesis data, assessment of risk factors, observation of the entire course of the childbirth, prediction of possible complications and ultrasound examination of the uterus during the first two postpartum hours and association of the obtained images with the existing clinical signs. The investigation of these changes in the uterine parameters enabled the researcher to notice the existing differences between the primiparous and multiparous women. The observation of the uterus in various planes helped to understand that various morphological uterine variants are a complete norm. Understanding these differences makes it easy to distinguish between physiological and pathological images, especially when differentiating whether or not enough the uterus is constricted after childbirth [*Mulic-Lutvica, 2007; Nicolaides et al., 2002*]. Most of the studies described so far have either not found or do not emphasize the correlation between the uterine involution and the number of previous deliveries (*Deans et al., 2006; Sokol et al., 2004; Mulic-Lutvica, 2007*], only in

2015 *Guedes-Martins* and co-authors published statistically significant correlations between the number of previous deliveries and the changes in the uterine arterial blood flow during the first 8 weeks postpartum, while *Olayemi* and his co-authors had provided statistically reliable data on the differences in the length, width, and the AP dimensions of the uterus in primiparous and multiparous African women [*Guedes-Martins et al., 2015; Olayemi et al., 2002*]. *Kristoschek* and his co-authors found a positive correlation between the number of childbirths in the past and the size of the uterus after childbirth [*Kristoschek et al., 2017*]. According to the results of our study, there was a significant difference between other parameters of the uterus in primiparous and multiparous women: the anterior and posterior uterine walls, width of the uterine cavity and inclusions, uterine artery blood flow rates. The uterus of the multiparous women contracts much more intensely, but it still remains larger from the first hours poatpartum to the end of the involution.

In the first month after childbirth, multiparous women have a significantly larger anteroposterior diameter (AP), later this difference decreases, but the AP remains larger even after the involution. According to Swedish scholar *Mulic-Lutvica*, who is the most involved in the studies of the uterine involution, the anteroposterior uterine dimension after delivery should be measured equally for everyone, so it is advisable to take the measurements on the basis of a certain reference point. This scientist suggested that the AP should be measured for all women at 5 cm below the uterine fundus, thus standardizing the data of all researchers and avoiding errors when comparisons are made [*Mulic-Lutvica et al., 2007; 2012*]. Based on the assumption of this researcher, we measured the AP for all 8 weeks at 5 cm below the uterine fundus and at the widest point of the longitudinal projection of the uterus. Comparing these measurements, we came to the conclusion that after childbirth, the AP should be measured in the widest part of the longitudinal projection of the uterus. Perhaps it would be more accurate to measure the AP diameter at 5 cm below the uterine fundus as there may be less subjectivity, but during the involution period, and especially at the end of it, the AP measurement would be incorrect, since during the course of the decrease of the uterus, this diameter would occur in the isthmus of the uterus or sometimes in the projection of the cervix.

In assessing the physiological features of the uterine involution, it would be appropriate to give special attention to the 10th day postpartum, however, we would be wrong to talk about just one particular day. Other authors observed the results similar to our findings after approximately 7-14 days postpartum (*Mulic-Lutvica et al., 2001; 2007; Al-Bdour et al., 2004; Edwards et al., 2000*]. Experience shows that during this period, most women call the in-patient reception department or, concerned about their condition, arrive at an outpatient health care institution because of more intensive uterine bleeding [*Fuller et al., 2015; Mulic-Lutvica et al., 2006*]. Equally, the

same complaints were recorded on the 10th day as indicated by all the participants after normal delivery and with normal uterine involution. In the uterine cavity, during the second week after childbirth, it is normal to see a broad hypoechoic band or mixed echogenicity inclusions without local blood flow due to accumulation of blood in the uterus. It is essential not to confuse this image with the true retained products of conception, the most common cause of secondary uterine hemorrhage, and to decide correctly on further tactics [*Rufener et al., 2008*]. According to our study, on the 10th postpartum day the uterine cavity in multiparous women is much wider if compared to the primiparous women (p=0.001), so these women are at greater risk of the uterine

cavity abrasion or the uterine content suction. Taking into account the seriousness of both anesthetic and gynecological complications, women may face unnecessary uterine cavity abrasions (uterine perforation, infection, Asherman syndrome, which can have long-term effects on fertility and other pregnancies and placenta, life-threatening uterine hemorrhage triggered by very rare arteriovenous uterine malformations), the diagnosis of RPOC should have some serious criteria: hyperechoic thickened endometrial echo complex (EEC) visible in the uterus or markedly limited mass in the uterus cavity + local vascularity [*Al-Bdour et al., 2004; Edwards et al., 2000; Adkins et al., 2016; Sellmyer et al., 2013; Doumouchtsis et al., 2014*]. By combining the grayscale echoscopy with doplerometry, it is much easier to diagnose the RPOC in the uterus [*Al-Bdour et al., 2004; Doumouchtsis et al., 2014*]. An additional criterion would be the thickness of the endometrium, which in a variety of sources varies from 8 mm to

13 mm. *Sellmyer* and his co-authors suggest that the endometrium thicker than 10 mm should be considered the reference point. The sensitivity of this criterion is about 80%, but the specificity is relatively low (20%), since the endometrium thicker than 10 mm may also be present in the absence of RPOC, however, if the endometrium (EEC) is thinner than 10 mm, the negative predictive RPOC value will be 63-80%. In severe cases, it is recommended to confirm the diagnosis by MRI [*Sellmyer et al., 2013*]. Even after diagnosing RPOC, but in the absence of life-threatening hemorrhage, there is no need for rush with intervention procedures. There are always several choices: expectant management, administration of uterotonics (prostaglandin E1 analogues). Only if conservative treatment is ineffective, dilation and curettage can be performed or safe removal of the existing retained placental tissues or membranes by hysteroscopy [*Guarino et al. 2015; Belfort et al., 2017*].

The results of the uterine artery blood flow examination after normal delivery obtained in our study have partly coincided with the results of other researchers [*Mulic-Lutvica et al., 2007; 2009; Sohn et al., 1988; Sokol et al., 2004; Van Schoubroeck et al., 2004; Reles et al., 1992*]: during the first postpartum hours, the uterine artery RI, PI, and S/D correspond to end-of-pregnancy rates, these rates consistently increase

between the 1st, 3rd and 10th day after childbirth, and start intensively increasing from the 10th to 30th day, and later, between 6 and 8 weeks after normal delivery, these indices do not change, although the parameters of the uterus size in this period (uterine length, width and AP dimension) are still decreasing. Unlike most other scholars, we found statistically significant differences between the primiparous and multiparous women in terms of their uterine arterial blood flow indices on the 1st → 3rd → 10th day postpartum ($p < 0.05$). Only two months after normal delivery, the blood flow indices in primiparous and multiparous are similar. Circulatory differences between these two groups of women are described by *Guedes-Martins* and co-authors, but unlike this author, we found larger RI, PI and S/D in multiparous women in primary postpartum period [*Guedes-Martins et al. 2015*]. Thus, when evaluating the condition of women for the first ten postpartum days, higher blood flow indices observed in multiparous women should not be considered pathological, and the indices should be estimated as delayed involution in primiparous women, if their rates are lower than those of multiparous subjects.

There is no doubt that the incidence of the diastolic peak in the uterine artery after delivery is due to the progression of involution, as confirmed by most of the published studies [*Mulic-Lutvica et al., 2009; 2012; Fuller et al., 2014; Van Schoubroeck et al., 2004*]. Our results of the diastolic peak observation correlate with the published research, but the absence of the diastolic peak can not be a negative indicator of the end of the involution, since, even after two months postpartum, the diastolic peak does not occur for all the primiparous and multiparous women [*Maulik et al., 2005*]. According to our study, on the 1st day postpartum (within two hours) there is no diastolic peak in all women after normal childbirth, but according to some other authors, the occurrence of the diastolic peak in the first days after normal delivery is observed in 13-22.5% of the subjects, but as it has already been mentioned, nobody has investigated the uterine blood flow after delivery as early as we did. According to other authors, after 8 weeks postpartum, the diastolic peak is already seen in over 90% of women, while the data obtained in our study show that this incidence is not achieved either in primiparous (87%) or in multiparous women (77.3%). The general tendency of the diastolic peak incidence and its occurrence rate at the end of the involution is slightly different from the data obtained by other researchers, but the results may not coincide due to the relatively small size of our study group and the different criteria for selecting the subjects, applied by us and other authors [*Mulic-Lutvica et al., 2009; 2012; Fuller et al., 2014; Van Schoubroeck et al., 2004*].

By initiating this three-year study, we had the primary goal of examining the uterus in women after normal delivery and observing its involution, but during the study period, with the emergence of some cases that became complicated and required additional treatment, interventions or even surgical treatment, as a result

of which a high-risk group of postpartum women was compiled. Undoubtedly, it would be too ambitious to make concrete, firm conclusions from such a small sample of subjects, but the results obtained in our study are highly useful from a scientific point of view, thus, it is worth discussing them.

During the study, we did not establish a specific or statistically significant difference between the morphological parameters of the uterine size after the normal and complicated delivery during the entire period of involution. The data of other authors correlate with our findings [*Mulic-Lutvica, 2012; Dosedla et al., 2012; Rufener et al., 2008; Bardin et al., 2017; Nalaboff et al., 2001*]. The AP diameter has become the same in both groups on the 42nd day of the involution, but it declined more steadily from the first day after childbirth to the end of the first month in the normal childbirth group. The comparison of the first day data with the data obtained by other authors (92.0 mm) showed that our subjects had a slightly lower AP after normal (89.5 mm) and pathological (82.0 mm) delivery [*Mulic-Lutvica et al., 2007*]; other uterine size indicators were very similar. Obviously, the results may be different as a result of the time of measurement, because, as we have already mentioned, other authors provide "first day" data, and we performed the first ultrasound examination very early – within two hours after placenta delivery or after C/S, when the uterus was especially strongly shrunk.

When evaluating the inclusions of the uterine cavity, mixed echogenicity inclusions were less common after normal and complicated childbirth. Hyperechoic inclusions are seen more frequently after normal delivery than after the pathological childbirth in the early postpartum stages (1st, 3rd day) and at the end of the involution period (42nd, 60th day). Fluid inclusions (pronounced hypoechoic band) begin to prevail from the 10th day in both the physiological and pathological groups. On the tenth day, no statistically significant difference was found between the subjects of these groups, but the uterus cavity was significantly broader on the 42nd day after high risk childbirth (a statistically significant difference, p=0.033). In a high-risk group, the wider cavity of the uterus remains longer than in the physiological group and only in the 6-8th week of involution is narrowing and on the 60th day is narrower than after normal delivery. Other authors emphasize the wider uterine cavity after pathological birth compared to normal, on the 7th and 14th days [*Mulic-Lutvica et al., 2012; Nalaboff et al., 2001*].

After manipulations in the uterus (manual or instrumental revision of the uterine cavity, manual placental separation), it is normal to see a specific image (gas inclusions) in the uterus due to air supply to the uterine cavity: bright hyperechogenic focal regions in the area of endometrium, shading down the shadows. This image visible even on the 3rd day after childbirth is not pathological and should be regarded as normal after manipulations, later during the involution, these changes disappear [*Wolman et al., 2009*].

The literature describes that after normal delivery [*Mulic-Lutvica et al., 2012*]

gas inclusions (described as multiple) can be seen in the uterine cavity of about 5% of women and even in up to 16-21% of subjects after complicated childbirth, which sometimes persist until the end of the involution [*Wachsberg et al., 1992; Piek et al., 1989*]. We observed gas inclusions in the uterine cavity of 10 percent of women after normal delivery and even in 44% of women after pathological childbirth. Although gas inclusions were diagnosed more frequently than those described in literature, these inclusions in our subjects disappeared much faster than described by other authors [*Mulic-Lutvica et al., 2012; Wachsberg et al., 1992; Piek et al., 1989*]. The risk factors for the occurrence of gas inclusions in the uterine cavity determined by us coincided with those highlighted by other researchers: most of these were surgical manipulations in the uterus (uterine cavity revision for RPOC or C/S). The former studies of the 1980 associated the presence of gas inclusions in the uterine cavity specifically with endometritis after childbirth [*Piek et al., 1989; Madrazo, 1985*], but later studies, including ours, deny this theory, since the results obtained indicate that gas inclusions in the uterine cavity may be a normal phenomenon after childbirth and certainly do not confirm the diagnosis of endometritis or RPOC [*Fuller et al., 2016; Mulic-Lutvica et al., 2007*]. In the case of endometritis, the uterus may look like that in a healthy woman, however, the only true symptom of endometritis is the slowed up involution, which in this case is best defined by a larger anteroposterior uterine diameter (AP) and the uterine inclination from the longitudinal axis of a woman's body (in the case of endometritis, uterine anteversion is less commonly observed than in the normal period after childbirth) [*Mulic-Lutvica et al., 2007; Deans et al., 2006*].

The largest difference in the position of the uterus (angle) in relation to the longitudinal axis of the woman's body between the normal and the physiological childbirth is observed on the first day after childbirth. On the 1st day after pathological childbirth, the uterus is more significantly found in retroverse position in than in normal delivery ($p=0.039$). However, after two hours of the uterine cavity revision (due to RPOC), the uterus is less inclined toward the sacrum (-59.90°± 17.11°) than in the rest group of subjects after complicated childbirth (-72.50°±15.77°) and the inclination almost coincides with the inclination seen after normal childbirth (-61.50°±14.36°). The results of the first day of the uterine inclination correlate directly with the data provided by other authors [*Mulic-Lutvica et al., 2007*]. During the rest of the involution time, the uterus inclination to the front (towards the pubic bone) is much less frequent after the pathological childbirth than after the normal childbirth.

Our results did not show a statistically significant difference between the uterine arterial blood flow indices (RI, PI, S/D) after normal and postpartum delivery, therefore, we recommend using the Doppler ultrasound force more frequently not for monitoring involution after pathological delivery but for diagnosis of retained

products of conception. More pronounced blood circulation in the uterine lining and the PI and RI of the uterine artery below 10 percentile show a high probability of RPOC. The absence of a hypervascularised area in the muscular surface of the uterus can not deny the diagnosis of RPOC, but its presence is most often associated with RPOC [*Adkins et al., 2016; Sellmyer et al., 2013; Mulic-Lutvica et al., 2009*].

The observation of the diastolic peak after both normal and complicated delivery was not valuable, although it is considered an indicator of uterine involution [*Mulic- Lutvica et al., 2009; 2012; Guedes-Martins et al. 2015*]. There was no statistically significant difference between this indicator in women after normal or complicated childbirth. The absence of the diastolic peak can not be considered as an indicator of subinvolution, since even two months after childbirth, it was present not in all of the two groups of subjects.

The overwhelming problem in obstetrics and gynecology is the increasing number of women who become pregnant after the previous C/S. The previous Caesarean section surgery is a risk factor for the uterine rupture during the following pregnancy or childbirth and the formation of the uterine artery pseudoaneurysm, which is considered a rare complication [*Bouchet et al., 2012*]. The question is: what indicators can help to measure the full recovery and firmness of the scar? We measured the length and thickness of the uterus in the longitudinal uterine projection and described the ultrasound images of the scar. The length of the scar in two months after the C/S shortens twofold, and the thickness is reduced by one-third. By the end of the first month after the C/S, the uterine scar is of heterogeneous, mixed or hyperechoic structure. It is difficult to compare this data with the data of other authors, since most of the data presented in the literature are collected during a much later period after childbirth. One of the most recently published studies comparing the uterus after natural childbirth via the birth canal with the uterus after a cesarean section presents slightly earlier measurements of the morphological parameters of the uterus size after cesarean section (1-3 days) but does not evaluate the ultrasound imaging of the uterine scar [*Bardin et al., 2017*]. We found that the length and width of the uterus were still changing between the 6th and 8th week after the C/S. According to other authors, the uterine scar completely heals six months after the caesarean section [*Yazicioglu et al., 2006*]. In terms of the observation of the uterine scar after the cesarean section, it is necessary to say that scientists are still looking for precise diagnostic methods for assessing the uterus scar healing and its full value, but there is no uniform pattern of observation and evaluation of the uterine scar, so the searches and studies continue, publications on this topic are only increasing [*Chen et al., 1990; Naji et al., 2012; Dosedla et al., 2012; Oser et al., 2009; 2010; Yazicioglu et al., 2006; Van der Voet et al., 2014; 2017*]. It is recommended not only to measure the quantitative parameters of the uterine scar, but also to evaluate the descriptive features of the scar. In the

uterine ultrasound images of our nine subjects, fluid frequently showed near the uterine scar from the early postoperative period (10-25%, in the first month) to the end of the involution (50-78%, in the second month). However, a hypoechoic band does not imply an imbalance in the myometrium. According to other authors, this hypoechogenic region should be called the niche of the scar and the niche's depth, length and thickness of the residual myometrium along with it should be measured [*Chen et al., 1990; Naji et al., 2012; Dosedla et al., 2012; Osser et al., 2009; Van der Voet et al., 2014*], but these indicators begin to emerge in the later period after C/S, usually six weeks later [*Van der Voet et al., 2014*]. Thus, we could not measure the parameters of the mentioned scar in our subjects immediately after cesarean section surgery, since the niche or cystic inclusions appeared only on the 30th-42nd postoperative day. Additional study methods (MRI) are often needed to assess the sufficiency of the uterine scar, but there are still no studies or standardized indices to absolutely precisely determine the firmness of the uterus (*Dicle et al., 1997; Yao et al., 2017*]. Our data on uterine scars after the C/S are only a tiny contribution to science, as the number of subjects is very small, but the data are useful and new in terms of their consistency and early observations.

When assessing the impact of neonatal birth weight on the uterine involution, we found that the uterine size of women who delivered babies weighing 4,000g and over was slightly larger after both, normal and complicated childbirth compared with women whose neonates weighed less than 4,000g but the difference was not statistically significant. We did not find any statistically significant neonate weight impact on the parameters of the uterine involution, either in primiparous or in women after normal childbirth, or in women after complicated childbirth. The great majority of other researchers also did not establish a statistically reliable relationship between the weight of the newborn and the uterine involution [*Mulic-Lutvica et al., 2012; Wachsberg et al., 1994; Al-Bdour et al., 2004*]. Only one of the recent publications claims that the uterine volume statistically reliably depends on the weight of the newborn, regardless of the period after delivery, but this study measured the uterus up to 7 days postpartum (*Kristoschek et al., 2017*).

The evaluation of early neonatal health indicators showed no cases of extremely severe conditions in newborns of our study groups that would be subjected to intensive treatment or reanimation measures in the later postpartum period. The lowest estimates by V.Apgar were 6 points 1 minute after birth in only two cases when acute fetal hypoxia was diagnosed during the second stage of delivery. Nevertheless, according to *Saling*, the evaluation by the V.Apgar scale is not sufficient to describe newborn hypoxia [*Saling, 2006*]. At uncomplicated childbirth, we found significantly lower umbilical cord blood artery pH and lactate levels in the group of primiparous women, which means that the inner in newborn infants of primiparous

women is much more acidic than that in neonates of multiparous women. This data correlate with the data described in the literature. The comparison of early neonatal conditions after normal and complicated childbirth does not show a statistically significant difference. It should be noted that the risk factors for a group of subjects after complicated delivery (postpartum bleeding, uterine cavity revision, cesarean section surgery, postpartum endometritis) more specifically define the risk for a woman rather than a newborn. According to the literature, maternal factors and method of delivery influence the umbilical cord artery blood gas composition in newborns. *Zhang* and his co-authors found that the first childbirth in the "modern population" lasts longer [*Zhang et al., 2010*]. Prolonged labour, and especially the second period, directly affects the amount of the umbilical cord artery lactates in the fetus: the longer the second period, the higher the lactate content. Another factor that indirectly affects the composition of the blood gas of the fetus is the epidural labour pain relief. Epidural analgesia may result in temporary uteroplacental circulatory failure due to the elevated maternal and fetal body temperature, leading to the reduced oxygen supply to the fetal tissues and the increased lactate levels in the fetal blood. Again, the increased use of epidural pain relief increases the frequency of instrumental termination of childbirth and cesarean sectional operations. The more frequent use of oxytocin causes more painful contractions, which promotes the need for epidural analgesia. Co-factors (oxytocin and epidural anesthetics) can increase the amount of arterial blood lactate levels in the umbilical cord of the newborn (*Wiberg et al., 2017*). Thus, primiparous women are at greater risk their newborns' acid-alkali balance will be indirectly affected by the factors of childbirth.

Based on literature data, considering the indicator (pH or lactate content) for assessing neonatal hypoxia and identifying health indicators, it could be argued that the investigation of umbilical arterial blood lactate levels is superior. In some newborns, lactate levels may be a prognostic indicator: hypoxic-ischemic encephalopathy, neonatal sepsis, necrotizing enterocolitis, bronchopulmonary dysplasia [*Ganetzky et al., 2017*]. The latter test requires a smaller amount of blood sample, simpler testing equipment, and has a higher value for predicting the future health of newborns [*Tuuli et al., 2014; Allanson et al., 2016; Malin et al., 2016; Wiberg et al., 2010; Borruto et al., 2006; East et al., 2015; Yeh et al., 2012*]. Taking into account that lactate is a very weak acid, and therefore it is particularly susceptible to the method of collecting blood from the umbilical cord artery, its storage and transportation. Even minimal hemolysis in cells can also increase lactate levels. Isolated lacticemia only reflects an increase in lactate levels without metabolic acidosis. Lacticemia can be caused by impaired pyruvate metabolism or gluconeogenesis defects in the newborn. The primary cause of lactic acidosis can be caused by more than 100 known genetic diseases. Thus, there are sources suggesting that the high levels of lactate in

the population can be false [*Ganetzky et al., 2017*]. Unfortunately, when investigating the newborns of subjects under analysis, due to the low number of newborns in severe condition, we failed to verify the hypothesis which of the indicators, ph or lactate, were superior, therefore the study of neonatal conditions was later extended into a study comprising a larger sample [*Einikyte, Snieckuviene, Ramasauskaite, Paliulyte, Opolskiene, Kazenaite, 2017*].

Thus, we found different umbilical cord blood gas parameters in newborn infants of primiparous and multiparous women, but we did not find a statistically significant difference between neonatal health indicators after normal and complicated childbirth.

Conclusions

1. After the uncomplicated childbirth, the ultrasound image of the uterus may be very diverse, but some of the features are specific for a specific period of uterine involution:
   - Immediately after birth, the uterus is tilted towards the female's sacrum, uterine size is 2.5 times larger than at the end of the involution, and the uterus cavity looks like a narrow hyperechoic or mixed echogenicity band;
   - From the 10th to the 30th day after childbirth, the uterus is closest to the longitudinal axis of the woman's body, the size of the uterus on the 10th day is twice as large as at the end of the involution, and on the 30th day is almost the same as after two months; hypoechoic (fluid) content prevails in the uterus during this period;
   - From the middle to the end of the second month, the uterus is inclined toward the pubic bone, the length of the uterus remains almost unchanged, and the uterine cavity is dominated by a hyperechoic band.

1.1. Importance of morphological parameters of the uterus size and relationships with the woman's health:
   - Physiological indices of the uterine size over a period of two months after childbirth vary according to the number of childbirths in the past and last for more than six weeks.
   - The woman's age, BMI, smoking, number of miscarriages, termination of pregnancy, anemia, gestational diabetes, GBS colonisation, childbirth duration, period of membrane rupture, amniotic fluid colour, placental location, blood leukocyte count, CRP do not have a statistically significant effect on the changes of size of the uterus after normal childbirth.
   - When examining women after childbirth, it is recommended to evaluate

the following ultrasound indicators of the uterine involution:
  - the longitudinal, transverse and anteroposterior (AP) diameters of the uterus;
  - the width and inclusions of the uterine cavity and their description;
  - uterine artery blood flow markers (RI, PI, S/D).
- The anteroposterior uterine diameter (AP) is recommended to be measured in the widest part of the longitudinal uterine projection, but not 5 cm from the uterus fundus, as recommended by other authors.

1.2. The morphological parameters of the uterus size in primiparous women after uncomplicated childbirth are smaller from the first hours to the end of the involution, compared to the uterus of multiparous women.
- The greatest differences in the size of the uterus between primiparous and multiparous women are observed on the 10th day after childbirth.
- The most pronounced differences in the blood flow of the uterine artery between primiparous and multiparous women are observed up to the 10th day after childbirth. In this period, the involution of the uterine artery blood flow is more intense in multiparous women. From the end of the first month to the 60th day after childbirth, blood flow indices are almost unchanged.
- The change in the position of the uterus from the sacrum towards the pubic bone with respect to the longitudinal axis of the woman's body is more intense in multiparous women. At the end of involution, the uterus of multiparous women remains more inclined toward the pubic bone compared to primiparous women.

1.3. The most intense changes in the uterine cavity occur in the second week after childbirth, so during this period it is normal to see it expanded, filled with fluid or mixed inclusions. Such ultrasound image of the uterus after normal childbirth can not be indicative of uterine abrasion or uterine contents suction for either primiparous or multiparous women.

2. Birth weight of the newborn has no statistically significant effect on the size of the uterus or on the uterine involution, both after multiparous childbirth, and after normal or complicated childbirth.
3. Evaluation of neonatal condition by points according to the V.Apgar scale did not show a statistically significant difference between primiparous and multiparous women in the case of uncomplicated childbirth. The rates of umbilical artery blood after normal childbirth depend on the number of woman's childbirths: the blood pH and lactate levels of the umbilical cord blood of the newborn are normal, but lower in primiparous women. The

factors hazardous to the maternal health as a result of complicated delivery (retained products of conception, postpartum bleeding, postpartum endometritis, childbirth through C/S) were not statistically significant for neonatal health indicators.

4. We did not establish a statistically significant difference between the morphological parameters of the uterus after normal and complicated delivery.
   - Specific features of ultrasound monitoring of women after complicated delivery:
     - Neither method of childbirth (natural childbirth via birth canal or via C/S), nor the early uterine intervention procedures after childbirth affect the morphological parameters of the uterus or the uterine artery blood flow indices during the involution period.
     - Hyperechoic inclusions without blood flow intensification are a normal ultrasound finding after manipulation in the uterus for the first three days after childbirth;
     - Gas inclusions in the uterine cavity are more commonly associated with delivery complications rather than uncomplicated childbirth, but this is not a specific indication of endometritis.

5. The morphological markers of the uterine size after C/S do not differ from the same parameters after normal childbirth.
   - The most intensive changes in the uterine scar length and thickness are observed in the first month after C/S, later more intensive changes are seen in the descriptive features of the scar.
   - Hypoechoic inclusions in the uterine scar area are normal for the first two months after C/S and they do not indicate the impairment/failure of the uterine scar.
   - One month after C/S, it is no longer sufficient to measure the length and thickness of the uterine scar in the longitudinal uterine projection; additional morphological criteria are already needed to evaluate the healing of the scar: uterine scar niche, its depth; the thickness of residual myometrium; thickness of the adjacent myometrium; descriptive parameters.

References

A list of references is provided in the manuscript of the Dissertation.

SANTRAUKA

Rezultatų apibendrinimas ir išvados

1. Po nekomplikuoto gimdymo gimdos ultragarsinis vaizdas gali būti labai įvairus, tačiau kai kurie požymiai yra specifiniai konkrečiam gimdos involiucijos laikotarpiui:
   - Iš karto po gimdymo gimda yra nukrypusi link moters kryžkaulio, gimdos dydis 2,5 karto didesnis nei involiucijos pabaigoje, o gimdos ertmė atrodo kaip siaura echopozityvi arba mišraus echogeniškumo juostelė;
   - Nuo 10-os iki 30-os dienos po gimdymo gimdos kūnas yra arčiausiai moters išilginės kūno ašies, gimdos dydis 10-ą dieną dar dvigubai didesnis nei involiucijos pabaigoje, o 30-ą dieną jau beveik toks pat kaip po dviejų mėnesių; gimdos ertmėje šiuo laikotarpiu vyrauja echonegatyvus (skysčio) turinys;
   - Nuo antrojo mėnesio vidurio iki pabaigos gimda pakrypsta link gaktikaulio, gimdos ilgis beveik nebekinta, o ertmėje vyrauja echopozityvi juostelė.

1.1. Gimdos dydžio morfologinių parametrų svarba ir sąsajos su moters sveikata:
   - Fiziologiniai gimdos dydžio rodikliai dviejų mėnesių laikotarpiu po gimdymo kinta priklausomai nuo gimdymų skaičiaus praeityje ir tai trunka ilgiau nei šešias savaites.
   - Moters amžius, KMI, rūkymas, persileidimų, nėštumo nutraukimų skaičius praeityje, anemija, gestacinis diabetas, BGS kolonizacija, gimdymo trukmė, bevandenio laikotarpio trukmė, vaisiaus vandenų spalva, placentos lokalizacija, leukocitų kiekis kraujyje, CRB vertė neturi statistiškai reikšmingos įtakos gimdos dydžio rodiklių pokyčiams po normalaus gimdymo.
   - Tiriant moteris po gimdymo, rekomenduotina įvertinti šiuos ultragarsinius gimdos involiucijos stebėsenos rodiklius:
     - gimdos išilginį, skersinį ir priekinį–užpakalinį (AP) matmenį;
     - gimdos ertmės plotį, intarpus ir juos aprašyti;
     - gimdos arterijos kraujotakos žymenis (RI, PI, S/D).
   - Priekinį–užpakalinį gimdos matmenį (AP) rekomenduojama matuoti plačiausioje išilginės gimdos projekcijos dalyje, o ne 5 cm atstumu nuo gimdos dugno, kaip tą rekomenduoja kiti autoriai.

1.2. Visi pirmą kartą gimdžiusių moterų gimdos dydžio morfologiniai parametrai po nekomplikuoto gimdymo yra mažesni nuo pirmųjų valandų iki pat involiucijos pabaigos lyginant juos su pakartotinai gimdžiusių moterų gimda.
   - Didžiausi gimdos dydžio rodiklių skirtumai tarp pirmą kartą ir pakartotinai gimdžiusių moterų stebimi 10-ą parą po gimdymo.
   - Ryškiausi gimdos arterijos kraujotakos rodiklių skirtumai tarp pirmą kartą ir pakartotinai gimdžiusių moterų matomi iki 10-os dienos po gimdymo. Šiuo laikotarpiu gimdos arterijos kraujotakos involiucija yra intensyvesnė pakartotinai gimdžiusioms moterims. Nuo pirmojo mėnesio pabaigos iki 60-os dienos po gimdymo kraujotakos indeksai beveik nekinta.

- Gimdos padėties kitimas nuo kryžkaulio link gaktikaulio moters išilginės kūno ašies atžvilgiu intensyvesnis pakartotinai gimdžiusioms moterims. Involiucijos pabaigoje pakartotinai gimdžiusių moterų gimda lieka labiau pakrypusi link gaktikaulio nei pirmakarčių.

1.3. Intensyviausi gimdos ertmės pokyčiai vyksta antrąją savaitę po gimdymo, todėl šiuo laikotarpiu yra normalu ją matyti išplėstą, pripildytą skysčio ar mišrių intarpų.
Toks ultragarsinis gimdos vaizdas po normalaus gimdymo negali būti indikacija gimdos abrazijai ar gimdos turinio siurbimui nei pirmą kartą, nei pakartotinai gimdžiusioms moterims.

2. Naujagimio gimimo svoris neturi statistiškai reikšmingos įtakos nei gimdos dydžiui, nei gimdos involiucijai tiek po pirmo, tiek po pakartotinio gimdymo, tiek po normalaus, tiek po komplikuoto gimdymo.
3. Naujagimių būklės įvertinimai balais pagal V.Apgar skalę statistiškai reikšmingai nesiskyrė tarp pirmą kartą ir pakartotinai gimdžiusių moterų, kai gimdymas buvo nekomplikuotas. Virkštelės arterijos kraujo įvertinimo rodikliai po normalaus gimdymo priklauso nuo moters gimdymų skaičiaus: naujagimio virkštelės arterijos kraujo pH ir laktatų rodikliai yra normalūs, bet mažesni, jei moteris gimdo pirmą kartą. Komplikuoto gimdymo veiksniai, sukėlę riziką motinos sveikatai (placentinio audinio likučiai gimdoje, kraujavimas po gimdymo, endometritas po gimdymo, gimdymas per CPO), statistiškai patikimai neturėjo įtakos naujagimių sveikatos rodikliams.
4. Nenustatėme statistiškai reikšmingo skirtumo tarp gimdos dydžio morfologinių parametrų po normalaus ir po komplikuoto gimdymo.
   - Moterų po komplikuoto gimdymo gimdos ultragarsinio stebėjimo ypatumai:
     - Nei gimdymo būdas (natūraliais takais ar per CPO), nei ankstyvas gimdos intervencinių procedūrų atlikimas po gimdymo neturi įtakos gimdos dydžio morfologinių parametrų ar gimdos arterijos kraujotakos indeksų kitimui involiucijos metu.
     - Echopozityvūs intarpai be kraujotakos suintensyvėjimo yra normalus ultragarsinis radinys po manipuliacijų gimdoje pirmąsias tris dienas po gimdymo;
     - Dujų intarpai gimdos ertmėje yra dažnesni buvus gimdymo komplikacijų nei po nekomplikuoto gimdymo, bet tai nėra specifinis endometrito požymis.
5. Gimdos dydžio morfologiniai žymenys po CPO nesiskiria nuo tokių pat parametrų po normalaus gimdymo.
   - Intensyviausiai gimdos rando ilgis ir storis keičiasi per pirmąjį mėnesį po CPO, vėliau labiau kinta rando aprašomieji požymiai.
   - Echonegatyvūs intarpai gimdos rando srityje pirmuosius du mėnesius po CPO yra normalūs ir jie nereiškia gimdos rando nepakankamumo.
   - Praėjus mėnesiui po CPO, nebeužtenka matuoti gimdos rando ilgio ir storio išilginėje gimdos projekcijoje, rando gijimui vertinti jau reikalingi papildomi morfologiniai kriterijai: gimdos rando niša, jos gylis; liekamojo miometriumo storis; šalia esančio miometriumo storis; aprašomieji parametrai.

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Augustas Beiša

THE SIGNIFICANCE OF THE BRAF V600E MUTATION AND THE CYTOMORPHOLOGICAL FEATURES FOR THE OPTIMIZATION OF PAPILLARY THYROID CANCER DIAGNOSTICS

BRAF V600E MUTACIJOS IR CITOMORFOLOGINIŲ POŽYMIŲ REIKŠMĖ PAPILINIO SKYDLIAUKĖS VĖŽIO DIAGNOSTIKOS OPTIMIZAVIMUI

Biomedical Sciences, Medicine (06B)
Biomedicinos mokslai, medicina (06B)

Abbreviations

BRAF – *B-type Rapidly Accelerated Fibrosarcoma*
FNA – fine needle aspiration biopsy
PCR – polymerase chain reaction
PTC – papillary thyroid cancer
TC – thyroid cancer
US – ultrasound
VUH SK – Vilnius University Hospital Santaros klinikos

Introduction

Thyroid nodules are detected in about 50% of adults who live in regions deficient in iodine. Although thyroid cancer is the most common oncological endocrine disorder, only 5% of the thyroid nodules are malignant. Papillary thyroid cancer (PTC) comprises 85 to 90% of cases of all histological types of thyroid cancer. Although PTC has a slow progression and good survival rates, 20% to 30% of patients have localized disease recurrences or distant metastases and about 1% of patients die from this disease.

A fine needle aspiration biopsy (FNA) performed under ultrasound (US) guidance and a cytological examination of nodules remains the gold standard for the differential diagnostics of thyroid cancer. However, due to a well-differentiated nature of most thyroid cancers, 10 to 26% of samples are ascribed to cytologically indeterminate diagnostic categories (categories III, IV, V, according to the Bethesda system). In order to determine the exact diagnosis, diagnostic surgery is usually proposed for these patients; however, a histological examination of resected tissues confirms the diagnosis of thyroid cancer in only one-third of patients. In the absence of reliable preoperative diagnostic criteria, there is a risk of general, surgical intervention-related and thyroid surgery-specific complications including temporary or permanent vocal cord paralysis and temporary or permanent decrease in blood calcium levels. After surgery, replacement therapy with thyroxine is usually required for the rest of patients' lives. Besides, psychological discomfort due to a 4 to 6 cm long scar in the neck may be experienced.

These causes lead to high socioeconomic costs and encourage to search for new options of diagnostics and prediction that could potentially optimize patient selection for surgical treatment.

Molecular genetic tests are among the most promising methods for the diagnostics of thyroid cancer and for the predictions of the disease course. The first works on the impact of *BRAF* mutations in oncological diseases were performed in

studies of skin, colon and lung tumors. The in 2003. A high V400E mutation specificity for PTC was noticed at that time. Therefore, Xing et al. had proposed BRAF V600E mutation testing for presurgical PTC diagnostics already in 2004. Although many publications on the applications of BRAF V600E mutation testing for PTC diagnostics and prognostication have appeared since that time, published data and recommendations remain controversial. Since the prevalence of BRAF V600E mutation in PTC tumors ranges from 30 to 80% depending on the geographical region, it can be stated that only the detection of mutation prevalence in the population under study can determine the applicability of this testing in clinical practice. The prevalence of BRAF V600E mutations in PTC in Lithuania has not been investigated so far.

Aim of the Study

The aim of this dissertation is to evaluate the significance of the Bethesda system, the cytomorphological features, the BRAF V600E mutation and the BRAF V600E mutation combined with the cytomorphological features for the optimization of papillary thyroid cancer diagnostics. Further aims are:

1. To define indications for surgical treatment vs. an active surveillance of thyroid nodules;
2. To evaluate the possibilities of predicting signs of histological PTC aggressiveness prior to surgery.

Tasks of the Study

The tasks of the study are the following:

1. To evaluate the significance of the Bethesda system for the diagnostics of PTC;
2. To determine the prevalence of the BRAF V600E mutation in the population of Lithuanian patients with PTC;
3. To evaluate the significance of the cytomorphological features for the diagnostics of PTC in cytologically indeterminate diagnostic categories;
4. To evaluate the significance of the BRAF V600E mutation for the diagnostics of PTC in cytologically indeterminate diagnostic categories;
5. To evaluate the significance of the BRAF V600E mutation combined with the cytomorphological features for the diagnostics of PTC in cytologically indeterminate diagnostic categories;
6. To evaluate the significance of the Bethesda system for the prognosti- cation of signs of histological PTC aggressiveness;

7. To evaluate the significance of BRAF V600E for the prognostication of signs of histological PTC aggressiveness.

Novelty of the Study

The following arguments that are laid out are in support of this study's novelty:

1. For the first time, the prevalence of the BRAF V600E mutation was determined in the population of patients with PTC in Lithuania;
2. The significance of the BRAF V600E mutation, combined with the cytomorphological features for the diagnostics of PTC in inde- terminate cytological categories, was determined, and a PTC risk calculator, adapted for use in clinical practice, was developed;
3. The significance of the Bethesda system for the prognostication of signs of histological PTC aggressiveness and PTC staging was determined.

Practical Significance

This study contributes to the worldwide search for the diagnostic and prognostic biomarkers of thyroid cancer and the evaluations of applications of established biomarkers in clinical practice. More accurate diagnostics of thyroid cancer have the potential to reduce the number of diagnostic surgeries and provide indications for an active surveillance of patients. The prognostication of PTC aggressiveness and disease staging has a potential to provide means for the estimation of the extent of surgical treatment. Individualized treatments may directly affect the physical health and psychological well-being of patients and reduce socioeconomic costs.

Statements to be Defended

The following two statements stand as those to be defended by this study:

1. The BRAF V600E mutation testing, combined with the analysis of the cytomorphological features, may improve the diagnostics of PTC in cytologically indeterminate diagnostic categories;
2. Based on the Bethesda system, it is possible to predict signs of histological PTC aggressiveness.

Methodolog y of the Study

A permission of the Lithuanian Bioethics Committee (No. 158200-04-475-137) was obtained on April 26, 2012. The location of the study was Vilnius University Hospital Santaros Klinikos (VUH SK). Patients who applied to VUL SK and met the inclusion criteria of the study were invited to participate in this study.

Criteria for Inclusion into the Study

The inclusion of patients into the study was based on the following criteria:

1. Patients were 18-years-old or older;
2. Thyroid nodules with signs of malignancy were detected on thyroid US;
3. A US-guided FNA of thyroid nodules with signs of malignancy was performed;
4. Cytological features, classified as categories III, IV, V, VI according to the Bethesda system, were determined.

Exclusion Criteria

Patients were excluded from the study based on the following criteria:

- Pregnancy;
- Cytological features classified as categories I or II according to the Bethesda system were determined in an FNA aspirate.
- The indication for surgery was not a suspected oncological disorder.

Testing and Diagnostics

All patients with a suspected thyroid disease underwent a US scan examination in accordance with the diagnostic standards of the VUH SK. When thyroid nodules with signs of malignancy were detected on a US scan, a routine US-guided FNA of the nodule with signs of malignancy was performed. Every FNA aspirate was sent for a cytological examination (all samples were evaluated by the same investigator). For every sample, 22 cytological features were evaluated and all samples were classified according to the Bethesda System for Reporting Thyroid Cytopathology. All patients were referred for endocrinological consultation. Patients with nodular cytological features classified as categories III, IV, V, VI were also referred for surgical consultation. For all patients having indications for surgery, surgical treatment was recommended. Patients who agreed to be operated on and met the inclusion criteria were invited to participate in the study and given informed consent forms approved by the Lithuanian Bioethics Committee. Responses to any additional questions raised by patients were given in verbal by researchers of VUH SK. Upon the familiarization with study conditions and informed consent forms and agreements to participate in the study, patients signed informed consent forms. These forms were also signed and left for storage by the senior researcher. A cytology laboratory researcher was informed about all the patients who agreed to participate in the study and the residues of FNA aspirates of these patients were transferred to the genetic testing laboratory for BRAF V600E mutation testing (all samples were tested by the same investigator). Patients were admitted to the Center of Abdominal Surgery for

surgical treatment. Surgically removed histological tissues were sent for a histological examination (all preparations were examined by the same investigator).

The staging of oncological disorders was performed according to the 7th ed. of TNM classification issued in 2009, as recommended by the American Joint Committee on Cancer (AJCC). When the final histological examination revealed PTC but no BRAF V600E mutation was detected in the cytological sample, an additional testing for a BRAF V600E mutation from histological samples was performed.

Thy roid Ultrasound (US) Examination and Fine Needle Aspiration Biopsy (FNA). Validated Procedures of VUH SK

The thyroid US examination was performed on patients lying on their back, shoulders elevated with a pillow, head reclined. Ultrasound equipment with a high frequency linear sensor (7–15 MHz), high resolution (0.7–1 mm) and high penetration was used. Images were evaluated in a gray real-time and color Doppler scales. The thyroid gland parenchyma, the size of the lobes, the size of nodules, the localization of nodules, nodular US characteristics as well as the changes of the central and lateral lymph nodes were evaluated. All thyroid nodules were classified as having high, moderate, low or very low risk of malignancy and being non-malignant according to US characteristics.

Nodules with a High Risk of Malignancy

Hypoechoic, solid nodules or nodules with small amounts of fluid with 1 or more signs of malignancy according to US characteristics:

- Uneven nodule margins;
- Microcalcifications;
- Breaks in calcified rings;
- Taller-than-wide shape of nodules;
- An extension of the nodule beyond the margins of the thyroid gland.

Nodules with an Intermediate Risk of Malignancy

These nodules are hypoechoic, solid or with small amounts of fluid, with even margins and without other US signs of malignancy.

Nodules with a Low Risk of Malignancy

These nodules are isoechoic or hyperechoic, solid or partially solid with the solid part lying excentrically, without US signs of malignancy.

Nodules with a Very Low Risk of Malignancy

These nodules are spongiform or partially cystic, without any US signs of malignancy.

NON-MALIG NANT NODULES – cystic thyroid nodules without any solid components.

Indications for punction are based on the US characteristics and size of the nodules:

- Thyroid nodules of 1 cm or more in size, with a high risk of malignancy (without extrathyroidal extension);
- Thyroid nodules of any size with a high risk of malignancy and extrathyroidal extension;
- Thyroid nodules of 1 cm or more in size with an intermediate risk of malignancy;
- Thyroid nodules of 1.5 cm or more in size with a low risk of malignancy;
- Thyroid nodules of 2 cm or more in size with a very low risk of malignancy.

Remarks:

- Multiple thyroid nodules of 1 cm or more in size were punctured based on the same indications as solitary nodules.
- In cases of a multinodular goiter, thyroid nodules with the highest risk of malignancy according to US signs or the largest nodules were punctured.
- In cases of multiple thyroid nodules with a low or very low risk of malignancy, the FNA was not performed and patients were offered active surveillance (an endocrinologist was responsible for the choice of the monitoring mode); in other cases, the largest (more than 2 cm in size) thyroid nodule was punctured.

When a nodule with indications for US-guided FNA was detected during the US examination of a thyroid gland, after the preparation of the hands, the field and the tools according to the approved procedure, a US sensor was placed on the neck in the projection of the thyroid gland, and the image of a to-be- suspicious nodule or pathological tissue of the thyroid gland was placed onto the screen. The procedure was performed by holding a US sensor in one hand and a syringe with a 21-27G needle in the other hand. The puncture was performed close to the sensor, perpendicularly or transversaly to the ultrasound beam. Following the tip, the needle was inserted into the desired location and punctured in the mode of pendulum. An aspirate was obtained by vacuuming the syringe. The nodules were punctured twice and more times if necessary. A part of the thyroid tissue obtained from the FNA was injected into a liquid-based citology vial, and the remaining part of the aspirated material was spread evenly in a thin layer onto the slide. Smears were air-dried fixed.

Cytological Examination of the Aspirate

FNA aspirates of every patient were examined as smears stained with the Papanicolau and automatic BD Prep Stain system. The smears were examined using a bright-field microscope Olympus CH30/31. Initially, the whole smear was examined with a 10x magnifying lens. The smear was considered to be suitable for further examination if at least six groups of thyroid epithelial cells (7 to 10 cells in each group) were present. The cytomorphological features of cells were evaluated with 40 or 100x magnifying lenses. A special form was created for the systematic collection and analysis of cytological data. Cases were classified according to the Bethesda System for Reporting Thyroid Cytopathology.

Genetic Testing of the BR AF V600E Mutation

Liquid specimens of a thyroid FNA aspirate (treated with an erythrocyte lysis solution) or material from the histological preparation were transferred to 1.5 ml tubes. Genomic DNA was isolated using the GeneJet Genomic DNA Purification Kit (Thermo Scientific, Vilnius, Lithuania). In order to detect a BRAF V600E mutation (GTG > GAG), a real-time PCR assay was developed. The first reaction was used to amplify a sequence flanking the BRAF codon V600 of both mutated and non-mutated codons (internal reference sample control). The second reaction was designed to specifically target the GTG > GAG mutation. Primers were predesigned manually and investigated *in silico* using the OligoCalc web tool. A BRAF V600E mutation specific forward primer was edited manually by introducing a relevant mismatch nucleotide at the 3' end according to the Amplification Refractory Mutation System primer design approach. The reverse primer was the same for both reactions. Primer sequences were as follows: BRAF_V600E_ Forward GTGATTTTGGTCTAGCTACGGAG; BRAF_Forward TAGGTGATTTTGGTCTAGCTACAG; BRAF_Reverse CATCCACAAAATGGATCCAGAC. Real-Time PCR reactions were performed in duplicates using a Maxima™ SYBR Green qPCR Master Mix, supplemented with 0.4 units per reaction of UNG (both Thermo Scientific, Vilnius, Lithuania), 300 nmol of each primer (Metabion, 82152 Planegg/ Steinkirchen, Germany), and 0.005-0.05 µg of DNA in a 20 µl reaction volume. The Real-Time PCR reaction conditions on a Bio-Rad CFX96 system (Bio-Rad Laboratories, Hercules, CA, US) were as follows: 2 minutes at 50°C, 10 minutes at 95°C, 37 cycles at 95°C for 15 seconds, and 1 minute at 60°C.

The first 20 BRAF V600E-positive and 20 wild type allele (Braf V600E- negative) cases were confirmed by sequencing histological samples according to the method described earlier. The analytical sensitivity of the Real-Time PCR assay was 2.5 log (or 0.2%). It was tested by serial dilutions of previously sequenced positive BRAF V600E DNA in negative DNA.

Surgical Treatment

All patients received timely and appropriate treatment according to the existing treatment standards of VUH SK. Participation in the biomedical study had no effect on the course, treatment or outcomes of their disease.

The operating surgeon was not familiar with the results of the BRAF V600E mutation testing and these results could not influence decisions on the indications for surgery or extent of surgical treatment. Patients underwent a resection of isthmus, one lobe, one lobe with isthmus or a resection of the entire thyroid gland. An urgent histological examination of resected tissues was usually performed during the operation. Part of the patients' cohort underwent prophylactic lymph node dissection (VI level) on one or both sides. When pathological US signs in the lateral lymph nodes were detected prior to surgery, a modified radical lymph node dissection (II-V levels) was also performed.

Statistical Analysis of Results

The sample size was calculated based on the number of newly diagnosed thyroid cancer cases per year in the Cancer Registry database, with a confidence level of 95% and a margin of error 5%. A database of study participants was created for the analysis of study results. All statistical analyses were performed using IBM SPSS STATISTICS, version 23.0. The significance of the Bethesda system, the cytomorphological features, the BRAF V600E mutation, the BRAF V600E mutation combined with cytological features for the diagnostics of papillary thyroid cancer were assessed in this study. The significance of the Bethesda system and the BRAF V600E mutation for predicting the histological aggressiveness of papillary thyroid cancer were also evaluated. The absolute values and percentages for categorical variables and mean averages with standard deviations or medians with ranges for continuous variables were given in the descriptions of patients' comparative groups. Continuous variables were checked for normal distribution using the Kolmogorov-Smirnov and the Shapiro-Wilk test. The statistical significance of differences between two groups of non-parametric data with non-normal distribution was assessed using the Mann-Whitney U test. The statistical significance of differences between the nominal and categorical variables was assessed using the Pearson χ^2 Chi-Square criterion; otherwise, the Fisher's Exact Test was used when the observed frequency of at least one value of the variable was <5. After establishing a statistically significant correlation between the factors under analysis, the strength of this correlation was also calculated using the correlation coefficient r. Cramer's V correlation coefficient was calculated for the assessment of the correlation between the nominal variables. For assessments regarding the strength of the correlation, when one of the variables was nominal or categorical and the other vari-

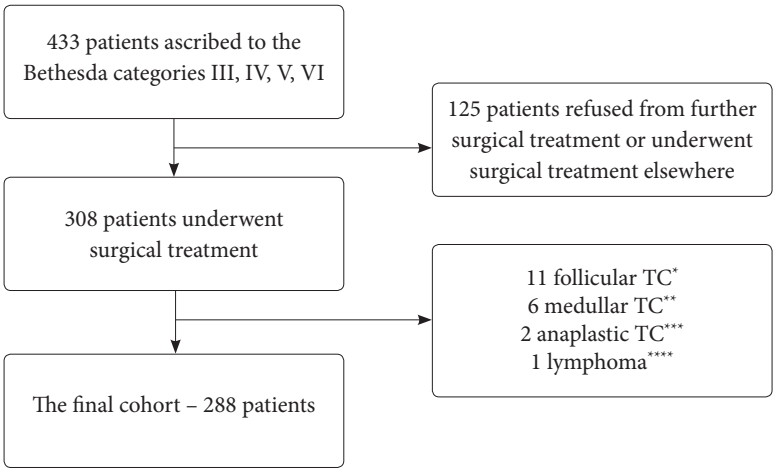
able was non-parametric, the Eta factor was calculated. For predictions of histological examination results when the results of other testings were known, a binary logistic regression model was developed. The cutoff of 0.5 was selected and patients were divided into two groups of PTC risk: the low risk group (when PTC risk <0.5) and the high risk group (when PTC risk ≥0.5). The statistical significance level $\alpha = 0.05$ was chosen, and the results were statistically significant when the p-value of the two-sided hypothesis was less than 0.05 ($p < 0.05$).

Results of the study

Composition of the Patients' Cohort

From January 1, 2012 to February 1, 2016, 433 patients were included into the biomedical study, while 145 patients were excluded from the study.

The final cohort consisted of 288 patients who participated in the study until its end; these patients underwent surgery because of suspected thyroid cancer and met the criteria for inclusion into the study. The scheme of patient inclusion and exclusion from the study is presented in Figure 1.



* 5 cases were ascribed to category IV, 5 – V, 1 – VI.
** 1 case was ascribed to category IV, 2 – V, 3 – VI.
*** 1 case was ascribed to category III, 1 – V.
**** 1 case was ascribed to category III.
All cases were negative for the BRAF V600E mutation.

FIGURE 1. The scheme of patient inclusion and exclusion from the study.

General Characteristics of Study Participants

A total of 288 patients participated in the study. There were 251 female (87.2%) and 37 male (12.8%) patients. The mean age of patients was 54.4 ± 13.1 years. An FNA of thyroid nodules prior to surgery was performed on all patients. All patients had the cytological examination of a thyroid FNA sample specimen; in every sample, 22 cytomorphological features were evaluated and all samples were classified according to the Bethesda System for Reporting Thyroid Cytopathology. Besides, a BRAF V600E mutation testing from the FNA aspirate was performed in all patients, and an additional BRAF V600E mutation testing was also performed on the histological specimens when the mutation was not detected in the initial FNA sample specimen testing. All study participants underwent surgical treatment. A histological examination of resected tissues was performed for all patients.

Characteristics of the Comparative Groups of Patients

Based on the results of histological examination, the patients were divided into two comparative groups: 143 patients (49.7%) entered the PTC group, 145 patients (50.3%) entered the benign nodular thyroid disease group.

General Data

Statistically significant differences were observed in the distribution of males and females in the abovementioned comparative groups: in the PTC group, there were 131 females (91.6%) and 12 males (8.4%), in the benign nodular thyroid disease group, there were 120 females (82.8%) and 25 males (17.2%), $p = 0.025$ ($p < 0.05$). There were no statistically significant differences in the distribution of patients by age: in the PTC group, the mean age of patients was 53.8 ± 12 years, in the benign nodular thyroid disease group, the mean age of patients was 54.9 ± 14.1 years, $p = 0.309$ ($p \geq 0.05$).

Cytomorphological Features

A high anisokaryosis, a streaked chromatin, a finely dispersed chromatin, nucleoli, a dark cytoplasm, papillary structures, atypia, intranuclear cytoplasmic inclusions, psammoma bodies, an absence of colloid, a dense colloid and eosinophilic colloid bodies were statistically significantly more frequent in the PTC group as compared to the benign nodular thyroid disease group ($p < 0.05$).

A low or intermediate anisokaryosis, a homogeneous chromatin, Hurthle cells, a normofollicular/macrofollicular structure, a microfollicular structure, a abundant

amount of colloid and liquid colloid were statistically significantly more frequent in the benign nodular thyroid disease group as compared to the PTC group ($p < 0.05$). The data on the frequencies of cytomorphological features and correlations between the comparative groups are presented in Table 1.

TABLE 1. Data of the cytological analysis of comparative groups, n = 288.

Cytomorphological features	Papillary thyroid cancer, n=143	Benign nodular thyroid disease, n=145	*p* value
Neuclei: Monomorphic Polymorphic	1 (0.7%) 142 (99.3%)	1 (0.7%) 144 (99.3%)	0,992*
Anisokaryosis: Not detected Low Intermediate High	0 (0%) 10 (7%) 74 (51.7%) 59 (41.3%)	2 (1.4%) 36 (24.8%) 94 (64.8%) 13 (9%)	0,498* **0,000**** **0,024**** **0,000****
Homogenous chromatin: Absent Present	88 (61.5%) 55 (38.5%)	14 (9.7%) 131 (90.3%)	0,000**
Streaked chromatin: Absent Present	31 (21.7%) 112 (78.3%)	89 (61.4%) 56 (38.6%)	0,000**
Coarsely dispersed chromatin: Absent Present	87 (60.8%) 56 (39.2%)	103 (71%) 42 (29%)	0,068**
Finely dispersed chromatin: Absent Present	61 (42.7%) 82 (57.3%)	88 (60.7%) 57 (39.3%)	0,002**
Nucleoli: Absent Present	13 (9.1%) 130 (90.9%)	28 (19.3%) 117 (80.7%)	0,013**
Amount of cytoplasm: Sufficient Abundant	76 (53.1%) 67 (46.9%)	84 (57.9%) 61 (42.1%)	0,414**
Intensity of cytoplasm: Light Dark	61 (42.7%) 82 (57.3%)	116 (80%) 29 (20%)	0,000**
Degeneration: Absent Present	80 (55.9%) 63 (44.1%)	83 (57.2%) 62 (42.8%)	0,824**

Cytomorphological features	Papillary thyroid cancer, n=143	Benign nodular thyroid disease, n=145	*p* value
Hurthle cells:			
Absent	119 (83.2%)	94 (64.8%)	0,000**
Present	24 (16.8%)	51 (35.2%)	
Lymphocytes:			
Absent	63 (44.1%)	73 (50.3%)	0,285**
Present	80 (55.9%)	72 (49.7%)	
Makrophages:			
Absent	54 (37.8%)	66 (45.5%)	0,182**
Present	89 (62.2%)	79 (54.5%)	
Normofollicular/ Macrofollicular structures:			
Absent	109 (76.2%)	82 (56.6%)	0,000**
Present	34 (23.8%)	63 (43.4%)	
Microfollicular structures:			
Absent	55 (38.5%)	38 (26.2%)	0,026**
Present	88 (61.5%)	107 (73.8%)	
Papillary structures:			
Absent	43 (30.1%)	131 (90.3%)	0,000**
Present	100 (69.9%)	14 (9.7%)	
Atypia:			
Absent	5 (3.5%)	34 (23.4%)	0,000**
Present	138 (96.5%)	111 (76.6%)	
Cytoplasmic inclusions:			
Absent	43 (30.1%)	128 (88.3%)	0,000**
Present	100 (69.9%)	17 (11.7%)	
Psammoma bodies:			
Absent	125 (87.4%)	143 (98.6%)	0,000*
Present	18 (12.6%)	2 (1.4%)	
Colloid:			
Absent	39 (27.3%)	23 (15.9%)	**0,018****
Small amount	88 (61.5 %)	80 (55.2%)	0,273**
Intermediate amount	9 (6.3%)	19 (13.1%)	0,051**
Abundant	7 (4.9%)	23 (15.9%)	0,002**
Consistency of colloid:			
No colloid	39 (27.3%)	23 (15.9%)	**0,018****
Liquid	34 (23.8%)	104 (71.7%)	**0,000****
Dense	70 (49%)	18 (12.4%)	**0,000****
Eosinophilic colloid bodies:			
Absent	98 (68.5%)	124 (85.5%)	0,001**
Present	45 (31.5%)	21 (14.5%)	

* Fisher's Exact Test; ** Pearson's Chi-Squared Test (χ^2); Values in bold represent statistically significant correlations.

Bethesda System Categories

A comparison of the distributions of the Bethesda system categories in the PTC and the benign nodular thyroid disease groups showed statistically significantly higher frequencies of categories III and IV in the benign nodular thyroid disease group ($p < 0.05$). Conversely, V and VI categories were statistically significantly more frequent in the PTC group ($p < 0.05$). The frequencies of the Bethesda system categories and correlations between comparative groups are presented in Table 2.

TABLE 2. The distribution of the Bethesda system categories in the comparative groups, n = 288.

Bethesda system categories	Papillary thyroid cancer, n=143	Benign nodular thyroid disease, n=145	*p* value
III	14 (9.8%)	28 (19.3%)	**0,022***
IV	22 (15.4%)	104 (71.7%)	**0,000***
V	41 (28.7%)	10 (6.9%)	**0,000***
VI	66 (46.2%)	3 (2.1%)	0,000**

* Pearson's Chi-Squared Test (χ^2); ** Fisher's Exact Test; Values in bold represent statistically significant correlations.

BRAF V600E mutation Testing

The testing performed in cytological specimens or in both cytological and histological specimens showed that the BRAF V600E mutation was statistically significantly more frequent in the PTC group ($p < 0.05$). The results of the BRAF V600E mutation testing and correlations between the groups are presented in Table 3.

TABLE 3. Results of the BRAF V600E mutation testing in the comparative groups, n = 288.

Results of mutation testing:	Papillary thyroid cancer, n=143	Benign nodular thyroid disease, n=145	*p* value
BRAF V600E (cytological specimens):			
Negative	52 (36.4%)	145 (100%)	
Positive	91 (63.6%)	0 (0%)	0,000*
BRAF V600E (cytological and histological specimens):			
Negative	33 (23.1%)	145 (100%)	
Positive	110 (76.9%)	0 (0%)	0,000*

* Fisher's Exact Test; Values in bold represent statistically significant correlations.

A BRAF V600E mutation was detected in 8 (57.1%) PTC cases of the Bethesda III category, 11 (50%) of Bethesda IV, 33 (80.5%) Bethesda V and 58 (87.9%) PTC cases of the Bethesda VI category.

In a classical PTC variant, a BRAF V600E mutation was detected in 88 (83%) cases, in an oncocytic PTC variant – 11 (78.6%), follicular PTC – 11 (47.8%) cases.

Data of Surgical Treatment

Results of the urgent intraoperative histological examination, types of surgeries and lymph node dissection modes were not compared. Detailed data of surgical treatments are shown in Table 4.

TABLE 4. Data of surgical treatments, n = 288.

Feature	Papillary thyroid cancer, n=143	Benign nodular thyroid disease, n=145
Report of urgent intraoperative histological examination:		
Performed	113:	145:
Non-informative	2 (1.8%)	1 (0.7%)
Benign nodular thyroid disease	12 (10.6%)	144 (99.3%)
Papillary thyroid cancer	99 (87.6%)	0 (0%)
Type of surgery		
Thyroidectomy	141(98.6%)	75 (51.7%)
Lobisthmectomy	2 (1.4%)	67 (46.2%)
Isthmectomy	0 (0%)	3 (2.1%)
Type of lymph node dissection:		
Performed	116:	4:
Central unilateral	99 (85.3%)	4 (100%)
Central bilateral	11 (9.5%)	0 (0%)
Modified radical unilateral	3 (2.6%)	0 (0%)
Central bilateral and modified radical unilateral	3 (2.6%)	0 (0%)

Results of the Final Histolog ical Examination in the Group of Papillary Thyroid Cancer (PTC)

The mean tumor size in the final histological examination of the 143 patients with PTC (87.7% of all thyroid cancer cases) was 1.5 (0.3–7) cm. A tumor extension beyond the thyroid capsule was observed in 59 patients (41.3%). Of the 116 patients in whom a lymph node dissection was performed, 49 patients (42.2%) had metastases in the neck lymph nodes. A lymphovascular invasion was detected in 52 patients (36.4%). Multifocal thyroid cancer was detected in 55 patients (38.5%). An

encapsulated tumor was detected in 47 patients (32.9%). All cases were classified according to the TNM Classification of Malignant Tumors. According to the evaluation of tumor size and the extension to the surrounding tissues, 39 cases (27.3%) were classified as T1a, 34 (23.8%) – as T1b, 11 (7.7%) – as T2, 59 (41.3%) – as T3. According to the evaluation of tumor spread into the regional lymph nodes, 27 cases (18.9%) were classified as Nx (no lymph node dissection), 67 (46.9%) – as N0, 45 (31.5%) – as N1a, 4 (2.8%) – as N1b. Distant metastases (M1) were not detected in any patient. According to clinical and histological data, 83 cases (58%) were diagnosed with stage I, 3 (2.1%) – stage II, 24 (16.8%) – stage III, 33 (23.1%) – IVA disease stage. 106 patients (74.1%) had a classical PTC variant, 23 (16.1%) – a follicular PTC, 14 (9.8%) – an oncocytic PTC variant.

Significance of the Bethesda System for the Diagnostics of PTC

Based on the results of 288 cytological analyses, all cases were classified according to the Bethesda System for Reporting Thyroid Cytopathology; 42 cases (14.6%) were classified as belonging to category III, 126 (43.8%) – to category IV, 51 (17.7%) – to category V, 69 (24%) – to category VI.

Out of 42 patients ascribed to category III according to the cytological analysis, PTC in the final histological examination was detected in 14 (33.3%) patients, while 28 (66.7%) patients had benign nodular thyroid disease. Out of 126 patients ascribed to category IV, PTC in the final histological examination was detected in 22

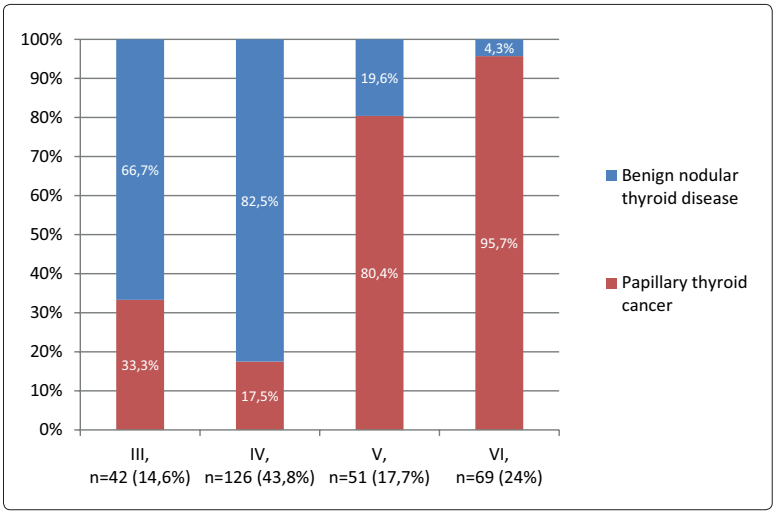


FIGURE 2. The Significance of the Bethesda System for PTC diagnostics, n = 288.

(17.5%) patients, while 104 (82.5%) patients had benign nodular thyroid disease. Of the 51 patients ascribed to category V, 41 (80.4%) patients had PTC and 10 (19.6%) patients had benign nodular thyroid disease. Out of 69 patients classified as category VI, 66 (95.7%) patients had PTC, 3 (4.3%) patients had benign nodular thyroid disease (Figure 2).

The highest frequency of PTC, 95.7%, was observed in the cytological category VI, and statistically significant differences as compared to the frequencies of PTC in the other three categories of the Bethesda system were observed. In comparison of PTC and the benign nodular thyroid disease in categories III and VI, $p = 0.000$ ($p < 0.05$), IV and VI, $p = 0.000$ ($p < 0.05$), V and VI, $p = 0.015$ ($p < 0,05$).

Following these results, it was decided that there are no diagnostic problems with Bethesda category VI; therefore, a further analysis of PTC diagnostics included only indeterminate diagnostic categories of PTC (Bethesda III, IV, V).

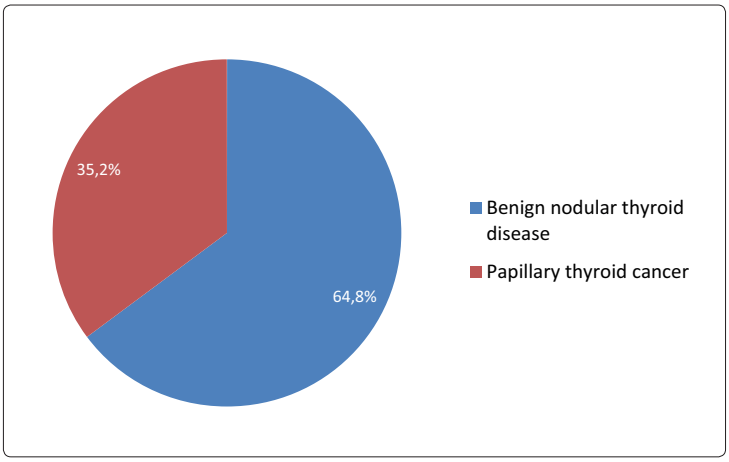


FIGURE 3. The distribution of the histological examination results in the cytologically indeterminate diagnostic categories, n = 219.

Out of 219 patients ascribed to indeterminate diagnostic categories according to the results of the cytological analysis, the final histological examination revealed PTC in 77 (35.2%) patients and the benign nodular thyroid disease in 142 (64.8%) patients (Figure 3).

The Significance of the Cytomor phological Features for PTC Diagnostics in the Indeterminate Diagnostic Categories

In order to differentiate PTC from the benign nodular thyroid disease in the cytologically indeterminate diagnostic categories, a binary logistic regression model that analyzes the effect of cytomorphological features on the results of the final histological examination was developed. The final binary logistic regression model was expressed by the equation:

z (probability ratio logit function) = 0,84 + (-1,14 × homogeneous chromatin) + + (-1,04 × liquid colloid) + (1,13 × eosinophilic colloid bodies) + (1,73 × papillary structures) + (1.74 x cytoplasmic inclusions).

Here the homogeneous chromatin, the liquid colloid, eosinophilic colloid bodies, papillary structures and cytoplasmic inclusions are expressed as the numerical values obtained in the study, with two values being 0 (absent) or 1 (present).

The logistic model was well-suited for the analyzed data and, in a way, was optimal, too: all coefficients significantly differed from 0, the adding of an additional variable did not improve the model, the log probability function of the model was the highest. The suitability of the model for the analyzed data was confirmed by the χ^2 statistics criterion of the probability ratio of the model, equal to 99.43, $p = 0.000$ (<0.05). The χ^2 statistics of the Hosmer-Lemeshow test was 2.72, $p = 0.744$ (> 0.05). The Cox & Snell R Square determination coefficient was 0.37, while Nagelkerke was equal to 0.50. According to the Wald criterion, all regressors were statistically significant, i.e., for all regressors, $p < 0.05$.

Out of 219 patients, the regression analysis of 154 (70.3%) patients classified them as having a low risk of PTC, while 65 (29.7%) were classified as having a high risk of PTC.

Out of 154 patients who were classified by the regression model as having a low risk of PTC, the final histological examination revealed PTC in 24 (15.6%) of the patients, and the benign nodular thyroid disease in 130 (84.4%) of the patients. Of the 65 patients who were classified by the regression model as having a high risk of PTC, the final histological examination revealed PTC in 53 of the (81.5%) patients, and the benign nodular thyroid disease in 12 (18.5%) of the patients (Figure 4).

According to the risk of having PTC and the results of the final histological examination, all cases were clasified as true positive, true negative, false positive and false negative. Fifty three cases were considered to be true positive, 130 were true negative, 12 were false positive, 24 were false negative. Calculated diagnostic accuracy measures of the model are shown in Table 5.

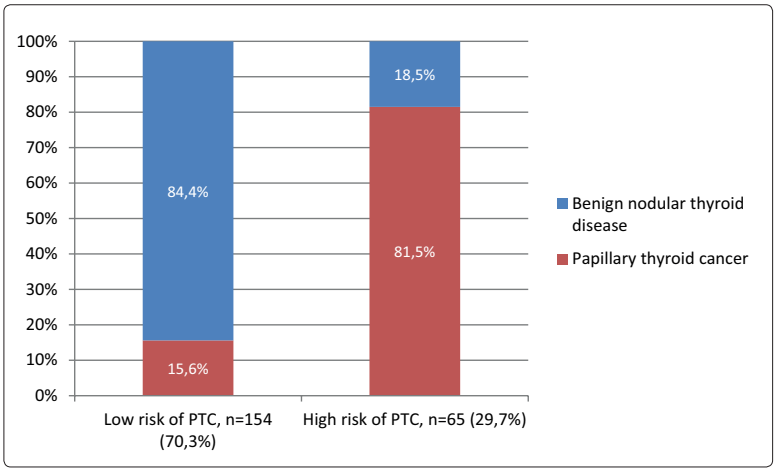


FIGURE 4. The diagnostic value of the regression model based on the analysis of cytomorphological features, n = 219.

TABLE 5. The diagnostic accuracy measures of the regression model based on the analysis of cytomorphological features, n = 219.

Measure	Value	95% confidence interval
Sensitivity	68.8%	57,3-78,9
Specificity	91.6%	85,7-95,6
Positive predictive value	81.5%	71,6-88,6
Negative predictive value	84.4%	79,5-88,3
Accuracy	83.6%	78-88,2

The Significance of the BRAF V600E Mutation Testing for PTC Diagnostics in the Indeterminate Diagnostic Categories

Of the 219 patients ascribed to indeterminate diagnostic categories, a BRAF V600E mutation was detected in 52 patients (23.7%). Of these, 37 patients (71.2%) had a BRAF V600E mutation in FNA specimens, 15 (28.8%) – in the histological specimens. A BRAF V600E mutation was not detected in 167 (76.3%) patients.

Of the 52 patients with a BRAF V600E mutation, the final histological examination revealed PTC in all 52 patients (100%). Of the 167 patients without a BRAF V600E mutation, the final histological examination revealed PTC in 25 (15%) patients and the benign nodular thyroid disease in 142 (85%) patients. Statistically significant differences of the BRAF V600E mutation distribution in the comparative groups were found, $p = 0.000$ ($p < 0.05$) (Figure 5).

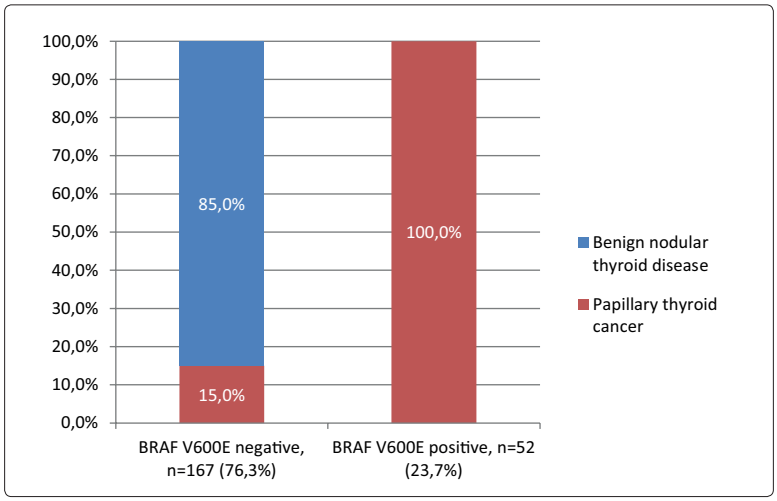


FIGURE 5. The diagnostic value of a BRAF V600E mutation status, n = 219.

The verification of the hypothesis that the results of the final histological examination depend on the mutation status for a BRAF V600E mutation revealed a statistically significant correlation between a BRAF V600E mutation and PTC detected in the final histological examination: $r = 0.76$, $p = 0.000$ ($p < 0{,}05$).

According to the mutation status for a BRAF V600E mutation and the results of the final histological examination, all cases were categorized to true positive, true negative, false positive and false negative. Fifty-two cases were true positive, 142 were true negative, 0 were false positive, 25 were false negative. Calculated diagnostic accuracy measures are presented in Table 6.

TABLE 6. The diagnostic measures of a BRAF V600E mutation testing.

Measure	Value	95% confidence interval
Sensitivity	67.5%	55,9–77,8
Specificity	100%	97,4–100
Positive predictive value	100%	–
Negative predictive value	85%	80,5–88,7
Accuracy	88.6%	83,6–92,5

The Significance of BRAF V600E Mutation Testing Combined with the Analysis of Cytomorphological Features for PTC Diagnostics in the Indeterminate Diagnostic Categories

In order to refine the diagnostics of PTC in the cytologically indeterminate diagnostic categories, we added the results of the BRAF V600E mutation testing and pre-operative clinical data (gender, age, the relevant Bethesda system category) to the results of the cytological analysis and developed an additional binary logistic regression model. The final binary logistic regression model was expressed by the equation:

z (probability ratio logit function) = 1.82 + (-1.46 × liquid colloid consistency) + + (1.57 × eosinophilic colloid bodies) + (2.16 × papillary structures) + (22.79 × ×BRAF V600E mutation).

Here liquid colloid consistency, eosinophilic colloid bodies, papillary structures, and the BRAF V600E mutation are expressed as the numerical values obtained in the study, with two values being 0 (absent) or 1 (present).

The logistic model was well suited for the analyzed data and, in a way, was optimal, too: all coefficients significantly differed from 0, the adding of an additional variable did not improve the model, the log probability function of the model was the highest. The suitability of the model for the analyzed data was confirmed by the χ^2 statistics criterion of the probability ratio of the model, equal to 177,2, p=0,000 (<0,05). The χ^2 statistics of the Hosmer-Lemeshow test was 0,14, p=0,998 (>0,05). The Cox & Snell R Square determination coefficient was 0,56, while Nagelkerke was equal to 0,76. According to the Wald criterion, all regressors were statistically significant, i.e., for all regressors, p <0.05, except for the BRAF V600E mutation, which was exempted due to its high significance for the final result of the regression equation.

Of the 219 patients, the regression model classified 156 patients (71.2%) as having a low risk of PTC, and 63 (28.8%) patients as having a high risk of PTC.

Of the 156 patients who were classified by the regression model as having a low risk of PTC, the final histological examination revealed PTC in 15 (9.6%) patients and the benign nodular thyroid disease in 141 (90.4%) patients. Of the 63 patients who were classified by the regression model as having a high risk of PTC, the final histological examination revealed PTC in 62 (98.4%) patients and the benign nodular thyroid disease in 1 (1.6%) patient (Figure 6).

According to the risk of having PTC and the results of the final histological examination, all cases were categorized into true positive, true negative, false positive and false negative. Sixty two cases were considered to be true positive, 141 – true

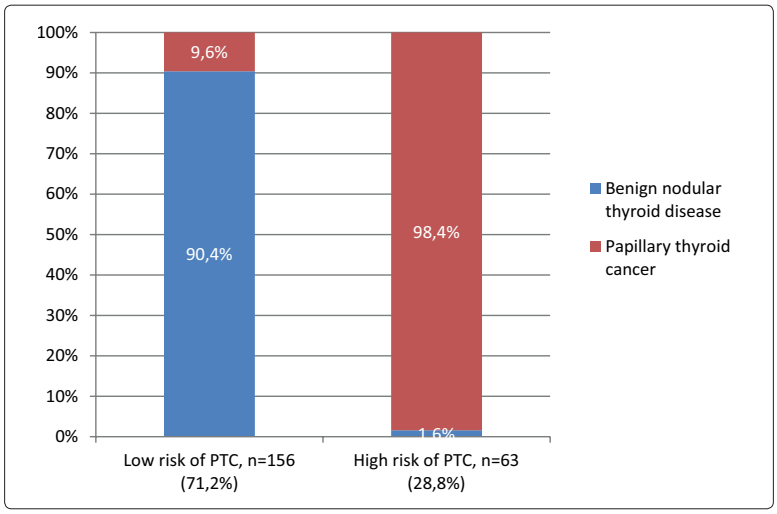


FIGURE 6. The diagnostic value of the regression model based on a BRAF V600E mutation and the analysis of cytomorphologic features, n = 219.

negative, 1 – false positive, 15 – false negative. Calculated diagnostic accuracy measures are presented in Table 7.

TABLE 7. The diagnostic accuracy measures of the regression model based on the analysis a BRAF V600E mutation and the analysis of cytomorphological features, n = 219.

Measure	Value	95% confidence interval
Sensitivity	80.5%	69,9–88,7
Specificity	99.3%	96,1–100
Positive predictive value	98.4%	89,8–99,8
Negative predictive value	90.4%	85,7–93,7
Accuracy	92.7%	88,4–95,8

The Significance of the Bethesda System for Predicting Signs of Histological PTC Aggressiveness

The comparisons of signs of histological aggressiveness in indeterminate diagnostic categories (III, IV, V) and category VI revealed that in group VI, tumors were statistically significantly larger, an extrathyroid tumor extension and a higher stage of cancer was observed more often ($p < 0.05$). There were no other statistically significant differences between the groups. Frequencies of signs, reliability levels and correlation indices are given in Table 8.

TABLE 8. The significance of the Bethesda system for predicting signs of aggressiveness, n = 143.

Feature	Bethesda III-V, n=77	Bethesda VI, n=66	*p* value	*r* correlation coefficient
Size (cm):	1,1 (0,3-3,8)	1,5 (0,3-7)	0,008*	0,24***
Extrathyroid extension: Absent Present	 54 (70.1%) 23 (29.9%)	 30 (45.5%) 36 (54.5%)	**	****
Metastases in lymph nodes (n=116): Absent Present	 34 (61.8%) 21 (38.2%)	 33 (54.1%) 28 (45.9%)	0,401**	-
Lymphovascular invasion: Absent Present	 53 (68.8%) 24 (31.2%)	 38 (57.6%) 28 (42.4%)	0,163**	
Multifocality: Absent Present	 52 (67.5%) 25(32.5%)	 36 (54.5%) 30(45.5%)	0,112**	-
Encapsulated tumor: Absent Present	 47 (61%) 30 (39%)	 49(74.2%) 17 (25.8%)	0,094**	-
Stage: I-II III-IVA	 53 (68.8%) 24 (31.2%)	 33 (50%) 33 (50%)	**	****

* Mann-Whitney-Wilcoxon Test; ** Pearson's Chi-Squared Test ($\chi 2$); *** Eta coefficient; **** Cramer's V coefficient; Values in bold represent statistically significant correlations.

The Significance of a BRAF V600E Mutation for Predicting the Signs of PTC Histological Aggressiveness

Comparisons of signs of the histological aggressiveness in BRAF V600E negative and BRAF V600E positive cases did not reveal any statistically significant differences ($p \geq 0.05$). Frequencies of signs and reliability levels are given in Table 9.

TABLE 9. The significance of a BRAF V600E mutation for predicting signs of PTC aggressiveness, n = 143.

Feature	BRAF V600E negative, n=33	BRAF V600E positive, n=110	*p* value
Size (cm):	0,9 (0,3-7)	1,3 (0,3-5)	0,073*

Feature	BRAF V600E negative, n=33	BRAF V600E positive, n=110	*p* value
Extrathyroid extension: Absent Present	 23 (69.7%) 10 (30.3%)	 61 (55.5%) 49 (44.5%)	 0,145**
Metastases in lymph nodes (n=116): Absent Present	 13(59.1%) 9 (40.9%)	 54 (57.4%) 40 (42.6%)	 0,888**
Lymphovascular invasion: Absent Present	 24 (72.7%) 9 (27.3%)	 67 (60.9%) 43 (39.1%)	 0,216**
Multifocality: Absent Present	 19 (57.6%) 14 (42.4%)	 69 (62.7%) 41(37.3%)	 0,594**
Encapsulated tumor: Absent Present	 18 (54.5%) 15 (45.5%)	 78 (70.9%) 32 (29.1%)	 0,079**
Stage: I-II III-IVA	 23 (69.7%) 10 (30.3%)	 63 (57.3%) 47 (42.7%)	 0,201**

* Mann-Whitney-Wilcoxon Test; ** Pearson's Chi-Squared Test (χ^2)

Conclusions

1. Patients' data analysis shows that the frequency of PTC in category III is 33.3%, in category IV – 17.5%, in category V – 80.4%, in category VI – 95.7%;
2. A BRAF V600E mutation was detected in 76.9% of the patients with PTC;
3. The PTC diagnostic sensitivity of the logistic regression model that is based on the analysis of the cytomorphological features and includes five cytolomorphological features, applied in the indeterminate diagnostic categories, is 68.8%, the specificity is 91.6%, the positive predictive value is 81.5%, the negative predictive value is 84.4%, accuracy is 83.6%;
4. The PTC diagnostic sensitivity of a BRAF V600E mutation in the indeterminate diagnostic categories is 67.5%, specificity is 100%, positive predictive value is 100%, negative predictive value is 85%, accuracy is 88.6%;
5. The PTC diagnostic sensitivity of the logistic regression model that is based on the BRAF V600E mutation testing combined with the analysis of the

cytomorphological features and includes three cytomorphological features, applied in the indeterminate diagnostic categories, is 80.5%, specificity is 99.3%, positive predictive value is 98.4%, negative predictive value is 90.4%, accuracy is 92.7%;

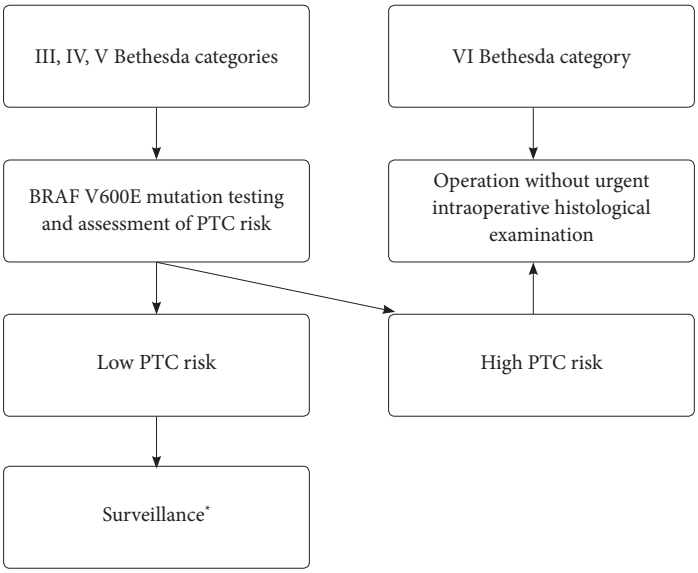
6. The categories of the Bethesda system have a prognostic value. Statistically significant differences were found with a weak or very weak direct correlation between category VI and tumor size ($p = 0.008$, $r = 0.24$), extrathyroid extension ($p = 0.003$, $r = 0.25$) and the stage of cancer ($p = 0.022$, $r = 0.19$);
7. A BRAF V600E mutation is not suitable for predicting histological aggressiveness. There were no statistically significant differences in the histological findings in BRAF V600E negative and BRAF V600E positive patient groups.

Recommendations

- For patients who are classified as category VI according to the Bethesda system, we recommend surgical treatment. Due to the high risk of the PTC in this category, an urgent, intraoperative, histological examination of resected tissues is not recommended in this category of patients.
- When samples are categorized as indeterminate diagnostic categories (III, IV, V) according to the Bethesda system, we recommend to test them additionally for a BRAF V600E mutation. The risk of PTC in these patients should be assessed by using a calculator based on the BRAF V600 mutation testing combined with the analysis of the cytomorphological features. A calculator developed for clinical applications may be found on the internet at http://ptc-calc.we2host.lt/.
- Active surveillance is recommended for patients who are ascribed to indeterminate diagnostic categories and in whom:
  1. On the US scan, there are no signs of disease spread;
  2. Calcitonin levels in the blood do not exceed normal ranges;
  3. There is a low risk of PTC based on the results of the calculator that combines the results of the BRAF V600 mutation testing and the analysis of the cytomorphological features;

     In these patients, indications for an ultrasound examination with a fine needle biopsy and a BRAF V600E mutation testing should be reevaluated based on disease progression.
- For patients who are ascribed to the group of high PTC risk according to the calculator that combines the BRAF V600E mutation testing and the analysis of the cytomorphological features, we recommend surgical treatment. Due to the high risk of the PTC in this category, an urgent, intraoperative, histological examination of resected tissues is not recommended in this group of patients.

An algorithm of diagnostic recommendations is presented in Figure 7.



* There are no signs of disease spread on the US scan; calcitonin levels in the blood do not exceed normal ranges.

FIGURE 7. The algorithm of diagnostic recommendations.

The Presentation and Approbation of the Results

Publications:

1. Beiša A, Beiša V, Stoškus M, Ostanevičiūtė E, Griškevičius L, Strupas K. "The Value of the Repeated Examination of BRAF V600E Mutation Status in Diagnostics of Papillary Thyroid Cancer." *Endokrynol Pol*. 2016;67(1):35–40.

2. Beiša A, Kvietkauskas M, Beiša V, Stoškus M, Ostanevičiūtė E, Jasiūnas E, Griškevičius L, Strupas K. "The Utility of the Bethesda Category and Its Association with BRAF Mutation in the Prediction of Papillary Thyroid Cancer Stage." *Langenbecks Arch Surg*. 2017 Mar;402(2):227–234.

3. Beiša A, Kvietkauskas M, Beiša V, Stoškus M, Ostanevičiūtė E, Jasiūnas E, Griškevičius L, Šeinin D, Šileikytė A, Strupas K. "Significance of BRAF V600E Mutation and Cytomorphological Features for the Optimization of Papillary Thyroid Cancer Diagnostics in Cytologically Indeterminate Thyroid Nodules." *Exp Clin Endocrinol Diabetes*, DOI: 10.1055/a-0588-4885.

Presentations:

1. Beiša A, Beiša V, Stoškus M, Ostanevičiūtė E, Juškevičius D, Petroška D, Griškevičius L, Strupas K. BRAF V600E. "Opportunity to Decrease the Amount of Thyroid Surgery." The 5th Workshop of the European Society of Endocrine Surgery (ESES). May 23–25, 2013. Berlin, Germany.

2. Beiša A, Beiša V, Ostanevičiūtė E, Stoškus M, Juškevičius D, Petroška D, Griškevičius L, Strupas K. "Cancer Papillaire de la Thyroid. Expression des Mutations BRAF V600E. Évaluation et de Leurs Methode de Determination." 13th Congrés de l' Association Francophone de Chirurgie Endocrinienne (AFCE). June 14, 2013. Lyon, France.

3. Beiša A, Beiša V, Ostanevičiūtė E, Stoškus M, Griškevičius L, Strupas K. "The Potential Utility of BRAF V600E Status Follow-up in FNA of Thyroid Nodule." The 6th Workshop of the European Society of Endocrine Surgery (ESES). May 28–30, 2015. Varna, Bulgaria.

4. Beiša A, Kvietkauskas M, Beiša V, Stoškus M, Ostanevičiūtė E, Griškevičius L, Strupas K. "The Utility of the BRAF Mutation Status and Bethesda Category in the Prediction of Papillary Thyroid Cancer Stage." The 3rd International Conference of Evolutionary Medicine: Pre-Existing Mechanisms and Patterns of Current Health Issues. June 14–19, 2016. Vilnius, Lithuania.

5. Beiša A, Kvietkauskas M, Beiša V, Stoškus M, Ostanevičiūtė E, Griškevičius L, Strupas K. "The Utility of the Bethesda Category and Its Association with BRAF Mutation in the Prediction of Papillary Thyroid Cancer Stage." The 7th Symposium of the European Society of Endocrine Surgery (ESES). April 6–8, 2017. Oxford, United Kingdom.

6. Beiša A, Kvietkauskas M, Beiša V, Stoškus M, Ostanevičiūtė E, Griškevičius L, Strupas K. "The Value of BRAF V600E Mutation Status Combined with Cytomorphological Features for Surgical Treatment Optimisation of Papillary Thyroid Cancer." A conference for young scientists, organized by the Biological, Medical and Geoscience Division of LAS. Biofuture: the Perspective of Nature and Life Sciences. December 7, 2017. Vilnius, Lithuania.

7. Beiša A, Kvietkauskas M, Beiša V, Stoškus M, Ostanevičiūtė E, Jasiūnas E, Šeinin D, Šileikytė A, Griškevičius L, Strupas K. "The Diagnostic Value of BRAF V600E Mutation Status Combined with Cytomorphological Features for Diagnosis of Papillary Thyroid Cancer in Cytologically Indeterminate Thyroid Nodules." The 8th Biennal Congress of the European Society of Endocrine Surgery (ESES). May 24–26, 2018. Amsterdam, the Netherlands.

Studies and Research Activities:

Posters and Presentations in Local and International Conferences

2013. Congress of European Endocrine Surgery in Berlin, Germany. BRAF V600E. "Opportunity to Decrease the Amount of Thyroid Surgery." Oral presentation.

2013. Conference of Endocrine Surgeons – Phrancophones association in Lille, France. "Cancer Papillaire de la Thyroid. Expression des Mutations BRA F V600E Évaluation et de Leurs Methode de Determination." Poster presentation.

2015. European Congress of Endocrine Surgery in Varna, Bulgaria. "The Potential Utility of BRAF V600E Status Follow-up in FNA of Thyroid Nodule." Poster presentation.

2016. The 3rd International Conference of Evolutionary Medicine: Pre- Existing Mechanisms and Patterns of Current Health Issues in Vilnius, Lithuania. "The Utility of the BRAF Mutation Status and Bethesda Category in the Prediction of Papillary Thyroid Cancer Stage." Oral presentation.

2017. The 7th Symposium of the European Society of Endocrine Surgery (ESES) in Oxford, United Kingdom. "The Utility of the Bethesda Category and Its Association with BRAF Mutation in the Prediction of Papillary Thyroid Cancer Stage." Poster presentation.

2017. A conference for young scientists organized by the Biological, Medical and Geoscience Division of LAS. Biofuture: the Perspective of Nature and Life Sciences in Vilnius,

Lithuania. "The Value of BRAF V600E Mutation Status Combined with Cytomorphological Features for Surgical Treatment Optimisation of Papillary Thyroid Cancer." Oral presentation.

2018. The 8th Biennal Congress of the European Society of Endocrine Surgery (ESES) in Amsterdam, the Netherlands. "The Diagnostic Value of BRAF V600E Mutation Status Combined with Cytomorphological Features for Diagnosis of Papillary Thyroid Cancer in Cytologically Indeterminate Thyroid Nodules." Poster presentation.

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1. Beiša V., Lagunavičius K., Beiša A., Strupas K. "Parathyroidectomia: Treatment of Secondary Hyperparathyroidism. Causes and Pro- phylaxis of Disease Relapse." *Acta Medica Lithuanica*. 2011. Vol. 18. No. 4. P. 170–174

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1. Beiša V, Beiša A, Ūselis S, Strupas K. "Parathyromatosis after Parathy- roidectomy because of Primary Hyperparathyroidism: A Case Report." *Cent Eur J Med*. 2012;7(3): 371–5.

2. Šileikis A, Beiša V, Beiša A, Samuilis A, Šerpytis M., Strupas K. "Minimally Invasive Retroperitoneal Necrosectomy in Management of Acute Necrotizing Pancreatitis." *Videosurgery Miniinv*. 2013;8(1):29– 35.

3. Šilekis A, Beiša A, Zdanytė ES, Jurevičius S, Strupas K. "Minimally Invasive Management of Pancreatic Pseudocysts." *Videosurgery Miniinv*. 2013;8(3):211–5.

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5. Beiša A, Beiša V, Stoškus M., Ostanevičiūtė E, Griškevičius L, Strupas K. "The Value of the Repeated Examination of BRAF V600E Mutation Status in Diagnostics of Papillary Thyroid Cancer." *Endokrynol Pol*. 2016;67(1):35–40. doi:10.5603/EP.2016.0005.

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7. Beiša A, Kvietkauskas M, Beiša V, Stoškus M, Ostanevičiute E, Jasiūnas E, Griškevičius L, Strupas K. "The Utility of the Bethesda Category and Its Association with BRAF Mutation in the Prediction of Papillary Thyroid Cancer Stage." *Langenbeck Arch Surg*. 2017; doi:10.1007/ s00423-017-1560-2.

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Bronius Buckus

ADIPOSE TISSUE COMPOSITION AND DISTRIBUTION DIFFERENCES IN PREDICTING METABOLIC DISORDERS IN OBESE PATIENTS

RIEBALINIO AUDINIO PASISKIRSTYMO IR RIEBALŲ SUDĖTIES SKIRTUMAI TARP METABOLINIŲ SUTRIKIMŲ TURINČIŲ IR NETURINČIŲ NUTUKUSIŲ PACIENTŲ

Biomedical sciences, medicine (06B)
Biomedicinos mokslai, medicina (06B)

Abbreviations

CI – confidence interval
CVD – cardiovascular diseases
DBP – diastolic blood preassure
HDL – highdensity lipoprotein cholesterol
HOMA-IR – a homeostasis model assessment for insulin resistance
^{1}H-MRS – hydrogen-1 magnetic resonance spectroscopy
^{1}H-NMR – hydrogen-1 nuclear magnetic resonance
MAO – metabolically abnormal obese
MHO – metabolically healthy obese
MS – metabolic syndrome
MUFA – monounsaturrated fatty acid
nonMS – metabolic syndrome is absent
LDL – lowdensity lipoprotein cholesterol
PAT – preperitoneal adipose tissue
PUFA – polyunsaturrated fatty acid
SAT – subcutaneous adipose tissue
SBP – systolic blood preassure
SFA – saturated fatty acid
TG – triglycerides
UA – uric acid
VAT – visceral adipose tissue

1. Introduction

The prevalence of obesity is increasing worldwide, and more than 20% of all human population are obese. Obesity's prevalence has tripled during the last three decades (WHO 2017). There has been an increase of 28.3% in the rate of deaths related to high BMI globally since 1990 (Collaborators 2017). Each 5 kg/m^2 higher than one's recommended BMI is associated with a 30% higher overall mortality. Cardiovascular disease was the leading cause of death, diabetes was the second one, accompanied by chronic kidney, hepatic diseases and cancer (MacMahon et al. 2009). Metabolic syndrome is a risk factor for CVD, type 2 diabetes and is diagnosed in 60% of obese patients (Park et al. 2003).

It has been shown that there exists a subset of the obese population without any metabolic abnormalities. This phenotype of obesity is termed as metabolically healthy obesity. The overall MHO prevalence ranges from 6% to 75% (ReyLópez et al. 2014); thus, MHO patients might be at a lower risk of developing cardiovascular events or allcause mortality (Lassale et al. 2017; Eckel et al. 2016). Despite the excessive BMI or total amount of adipose tissue, these patients remain insulinsensitive and have a normal lipid profile and blood pressure. It was determined that MHO has a distinct fat distribution pattern, a favorable inflammatory profile as well as different adipose tissue metabolism and composition (Mathew et al. 2016).

Until the 1950s adipose tissue was characterized as a connective tissue that contained lipid droplets (Rosen & Spiegelman 2014). Over the past decade and a half,

adipose tissue became to be considered as an endocrine organ that plays a critical role in the maintenance of energy homeostasis in the autocrine, paracrine and endocrine levels (Harwood 2012; Wozniak et al. 2009; Galic et al. 2010; Esteve Ràfols 2014).

Obesity is characterized by an excessive storage of fatty acids in an expanding adipose tissue mass. In the situation of overfeeding, individual adipose tissue has an increased demand for lipid storage. It was shown that a healthy adipose tissue storage capacity involves adipocyte hyperplasia, and dysfunctional remodeling involves hypertrophy. White adipose tissue expansion through hyperthrophy is associated with insulin resistance and cardiometabolic complications (Engin & Engin 2017). Studies revealed that the SAT has a finite capacity to expand, which is called *adipose expandability hypothesis*. When the personal fat threshold is reached, the lipid excess is deposited as an ectopic fat (Gesta & Kahn 2017; Cuthbertson et al. 2017; Dobson et al. 2016).

Visceral fat is a strong, independent predictor of all-cause mortality in men (Kuk et al. 2006). Recent studies demonstrated that it has a strong correlation with insulin resistance, type 2 diabetes, CVD, metabolic syndrome and cancer (Preis et al. 2011; Fox et al. 2007; Neeland et al. 2013; Liu et al. 2010; Britton et al. 2014; Smith et al. 2012; Oka et al. 2010; Shiina & Homma 2013; GletsuMiller et al. 2013). Nevertheless, it is still unknown what is the critical VAT threshold for the development of metabolic disturbances and a transition from the MHO to the MAO phenotype.

It is possible to quantify the amount of adipose tissue by various techniques, while anthropometry, ultrasonography, CT and MRT are used the most. It is possible to estimate the obesity by skinfold measurements, BMI or WHR; however, it is impossible to distinguish the different fat depots by these determinants. CT and MRI are the standard techniques for determining the distribution of adipose tissue. However, their limited availability, price and time required to perform the examination, the possible contraindications and radiation exposure during a CT scan are limiting the usage of these research tools in large epidemiologic studies. Meanwhile, the ultrasonography is easily performed, widespread, patients are not exposed to radiation during it and its results are comparable with those obtained by CT and MRI (Fox et al. 2007; Kim et al. 2004; Leite et al. 2002; RibeiroFilho et al. 2001; Bazzocchi et al. 2016) and between different examiners (Gouvea et al. 2013; Stoner et al. 2015; Philipsen et al. 2013). An ultrasonography can measure intra-abdominal fat more accurately than anthropometric measurements (Stolk et al. 2003; Bertoli et al. 2015; Meriño-Ibarra et al. 2005) and can be used in the obese population (Pontiroli et al. 2002; Sabir et al. 2001; Philipsen et al. 2015). Visceral fat thickness, measured by ultrasonography, is strongly associated with all MS components (Kim et al. 2004; Guldiken et al. 2006; Sogabe et al. 2012; Miranda Prado 2012; Soattin et al. 2013; Roever et al. 2016) and it is proposed that it could be used for the diagnosis of MS (RibeiroFilho et al. 2001; MeriñoIbarra et al. 2005; Chiba et al. 2007; Vasilescu et al. 2011).

Needle aspiration biopsy was the preferable method for obtaining adipose tissue samples in most of the human adipose tissue fatty acid composition studies. Recently, a non-invasive research tool was developed for measuring adipose tissue unsaturation *in vivo* – magnetic resonance spectroscopy. It is possible to distinguish different fat locations during the examination. ^{1}H-MRS (1.5 or 3 Tesla) used *in vivo* reduces the spectral dispersion of lipids and limits the usage of this tool and is not capable of distinguishing individual fatty acids. The low magnetic field 7 Tesla ^{1}H-MRS was introduced recently, but its clinical usage is still limited in clinical practice. However, ^{1}H-MRS allows to perform a repeated sampling of the same adipose tissue location, determine fatty acid composition (MUFA, PUFA and SFA ratios) and estimate its metabolic activity (Lundbom et al. 2016). Thus, it is possible to measure the total amount of lipids in various depots and its saturation ratio (Calderan et al. 2006; Velan et al. 2008; Lundbom et al. 2011; Naukkarinen et al. 2014; Lundbom et al. 2016). A recent study has shown that adipocyte insulin sensitivity correlates positively with their saturated fat content, and that the fat unsaturation degree is different in various fat localizations (Lundbom et al. 2013). It was performed with ^{1}H-MRS, but the results were not validated by the analysis of adipose tissue biopsies.

Nuclear magnetic resonance spectroscopy is widely used as a method in lipid analysis *in vitro*. It allows for structure elucidation as well as a qualitative and quantitative analysis of lipid molecules. NMR has several advantages compared to the commonly used chromatographic analysis. It is a nondestructive method, and, for quantification, no specific standards are necessary. For lipid investigation, ^{1}H, ^{13}C and ^{31}P nuclei are used for routine NMR. The ^{1}H nucleus with a spin quantum number I=1/2, a high gyromagnetic ratio and a natural abundance of 99.985% is the most sensitive NMR probe and, thus, appropriate for investigating even minor unsaturated lipids within a short experimental time (Alexandri et al. 2017; Knothe & Kenar 2004).

This study uses anthropometric, ultrasonographic and ^{1}H-MRS tools to determine whether superficial and deep subcutaneous, preperitoneal and visceral adipose tissue depots differ in their distribution and fatty acid composition in metabolically healthy and unhealthy obese populations.

2. The aim of the study

The aim of this study was to determine the distribution and composition of adipose tissue and its predictive value for metabolic disorders in obese patients.

3. Objectives

1. To evaluate the distribution of adipose tissue by anthropometric and ultrasonographic methods in obese patients with and without metabolic syndrome.
2. To determine the composition of superficial and deep subcutaneous, preperitoneal and visceral adipose tissues by ^{1}H NMR in obese patients with and without metabolic syndrome.
3. To evaluate the influence of superficial and deep subcutaneous, preperitoneal and visceral adipose tissue distributions and compositions on the emergence of metabolic disturbances in obese patients.

4. Scientific novelty of the study

1. The present study is the first prospective study determining the distribution of superficial, preperitoneal and visceral adipose tissues using ultrasonography and determining the composition of these tissues with ^{1}H NMR in obese patients.
2. The composition of preperitoneal adipose tissue was determined by ^{1}H NMR spectroscopy in obese patients for the first time.
3. This is the first prospective study evaluating the impact of preperitoneal adipose tissue ^{1}H NMR composition on metabolic disturbances in obese patients.
4. This study has identified the independent prognostic factors determining the occurrence of metabolic disturbances in obese patients.

5. Practical significance

1. The present study demonstrates that waist circumference should be measured as the narrowest waist circumference in obese patients.
2. The data of this study provides anthropometric measurement thresholds for metabolic syndrome in obese patients: the cutoff value for weight is 123.90 kg, and the cutoff value for waist circumference at midpoint is 123.40 cm, and the cutoff value for the narrowest waist circumference is 117.60 cm (calculated for an average man's height of 1.79 m and woman's height of 1.65 cm).

3. This study demonstrates that visceral adipose tissue thickness should be measured up to the lumbar vertebra using an ultrasonography in obese patients.
4. The data of this study provides ultrasonographic thresholds for metabolic syndrome in obese patients: the cutoff value for adipose tissue thickness up to the aorta is 8.93 cm, and the cutoff value for adipose tissue thickness up to the lumbar vertebra is 11.53 cm.
5. The present study demonstrates that the composition of superficial and deep subcutaneous adipose tissue should be evaluated separately in obese patients.
6. The present study demonstrates that preperitoneal adipose tissue should be distinguished from visceral adipose tissue in studies concerned with visceral adipose tissue.
7. The data of this study enabled us to build a prognostic model for distinguishing metabolic disturbances in obese patients.

6. Study hypotheses

1. There is no essential difference between skin thickness measurements in obese patients with and without metabolic syndrome.
2. Ultrasonography is an effective method for distinguishing obese patients with and without metabolic syndromes.
3. The ^{1}H NMR composition of adipose tissue is different between obese patients with and without metabolic syndrome.

7. Material and methods

7.1 Subjects and Study Design

The prospective cross-sectional study was approved by the Lithuanian Bioethics Committee on March 12, 2014.

7.2 Recruitment of Subjects

All the subjects included in our study were recruited at the Department of General Surgery of the Republican Vilnius University Hospital, where they were referred for surgical treatment. Inclusion criteria were as follows: aged between 18 and 65 years with the BMI more than 30 kg/m^2. The exclusion criteria for the study were pregnancy, mental diseases, contraindications for operative treatment. All participants signed a form of informed consent.

7.3 Assessment of the Subjects

All the participants underwent the standard examination, which included anthropometric measurements, blood tests, an ultrasonography of adipose tissue and an ^{1}H NMR of adipose tissue samples according to the study protocol.

The standard anthropometric measurements were those of height and weight, waist measurements (narrowest waist, midpoint between the lowest rib and the iliac crest and above the uppermost border of the iliac crest), hip circumference and skinfolds. The body mass index (BMI) was calculated as an individual's weight (kg) divided by height squared (m^2).

Blood samples for laboratory analyses were taken after a 12 h fasting. Laboratory tests included an assessment of fasting plasma glucose, total cholesterol, HDL, LDL, TG, UA, insulin, leptin, adiponectin concentrations. Blood tests were performed at the Centers of Laboratory Diagnostics of the Republican Vilnius University Hospital and Vilnius University Hospital Santaros Klinikos using standard laboratory methods. A homeostasis model assessment for insulin resistance (HOMA-IR) was used as an indicator for insulin resistance.

All ultrasonographic examinations were performed by a single investigator. Ultrasonography was performed with the Aloka ultrasonography machine (Japan). A modified method of Stolk was used to assess the subcutaneous, preperitoneal and visceral adipose tissue thickness (Figure 1). All adipose tissue measurements were performed on a supine subject following a strict protocol. All measurements were performed three times at the end of the quiet expiration without compression applied to the transducer. The mean values were taken for analysis.

Adipose tissue biopsies were taken at the beginning of surgery from superficial and deep subcutaneous (separated by subsutaneous fascial plane), preperitoneal (lo-

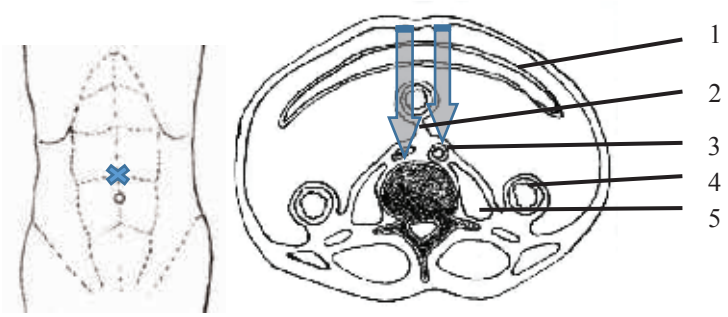

FIG. 1. Visceral adipose tissue thickness measurements by modified method of Stolk.
X – position of the ultrasound probe – 2 cm above umbilicus.
1. *omentum majus* 2. *mesenterium* 3. *aorta abdominalis* 4. *colon descendens* 5. *m. psoas major*

cated between parietal peritoneum and the transversal fascia) and visceral (omental) adipose tissues, which, in turn, were washed out in a normal saline solution and frozen immediately. Adipose tissue samples were stored in –70°C temperature before the chemical analyses were performed. The adipose tissue samples were homogenized and extracted using a modified Folch extraction procedure. ^{1}H NMR spectra were recorded on a Bruker Adipose tissue biopsies were taken at the beginning of surgery Ascend 400 spectrometer operating at 9.4 Tesla, corresponding to the from superficial and deep subcutaneous (separated by subsutaneous resonance frequency of 400 MHz for the ^{1}H nucleus, equipped with a fascial plane), preperitoneal (located between parietal peritoneum direct detection fournuclei probe head and field gradients on axis z. and the transversal fascia) and visceral (omental) adipose tissues, The subjects were classified according to the NCEP:ATPIII criteria into two groups, namely a group with metabolic syndrome and one without metabolic syndrome. High blood pressure was defined as SBP≥130 mmHg and/or DBP≥85 mmHg or higher and/or a patient using antihypertensive drugs; hypertriglyceridemia was defined as TG≥1,7 mmol/l; HDL cholesterolemia was defined as <1,03 mmol/l for men and <1,29 mmol/l for women and/or a patient using anticholesteremic drugs; high fasting plasma glucose was defined as ≥5,6 mmol/l; waist circumference was defined as abnormal >102 cm for men and >88 cm for women. MS was diagnosed for those who had 3 or more of the above criteria.

7.4 Statistical Analysis

The primary effect variable used for power and sample size calculation analysis was the thickness of visceral adipose tissue. Assuming the mean difference to be 2.3, the SD of 0.3 for groups, and aiming for the power of 0.90 and the type 1 error of 0.05, 29 patients were required in each group.

The Shapiro-Wilk test was used to examine the normality of data. The central tendency and dispersion of normally distributed data were described by mean and standard deviations. Student *t* and Mann-Whitney-Wilcoxon tests were used to compare parametric and nonparametric data, respectively. Pearson or Spearman correlations were computed among all measurements. Multivariate linear regression was used to evaluate the associations of the factors with the MS. Cutoff thresholds were obtained using a univariate analysis with receiver operating characteristic curves (ROC). Multiple logistic regression was used to determine the variables with a major effect on the MS. The odds ratio and 95% CI were calculated using the β coefficient from the logistic regression models.

The statistical software R-commander (version 3.2.4) and Excel (Microsoft Office 365) were used for data analysis. A p value of <0.05 was considered statistically significant.

8. Results

70 patients were recruited in the study between March 2014 and January 2018. Fourty eight participants (68.57%) were female. The mean age of the study population was 42.7±11.56 years. The mean BMI was 41.47±8.42 kg/m^2 (Tables 1, 2). In the MS group, participants were heavier and had higher BMIs than in the nonMS group.

TABLE 1. Baseline characteristics of the study population.

	Men (n=22)	Women (n=48)	*p* value
Age (years)	45.36±9.54	41.47±12.28	0.16
Height (m)	1.79±0.06	1.65±0.05	<0.001
Weight (kg)	139.83±22.54	109.66±23.77	<0.001
BMI (kg/m^2)	43.79±7.88	40.41±8.53	0.07

BMI – body mass index.

TABLE 2. Baseline characteristics of the MS and nonMS obese study subjects.

	MS (n=40)	nonMS (n=30)	*p* value
Age (years)	44.0±11.56	40.96±11.54	0.28
Height (m)	1.70±0.09	1.68±0.08	0.28
Weight (kg)	127.71±26.87	107.71±23.44	0.001
BMI (kg/m^2)	43.98±8.81	38.12±6.63	0.002

MS – metabolic syndrome is present; nonMS – metabolic syndrome is absent; BMI – body mass index.

All three waist circumferences were wider between MS and nonMS groups. The waisttohip ratio was significantly higher in the MS group. No difference was observed in skinfold thickness measurements between these groups (Table 3).

TABLE 3. Anthropometric characteristics of the MS and nonMS obese study subjects.

	MS (n=40)	nonMS (n=30)	*p* value
WC 1 (cm)	124.81±16.20	110.47±13.38	<0.001
WC 2 (cm)	132.88±17.13	116.62±15.47	<0.001
WC 3 (cm)	133.86±16.93	121.66±13.92	0.001
Hip circumference (mm)	125.97±12.80	120.33±12.50	0.07
Waist hip ratio	1.06±0.09	0.97±0.09	<0.001
Biceps skinfold thickness (mm)	21.71±9.39	24.10±12.24	0.59

	MS (n=40)	nonMS (n=30)	*p* value
Triceps skinfold thickness (mm)	30.43±13.07	31.61±11.11	0.69
Subscapular skinfold thickness (mm)	41.10±13.38	44.77±15.72	0.48
Suprailiac skinfold thickness (mm)	31.65±12.70	29.84±12.40	0.42
Abdominal skinfold thickness (mm)	45.86±14.04	41.81±12.54	0.23
Mid-thight skinfold thickness (mm)	44.69±21.97	55.98±18.41	0.07

MS – metabolic syndrome is present; nonMS – metabolic syndrome is absent; WC 1 – narrowest waist circumference; WC 2 – waist circumference at the midpoint between the lowest rib and the iliac crest; WC 3 – waist circumference above the uppermost border of the iliac crest.

No difference was found in the ultrasonographic measurements of subcutaneous and preperitoneal adipose tissues between MS and nonMS groups; however, visceral fat thickness was significantly higher in the MS group (Table 4).

TABLE 4. Ultrasonographic characteristics of the MS and nonMS obese study subjects.

	MS (n=40)	nonMS (n=30)	*p* value
SAT_{epi} (cm)	3.76±1.30	3.55±1.19	0.42
SAT_{umb} (cm)	4.77±1.49	4.52±1.31	0.47
PAT (cm)	2.25±0.77	1.96±0.78	0.13
VAT_{ao} (cm)	10.03±3.68	7.01±2.72	<0.001
VAT_{vert} (cm)	12.45±3.96	9.23±2.86	<0.001

MS – metabolic syndrome is present; nonMS – metabolic syndrome is absent; SAT_{epi} – subcutaneous adipose tissue thickness in epigastrium; SAT_{umb} – subcutaneous adipose tissue thickness in the paraumbilical area; PAT – preperitoneal adipose tissue thickness; VAT_{ao} – visceral adipose tissue thickness up to the superior wall of the aorta; VAT_{vert} – visceral adipose tissue thickness up to the upper border of the vertebral body.

As subjects were classified using the NCEP:ATPIII criteria into two groups, the significant differences in fasting plasma glucose, TG, HDL, SBP and DBP were as expected. The UA, insulin concentration and HOMAIR were significantly higher in the MS group. Adiponectin concentration was higher in the nonMS group (Table 5).

Fat tissue samples obtained during the surgery were examined using the ^{1}H NMR. The amount of monounsaturated fatty acids in superficial subcutaneous adipose tis-

sue was significantly higher in the nonMS group. The amount of polyunsaturated fatty acid composition of superficial and preperitoneal subcutaneous adipose tissue was significantly higher in the nonMS group. There was no difference in saturated fatty acid composition between the MS and nonMS groups (Tables 6, 7 and 8).

TABLE 5. Clinical and laboratory data of the MS and nonMS obese study subjects.

	MS (n=40)	nonMS (n=30)	*p* value
Glu (mmol/l)	6.28±1.36	5.41±0.59	<0.001
TG (mmol/l)	2.77±1.31	1.41±0.65	<0.001
HDL (mmol/l)	1.11±0.28	1.30±0.28	0.004
LDL (mmol/l)	3.67±0.78	3.74±0.80	0.72
Chol (mmol/l)	5.74±1.08	5.71±0.95	0.91
Uric acid (μmol/l)	378.88±95.58	329.33±80.59	0.021
Adiponectin (μg/ml)	5.87±2.34	9.39±6.09	0.002
Leptin (ng/ml)	43.79±28.42	42.69±31.30	0.70
Insulin (pmol/l)	121.67±59.66	82.17±33.25	0.003
HOMA-IR	2.36±1.14	1.55±0.63	0.001
SBP (mmHg)	141.27±20.84	125.5±13.73	<0.001
DBP (mmHg)	85.41±9.8	79.07±8.26	0.003

MS – metabolic syndrome is present; nonMS – metabolic syndrome is absent; Glu – fasting plasma glucose; TG – triglycerides; HDL – high density lipoprotein cholesterol; LDL – low density lipoprotein cholesterol; Chol – total cholesterol; HOMAIR – a homeostasis model assessment for insulin resistance; SBP – systolic blood pressure; DBP – diastolic blood pressure.

TABLE 6. The ^{1}H NMR monounsaturated fatty acid composition of adipose tissue triglycerides of the MS and nonMS obese study subjects.

	MS (n=40)	nonMS (n=30)	*p* value
MUFA SAT 1 (%)	48.52±8.41	53.88±7.97	0.009
MUFA SAT 2 (%)	50.58±8.58	54.23±6.69	0.11
MUFA PAT (%)	47.58±9.50	52.18±8.84	0.15
MUFA VAT (%)	52.32±8.74	54.56±6.84	0.77

MS – metabolic syndrome is present; nonMS – metabolic syndrome is absent; MUFA – monounsaturated fatty acids; SAT 1 – superficial subcutaneous adipose tissue; SAT 2 – deep subcutaneous adipose tissue; PAT – preperitoneal adipose tissue; VAT – visceral adipose tissue.

TABLE 7. ^{1}H NMR polyunsaturated fatty acid composition of adipose tissue triglycerides of the MS and nonMS obese study subjects.

	MS (n=40)	nonMS (n=30)	*p* value
PUFA SAT 1 (%)	20.37±6.89	17.26±5.65	0.025
PUFA SAT 2 (%)	17.34±6.18	15.92±5.61	0.25
PUFA PAT (%)	20.40±6.65	15.91±6.39	0.014
PUFA VAT (%)	17.53±5.40	16.45±4.25	0.37

MS – metabolic syndrome is present; nonMS – metabolic syndrome is absent; PUFA – polyunsaturated fatty acids; SAT 1 – superficial subcutaneous adipose tissue; SAT 2 – deep subcutaneous adipose tissue; PAT – preperitoneal adipose tissue; VAT – visceral adipose tissue.

TABLE 8. ^{1}H NMR saturated fatty acid composition of adipose tissue triglycerides of the MS and nonMS obese study subjects.

	MS (n=40)	nonMS (n=30)	*p* value
SFA SAT 1 (%)	31.08±5.04	28.98±4.83	0.18
SFA SAT 2 (%)	32.08±7.90	30.31±5.53	0.55
SFA PAT (%)	32.05±5.67	32.01±5.74	0.97
SFA VAT (%)	30.21±6.93	29.04±3.99	0.83

MS – metabolic syndrome is present; nonMS – metabolic syndrome is absent; SFA – saturated fatty acids; SAT 1 – superficial subcutaneous adipose tissue; SAT 2 – deep subcutaneous adipose tissue; PAT – preperitoneal adipose tissue; VAT – visceral adipose tissue.

VAT thickness, measured with ultrasonography in the MS group, showed a statistically significant linear correlation with height, weight, BMI, the narrowest waist circumference, waist circumference at the midpoint, waist circumference above the uppermost border of the iliac crest, hip circumference, waist-hip ratio, suprailiac and abdominal skinfold thicknesses, SBP and DBP, glucose and UA concentrations in the blood, pancreatic βcell function (r=0.32–0.81). VAT thickness negatively correlated with SFA concentration in PAT and VAT (r=-0.53–-0.39).

SAT thickness in the epigastrium, measured with ultrasonography in the MS group, showed a statistically significant linear correlation with BMI, waist circumference above the uppermost border of the iliac crest, the triceps, subscapular, abdominal and mid-thigh skinfold thicknesses, HDL, leptin concentration, pancreatic βcell function (r=0.33–0.61) and had a negative correlation with height and TG (r=-0.47–-0.39). SAT thickness in the epigastrium, measured using ultrasonography in the nonMS group, showed a statistically significant linear correlation with BMI, the narrowest waist circumference, hip circumference, biceps, suprailiac and mid-thigh skinfold thicknesses, leptin and insulin concentrations (r=0.39–0.62) and showed a negative correlation with LDL and total cholesterol concentration in blood (r=-0.40–-0.39).

SAT thickness in the supraumbilical region, measured using ultrasonography in the MS group, showed a statistically significant linear correlation with hip circumference, subscapular, iliac and midthigh skinfold thicknesses, leptin concentration in the blood (r=0.39–0.79) and showed a negative correlation with age, height, glucose and TG concentrations in the blood (r=-0.43–-0.34). SAT thickness in the supraumbilical region, measured using ultrasonography in the nonMS group, showed a statistically significant negative linear correlation with deep SAT SFA concentration (r=-0.41)

PAT thickness, measured using ultrasonography in the MS group, showed a statistically significant linear correlation with waist circumference and UA concentration in the blood (r=0.32–0.40). PAT thickness, measured using ultrasonography in the nonMS group, showed a statistically significant linear correlation with weight, triceps, subscapular, suprailiac, abdominal and mid-thigh skinfold thicknesses, leptin and insulin concentrations and HOMA-IR (r=0.38–0.46). It showed a negative correlation in the same group with age (r=-0.48).

A fatty acid concentration in the adipose tissue showed multiple statistically significant linear correlations with anthropometric, blood test and clinical determinants in the group with MS (Table 9). SFA concentration in deep SAT showed a negative correlation with almost all of them except the HDL, UA and insulin concentrations in the blood.

TABLE 9. Linear correlations of fatty acid ^{1}H-NMR composition with anthropometric, blood test and clinical determinants in the group with MS.

Determinant	Fatty acids	r	*p* value
Weight	MUFA PAT	0.33	0.042
	SFA PAT	-0.38	0.017
	SFA VAT	-0.36	0.025
BMI	SFA SAT 2	-0.38	0.018
	SFA PAT	-0.39	0.019
	SFA VAT	-0.38	0.017
WC1	SFA SAT 2	-0.35	0.034
	SFA PAT	-0.37	0.019
	SFA VAT	-0.38	0.016
WC2	SFA SAT 1	-0.37	0.02
	SFA SAT 2	-0.41	0.011
	SFA PAT	-0.41	0.009
	SFA VAT	-0.47	0.002
WC3	SFA SAT 2	-0.35	0.034
	SFA PAT	-0.37	0.019
	SFA VAT	-0.38	0.016
Hip circumference	SFA SAT 2	-0.35	0.032

Determinant	Fatty acids	r	*p* value
Waist-hip ratio	MUFA VAT	0.32	0.046
	SFA PAT	-0.35	0.028
	SFA VAT	-0.34	0.034
Glucose	MUFA SAT 2	0.34	0.035
	SFA SAT 2	-0.34	0.038
HDL	MUFA SAT 2	-0.34	0.037
Uric acid	MUFA SAT 1	0.36	0.025
	MUFA SAT 2	0.44	0.005
Insulin	MUFA PAT	0.37	0.019
	SFA VAT	-0.33	0.039
HOMA-IR	MUFA PAT	0.37	0.022
	SFA SAT 2	-0.34	0.038
	SFA PAT	-0.33	0.038

WC 1 – narrowest waist circumference; WC 2 – waist circumference at the midpoint between the lowest rib and the iliac crest; WC 3 – waist circumference above the uppermost border of the iliac crest; MUFA – monounsaturated fatty acids; SFA – saturated fatty acids; SAT 1 – superficial subcutaneous adipose tissue; SAT 2 – deep subcutaneous adipose tissue; PAT – preperitoneal adipose tissue; VAT – visceral adipose tissue; HDL – high density lipoprotein cholesterol; HOMA-IR – a homeostasis model assessment for insulin resistance.

SFA in all adipose tissue layers showed a strong linear correlation with waist circumference at the midpoint. SFA concentration in the PAT layer showed a negative correlation with weight, BMI, the narrowest waist circumference, waist circumference at the midpoint, waist circumference above the uppermost border of the iliac crest, waist-hip ratio, insulin concentration in the blood and HOMA-IR. There were no statistically significant correlations between the studied determinants and PUFA concentration in the adipose tissue layers.

The fatty acid concentration in the adipose tissue showed multiple statistically significant linear correlations with anthropometric, blood test and clinical determinants in the nonMS group (Table 10). MUFA and PUFA in the layers of adipose tissue showed a correlation with the triceps, subscapular and mid-thigh skinfold thicknesses. Truncal skinfold thicknesses showed a positive correlation with MUFA and negative concentration with PUFA concentrations in superficial SAT. Peripheral adipose tissue in the extremities showed a positive correlation with PUFA concentration in deep SAT. The triceps and mid-thigh skinfold thicknesses showed a positive correlation with PUFA concentration in VAT. SFA concentration in the deep SAT, PAT and VAT showed a negative correlation with mid-thigh skinfold thickness.

PUFA concentration in the deep SAT showed a positive correlation with adiponectin and leptin concentration in the blood.

SFA concentration in deep SAT showed a negative correlation with insulin concentration in the blood, HOMAIR and pancreatic βcell function.

In a ROC analysis, we further defined the anthropometric, ultrasonographic, adipose tissue ^{1}H NMR and blood test determinants, which were statistically significant for the MS prediction in obese patients and determined the optimal cutoff values (Table 11).

TABLE 10. Linear correlations of fatty acid ^{1}H-NMR composition with anthropometric, blood test and clinical determinants in the group without MS.

Determinant	Fatty acids	R	*p* value
Age	MUFA SAT 1	-0.38	0.039
	MUFA SAT 2	-0.47	0.009
	SFA SAT 2	0.46	0.011
Weight	SFA SAT 2	-0.43	0.018
BMI	PUFA SAT 2	0.43	0.018
WC1	MUFA SAT 1	0.41	0.025
	SFA SAT 1	-0.37	0.045
	SFA PAT	-0.39	0.034
WC2	MUFA SAT 1	0.47	0.008
	SFA SAT1	-0.40	0.027
Biceps skinfold thickness	PUFA SAT 2	0.38	0.036
Triceps skinfold thickness	PUFA SAT 2	0.40	0.03
	PUFA VAT	0.43	0.02
Subscapular skinfold thickness	MUFA SAT 1	0.51	0.004
	PUFA SAT 1	-0.38	0.038
Mid-thigh skinfold thickness	MUFA SAT 2	-0.47	0.048
	MUFA PAT	-0.44	0.035
	MUFA VAT	-0.44	0.038
	PUFA SAT 2	0.57	0.005
	PUFA VAT	0.44	0.034
Adiponectin	PUFA SAT 1	0.50	0.005
	PUFA SAT 2	0.42	0.022
Leptin	PUFA SAT 2	0.54	0.002
Insulin	SFA SAT 2	-0.43	0.017
HOMA-IR	SFA SAT 2	-0.41	0.024
%B	SFA SAT 2	-0.45	0.013

WC 1 – narrowest waist circumference; WC 2 – waist circumference at the midpoint between the lowest rib and the iliac crest; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; SFA – saturated fatty acids; SAT 1 – superficial subcutaneous adipose tissue; SAT 2 – deep subcutaneous adipose tissue; PAT – preperitoneal adipose tissue; VAT – visceral adipose tissue; HOMA IR – a homeostasis model assessment for insulin resistance.

TABLE 11. An ROC analysis on anthropometric, ultrasonographic, adipose tissue fatty acid composition and blood test data to predict metabolic syndrome in obese patients.

Determinant	AUC (CI)	Cutoff values (AUC CI)
Weight (kg)	0.73 (0.60, 0.90)	123.90 (0.60, 0.90)
BMI (kg/m^2)	0.71 (0.59, 0.84)	43.86 (0.59, 0.84)
WC 1 (cm)	0.75 (0.64, 0.87)	117.60 (0.70, 0.80)
WC 2 (cm)	0.76 (0.64, 0.88)	123.40 (0.72, 0.77)
VRA_{ao} (cm)	0.74 (0.62, 0.86)	8.93 (0.62, 0.77)
VRA_{vert}(cm)	0.74 (0.63, 0.86)	11.53 (0.60, 0.80)
Insulin (pmol/l)	0.71 (0.59, 0.83)	-
HOMA-IR	0.73 (0.61, 0.85)	-
MUFA SAT 1 (%)	0.67 (0.54, 0.80)	52 (0.62, 0.70)
PUFA PAT (%)	0.67 (0.55, 0.80)	20 (0.51, 0.77)

AUC – area under the curve; CI – confidence interval; BMI – body mass index; WC 1 – narrowest waist circumference; WC 2 – waist circumference at the midpoint between the lowest rib and the iliac crest; VAT_{ao} – visceral adipose tissue thickness up to superior wall of aorta; VAT_{vert} – visceral adipose tissue thickness up to the upper border of vertebral body; HOMA-IR – a homeostasis model assessment for insulin resistance; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; SAT 1 – superficial subcutaneous adipose tissue; PAT – preperitoneal adipose tissue; "-" – non significant.

In order to predict MS, we measured its correlation with multiple anthropometric, ultrasonographic, adipose tissue ^{1}H NMR data and blood test determinants. The parameters that had strong correlation with MS and uncorrelated with each other were used to build an MSpredicting multiple linear regression model. Our study showed that in all obese patients under the study adiponectin, VAT thickness measured using ultrasonography up to the upper border of the vertebral body and MUFA concentration in superficial SAT are independent determinants of MS (Table 12). The odds ratio was also calculated (Table 13).

TABLE 12. Indices in the MS predicting model in obese patients

Predicting parameter	B	p	R^2
Adiponectin	-0.27±0.11	0.02	0.34
VAT_{vert}	0.29±0.1	0.004	
MUFA SAT 1	-1.48±0.5	0.004	

VAT_{vert} – visceral adipose tissue thickness up to the upper border of vertebral body; MUFA – monounsaturated fatty acids; SAT 1 – superficial subcutaneous adipose tissue; β – beta coefficient; R^2 – determination coefficient.

TABLE 13. The odds ratio for adiponectin, VAT thickness measured using ultrasonography up to the upper border of the vertebral body and MUFA concentration in superficial SAT to predict MS in obese patients.

Predicting parameter	Odds ratio	95% CI
Adiponectin	0.76	0.59, 0.92
VAT_{vert}	1.34	1.12, 1.68
MUFA SAT 1	0.24	0.08, 0.57

VAT_{vert} – visceral adipose tissue thickness up to the upper border of vertebral body; MUFA – monounsaturated fatty acids; SAT 1 – superficial subcutaneous adipose tissue; CI – confidence interval.

The final regression model was:

$$P\ (MS=1)=\frac{e^{6,53-0,27*Adiponektinas\ (\mu g/ml)+0,29*\mathrm{VRA}_{sl}\ (cm)-1,43*MNRR\ PRA1\ (\%)}}{1+e^{6,53-0,27*Adiponektinas\ (\mu g/ml)+0,29*\mathrm{VRA}_{sl}\ (cm)-1,43*MNRR\ PRA1\ (\%)}}$$

The regression model predicted MS in 85.5 % of all patients. An ROC curve analysis on the regression model to predict MS reached the sensitivity and specificity of 87.2% and 83.3%. The area under the ROC curve was 0.88 (95% CI 0.79, 0.97).

9. Study limitations

Our study has limitations that need to be mentioned. First, our findings are limited by the small sample size, especially in the nonMS men's group. A greater number of samples could lead to a higher generalization of our results in the obese population. Second, the BMI and weight were different in the MS and nonMS groups in the study. However, after data correction for these variables and in regression models, the BMI and weight did not have any significant impact on the determinants of metabolic health or the prediction of MS. Third, we studied only the Lithuanian obese population. Thus, populationbased studies are therefore required to generalize our findings.

10. Discussion

One of the main goals of this research was an attempt to find a way to predict metabolic disturbances in obese individuals. While not all results were significant, the overall direction of results showed trends that could be helpful in learning about who is more likely to be metabolically healthy or unhealthy if obese. To the best of our

knowledge, this study is the first that complexisively examined the distribution of subcutaneous, preperitoneal and visceral adipose tissue by means of an ultrasonography and its composition using ^{1}H-NMR in the obese population. To date, no work was published on the ^{1}H-NMR composition of preperitoneal adipose tissue or its impact on metabolic disturbances in obese individuals.

Our data suggests that the MS group has a lower BMI, WC and WHR than nonMS individuals. Schröder (Schröder et al. 2014) found that the MHO were younger, more highly educated, smoked more, adhered to a less healthy diet, possessed lower BMIs and lower WCs than the MAO phenotype group. However, there is no direct link between BMI and metabolic disturbances. Our study contributes that weight and BMI did not have any direct impact on the emergence of MS.

WC has a strong correlation with cardiometabolic disturbances and is an accurate surrogate marker for visceral adiposity (Borruel & Molto 2014). In our study, the WCs of all men were more than 102 cm, and for women – more than 88 cm. Furthermore, WHR exceeded the WHO recommended values (1,11±0,06 in men and 0,98±0,09 in women). We can conclude that all participants in our study had central adiposity. However, the metabolic activity of SAT and VAT is different. WC and WHR cannot differentiate between these two fat depots in the abdominal area; hence, more accurate markers should be used.

Our present results indicate that there is no difference between skinfolds in the MS and nonMS groups in the obese population. However, the triceps and mid-thigh skinfolds were thicker in women in comparison with men in both the MS and nonMS groups. We have faced the same technical problems as Gray (Gray et al. 1990) did while performing anthropometric measurements. It was impossible to measure abdominal (BMI ≥35 kg/m^2) and thigh (BMI ≥40 kg/m^2) skinfold thickness in some study participants. On the one hand, it was difficult to raise an adequate skinfold for measurement, and on another – the used caliper could not accommodate some skinfolds in obese individuals.

In our study, glucose, TG and insulin concentrations were higher, and HDL was lower in the MS group. Our data was in agreement with the NCEP-ATPIII recommended values for MS evaluation. Several studies have shown that MHO individuals have lower TG and higher HDL concentrations (Brochu et al. 2001; Karelis et al. 2005; Kim et al. 2013; Manu et al. 2012) and that they have better insulin sensitivity; therefore, lower glucose and insulin concentrations in serum (Brochu et al. 2001; Karelis et al. 2005; Stefan et al. 2008). We observed that adiponectin serum concentration was higher in the nonMS group and there were no differences in leptin serum concentration between MS and nonMS groups. These findings are consistent with the data of various authors who had previously conducted metabolical health research in obesity (Alfadda 2014; Eglit et al. 2013). Our study suggests that patients with MS have

a higher UA concentration in the serum than nonMS individuals. It is in consistency with the previous report of Mangge (Mangge et al. 2013). He found that the UA levels in serum was a significant predictor of obesity with metabolic disturbances in juveniles and adults. It was shown in other studies that the UA concentration in serum was independently associated with the presence of metabolic and proinflammative disorders in MS (Tamba et al. 2008; Nagahama et al. 2015; Li et al. 2015; Chen et al. 2017). There was no difference in serum UA concentration between MS and nonMS groups in men, although it almost reached statistical significance between MS and nonMS women (p=0.052) in our study. Iacobellis et al. (Iacobellis et al. 2005) suggested a UA concentration in serum <333 μmol/l in women and <416 μmol/l in men as a threshold for differentiating MHO and MAO phenotypes. Primeau et al. (Primeau et al. 2011) reviewed 15 studies that report MHO prevalence and the UA concentration in serum as the predicting factors of metabolic health were used only in the study of Iacobellis et al. HOMA-IR was statistically different in our study groups and reached the mean of 2.36±1.14 in the group with MS. Qu (Qu et al. 2011) suggested HOMAIR <2.6 as a normal range, HOMAIR 2.63.8 as a transitional range without insulin resistance and HOMAIR >3.8 as the best cutoff value for the definition of insulin resistance. However, these reference values are calculated for Mexican Americans. In fact, there are other HOMAIR thresholds suggested: ≤1,95 (Karelis et al. 2004; Shin et al. 2006; Jennings et al. 2008), ≤2,5 (Kuk & Ardern 2009), ≤2,7 (Karelis et al. 2005), <2,8 (Bonora et al. 1998), <5,1 (Wildman et al. 2008; Messier et al. 2010). The lack of standardized reference range for the HOMA-IR, adipokines and UA shows that more prospective population-based studies should be conducted in the future.

We used a modified method of Stolk to assess subcutaneous, preperitoneal and visceral adipose tissue thickness by ultrasonography. In our systematic literature review we concluded that SAT and VAT should be measured 1-2 cm above the umbilicus and VAT should be measured up to the anterior wall of aorta or up to the lumbar vertebra in obese patients (Buckus & Brimas 2018). SAT and PAT thickness in epigastrium were measured 2 cm below the xiphoid process. PAT was measured as a distance between *linea alba* and the peritoneum of the liver. Kim et al. (Kim et al. 2004) reported that the measurements of PAT thickness are unreliable because of the irreproducibility or from differences in measuring sites. Even though it is difficult to evaluate the exact ultrasound probe placement site, our study shows that PAT is involved in the change of metabolic health in obesity. We believe more prospective studies should be done to find the best measurement site for PAT and to verify it using CT or MRT.

Our data suggests that SAT was thicker in women and VAT was thicker in men. In the nonMS group, VAT thickness measured using ultrasonorgaphy was less than in the MS group. This was still the case after correcting the data for sex, body weight and BMI (VAT_{ao} p<0.039, VAT_{vert} p=0.042). Therefore, we suggest that VAT plays a significant role in the evaluation of MS in obese population.

In the present study, pancreatic βcell function had a significant negative correlation with VAT thickness and positive correlation with SAT thickness in obese individuals with MS. Therefore, this data suggests that SAT has a protective role against the evaluation of carbohydrate metabolism disturbances.

Our research proved that VAT thickness measured using ultrasonography has a strong correlation with the determinants of metabolic health, such as WC, WHR, abdominal and suprailiac skinfolds, SBP and DBP, glucose and UR concentrations in serum in obese individuals. The same correlations were observed in both sexes with MS. Our data agreed with the previous reports where VAT thickness correlated with all components of MS (Kim et al. 2004; Guldiken et al. 2006; Sogabe et al. 2012; Miranda Prado 2012; Soattin et al. 2013; Roever et al. 2016) and ultrasonography was suggested as a diagnostic tool for MS diagnosis (RibeiroFilho et al. 2001; MeriñoIbarra et al. 2005; Chiba et al. 2007; Vasilescu et al. 2011). Our present study suggests that VAT thickness measurements, taken using ultrasonography, are a useful tool for the evaluation of VAT distribution and accompanied metabolic disturbances in the obese population.

The overall sensitivity of an ultrasonography up to the aorta to detect MS was 78%, specificity 60%, and the summary area under the ROC curve was 0,74 (95% CI 0.62-0.86) and sensitivity up to the lumbar vertebra to detect MS was 60%, specificity 80%, and the summary under the ROC curve was 0,69 (95% CI 0.63-0.86). We further examined the lower cutoffs for the detection of MS and found that VAT thickness up to the aorta and VAT thickness up to the lumbar vertebra measured using ultrasonography was 8.93 cm and 11.53 cm, respectively.

VAT thickness was measured using various techniques in other studies. Therefore, it is very difficult to compare the results, as measurements are performed in different ethnical populations and in the groups with different weight or BMI values. Kim et al. (Kim et al. 2004) suggested the cutoff value of VAT for predicting the presence of MS to be 4.76 cm and 3.55 cm in men and women, respectively. However, the study was performed only within the Korean population. Leite et al. (Leite et al. 2002) suggested the cutoff value of intraabdominal thickness to predict risk factors for CVD to be 7 cm for moderate risk group and 9 cm for men and 8 cm for women in high-risk groups. Thus, the exact cutoff values for predicting metabolic disturbances are warranted in longitudinal studies.

PAT thickness did not show significant difference between our study groups. However, it had a strong correlation with the triceps, subscapular, suprailiac, abdominal and mid-thight skinfold thicknesses in the nonMS women group and with the subscapular skinfold thickness in MS men group. Excessive energy is stored in SAT at first. When the personal fat threshold is reached, the lipid excess is deposited as an ectopic fat (Gesta & Kahn 2017; Cuthbertson et al. 2017; Dobson et al. 2016). PAT is analyzed

as a part of VAT in the literature (Fukuda et al. 2015). Our data suggests that PAT is a distinctive fat depot with different metabolic activity and fat deposition pattern.

Needle aspiration biopsy was the preferable method for obtaining adipose tissue samples in most of the human adipose tissue fatty acid composition studies. However, adipose tissue samples were taken from different sites, e.g., the thighs, buttocks, SAT from the abdominal wall, VAT as omental adipose tissue etc. (Malcom 1989; Calder et al. 1992; Tjønneland & Overvad 1993; Phinney et al. 1994; Thomas et al. 1998; Pedersen et al. 2000; Abraitienė et al. 2008; Lundbom et al. 2010; Giuliani et al. 2014). That is why the results could not be generalized in most cases. Diet, ethnicity, lifestyle, the region of residence and age of the individual could affect the composition of adipose tissue fatty acids. To our best knowledge, this is the first study where adipose tissue fatty acid composition from four depots (superficial SAT, deep SAT, PAT and VAT) in the obese population is examined. There is a lack of information about the exact fatty acid composition in these specific depots. Some studies have compared the fatty acid composition of the superficial and deep SAT (Lundbom et al. 2013); however, there is no such studies on the superficial or deep SAT and VAT composition differences. We did not find any studies on the fatty acid composition of PAT in our systematic review.

Our data suggests that the SAT and VAT fatty acid composition of MUFA, PUFA and SFA is in consistency with other authors' data (Garaulet et al. 2001). Men had more MUFA, and women had more SFA in PAT in our study. SFA concentration was bigger in PAT in comparison with superficial SAT (the difference was observed in all research participants and in the MS group), although the difference was not statistically significant. PAT has a significantly bigger concentration of SFA than VAT. It could be related to the differences of SFA/MUFA ratio, which, in turn, is related to *de novo* lipogenesis, desaturase activity and a turnover of fatty acids (Roberts et al. 2009; Sjögren et al. 2008; Votruba & Jensen 2007). However, it is still unknown how these processes impact the amount of SFA and MUFA in adipose tissue. Lundbom et all. (Lundbom et al. 2013) proposed that more saturated adipose tissue has a bigger metabolic activity. We observed a statistically significant difference in SFA concentration in superficial SAT, PAT and VAT in the nonMS group. It was proposed earlier that PAT has lessproinflammatory properties than SAT and VAT in obese individuals (Silva et al. 2017) and that PAT has a different lipolytic activity in comparison with superficial SAT and VAT (Enevoldsen et al. 2001). Our research proved that PAT is a distinct fat depot with different metabolic activity and fatty acid composition, although it is an internal fat depot.

In the present study, we observed a statistically significant difference in superficial SAT MUFA (p=0.009), PUFA (p=0.025) and PAT PUFA (p=0.014) concentrations between MS and nonMS groups. The same significance was observed after correcting the data for sex, age, weight, height and WC at the midpoint between the lowest

rib and the iliac crest (superficial SAT MUFA p=0.003 and PUFA p=0.03, PAT PUFA p=0.046). Therefore, we suggest that the composition of fatty acids in superficial SAT and PAT is suitable for studying metabolic health in the obese population.

In the present study, we observed a statistically significant difference in superficial SAT MUFA (p=0.005), PUFA (p=0.004) and PAT MUFA (p=0.0046), PUFA (p=0.011) concentrations between MS and nonMS groups in women. The same significance was observed after correcting the data for age, weight, height, BMI and WC at the midpoint between the lowest rib and the iliac crest (superficial SAT MUFA p=0.0046, PUFA p=0.008 and PAT MUFA p=0.031, PUFA p=0.027). Therefore, we suggest that the composition of fatty acids in superficial SAT and PAT is suitable for studying metabolic health in the obese women population.

In the present study, we found that deep SAT SFA and MUFA concentrations have a significant relationship with the MS determinants in MS group. We did not find the same correlation with superficial SAT or PUFA concentrations in different fat depots. However, SFA, MUFA and PUFA concentrations in superficial and deep SAT depots significantly correlate with anthropometric and laboratory data in the nonMS group. PUFA concentration in the superficial SAT correlates with leptin and adiponectin concentrations in serum. SFA concentration in deep SAT has a negative correlation with insulin concentration in the serum, HOMAIR and βcell function. The thickness of different skinfolds positively correlated with MUFA and PUFA concentrations in the superficial and deep SATs in the nonMS group. Enevoldsen et al. (Enevoldsen et al. 2001) suggest that superficial and deep SATs *in vivo* have different lipolytic activities in humans. Kim et al. propose that deep SAT is closely related with obesity-induced metabolic complications (Kim et al. 2016). However, Walker et al. (Walker et al. 2014) suggested that superficial SAT has independent effects that associate with the metabolic complications of obesity. Therefore, the exact association between SAT layers and the complications of obesity is still unclear. Our data suggests that deep SAT correlates with the determinants of the adverse effects of obesity. We suggest that more prospective studies should be conducted to evaluate the impact of SAT layers on metabolic health in the obese population.

In the present study, UA concentration in serum had a positive correlation with MUFA and a negative correlation with SFA concentrations in deep SAT and VAT in the women's group with MS. The opposite was observed in the nonMS women's group – a positive correlation with PUFA and SFA concentrations in VAT and a negative correlation with MUFA concentration in deep SAT and VAT. UA concentration in the serum has an association with the adverse effects of central adiposity; therefore, its relations with fatty acid patterns in fat depots suggests that adipose tissue composition plays a significant role in the metabolic health of the obese population.

Adiponectin, insulin and UA concentrations in the serum, HOMAIR, BMI, weight, MUFA or PUFA concentrations in superficial SAT, PUFA concentration in PAT, VAT thickness measured using ultrasonography up to the aorta or VAT thickness up to the lumbar vertebra were found as variables that had a strong correlation with MS and uncorrelated with each other. The variables used for the diagnosis of MS were not included in the analysis. Based on P values, four linear regression models with MS as a dependent variable in the studied population were performed. The linear regression models showed that in all obese patients under the study, adiponectin concentration in serum, VAT thickness measured using ultrasonography up to the upper border of the vertebral body and MUFA concentration in superficial SAT are independent determinants of MS. The best regression model predicted MS in 85.5% of all patients. An ROC curve analysis on the regression model to predict MS reached the sensitivity and specificity of 87.2% and 83.3%, AUC 0,88 (CI 95% 0.79, 0.97). For every 1 cm increase in VAT thickness measured with an ultrasonoraphy up to the upper border of the vertebral body, the obese patient had a 1.34-fold (95% CI, 1.12, 1.68) greater MS risk. For every 1 μg/ml increase in adiponectin concentration, the obese patient had a 1.3-fold (95% CI, 0.59, 0.92), and for every 0.1% increase in MUFA concentration in superficial SAT – a 4.17fold (95% CI, 0.08, 0.57) lower MS risk.

In the final linear regression model, weight and BMI did not have a significant impact on the prediction of MS. We believe these two variables do not have any essential impact on the evaluation of MS in the obese population. However, larger population-based studies are required to confirm our findings.

Despite the increased interest in the metabolically normal obesity, there are no unique criteria to define metabolic health (Primeau et al. 2011; Roberson et al. 2014). A lack of knowledge of the determinants and molecular mechanisms associated with MHO indicates the need to investigate the composition and distribution of adipose tissue and its association with metabolic disturbances. A better understanding of the underlying causes of metabolic health would help to distinguish the obesity phenotypes, aid in the development of new preventive strategies for those who are at a greater risk of developing metabolic abnormalities and prevent any excessive dieting, pharmacological and surgical treatments in healthy obese individuals. Future population-based studies should be performed to consolidate the adiponectin concentration in serum, VAT thickness measured using ultrasonography up to the upper border of the lumbar vertebra and the MUFA concentration in superficial SAT as the relevant predictors of metabolic syndrome in the obese population.

11. Conclusions

1. The thickness of the visceral adipose tissue measured using ultrasonographic and anthropometric determinants (waist circumference and waist hip ratio) were significantly higher in the group of obese patients with metabolic syndrome.
2. The amount of polyunsaturated fatty acids in superficial subcutaneous adipose tissue and in preperitoneal adipose tissue were significantly higher, while that of monounsaturated fatty acids in superficial subcutaneous tissue was significantly lower in the group of obese patients with metabolic syndrome.
3. Adiponectin concentration, visceral adipose tissue thickness measured using ultrasonography up to the lumbar vertebra and monounsaturated fatty acid concentration in superficial subcutaneous adipose tissue are independent prognostic factors in metabolic syndrome determination in obese patients.

12. Further directions

Adipose tissue fatty acid composition and its relationship with adipose tissue metabolism will be evaluated in further studies.

The morphological structure of adipose tissue will be identified by running electron microscopy scans, conducting genetic research and carrying out studies of proinflammatory determinants.

Further population studies should be done to evaluate the significance of the prognostic model and the thresholds of its determinants.

Patients without metabolic disturbances should be evaluated in long-term follow-up studies so as to determine their metabolic health changes.

13. References

A list of references is provided in the manuscript of the Dissertation.

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SANTRAUKA

Sutrumpinimai

AKS – arterinis kraujo spaudimas
CD – cukrinis diabetas
DTLch – didelio tankio lipoproteinų cholesterolis
IR – rezistentiškumas insulinui
JA – juosmens apimtis
JKS – juosmens klubų santykis
KM – kūno masė
KMI – kūno masės indeksas
KT – kompiuterinė tomografija
^{1}HMBR – vandenilio jonų magnetinių branduolių rezonansas
MetS – metabolinis sindromas
MNRR – mononesočiosios riebalų rūgštys
MTLch – mažo tankio lipoproteinų cholesterolis
PNRR – polinesočios riebalų rūgštys
PRA – poodinis riebalinis audinys
PreRA – preperitoninis riebalinis audinys
RA – riebalinis audinys
RR – riebalų rūgštys
SRR – sočiosios riebalų rūgštys
ŠR – šlapimo rūgštis
TAG – trigliceridai
UGT – ultragarsinis tyrimas
VRA – visceralinis riebalinis audinys

1. Darbo tikslas

Nustatyti riebalinio audinio pasiskirstymo ir sudėties ypatumus, leidžiančius prognozuoti metabolinius sutrikimus nutukusiems pa cientams.

2. Darbo uždaviniai

1. Tiriant antropometriniais ir ultragarsiniais metodais, nustatyti metaboliniu sindromu sergančių ir juo nesergančių nutukusių pacientų riebalinio audinio (poodinio paviršinio, poodinio giliojo, preperitoninio ir centrinio) pasiskirstymo skirtumus.
2. Tiriant ^{1}H-MBR spektroskopijos metodu, nustatyti metaboliniu sindromu sergančių ir juo nesergančių nutukusių pacientų riebalinio audinio (poodinio paviršinio, poodinio giliojo, preperitoninio ir centrinio) sudėties skirtumus.
3. Įvertinti riebalinio audinio pasiskirstymo ir sudėties ypatumų ryšį su metaboliniu sindromu ir nustatyti nepriklausomus prognostinius veiksnius, lemiančius metabolinių sutrikimų atsiradimą nutukusiems pacientams.

3. Darbo naujumas

1. Nutukusių ligonių periferinio, preperitoninio ir centrinio riebalinio audinio pasiskirstymas, nustatomas ultragarsiniu metodu, šių riebalinio audinio sričių riebalų rūgščių sudėties tyrimas ^{1}H-MBR spektroskopijos metodu ir jų sąsajos su metaboliniais sutrikimais kompleksiškai nebuvo tirta ne tik Lietuvoje, bet ir tarptautiniu mastu.

2. Pirmą kartą ^{1}HMBR spektroskopijos metodu tiriama nutukusių ligonių preperitoninio riebalinio audinio sudėtis. Taip pat pirmą kartą įvertinta preperitoninio riebalinio audinio sudėties įtaka nutukusių asmenų metaboliniams sutrikimams atsirasti.
3. Nustatyti nepriklausomi prognostiniai veiksniai, lemiantys metabolinių sutrikimų atsiradimą nutukusiems pacientams.

4. Praktinė reikšmė

1. Nutukusių asmenų juosmens apimtį geriausia matuoti siauriausioje juosmens vietoje tarp apatinių šonkaulių ir klubinių skiauterių.
2. Metabolinio sindromo diagnostikos nutukusiems ligoniams kūno masės slenkstinė riba – 123,90 kg, juosmens apimties vidurio taške slenkstinė riba – 123,40 cm, siauriausio juosmens matmens slenkstinė riba – 117,60 cm (apskaičiuota pagal vidutinį vyrų (1,79 m) ir moterų (1,65 m) ūgį).
3. Nutukusių asmenų visceralinį riebalinį audinį UGT metu reikia matuoti iki juosmeninio slankstelio viršutinio krašto.
4. Metabolinio sindromo diagnostikos nutukusiems ligoniams ultragarsinio tyrimo metu išmatuoto visceralinio riebalinio audinio iki aortos priekinės sienelės slenkstinė riba – 8,93 cm, visceralinio riebalinio audinio iki juosmens slankstelio viršutinio krašto – 11,53 cm.
5. Atliekant nutukusių asmenų poodinio riebalinio audinio tyrimus, reikia atskirai tirti paviršinį ir gilųjį sluoksnius.
6. Tiriant nutukusių asmenų visceralinį riebalinį audinį, turi būti išskiriamas ir preperitoninis riebalinis audinys bei jo įtaka metabolinei sveikatai.
7. Prognostiniu modeliu galima išskirti nutukusių asmenų rizikos grupes ir joms pritaikyti individualius diagnostikos ir nutukimo gydymo algoritmus.

5. Ginamieji disertacijos teiginiai

1. Metaboliniu sindromu sergančių ir juo nesergančių nutukusių asmenų odos klosčių matavimai nesiskiria.
2. Ultragarsiniai riebalinio audinio matavimai yra efektyvus būdas atskirti metaboliniu sindromu sergančius ir juo nesergančius nutukusius asmenis.
3. Metaboliniu sindromu sergančių ir šiuo sindromu nesergančių nutukusių asmenų ^{1}HMBR riebalinio audinio sudėtis skiriasi.

6. Darbo metodologija

6.1 Tyrimo modelis

Šis darbas yra perspektyvusis skerspjūvio tipo biomedicininis tyrimas, kuriame tiriami MetS rizikos veiksniai nutukusių ligonių populiacijoje, naudojant antropometrines, ultragarsines ir ^{1}HMBR vaizdinimo priemones. Tyrimą 2014 m. kovo 12 d. patvirtino Lietuvos bioetikos komitetas.

6.2 Tiriamųjų atranka

Tyrime dalyvavo VšĮ Respublikinės Vilniaus universitetinės ligoninės Bendrosios chirurgijos centro ligoniai. Nuo 2014 m. kovo 12 d. iki 2018 m. sausio 1 d. tyrime buvo siūloma dalyvauti pacientams, kuriems buvo numatytas operacinis gydymas Bendrosios chirurgijos centre ir jie atitiko atrankos kriterijus.

Ligonių įtraukimo kriterijai:

1. Ligonis yra nutukęs (KMI >30 kg/m^2)
2. Ligoniui 1865 metai
3. Ligoniui atliekama planinė pilvo ertmės operacija
4. Ligonis pasirašė asmens informavimo ir informuoto asmens sutikimo formą.

Ligonių atmetimo kriterijai:

1. Ligonis jaunesnis nei 18 ir vyresnis nei 65 metų amžiaus
2. Ligonis serga psichikos liga
3. Ligoniui kontraindikuotinas operacinis gydymas
4. Ligonis nepasirašė informuoto asmens sutikimo formos.

Visiems biomedicininio tyrimo dalyviams buvo pateiktos Lietuvos bioetikos komiteto patvirtintos asmens informavimo formos, atsakyta į visus klausimus ir jie pasirašė informuoto asmens sutikimo formas. Kiekvienam ligoniui suteiktas kodas, naudojant sugeneruotą atsitiktinių skaičių seką.

6.3 Tyrimo eiga

1. Pacientui sutikus dalyvauti tyrime, prieš operaciją buvo surenkami reikalingi duomenys (amžius, ūgis, svoris, KMI, juosmens, klubų apimtys ir odos klosčių apimtys) ir užpildoma duomenų anketa.
2. Atliekami paciento kraujo tyrimai – nustatoma lipidograma, TAG, insulinas, gliukozė, ŠR, leptinas, adiponektinas.
3. Atliekamas RA ultragarsinis tyrimas, kurio metu nustatomas PRA, PreRA ir VRA pasiskirstymas.
4. Operacijos metu, nenaudojant papildomų pjūvių, ligoniams paimta po gabalėlį RA (~5 g iš priekinės pilvo sienos paviršinio ir giliojo sluoksnių, preperitoninio bei visceralinio RA).
5. Dalis kraujo tyrimų atlikta VšĮ Respublikinės Vilniaus universitetinės ligoninės laboratorinės diagnostikos skyriuje, kita dalis kraujo ir RA audinys buvo užšaldyti esant –70 ^{O}C temperatūrai. Sukaupus pakankamą tiriamosios medžiagos kiekį, ji transportuota į VšĮ Vilniaus universiteto ligoninės Santaros klinikų Laboratorinės diagnostikos centrą atlikti likusių kraujo rodiklių tyrimų, o RA – į Vilniaus universiteto Chemijos fakultetą, kur atlikta jo homogenizacija ir riebalų ekstrakcija. RA ^{1}H-MBR tyrimas atliktas pagal Vilniaus universiteto Chemijos fakultete priimtas metodikas.

6.4 Statistinė analizė

Statistinė duomenų analizė atlikta naudojant „Excel“ (Microsoft Office 365) ir „Rcommander“ (versija 3.2.4) [329] standartinių programų paketus. Atlikta aprašomoji statistika: apskaičiuoti kiekybinių kintamųjų rodiklių vidurkiai, vidurkių standartinės paklaidos ir pasikliautiniai intervalai, standartiniai nuokrypiai, duomenų aibių pločiai (min–max). Apskaičiuotos kokybinių kintamųjų absoliučios ir procentinės vertės. Kintamųjų pasiskirstymas pagal normalųjį dėsnį tikrintas pritaikius Šapiro ir Vilko normalumo testą. Nepriklausomų imčių, kurių intervaliniai kintamieji buvo su normaliuoju pasiskirstymu, vidurkių lygybės hipotezei tikrinti naudotas Stjudento t testas, kai buvo lyginami dviejų grupių vidurkiai, ir vienfaktorinės dispersinės analizės (ANOVA) metodas, kai buvo lyginami kelių grupių vidurkiai. Nepriklausomų imčių, kurių intervaliniai kintamieji buvo su nenormaliuoju pasiskirstymu, vidurkių lygybės hipotezei tikrinti naudotas Mano, Vitnio ir Vilkoksono kriterijus. Tiesinei priklausomybei tarp kintamųjų įvertinti buvo naudojamas Pirsono koreliacijos koeficientas, jei duomenys buvo pasiskirstę pagal normalųjį dėsnį, ir Spirmeno, jei ne. Koreliuojančių veiksnių įtakos vertinimui naudota tiesinė ir logaritminė regresija. Prognostinių veiksnių, turinčių įtakos MetS, paieškai sukurtas regresijos modelis, jame paliekant reikšmingus ir tarpusavyje nekoreliuojančius kintamuosius. Skirtumas tarp dviejų imčių laikytas statistiškai reikšmingu, kai $p < 0,05$.

7. Tyrimų rezultatai

Nuo 2014 m. kovo 3 d. iki 2018 m. sausio 1 d. į biomedicininį tyrimą įtraukta 70 ligonių – 48 (68,57 %) moterys ir 22 (31,43 %) vyrai. Vidutinis amžius buvo 42,7±11,56 metai (nuo 18 iki 63), vidutinė KM – 119,14±27,18 kg (nuo 74 iki 185,55). Tiriamųjų demografiniai rodikliai pateikiami 10 ir 11 lentelėse. Vyrų ūgis ir KM buvo didesni negu moterų. Pagal amžių ir KMI vyrų ir moterų grupės nesiskyrė.

Nutukusiųjų, kurie serga MetS, kūno masė ir KMI buvo didesni negu nesergančiųjų MetS.

Visos trys JA (siauriausias juosmens matmuo, JA vidurio taške, JA virš klubinių skiauterių) ir JKS statistiškai reikšmingai buvo didesni pacientų, kuriems diagnozuotas MetS, grupėje, palyginti su pacientų, kurie nesirgo MetS, grupe. Odos klosčių matmenys tarp sergančiųjų ir nesergančiųjų MetS statistiškai reikšmingai nesiskyrė. Nors šlaunies odos klostės nesergančiųjų MetS buvo storesnės, tačiau reikšmingo skirtumo negavome.

Ligonių, kuriems diagnozuotas MetS, kraujo gliukozės, TAG, ŠR, insulino, HOMA IR rodikliai ir SAKS bei DAKS buvo statistiškai reikšmingai didesni negu ligonių, kuriems nepasireiškė MetS. Ligonių, kurie neserga MetS, adiponektino ir DTLch koncentracija buvo statistiškai reikšmingai didesnė negu ligonių, kurie serga MetS

Tiriamųjų, sergančių MetS, VRA buvo statistiškai reikšmingai didesnis negu ligonių, nesergančių MetS, tačiau nei PRA, nei PrePRA storiai, matuojant UGT, tarp šių grupių nesiskyrė.

Sergančiųjų MetS ir nesergančiųjų šiuo sindromu grupėse statistiškai reikšmingai skyrėsi paviršinio PRA MNRR ir PNRR bei preperitoninio RA PNRR kiekis. Lyginant nutukusių

ligonių RR sudėtį RA sluoksniuose, nustatyta, kad paviršinio PRA sluoksnio PNRR kiekis didesnis negu giliajame PRA ir VRA, SRR kiekis mažesnis negu PreRA, o MNRR kiekis mažesnis negu VRA. Giliojo PRA MNRR kiekis mažesnis, o SRR kiekis didesnis negu VRA. PreRA MNRR kiekis mažesnis negu VRA, o PNRR ir SRR kiekis didesnis negu VRA.

VRA UGT matavimai iki aortos ir iki juosmeninio slankstelio sergančiųjų MetS grupėje statistiškai reikšmingai koreliavo su ūgiu, KM, KMI, siauriausiu juosmens matmeniu, JA vidurio taške, JA virš klubinių skiauterių, klubų apimtimi, JKS, klubo ir pilvo odos klostėmis, SAKS ir DAKS, gliukozės ir ŠR kiekiu kraujyje, kasos β ląstelių funkcija (r = 0,32–0,81). Abu centrinio riebalinio audinio UGT matmenys neigiamai koreliavo su PreRA ir VRA SRR kiekiu nesergančiųjų MetS grupėje (r = –0,53–0,39).

UGT matavimai epigastriumo srityje sergančiųjų MetS grupėje statistiškai reikšmingai koreliavo su ūgiu, KMI, JA virš klubinių skiauterių, užpakaline žasto, pomentine, pilvo ir šlaunies odos klostėmis, DTLch, leptinu, kasos β ląstelių funkcija (r = 0,33–0,61) bei neigiamai koreliavo su ūgiu ir TAG (r = –0,47–0,39). PRA UGT matavimai epigastriumo srityje nesergančiųjų MetS grupėje statistiškai reikšmingai koreliavo su KMI, siauriausiu juosmens matmeniu, klubų apimtimi, žasto priekine, klubo ir šlaunies odos klostėmis, leptinu, insulinu (r = 0,39–0,62) bei neigiamai koreliavo su amžiumi, MTLch ir bendro cholesterolio kiekiu (r = –0,40–0,39).

PRA UGT matavimai virš bambos sergančiųjų MetS grupėje statistiškai reikšmingai koreliavo su klubų apimtimi, pomentine, klubo odos, šlaunies odos klostėmis, leptinu (r = 0,39–0,79) bei neigiamai koreliavo su amžiumi, ūgiu, gliukozės ir TAG kiekiu (r = –0,43–0,34). PRA UGT matavimai virš bambos neigiamai koreliavo su giliojo PRA sočiųjų RR kiekiu nesergančiųjų MetS grupėje (r = –0,41).

PreRA UGT matavimai sergančiųjų MetS grupėje statistiškai reikšmingai koreliavo su klubų apimtimi ir ŠR kiekiu kraujyje (r =0,32–0,40). PreRA UGT matavimai nesergančiųjų MetS grupėje statistiškai reikšmingai koreliavo su KM, žasto užpakaline, pomentine, klubo, pilvo ir šlaunies odos klostėmis, leptino, insulino kiekiu kraujyje ir HOMA IR (r = 0,38–0,46) bei neigiamai koreliavo su amžiumi (r = –0,48).

Apskaičiavus RA RR sudėties koreliacijas su antropometriniais, kraujo tyrimų ir klinikiniais rodikliais, tiriamųjų, kurie serga MetS, grupėje nustatyta, kad RR sudėtis statistiškai reikšmingai koreliuoja su KM, KMI, siauriausiu juosmens matmeniu, JA vidurio taške, JA virš klubinių skiauterių, klubų apimtimi, JKS, gliukozės, DTLch, ŠR ir insulino koncentracija kraujyje, jautrumu insulinui.

Giliojo PRA sluoksnio SRR kiekis neigiamai koreliavo beveik su visais išvardytais komponentais, išskyrus DTLch, ŠR ir insulino kiekiu kraujyje.

Tarp JA vidurio taške ir visų RA sluoksnių SRR kiekio pastebėta statistiškai reikšminga neigiama koreliacija.

PreRA SRR sudėtis statistiškai reikšmingai neigiamai koreliavo su KM, KMI, siauriausiu juosmens matmeniu, JA vidurio taške, JA virš klubinių skiauterių, JKS ir insulino koncentracija bei jautrumu insulinui.

Tiriamųjų, kurie serga MetS, grupėje statistiškai reikšmingų koreliacijų tarp tiriamų rodiklių ir PNRR skirtingose RA srityse nepastebėta.

Apskaičiavus RA RR sudėties koreliacijas su demografiniais, an tropometriniais, kraujo tyrimų ir klinikiniais rodikliais, tiriamųjų, kurie neserga MetS, grupėje nustatyta, kad RR sudėtis statistiškai reikšmingai koreliuoja su amžiumi, KM, KMI, siauriausiu juosmens matmeniu, JA vidurio taške, žasto užpakaline, žasto priekine, pomentine ir šlaunies odos klostėmis, adiponektino, leptino ir insulino koncentracija kraujyje, jautrumu insulinui ir kasos β ląstelių funkcija.

Amžius reikšmingai neigiamai koreliuoja su paviršinio ir giliojo PRA sluoksnių MNRR ir teigiamai su SRR kiekiu giliajame PRA.

Skirtingai negu sergančiųjų MetS grupėje, nesergančių MetS tiriamųjų grupėje stebima RA MNRR bei PNRR koreliacija su žasto užpakalinės, žasto priekinės, pomentinės ir šlaunies odos klosčių storiu. Liemens odos klostės storis reikšmingai teigiamai koreliuoja su paviršinio PRA MNRR ir neigiamai – su PNRR kiekiu, o galūnių odos klostės reikšmingai teigiamai koreliuoja su giliojo PRA PNRR. Žasto užpakalinės ir šlaunies odos klosčių storis reikšmingai teigiamai koreliuoja su VRA PNRR sudėtimi, o šlaunies odos klostės storis reikšmingai neigiamai koreliuoja su giliojo PRA, PreRA ir VRA SRR kiekiu.

Adiponektino ir leptino koncentracijos kraujyje reikšmingai teigiamai koreliuoja su giliojo PRA PNRR, o adiponektino koncentracija dar ir su paviršinio PRA PNRR kiekiu.

Insulino koncentracija kraujyje, jautrumo insulinui modelis HOMA IR ir kasos β ląstelių funkcija reikšmingai neigiamai koreliuoja su giliojo PRA SRR kiekiu.

ROC kreivių analizė parodė, kad nutukusių ligonių KM, KMI, siauriausio juosmens matmens, JA vidurio taške, UGT iki aortos ir juosmeninio slankstelio, insulino koncentracija kraujyje ir jautrumas insulinui, paviršinio PRA MNRR bei PreRA PNRR kiekių plotų po kreive (angl. *area under the curve*, AUC) reikšmės statistiškai reikšmingai skyrėsi nuo atskaitos linijos ir yra tinkamos prognozuoti MetS pasireiškimą. Taip pat išskirtos nutukusių asmenų MetS prognozavimo rodiklių slenkstinės reikšmės.

Rodikliai, reikšmingai koreliuojantys su MetS, toliau panaudoti sudarant prognostinius regresijos modelius:

$$P\ (MS=1)=\frac{e^{6,53-0,27*Adiponektinas\ (\mu g/ml)+0,29*\mathrm{VRA}_{sl}\ (cm)-1,43*MNRR\ PRA1\ (\%)}}{1+e^{6,53-0,27*Adiponektinas\ (\mu g/ml)+0,29*\mathrm{VRA}_{sl}\ (cm)-1,43*MNRR\ PRA1\ (\%)}}$$

Regresijos modelio bendras teisingai suklasifikuotų pacientų procentas sudarė 85,5 proc. ROC kreivės analizė parodė, jog šio regresinio modelio plotas po kreive yra statistiškai reikšmingas (AUC = 0,88, PI 0,79–0,97), jo specifiškumas siekia 83,3 proc., jautrumas – 87,2 proc.

Nustatyta, kad 1 cm ultragarsu išmatuoto VRA storio iki juosmeninio slankstelio padidėjimas metabolinio sindromo išsivystymo tikimybę nutukusiam ligoniui padidina 1,34 (95 % PI, 1,12–1,68) karto, o 1 μg/ml adiponektino koncentracijos bei 0,1 proc. mononesočiųjų riebalų rūgščių koncentracijos padidėjimas metabolinio sindromo išsivystymo tikimybę nutukusiam ligoniui sumažina 1,3 (95 % PI, 0,59–0,92) ir 4,17 (95 % PI, 0,08–0,57) karto.

8. Išvados

1. Metabolinį sindromą turinčių nutukusių ligonių grupėje centrinio riebalinio audinio storis, matuojant ultragarsu, ir antropometrinių tyrimų rodikliai (juosmens apimtis bei juosmens ir klubų santykis) yra statistiškai reikšmingai didesni negu nutukusių ligonių, neturinčių metabolinio sindromo.
2. Tiriant ^{1}H-MBR spektroskopijos metodu, nustatyta, kad metabolinį sindromą turinčių nutukusių ligonių grupėje mononesočių riebalų rūgščių kiekis paviršiniame poodiniame audinyje yra statistiškai reikšmingai mažesnis, o polinesočių riebalų rūgščių kiekis paviršiniame poodiniame bei preperitoniniame riebaliniame audiniuose didesnis negu metabolinio sindromo neturinčių ligonių grupės.
3. Adiponektinas, visceralinio riebalinio audinio storis iki juosmeninio slankstelio viršutinio krašto ir mononesočiųjų riebalų rūgščių kiekis paviršiniame poodiniame riebaliniame audinyje yra nepriklausomi prognostiniai veiksniai, turintys didžiausią įtaką nutukusių asmenų metaboliniams sutrikimams atsirasti.

9. Darbo tąsos kryptys

1. Tikslingi nutukusių asmenų riebalinio audinio riebalų rūgščių sudėties ir metabolizmo sąsajų su metaboliniais sutrikimais tolesni tyrimai.
2. Reikalingas riebalinio audinio morfologinės sandaros nustatymas moderniomis vaizdinimo priemonėmis, svarbu genetiniai tyrimai ir uždegiminių rodiklių paieška.
3. Tikslingi tolesni populiaciniai tyrimai prognostinės formulės reikšmingumo lygmeniui ir prognostinių veiksnių ribinėms reikšmėms nustatyti.
4. Reikalingas metabolinių sutrikimų neturinčių ligonių ilgalaikis sekimas, vertinant šių ligonių metabolinės sveikatos pokyčius.

Žygimantas Juodeikis

ADJUSTABLE GASTRIC BANDING: A RANDOMIZED CLINICAL TRIAL COMPARING 5-YEAR RESULTS OF TWO DIFFERENT BANDS

CHIRURGINIS NUTUKIMO GYDYMAS APJUOSIANT SKRANDĮ REGULIUOJAMA JUOSTA: PERSPEKTYVINIS ATSITIKTINĖS ATRANKOS IMČIŲ DVIEJŲ JUOSTŲ PALYGINAMASIS TYRIMAS 5 METŲ LAIKOTARPIU

Biomedical sciences, medicine (06 B)
Biomedicinos mokslai, medicina (06 B)

Abbreviations

%EWL – percentage excess weight loss
%EBMIL – percentage excess body mass index loss
%TWL – percentage total weight loss
AHT – arterial hypertension
BAROS – Bariatric Analysis and Reporting Outcome System
BMI – body mass index
BMIL – body mass index loss
CVD – cardiovascular diseases
DJD – degenerative joint disease
DM – diabetes mellitus
EBMI – excess body mass index
EW – excess weight
GERD – gastroesophageal reflux disease
HDL – high density lipoprotein
LAGB – laparoscopic adjustable gastric banding
LDL – low density lipoprotein
MS – metabolic syndrome
SAGB – Swedish Adjustable Gastric Band
SPSS – Statistical Package for the Social Sciences
TWL – total weight loss

Introduction

Laparoscopic adjustable gastric banding (LAGB) is now a well-established and standardized procedure, which is considered to be the least invasive, easily reversible, and the safest bariatric operation in regard to perioperative complication and mortality rates. Many different adjustable gastric bands are used worldwide. These bands differ in design, filling volume, internal pressure, and fixation mechanism. Hypothetically, these differences could influence the long-term outcomes. There are many publications comparing different gastric bands; however, most are focused on Swedish Adjustable Gastric Band (SAGB) and LAP-BAND devices. The main difference between SAGB and MiniMizer Extra bands is their distinct fixation method (with and without plication), which theoretically could contribute to different postoperative complication rates. Only one randomized study comparing early postoperative period outcomes of SAGB and MiniMizer Extra bands was found. This lack of research indicates the need to compare the long-term performance of these two bands.

The aim of the study

The aim of this study was to evaluate 5-year results comparing two different adjustable gastric bands.

Objectives

1. To evaluate 5-year results in terms of complications, weight loss, resolution of comorbidities and quality of life.
2. To compare 5-year results using two different bands.
3. To evaluate the influence of preoperative factors on the results of weight loss.

Scientific novelty of the study

1. The present study is the first randomized clinical trial comparing SAGB and MiniMizer Extra bands and providing 5-year results.
2. This is the first randomized controlled study performed in Baltic countries that provides 5-year results of LAGB in terms of weight loss, comorbidities, and quality of life.
3. For the first time, two different gastric band fixation methods (with and without plication) are compared.

Practical significance

1. The present study provides 5-year results of LAGB in terms of weight loss, improvement of obesity related comorbidities, and quality of life.
2. This study demonstrates that in the 5-year postoperative period two different fixation methods (with and without plication) are equally safe and efficient.
3. The data of this study enabled us to build %EWL predicting equations.
4. Based on the findings of this study, the modified band fixation method (MiniMizer Extra) was proposed.

Defensive statements

1. LAGB is a safe and effective bariatric procedure for weight loss.
2. LAGB is a safe and effective bariatric procedure for obesity related comorbidities.
3. There are no essential differences between SAGB and MiniMizer bands.

Materials and methods

The study was reviewed and approved by the Lithuanian Bioethics Committee. All patients seeking bariatric surgery were invited to participate in the study. From December 2009 to January 2010, a total of 103 morbidly obese patients underwent operations at the Centre of Abdominal Surgery of Vilnius University Hospital Santaros Klinikos.

Patients were eligible if they were aged between 18 and 70 years, with a BMI more than 40 kg/m2 or a BMI between 35 and 40 kg/m2 with obesity-related comorbidities.

Candidates were excluded if they had a history of previous bariatric surgery, were currently pregnant, or displayed other contraindications for laparoscopic surgery.

Patients were equally randomized (1:1) to one of two parallel groups to receive either the Swedish Adjustable Gastric Band (SAGB; Obtech Medical, Switzerland) or the MiniMizer Extra (Bariatric Solutions GmbH, Switzerland) band.

Laparoscopic gastric banding was performed using a *pars flaccida* technique. The SAGB was fixed by creating a gastric fold over the band (plication) with 3 to 4 gastro- gastric 2–0 silk stitches, and MiniMizer Extra retaining loops were fixed directly to the anterior gastric wall using 5 (two on the upper edge and three on the lower) interrupted 2–0 silk stitches (Fig. 1).

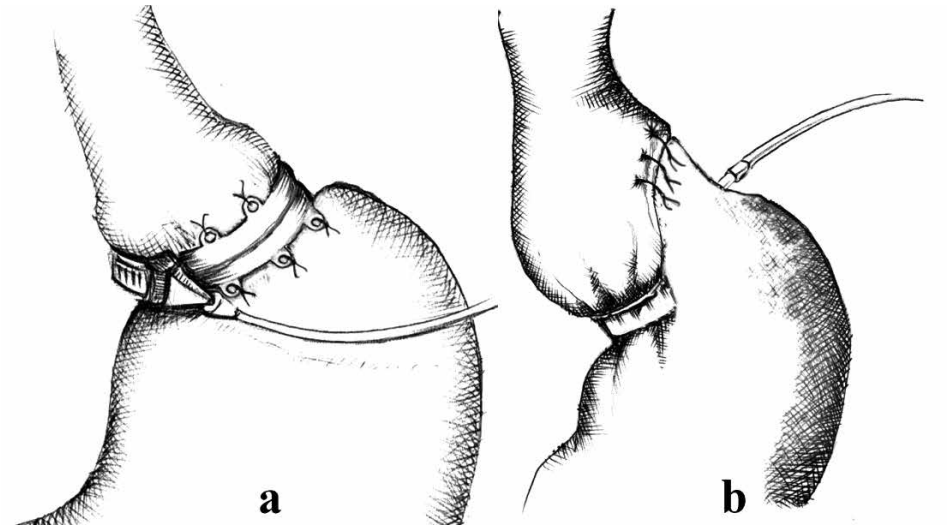


FIG. 1 Fixation without plication (MiniMizer Extra) (a) and fixation with plication (SAGB) (b)

The primary endpoint was weight loss, and secondary endpoints were complication rate, improvement of comorbidities, and quality of life.

Preoperative evaluation was performed by a multidisciplinary team, consisting of an endocrinologist, gastroenterologist, dietitian, cardiologist, and bariatric surgeon.

Assessed comorbidities included diabetes mellitus (DM), arterial hypertension (AHT), cardiovascular diseases (CVD), dyslipidemia, metabolic syndrome (MS), gastroesophageal reflux disease (GERD), and degenerative joint disease (DJD).

Comorbidities were determined based on information given by the patients, clinical examination, and blood samples, obtained after an overnight fast. Moreover, all patients underwent upper gastrointestinal endoscopy, abdominal sonography, and upper gastrointestinal tract radiography. The resolution and improvement criteria of comorbidities were adapted according to the Bariatric Analysis and Reporting Outcome System (BAROS).

Patients were defined as diabetic if diabetes has been diagnosed previously or fasting plasma glucose concentration was ≥7 mmol/L. Arterial hypertension was denoted if a resting blood pressure exceeded 140/90 mmHg or the patient was using antihypertensive medication. The diagnosis of cardiovascular diseases was based on previously documented evidence of coronary artery disease, peripheral vascular disease or congestive heart failure.

Dyslipidemia was defined as fasting concentrations of LDL of ≥3.3 mmol/L, HDL <1.03 mmol/L (males) and HDL <1.29 mmol/L (females), triglycerides ≥1.7 mmol/L, or the use of lipid-lowering medication, while metabolic syndrome was diagnosed according to the Adult Treatment Panel III guidelines. GERD was diagnosed by upper gastrointestinal endoscopy, self-reported symptoms, and the use of anti-reflux medication. DJD was diagnosed based on self-reported symptoms and previously documented radiographic studies. Quality of life was assessed using a modified Moorehead-Ardelt quality of life (M- AQoLII) questionnaire. It's important to mention that changes in obstructive sleep apnea and infertility were not included in the final score, as it was not possible to conduct polysomnography and all the hormonal studies suggested by BAROS.

Statistical analysis was performed using SPSS version 21.0 (SPSS Inc., Chicago). The Pearson χ^2 and Fisher exact tests were used to test for differences in categorical variables, and either the *t*-test or Mann-Whitney two-sample tests for continuous variables were used depending on distribution. A *p* value less than 0.05 was considered to be statistically significant. The variables strongly correlating with %EWL and not correlating with each other were used to build EWL-predicting linear regression models.

Results

A total of 103 morbidly obese patients were randomly assigned to either the SAGB or MiniMizer Extra groups. Their baseline characteristics are depicted in Table 1.The mean preoperative age in both groups was 45.9 ± 11.7 years, 69 (67%) patients were women, and 34 (33%) were men. Preoperative BMI was 47.5 ± 7.3 kg/m^2.

TABLE 1 Baseline characteristics

	All patients	SAGB	MiniMizer	*p*-value
Number of patients, N	103	49	54	0.622
Age (years)	45.9 ±11.7	46.1 ±11.5	45.8 ±11.9	0.869
Gender:				
Female (%)	69 (67)	31 (63.6)	38 (70.4)	0.530
Male (%)	34 (33)	18 (36.7)	16 (29.6)	0.942
Body weight (kg)	137.6 ±24.4	141.8 ±24.2	133.8 ±24.0	0.093
BMI (kg/m^2)	47.5 ±7.3	48.6 ±7.9	46.5 ±6.7	0.157
EBMI (kg/m^2)	22.5 ±7.4	23.6 ±7.9	21.5 ±6.7	0.157
EW (kg)	64.9 ±21.2	68.5 ±22.1	61.8 ±20.0	0.107

BMI – body mass index, EBMI – excess BMI, EW – excess weight

A total of 90 of 103 patients (87.3%) completed the 5-year follow-up; 4 (3.8%) patients died from unrelated causes, 6 (5.8%) had their band removed, and 3 (2.9%) patients were unable to attend the follow-up assessment. One death was caused by lung cancer, and the others were the result of acute cardiovascular events (1 to 4 years postoperatively). Patients whose bands had to be removed were excluded from further weight loss analysis.

On average, patients received 6.16 (7.1 in SAGB and 5.5 in MiniMizer groups; $p = 0.08$) band adjustments over the 5-year period.

All weight loss parameters at 5 years are depicted in Table 2. The average %EWL after 5 years was 47.3%. A %EWL of more than 50 % was observed in 34 (38.6%) patients. The proportion of patients who reached a BMI <35 kg/m2 at year 5 was significantly higher in the MiniMizer group ($p = 0.011$). There was no difference between bands in terms of %EWL in patients younger than 40 years; however, the MiniMizer Extra patients in over-40 group tended to lose more weight (51.2 ± 25.7 vs 38.9 ± 27.0%; $p = 0.035$).

TABLE 2 Weight parameters 5 years after LAGB

Weight variable	All patients	SAGB	MiniMizer	*p*-value
%EWL	47.3 ±29.7	44.1 ±31.9	50.3 ±27.6	0.148
Weight (kg)	107.6 ±26.2	110.7 ±25.7	104.8 ±26.6	0.290
BMI (kg/m^2)	37.1 ±8.3	38.3 ±8.8	36.0 ±7.8	0.092

Weight variable	All patients	SAGB	MiniMizer	*p*-value
BMIL (kg/m^2)	10.1 ±6.7	9.5 ±6.7	10.6 ±6.6	0.325
TWL (kg)	28.8 ±18.3	27.0 ±18.6	30.5 ±18.1	0.372
%TWL	21.2 ±13.2	19.7 ±13.5	22.6 ±13.0	0.309
BMI <35, N (%)	38 (40.4)	11 (25.5)	27 (52.9)	0.011*
%EWL >50%, N (%)	34 (38.6)	13 (30.2)	21 (46.6)	0.130

%EWL – percentage excess weight loss, N – number of the patients, TWL – total weight loss, BMIL – BMI loss; *p <0.05

The preoperative distribution of comorbidities is shown in Table 3. Five years following LAGB, the resolution or improvement of type 2 diabetes was observed in 14 (56%) patients, arterial hypertension improved or resolved in 35 (53%), cardiovascular disease in 7 (50%), dyslipidemia in 44 (78.5%), GERD in 21 (67.7%), and degenerative joint diseases improved in 23 (40.3%) patients. Only 26 out of 69 patients with MS before operation met MS criteria 5 years following LAGB, and a resolution was observed in 62.3% of the patients.

TABLE 3 Comorbidities at the baseline

Comorbidities	Total N (%)	SAGB N (%)	MiniMizer N (%)	*p*-value
DM	36 (34.9)	21 (42.8)	15 (27.7)	0.111
AHT	82 (79.6)	35 (71.4)	47 (87.0)	0.055
CVD	21 (20.3)	11 (22.4)	10 (18.5)	0.621
Dyslipidemia	69 (66.9)	36 (73.4)	33 (62.2)	0.227
MS	69 (66.9)	33 (67.3)	36 (66.6)	0.634
GERD	45 (43.6)	21 (42.8)	24 (44.4)	0.871
DJD	71 (68.9)	35 (71.4)	36 (66.6)	0.602

DM – diabetes mellitus, AHT – arterial hypertension, CVD – cardiovascular disease, MS – metabolic syndrome, GERD – gastroesophageal reflux disease, DJD – degenerative joint diseases

No significant difference regarding resolution and improvement of comorbidities was found between the compared bands (Tables 4-5).

TABLE 4 Resolution of comorbidities at 5 years

Comorbidities	SAGB N (%)	MiniMizer N (%)	*p*-value
DM	3 (20)	2 (20)	0.951
AHT	5 (15.6)	7 (15.2)	0.722
CVD	1 (11.1)	0 (0)	0.281
Dyslipidemia	25 (86.2)	19 (70.3)	0.149
MS	20 (60.6)	23 (63.8)	0.793
GERD	7 (36.8)	5 (20.8)	0.379
DJD	3 (9.3)	7 (20)	0.813

DM – diabetes mellitus, AHT – arterial hypertension, CVD – cardiovascular disease, MS – metabolic syndrome, GERD – gastroesophageal reflux disease, DJD – degenerative joint diseases

TABLE 5 Improvement of comorbidities at 5 years

Comorbidities	SAGB N (%)	MiniMizer N (%)	*p*-value
DM	7 (46.6)	2 (20)	0.182
AHT	11 (34.3)	12 (26)	0.881
CVD	2 (22.2)	4 (40)	0.506
GERD	6 (31.5)	3 (12.5)	0.202
DJD	5 (15.6)	9 (25.7)	0.384

DM – diabetes mellitus, AHT – arterial hypertension, CVD – cardiovascular disease, GERD – gastroesophageal reflux disease, DJD – degenerative joint diseases

TABLE 6 Complications 5 years post-LAGB

Adverse event	Total N (%)	SAGB N (%)	MiniMizer N (%)	*p*-value
Band erosion	5 (4.8)	0 (0)	5 (9.2)	0.058
Band slippage	4 (3.8)	2 (4.0)	2 (3.7)	0.921
Band intolerance	3 (2.9)	1 (2)	2 (3.7)	0.609
Port related	4 (3.8)	1 (2.0)	3 (5.5)	0.356
Total	16 (15.5)	4 (8.1)	12 (22.2)	0.060

The overall complication rate at 5 years was 15.5%. There was no significant difference between the MiniMizer Extra and SAGB groups. Complications consisted of 5 band erosions, 4 band slippages, 4 port-related complications, and 3 band intolerances (Table 6). Port-related complications consisted of 3 port-site infections and one port inversion. None of the port site infections were related to band erosion.

In 13 (12.6%) patients the complications had to be managed surgically. Six bands had to be removed (1 in SAGB and 5 in MiniMizer group; $p = 0.13$): 3 bands were removed due to erosions (3 MiniMizer patients) and 3 due to patient psychological intolerance and insufficient weight loss (1 SAGB and 2 MiniMizer patients). All three band slippages were managed by laparoscopic band reposition.

The average M-AQoLII questionnaire score significantly improved from 0.02 ± 1.2 points at baseline to 1.0 ± 1.2 after 5 years ($p < 0.001$). No significant differences were found between the SAGB and MiniMizer groups. All scores, except the social activity score ($p = 0.08$), significantly improved after 5 years. The average BAROS score after 5 years was 2.92 ± 2.59 in the SAGB group and 3.22 ± 2.19 in the MiniMizer Extra group ($p = 0.47$), which is considered a good outcome.

In order to predict %EWL, we measured its correlation with multiple preoperative anthropometric and blood test indices. Parameters strongly correlated with %EWL at 5 years and uncorrelated with each other were used to build a %EWL predicting linear regression models (Table 7).

TABLE 7 Preoperative indices in the %EWL predicting models

Group	Predicting parameter	β	R^2
SAGB and MiniMizer	Waist circumference HbA1c Gender	-0.298 -0.172 0.201	0.227
SAGB	Waist circumference Gender Cortisol	-0.449 0.285 0.358	0.442
MiniMizer	HbA1c	-0.328	0.108

HbA1c – glycated hemoglobin, β – beta coefficient, R^2 – determination coefficient

In the overall patient group, the %EWL was predicted by gender, waist circumference and the concentration of HbA1c. The final regression model was:

%EWL = 121.239 – 0.515 × waist circumference (cm) – 4.21 × HbA1c + 12.73 × gender (male – 1, female – 2)

In the SAGB patient group, the %EWL was predicted by preoperative waist circumference, gender, and cortisol concentration, resulting in this final regression model:

$$\%EWL = 87.118 - 0.8 \times \text{waist circumference (cm)} + 19.663 \times$$
$$\times \text{gender (male – 1, female – 2)} + 0.109 \times \text{cortisol concentration}$$

The determination coefficient R^2 was 0.442.

In the MiniMizer Extra patient group, the %EWL was only predicted by the preoperative concentration of HbA1c. The final regression model here was:

$$\%EWL = 110.374 - 10.017 \times \text{HbA1c concentration}$$

However, because the determination coefficient R^2 was 0.108, the predicting value of this model is uncertain.

Conclusions

1. LAGB is a safe and effective bariatric procedure; after 5 years the average %EWL was 47.3 ± 29.7%; the overall complication rate was 15.5%, and the mean BAROS and Moorhead-Ardelt quality of life questionnaire scores indicated good results.
2. After 5 years a significantly higher proportion of patients reached a BMI below 35 in the MiniMizer band group; therefore, the %WL and %EBMIL were also significantly higher in the MiniMizer group. The remaining parameters regarding weight loss were comparable between the two groups.
3. There were no differences between the two bands regarding the resolution of comorbidities, morbidity, and quality of life.
4. The derived predicting equations enable us to predict %EWL for the individual patient.

Further directions

The patients in this study will be followed in order to obtain 10-year postoperative results.

Publications on the subject of the doctoral dissertation

1. Juodeikis Ž, Abalikšta T, Brimienė V, Brimas G. Laparoscopic Adjustable Gastric Banding: a Prospective Randomized Clinical Trial Comparing 5-Year Results of two Different Bands in 103 Patients. Obes Surg. 2017 Apr;27(4):1024-1030.

2. Juodeikis Ž, Brimas G. Preoperative prognostic factors for weight-loss after adjustable gastric banding: 5-year results of randomized controlled trial. Liet Chir. 2017 Mar 21;17(1):57.

3. Juodeikis Ž, Brimas G. Long-term results after sleeve gastrectomy: A systematic review. Surg Obes Relat Dis. 2016 Oct 17. pii: S1550-7289(16)30745-6.

4. Urbanavicius V, Juodeikis Z, Dzenkeviciute V, Galkine A, Petrulioniene Z, Sapoka V, Brimiene V, Vitkus D, Brimas G. A prospective 4-year study of insulin resistance and adipokines in morbidly obese diabetic and non-diabetic patients after gastric banding. Wideochir Inne Tech Maloinwazyjne. 2017 Jun;12(2):147-153.

Presentations

8th Congress of Baltic Association of Surgeons 2015.09.10-12 Tallinn, Estonia.

1. Žygimantas Juodeikis, Bronius Buckus, Edvardas Brimas, Gintautas Brimas.

Laparoscopic adjustable gastric banding: a prospective randomized study comparing long term results of two different bands in 103 patients.

2. Žygimantas Juodeikis, Bronius Buckus, Gintautas Brimas. Laparoscopic adjustable gastric banding: long-term results of a prospective study.

Moscow international bariatric congress 2016.04.20-22 Moscow, Russia.

3. Žygimantas Juodeikis, Vilma Brimienė, Gintautas Brimas. Laparoscopic adjustable gastric banding: A prospective randomized study comparing 5-year results of two different bands in 103 patients.

4. Gintautas Brimas, Edvardas Brimas, Žygimantas Juodeikis. Adjustable gastric band slippage: A single centre experience of laparoscopic band repositioning.

22nd World Congress of the International Federation for the Surgery of Obesity and Metabolic Disorders, 2017.08.29-09.02 London, Great Britain

5. Žygimantas Juodeikis, Gintautas Brimas. Laparoscopic adjustable gastric banding: a prospective randomized study comparing 6-year results of two different bands in 103 patients.

SANTRAUKA

Darbo tikslas

Įvertinti skirtingų skradį apjuosiančių reguliuojamų juostų (SARJ) įtaką 5 metų gydymo rezultatams.

Darbo uždaviniai

1. Įvertinti skradžio apjuosimo reguliuojama juosta operacijos (SARJO) 5 metų gydymo rezultatus, atsižvelgiant į komplikacijų dažnį, kūno masės, gretutinių ligų ir gyvenimo kokybės pokyčius;
2. Palyginti 5 metų chirurginio gydymo rezultatus, naudojant skirtingas SARJ;
3. Įvertinti priešoperacinių veiksnių įtaką kūno masės rodikliams 5 metų laikotarpiu ir nustatyti prognostinius rodiklius.

Darbo naujumas

1. Pirmą kartą Baltijos šalyse perspektyvios atsitiktinės atrankos tyrime pateikiami 5 metų nutukimo gydymo, gretutinių ligų dinamikos ir gyvenimo kokybės rezultatai po SARJO;
2. Pirmą kartą pasaulyje palygintos dviejų skirtingų tipų (tvirtinamų su plikacija ir be plikacijos) juostos – SAGB-VC ir Minimizer Extra 5 metų pooperaciniu laikotarpiu;
3. Pirmą kartą pasaulyje pateikiami 5 metų chirurginio nutukimo gydymo, gretutinių ligų dinamikos ir gyvenimo kokybės rezultatai, operacijos metu panaudojus SARJ be plikacijos (Minimizer Extra).

Praktinė reikšmė

1. Darbe įvertinti 5 metų SARJO rezultatai gydant nutukimą, su juo susijusias gretutines ligas ir pooperacinė gyvenimo kokybė;
2. Perspektyviuoju atsitiktinių imčių tyrimu įrodyta, kad be plikacijos tvirtinama juosta (Minimizer Extra) 5 metų pooperaciniu laikotarpiu yra tokia pat saugi ir efektyvi gydant nutukimą bei su juo susijusias gretutines ligas ir gerinant gyvenimo kokybę, kaip ir juosta, fiksuojama su plikacija (SAGB-VC);
3. Remiantis darbo rezultatų analize, sukurti prognostiniai modeliai, galintys numatyti procentinį perteklinės kūno masės sumažėjimą po 1 ir 5 metų;
4. Remiantis darbo rezultatų analize, pasiūlytas modifikuotas SARJO, naudojant SARJ be plikacijos (Minimizer Extra) būdas.

Ginamieji disertacijos teiginiai

1. SARJO yra efektyvus ir saugus būdas gydyti morbidinį nutukimą;
2. SARJO yra efektyvus ir saugus būdas gydyti gretutines ligas morbidiniu nutukimu sergantiems pacientams;

3. Esminių skirtumų operacijai naudojant SAGB-VC ir Minimizer Extra juostas 5 metų pooperaciniu laikotarpiu nėra.

Darbo metodologija

Šis darbas yra tęstinis perspektyvusis atsitiktinės atrankos imčių lyginamasis biomedicininis tyrimas, kuriame lyginami 5 metų chirurginio nutukimo gydymo rezultatai, naudojant dvi skirtingas SARJ. Tyrimą patvirtino Lietuvos bioetikos komitetas.

Remiantis šio tyrimo vienerių metų rezultatais, Vilniaus universitete T. Abalikšta apgynė daktaro disertaciją, kurioje išsamiai aprašyta šio tyrimo metodologija.

Į tyrimą buvo įtraukti Vilniaus universiteto ligoninės Santaros klinikų pacientai. Nuo 2009 m. sausio 1 d. iki 2010 sausio 31 d. dalyvauti buvo siūloma visiems patologiniu nutukimu sergantiems pacientams, kurie atitiko atrankos kriterijus.

Pacientų įtraukimo kriterijai:

1) amžius nuo 18 iki 70 metų;

2) KMI ≥ 40 kg/m^2;

3) KMI ≥ 35 kg/m^2, esant su nutukimu susijusių gretutinių ligų.

Pacientų atmetimo kriterijai:

1) kontraindikacijos laparoskopinei operacijai;

2) anksčiau atliktos bariatrinės operacijos;

3) nėštumas.

Visiems tyrimo dalyviams pateiktos Lietuvos bioetikos komiteto patvirtintos asmens informavimo ir informuoto asmens sutikimo formos. Tyrimas buvo atsitiktinės atrankos imčių – pacientai turėjo pasirinkti vieną iš dviejų vienodų vokų, kuriuose buvo skirtingų SARJ pavadinimai:

1) SAGB (angl. *Swedish Adjustable Gastric Band*), Obtech Medical;

2) MiniMizer Extra, Bariatric Solutions GmbH.

Priešoperacinis pacientų tyrimas

Visus pacientus prieš operaciją ištyrė multidisciplininė gydytojų komanda, kurią sudarė chirurgas, gastroenterologas, endokrinologas, kardiologas ir dietologas. Pacientai buvo pasverti, išmatuotos juosmens, klubų, žasto apimtys ir ūgis, įvertintos gretutinės su nutukimu susijusios ligos ir metabolinio sindromo kriterijai. Kiekvienas ligonis užpildė antrąjį Moore-heado–Ardelto gyvenimo kokybės klausimyną, be to, visiems ligoniams atlikti tie patys kraujo ir instrumentiniai tyrimai:

- Pilvo organų echoskopija;
- Elektrokardiografija;
- Ultragarsinis širdies ir kraujagyskių tyrimas;
- Viršutinės virškinamojo trakto dalies endoskopija;
- Stemplės ir skrandžio rentgenoskopija.

Operacija

Pacientams atlikta laparoskopinė skrandžio apjuosimo reguliuojama juosta operacija, taikant *pars flaccida* metodiką. Visas operacijas atliko vienas chirurgas.

Pakartotinis pacientų tyrimas ir rezultatų vertinimas

Pagal tyrimo protokolą visi pacientai buvo pakartotinai iškviesti kontroliniams tyrimams praėjus 1 ir 5 metams po operacijos.

Vertinant gydymo rezultatus, pirminis gydymo tikslas buvo procentinis perteklinės kūno masės sumažėjimas (%PKMS). Antriniai kriterijai – gyvenimo kokybės ir gretutinių ligų pokyčiai, pooperacinės komplikacijos ir pakartotinės operacijos.

Visi ligoniai pasverti, išmatuotos klubų, juosmens ir žasto apimtys, įvertinti metabolinio sindromo kriterijai ir gretutinių ligų dinamika. Atlikti tie patys kraujo ir instrumentiniai tyrimai.

Pacientai, kuriems 5 metų laikotarpiu pašalinta SARJ, į kūno masės kitimo analizę neįtraukti.

Į cukriniu diabetu sergančių pacientų grupę įtraukti ligoniai, kuriems cukrinis diabetas diagnozuotas anksčiau arba kurių alkio glikemija buvo ≥7 mmol/l. Metabolinio sindromo diagnostikai pasitelkti ATP-III (angl. *Adult Treatment Panel III Guidelines*) diagnostiniai kriterijai. Gyvenimo kokybė vertinta naudojant modifikuotą Moorehead– Ardelt gyvenimo kokybės klausimyną. Tiriant ligonius buvo pildoma tyrimo dalyvio duomenų ir paciento stebėjimo anketos.

Gydymui vertinti taikėme BAROS (angl. *Bariatric Analysis and Reporting Outcome System*) sistemą, tačiau iš septynių didžiųjų gretutinių ligų vertinome tik arterinę hipertenziją, širdies ir kraujagyslių ligas, dislipidemiją ir cukrinį diabetą. Obstrukcinė miego apnėja, osteoartritas ir nevaisingumas nevertinti, nes nebuvo galimybės atlikti polisomnografijos, radiologinio sąnarių tyrimo ir nevaisingumo diagnostikai rekomenduojamų hormonų tyrimų.

Statistinė analizė

Duomenų analizė atlikta taikant SPSS Statistics 21.0 (angl. *Statistical Package for Social Sciences*) statistikos programų paketą. Lygindami grupes, apskaičiavome intervalinių kintamųjų vidurkius ir standartinius nuokrypius, o kategorinių kintamųjų – absoliučias ir procentines vertes. Normalusis kintamųjų pasiskirstymas tikrintas pasitelkus Kolmogorovo ir Smirnovo testą. Lygindami dvi nepriklausomas imtis, kuriose intervaliniai kintamieji buvo su normaliuoju pasiskirstymu, naudojome Studento *t* testą. Lygindami imtis, kuriose intervaliniai kintamieji buvo su nenormaliuoju pasiskirstymu, taikėme Mann–Whitney U testą. Skirtumui tarp kategorinių kintamųjų nustatyti pasitelktas Chi kvadrato arba tikslusis Fisherio testas. Lyginant dvi imtis, skirtumas laikytas statistiškai reikšmingu, kai $p < 0{,}05$. Koreliacijai nustatyti naudotas Pearsono koreliacijos koeficientas. Atlikdami prognostinių veiksnių, turinčių įtakos %PKMS, paiešką, sukūrėme regresijos modelius, juose palikdami statistiškai reikšmingus ir tarpusavyje nekoreliuojančius kintamuosius.

Tyrimų rezultatai

Pacientų įtraukimas į tyrimą vyko nuo 2009 m. sausio 1 d. iki 2010 m. sausio 31 d. Vilniaus universiteto ligoninės Santariškių klinikose. Iš viso įtraukti 103 tiriamieji, iš kurių 34 (33 %) buvo vyrai ir 69 (67 %) moterys. Amžiaus vidurkis buvo 46,1 metai (nuo 21 iki 70), vidutinis svoris – 141,9 kg (nuo 87,4 iki 199,3). MiniMizer Extra SARJ naudota 54 pacientams, o SAGB – 49. Pagal demografinius ir kūno masės rodiklius SAGB ir MiniMizer grupės prieš operaciją nesiskyrė.

Prieš operaciją 82 (79,6 %) ligoniai sirgo arterine hipertenzija, 71 (68,9 %) degeneracinėmis sąnarių ligomis, 69 (66,9 %) hipercholesterolemija, 45 (43,6 %) gastroezofaginio refliukso liga (GERL), 34 (33 %) kojų venų varikoze, 36 (34,9 %) antro tipo cukriniu diabetu, 28 (27,2 %) obstrukcine miego apnėja, 28 (27 %) tulžies pūslės akmenlige, 21 (20,4 %) ezofagitu, 21 (20,3 %) kardiovaskulinėmis ligomis. Reikšmingo skirtumo tarp SARJ grupių nebuvo.

Praėjus 1 metams po operacijos ištirta 90 (87,3 %) ligonių. Tolesniame tyrime nedalyvavo 13 pacientų: 10 negalėjo atvykti, 1 ligonė mirė (dėl miokardo infarkto), 1 neatvyko dėl nėštumo ir 1 neįtrauktas dėl SARJ penetracijos ir juostos pašalinimo.

Praėjus 5 metams ištyrėme 90 (87,3 %) ligonių. Iš 13 prarastų pacientų, 3 ligoniai negalėjo atvykti (su 1 nepavyko susisiekti, 1 atsisakė nuo tolesnio tyrimo), 4 ligoniai mirė [1 dėl plaučių vėžio (3 metai po SARJO), 3 – dėl miokardo infarkto (1-4 metais po SARJO)]. Viršutinės virškinamojo trakto dalies endoskopinis tyrimas atliktas 67 (74,4 %) pacientams. Šeši pacientai, kuriems 5 metų laikotarpiu pašalinta SARJ (5 MiniMizer ir 1 SAGB), į tolesnį stebėjimą neįtraukti.

Lyginant priešoperacinius kūno masės rodiklius su 1 ir 5 metų rodikliais ir vienerių metų su penkerių metų rodikliais, stebėtas reikšmingas kūno masės (KM) rodiklių mažėjimas. MiniMizer Extra grupėje buvo reikšmingai didesnė ligonių dalis, pasiekusi KMI < 35 (27 (52,9 %)), palyginti su 11 (25,5 %) KMI SAGB grupėje ($p = 0,011$). Kitų reikšmingų skirtumų tarp grupių nenustatyta.

Po 1 metų visi gretutiniai susirgimai, išskyrus hipercholesterolemiją, reikšmingai pagerėjo, palyginti su priešoperaciniais duomenimis. Lyginant priešoperacinius ir 5 metų duomenis, statistiškai reikšmingai pagerėjo visi gretutiniai susirgimai, išskyrus hipercholesterolemiją ir ezofagitą. Lyginant rezultatus po 1 ir 5 metų, reikšmingai nesumažėjo tik kardiovaskulinės ligos, hipercholesterolemija ir metabolinio sindromo dažnis.

Beveik pusei antro tipo cukriniu diabetu, arterine hipertenzija ar kardiovaskulinėmis ligomis sergančių tiriamųjų konstatuotas ligos pagerėjimas ar išnykimas. GERL simtomai pagerėjo ar išnyko beveik 70 % ligonių. Degeneracinės sąnarių ligos pagerėjo ar išnyko 40 % ligonių. Hipercholesterolemija išnyko 10 % ligonių. Praėjus 5 metams echoskopiškai nustatyti 5 (4,9 %) nauji tulžies pūslės akmenligės atvejai, o 14 ligonių, kuriems tulžies pūslės akmenligė diagnozuota prieš operaciją, 5 metų laikotarpiu atlikta laparoskopinė cholecistektomija.

Reikšmingų skirtumų tarp lygintų grupių 5 metų laikotarpiu nenustatyta.

Per pirmuosius metus metabolinio sindromo dažnis sumažėjo nuo 73,3 % iki 52,5 %, o po 5 metų metabolinis sindromas išnyko ar pagerėjo beveik dviem trečdaliams (62 %) ligonių. Po 1 metų vidutinis metabolinio sindromo kriterijų skaičius statistiškai reikšmingai sumažėjo, tačiau lyginant 1 ir 5 metų duomenis reikšmingo skirtumo nebuvo ($p = 0,75$).

Per penkerius metus SARJ vidutiniškai reguliuota 7,12 ± 5,1 kartų SAGB grupėje ir 5,53 ± 3,2 MiniMizer Extra grupėje (p = 0,08). Nustatyta teigiama koreliacija tarp juostos reguliavimų skaičiaus ir procentinio kūno masės sumažėjimo (%KMS) (r = 0,388; p < 0,001), procentinio perteklinės kūno masės sumažėjimo (%PKMS) (r = 0,309; p = 0,004) ir kūno masės indekso sumažėjimo (KMIS) (r = 0,42; p < 0,001).

Iš viso buvo nustatyta 16 (15,5 %) komplikacijų. Reikšmingų skirtumų tarp lyginamų grupių nepastebėta. Iš viso endoskopiškai nustatytos 5 (4,8 %) SARJ penetracijos į skrandį, visos nustatytos MiniMizer Extra tiriamųjų grupėje, tačiau reikšmingo skirtumo tarp juostų nebuvo (9,2 % ir 0 %; p = 0,058). Trys penetravusios SARJ buvo pašalintos: dvi laparoskopiškai, viena endoskopiškai. Likusios 2 paliktos stebėti. Viena juosta laparoskopiškai šalinta dėl susiformavusio pilvo ertmės pūlinio, kita – po nepavykusio endoskopinio šalinimo. Juostos dislokacija nustatyta 4 ligoniams (po dvi kiekvienoje grupėje), visais atvejais atlikta pakartotinė laparoskopija ir juostos repozicija (regastroplastika). Šešiems ligoniams dėl komplikacijų (3 penetracijos, 3 netoleravimai) SARJ buvo pašalinta. Praėjus 5 metams, rentgenolgiškai nustatyti 5 (4,8 %) stemplės išsiplėtimai (3 MiniMizer ir 2 SAGB juostų grupėse; p = 0,72). Kadangi stemplės išsiplėtimas yra juostos reguliavimų pasekmė, o ne tiesiogiai su SARJO susijusi komplikacija, stemplės dilatacija į bendrą komplikacijų skaičių nebuvo įtraukta.

Gyvenimo kokybė vertinta pasitelkus antrąjį Mooreheado-Ardelto gyvenimo kokybės klausimyną (M-AQoLQII).

Pagal M-AQoLQII vertinimo skalę balų suma visais vertinimo laikotarpiais atitiko patenkinamą rezultatą. Po 5 metų vidutinis įvertinimas visoje tiriamųjų grupėje pagerėjo lyginant su 1 metų rezultatais, tačiau statistiškai reikšmingas nebuvo (p = 0,27). Praėjus 5 metams reikšmingų gyvenimo kokybės skirtumų tarp lygintų juostų grupių nenustatyta. Palyginti su priešoperaciniais rezultatais, visų vertinimų, išskyrus socialinio gyvenimo (p = 0,08), reikšmingas pagerėjimas buvo regimas ir po 5 metų. Reikšmingai pagerėjo tik seksualinio aktyvumo įvertinimas (p = 0,04).

Po 1 metų vidutinis BAROS balas (2,5 ±2,0) atitiko patenkinamą, o praėjus 5 metams (3,0 ±2.3) - gerą rezultatą, tačiau skirtumas nebuvo reikšmingas (p = 0,17). Po 5 metų vidutinis BAROS balas SAGB ir MiniMizer Extra juostų grupėse atitinkamai buvo 2,92 ±2,6 ir 3,18 ±2,1 (p = 0,63), tačiau pagal vertinimo skalę, SAGB grupės balas atitiko patenkinamą, o MiniMizer grupės – gerą rezultatą.

Ieškodami prognostinių veiksnių, apskaičiavome %PKMS koreliaciją su juostos tipu, pacientų amžiumi, lytimi, kūno masės rodikliais, ūgiu, klubų, juosmens ir žasto apimtimis, juosmens ir klubų santykiu, įvairiais kraujo tyrimų duomenimis. Apskaičiavome šių rodiklių koreliaciją su %PKMS praėjus 1 ir 5 metams po SARJO visoje tiriamųjų grupėje ir skirtingų juostų grupėse atskirai. Iš rodiklių, reikšmingai koreliuojančių su %PKMS ir nekoreliuojančių tarpusavyje, sudarėme prognostinius regresijos modelius.

Po 5 metų visoje tiriamųjų grupėje %PKMS nustatyta statistiškai reišminga koreliacija su lytimi, KM, PKM, juosmens apimtimi, šlapimo rūgšties, gliukozės, glikuoto hemoglobino, APO B, ir DTL cholesterolio koncentracijomis. SAGB grupėje %PKMS reikšmingai koreliavo su lytimi, KM, juosmens apimtimi, šlapimo rūgšties, DTL cholesterolio ir kortizolio koncentracijomis. MiniMizer grupėje %PKMS reišmingai koreliavo su glikemija, glikuoto hemoglobino (HbA1c) ir APO B koncentracijomis.

Priešoperaciniai rodikliai, reikšmingai koreliuojantys su %PKMS, panaudoti sudarant prognostinius regresijos modelius.

Praėjus 5 metams, visos tiriamųjų grupės %PKMS įtakos darė lytis, juosmens apimtis ir HbA1c koncentracija. Prognostinis modelis: **%PKMS = 121,239 – 0,515 × juosmens apimtis** (cm) **– 4,21 × HbA1c + 12,73 × lytis** (vyras – 1, moteris – 2). Kadangi šio modelio determinacijos koeficientas R^2 = 0,227, jo taikymas pronozuojant %PKMS yra abejotinas.

Po 5 metų SAGB grupės pacientų %PKMS darė įtakos priešoperacinė juosmens apimtis, lytis ir kortizolio koncentracija. Labiausiai %PKMS veikė juosmens apimtis (β – 0,449). Prognostinis modelis: **%PKMS = 87,118 – 0,8 × juosmens apimtis (cm) + 19,663 × lytis** (vyras – 1, moteris – 2) **+ 0,109 × kortizolio koncentracija**. Determinacijos koeficientas R^2 = 0,442.

MiniMizer grupės ligonių %PKMS įtakos darė tik priešoperacinė glikuoto hemglobino koncentracija. Prognostinis modelis: **%PKMS = 110,374 – 10,017 × HbA1c koncentracija**. Kadangi šio modelio determinacijos koeficientas R^2 = 0,108, jis %PKMS prognozavimui taip pat yra netinkamas.

Kadangi %PKMS reikšmingai koreliavo su amžiumi, palyginome įvairių amžiaus grupių rezultatus. Praėjus 5 metams jaunesnių nei 40 metų pacientų grupėje KM rodikliai tarp lygintų grupių nesiskyrė. Vyresnių nei 40 metų MiniMizer Extra tiriamųjų grupėje reikšmingai didesni išliko %PKMS (p = 0,035) ir %PKMIS (p = 0,012), o KMI buvo reikšmingai mažesnis (p = 0,032).

Kadangi nustatėme reikšmingą koreliaciją tarp %PKMS ir KMI, palyginome įvairias KMI grupes. Paaiškėjo, kad praėjus vien eriems meta ms MiniMizer Extra grupės ligonių, kurių KMI ≤ 47, KM mažėjimo rodikliai geresni.

MiniMizer Extra juostos grupėje %PKMS, KMS ir %KMS buvo reikšmingai didesni.

Tačiau po 5 metų skirtumo tarp lygintų juostų grupių pacientų nebuvo.

Išvados

1. SARJO yra efektyvus ir saugus nutukimo gydymo būdas: po 5 metų vidutinis %PKMS buvo 47,3 ± 29,7 %, bendras komplikacijų dažnis – 15,5 %, o vidutinė BAROS (3,06 ± 2,3) ir Mooreheado-Ardelto gyvenimo kokybės klausimyno (1,0 ±1,2) balų suma atitiko gerus rezultatus.

2. Praėjus 5 metams po operacijos, MiniMizer Extra grupėje statistiškai reikšmingai didesnė ligonių dalis pasiekė KMI < 35 (52,9 % ir 25,5 %, p = 0,011), o vyresnių nei 40 metų MiniMizer Extra grupės pacientų %PKMS (p = 0,035) ir %PKMIS (p = 0,012) buvo statistiškai reikšmingai didesni. Kiti kūno masės rodikliai tarp lygintų SARJ grupių reikšmingai nesiskyrė.

3. Gretutinių ligų dinamika, komplikacijų dažnis ir gyvenimo kokybė tarp lygintų SARJ pacientų grupių reikšmingai nesiskyrė.

4. Taikant išvestas %PKMS prognozuojančias lygtis, galima kiekvienam pacientui apskaičiuoti tikėtiną kūno masės sumažėjimą 5 metų laikotarpiu.

Darbo tąsos kryptys

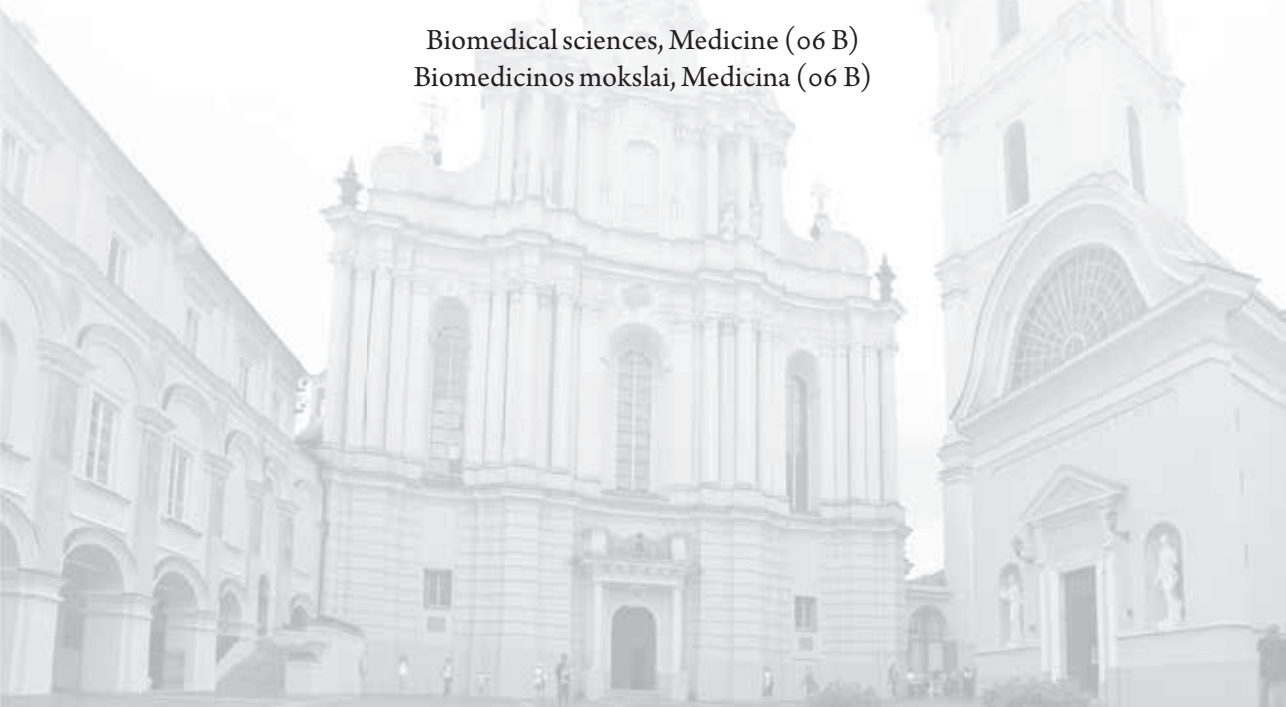
Pagal tyrimo protokolą šios studijos dalyvius planuojama ištirti praėjus 10 metų po operacijos.

Agnė Laučytė-Cibulskienė

ARTERIJŲ STANDUMO IR KITŲ BIOŽYMENŲ PROGNOSTINĖ REIKŠMĖ VERTINANT KRAUJAGYSLIŲ REMODELIACIJĄ IR KARDIOVASKULINĘ RIZIKĄ TARP PAKAITINE INKSTŲ TERAPIJA GYDOMŲ PACIENTŲ

ARTERIAL STIFFNESS AND OTHER BIOMARKERS AS VASCULAR REMODELING AND CARDIOVASCULAR RISK PREDICTORS IN PATIENTS ON RENAL REPLACEMENT THERAPY

Biomedical sciences, Medicine (06 B)
Biomedicinos mokslai, Medicina (06 B)



Santrumpos

AoLK – aortos lanko kalcifikacija
cMV – citomegalo virusas
GSIL – galutinės stadijos inkstų liga
HD – hemodializė
IT – inkstų transplantacija
KMI – kūno masės indeksas
KV – kardiovaskulinis (-ė)
KS – kraujo spaudimas
LIL – lėtinė inkstų liga
MAcE – didieji kardiovaskuliniai įvykiai
mkPBG – miego ir kirkšnies arterijų pulsinės bangos greitis
msPBG – miego ir stipininės arterijų pulsinės bangos greitis
OR – galimybių santykis
p – patikimumas
PBG – pulsinės bangos greitis
PD – peritoninė dializė
PI – pasikliautinasis intervalas
PS – pulsinis spaudimas
PTH – parathormonas
RAAS – renino–angiotenzino–aldosterono sistema
SN – standartinis nuokrypis
β – krypties koeficientas

1. Įžanga

Lėtinė inkstų liga (LIL) – tai pasaulinė sveikatos problema, būdinga apie 10,6–13,4 proc. populiacijos (1) ir lemianti pakitimus, susijusius su neįgalumu. LIL, kaip ir piktnaudžiavimas alkoholiu ar kitomis medžiagomis, kepenų cirozė, bronchų astma, Alzheimerio liga ir kitos demencijos, pjautuvo pavidalo ląstelių liga ir podagra, yra priskiriama prie „užleistų“ neužkrečiamųjų ligų (2). Nuo 2005 iki 2015 metų mirtingumas dėl LIL išaugo 31,7 proc. Vertinant gyvenimo metų trumpėjimo globalias priežastis galima teigti, kad LIL pakilo iš 25-tos vietos (1990 m.) į 21-ą (2005 m.) ir galiausiai į 17-tą (2015 m.) vietą (3).

Širdies ir kraujagyslių sistemos būklė yra glaudžiai susijusi su inkstų veikla. Blogėjant inkstų funkcijai didėja kardiovaskulinių ligų rizika (4). Tai literatūroje vadinama lėtiniu renokardialiniu sindromu, arba 4 tipo kardiorenaliniu sindromu (5). Pacientai, nuolat priklausomi nuo dializių, turi 50 kartų didesnę riziką mirti dėl kardiovaskulinės (KV) patologijos (6, 7).

Šiuo metu pats geriausias galutinės stadijos inkstų ligos (GSIL) gydymo metodas yra inkstų transplantacija (IT). Tačiau net ir po sėkmingos inkstų transplantacijos KV ligų rizika išlieka padidėjusi (8). Pagrindiniai veiksniai, turintys įtakos dažnam KV įvykių pasireiškimui po IT, yra donoro lytis, imunosupresinių vaistų vartojimas, uždegimas, transplantuoto inksto funkcija, homocisteino koncentracija, anemija ir mitybos būklė (9, 10). Kruopštus KV pacientų ištyrimas yra būtinas norint sumažinti GSIL sergančių ligonių sergamumo ir mirtingumo apimtis.

KV sistemos ir inkstų patogenetiniai ryšiai yra labai sudėtingi (5). Tokie tradiciniai KV rizikos veiksniai kaip amžius, rasė, lytis, hipertenzija, cukrinis diabetas, dislipidemija, nutukimas ir šeiminė anamnezė (11) negali visiškai paaiškinti KV ligų paplitimo tarp sergančiųjų LIL. Visa grupė netradicinių su LIL susijusių rizikos veiksnių, tokių kaip uždegimas, endotelio funkcijos sutrikimas, oksidacinis stresas, skysčių perkrova, sutrikusi kaulų ir mineralinių medžiagų apykaita, renino–angiotenzino–aldosterono sistema (RAAS), (12–14) padeda geriau suvokti didelę KV riziką šioje populiacijoje. Be to, kraujagyslių struktūros persitvarkymas (remodeliacija) sergant LIL apima pakitimus nuo aterosklerozės iki arteriosklerozės ir kraujagyslių kalcifikacijos (13–16), kurie ilgainiui sukelia kraujagyslių standėjimą. Šie procesai dažnai įvardijami kaip „paspartintas arterijų senėjimas" (17, 18).

Palaipsniui standėjant arterijoms mažėja jų elastingumas (19), prasčiau kompensuojama sistolinė banga, todėl padidėja sistolinis KS ir pulsinis spaudimas. Šie arterinio kraujospūdžio (KS) pokyčiai ilgainiui sukelia kairiojo skilvelio hipertrofiją, sutrikdo koronarų kraujotaką ir mikrocirkuliaciją tokiuose organuose kaip inkstai ir smegenys (18, 20). Minėti veiksniai yra tiesiogiai susiję su didesne KV (18, 21, 22), smegenų kraujotakos sutrikimo (23) ir periferinių arterijų okliuzinės ligos rizika (24).

Dar 1990 metais M. Londonas ir kt. (25) aprašė padidėjusį aortos pulsinės bangos greitį (PBG), kaip arterijų standumo įvertį, tarp dialize gydomų pacientų, lyginant su sveikų žmonių kontrole. Nuo to laiko mokslinė bendruomenė pradėjo aktyviau domėtis PBG vertinimo svarba LIL kontekste.

Auksiniu standartu įvardijamas miego ir kirkšnies arterijų PBG (mkPBG) yra išsamiai ištirtas bendroje populiacijoje (26, 27), tačiau duomenys apie mkPBG sergant LIL, GSIL ir po inksto transplantacijos nevienareikšmiai.

Kai kurie moksliniai straipsniai nurodo ir periferinių raumeninių kraujagyslių standėjimo svarbą. Periferinį arterijų standumą galima įvertinti matuojant PBG tarp miego arterijos ir stipininės arterijos (msPBG). Pastaruoju metu ir arterijų standumo gradientas (ASG), arba vadinamasis PBG santykis (PBG santykis = mkPBG / msPBG), taip pat įvardijamas kaip naujas ir svarbus arterijų standumą atspindintis įvertis (28). Įvertinus tokį aprašytų mokslinės bendruomenės debatų netolygumą, šioje disertacijoje buvo iškeltos tokios hipotezės:

1. Arterijų standumas ir su juo susiję biožymenys gali atspindėti kraujagyslių kalcifikacijos laipsnį ir dvejų metų kardiovaskulinę riziką pacientams, gydomiems pakaitine inkstų terapija;
2. Tiek hemodializė, tiek inkstų transplantacija yra susijusios su arterijų standumo pokyčiu dvejų metų laikotarpiu.

Disertacijos tikslas:

1. Įvertinti elastinių ir raumeninių kraujagyslių standumo ir kitų biožymenų pokytį bei jų reikšmę prognozuojant aortos lanko kalcifikacijos apimtį ir kardiovaskulinę riziką pacientams, gydomiems pakaitine inkstų terapija.

Uždaviniai:

1. Nustatyti elastinių ir raumeninių kraujagyslių standumo ir kitų biožymenų reikšmę prognozuojant aortos lanko kalcifikacijos apimtį ir dvejų metų kardiovaskulinę riziką pacientams, gydomiems pakaitine inkstų terapija;
2. Ištirti elastinių ir raumeninių kraujagyslių standumo ir kitų biožymenų pokyčius dvejų metų laikotarpiu tarp pacientų, gydomų hemodialize;
3. Įvertinti elastinių ir raumeninių kraujagyslių standumo ir aortos lanko kalcifikacijos pokytį po sėkmingos inkstų transplantacijos.

Ginamieji teiginiai:

1. Kraujagyslių persitvarkymas ir kalcifikacija – tai vieni pagrindinių patogenetinių veiksnių, sukeliančių arterijų standėjimą, susijusį su didesne kardiovaskuline rizika, pacientams, gydomiems pakaitine inkstų terapija;
2. Inksto transplantacija – tai pirmo pasirinkimo gydymo metodas pacientams, sergantiems galutinės stadijos inkstų liga, nes jis susijęs su geresne stambiųjų kraujagyslių funkcija.

2. Naujumas

Tai yra pirmoji studija, kurioje PBG santykis susietas su aortos kalcifikacijos apimtimi ir nustatyti kiti žymenys, susiję su PBG santykiu. Šiame tyrime buvo palygintos kelios arterijų standumo matavimo metodikos. Šis tyrimas yra vienas iš nedaugelio, kuriuo nustatyta, kad po sėkmingos inkstų transplantacijos sumažėja elastinių kraujagyslių standumas, bet nėra jokio raumeninių kraujagyslių standumo pokyčio. Be to, ši studija pateikia duomenis apie iš dalies „sveikesnius“ GSIL pacientus, nes į tyrimą buvo įtraukti tik asmenys, neturėję iki tol KV įvykių, smegenų kraujotakos sutrikimo ir periferinių arterijų okliuzinės ligos.

3. Metodologija

3.1. Tiriamieji

Nuo 2014 m. gruodžio mėn. iki 2017 m. liepos mėn. buvo atliktas vienacentris perspektyvusis kohortinis tyrimas. Atskirai buvo analizuojami dialize gydomi pacientai ir pacientai po inksto transplantacijos.

3.1.1. Dialize gydomų pacientų populiacija

Iš 200 nuolat dialize Vilniaus universiteto ligoninės Santaros klinikose, Nefrologijos centre, gydomų pacientų iš pradžių buvo atrinkta 130 tiriamųjų. Netinkamumo studijai kriterijai: jaunesni nei 18 metų, anksčiau buvę KV arba su smegenų kraujotaka susiję įvykiai, kliniškai reikšminga periferinių arterijų okliuzinė liga (žasto ir kulkšnies indeksas mažesnis nei 0,9), mažiau nei 3 mėnesius gydomi hemodialize arba peritonine dialize, ūminis susirgimas (infekcija, kraujavimas), prieširdžių virpėjimas, visiška širdies blokada, neišsamūs duomenys, nepasirašytas informuoto asmens sutikimas.

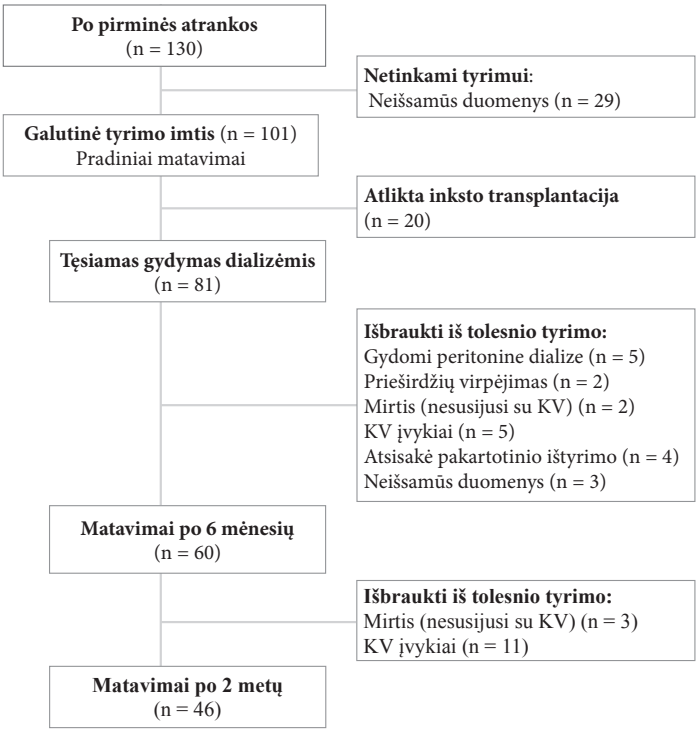
Iš 130 tiriamųjų tik 101 buvo įtrauktas į baigiamąją analizę. 20 pacientų beveik iškart po įtraukimo buvo pakviesti atlikti inkstų transplantacijos, o 81 toliau tęsė gydymą dializėmis.

Šiame tyrime taip pat siekta įvertinti arterijų standumo pokytį per tam tikrą laiko tarpą. Iš 81 paciento buvo atrinkta 60 tiriamųjų, kurie visi buvo gydomi išskirtinai hemodialize ir kuriems buvo atlikti pakartotiniai PBG matavimai po 6 mėnesių. Pakartotiniam tyrimui po 2 metų mums pavyko surinkti 46 pacientus. Detalesnė informacija pateikta 1 paveiksle.

3.1.2. Pacientai po inksto transplantacijos

37 mirusių donorų inksto recipientai iš 60 atitiko įtraukimo į tyrimą kriterijus, kurie išvardyti 3.1.1 skyrelyje. Visi 37 tiriamieji buvo stebimi prieš IT ir praėjus vidutiniškai metams bei dvejiems metams po sėkmingos IT.

Mūsų tyrimas neturėjo jokios įtakos indukcinės bei ilgalaikės imunosupresijos pasirinkimui. Visiems pacientams buvo taikomas standartinis ilgalaikis imunosupresinis gydymas po transplantacijos, t. y. kalcineurino inhibitoriaus (ciklosporinas A arba takrolimas), mofetilio mikofenolato ir metilprednizolono derinys. Transplantuoto inksto veikla buvo stabili praėjus 6 mėnesiams po transplantacijos.



1 PAV. Pacientų atranka; KV – kardiovaskuliniai

3.2. Informuoto asmens sutikimas

Tyrimui atlikti buvo gautas Vilniaus regioninio biomedicininių tyrimų etikos komiteto leidimas Nr. 158200-14-750-268. Visi pacientai pasirašė šio komiteto patvirtintą sutikimo formą. Taip pat atliekant šį tyrimą buvo atsižvelgta į 1975 m. Helsinkio deklaraciją ir jos vėlesnius pakeitimus.

3.3. Tyrimo protokolas

3.3.1. Dialize gydomų pacientų tyrimo protokolas

101 pacientas buvo apklaustas, visiems jiems buvo atlikti kraujo tyrimai, priekinė krūtinės ląstos rentgenograma ir PBG matavimai. 60 tiriamųjų, gydomų lėtine hemodialize, buvo pakartotinai tiriami po 6 mėnesių; iš jų 46 tiriamieji galėjo atlikti pakartotinius tyrimus po dvejų metų.

Kraujas tyrimams buvo imamas tarp 7 ir 12 val. ryto. Tą pačią dieną buvo vertinami ir hemodinamiką atspindintys įverčiai (arterinis kraujo spaudimas, PBG) bei krūtinės ląstos rentgenograma.

Buvo renkami duomenys apie amžių, lytį, LIL priežastį, rūkymą, hipertenziją, dializių trukmę, dializių rūšį, vartojamus vaistus, cukrinį diabetą tiesiogiai bendraujant su pacientu ir iš medicininės dokumentacijos.

Remiantis B. Ghoulo ir kt. (29) publikuotu straipsniu LIL priežastys buvo suskirstytos į diabetinę nefropatiją, nediabetinę glomerulopatiją, kraujagyslinės kilmės inkstų ligą, tubulointersticinę nefropatiją, paveldimą nefropatiją ir nepatikslintos etiologijos GSIL.

3.3.2. Tyrimo protokolas, skir tas pacientams po inkstų transplantacijos

Tiriamieji (n = 37) buvo apklausti, jiems buvo atlikti kraujo tyrimai ir PBG matavimai prieš pat IT, praėjus vieniems metams (n = 37) ir dvejiems metams (n = 20) po IT. Priekinė krūtinės ląstos rentgenograma buvo atliekama prieš pat IT ir praėjus dvejiems metams po IT.

Kraujas tyrimams buvo imamas prieš pat IT ir vėliau praėjus vieniems ir dvejiems metams po IT tarp 7 ir 12 val. ryto. Tą pačią dieną buvo vertinami ir hemodinamiką atspindintys įverčiai (arterinis kraujo spaudimas, PBG) bei krūtinės ląstos rentgenograma.

Buvo renkami duomenys apie amžių, lytį, LIL priežastį, rūkymą, hipertenziją, cukrinį diabetą, dializių trukmę, dializių rūšį, donoro ir recipiento citomegolovirusinę infekciją, vartojamus vaistus tiesiogiai bendraujant su pacientu ir iš medicininės dokumentacijos.

3.4. Antropometriniai matavimai

Naudojant sukalibruotus prietaisus, tiriamiesiems buvo matuojama ūgis (cm) ir svoris (kg). Kūno masės indeksas (KMI) apskaičiuotas pagal formulę KMI = svoris/ūgis^2 (kg/m^2). Kūno paviršiaus plotas apskaičiuotas pagal DuBois formulę: kūno paviršiaus plotas = (svoris^{0,425} × ūgis^{0,725}) × 0,007184 (m^2).

3.5. Kraujo mėginių tyrimas

β2-mikroglobulino, serumo cistatino c, kreatinino, šlapalo, kalcio, jonizuotojo kalcio, fosforo, albumino, bendrojo cholesterolio, šlapimo rūgšties, c reaktyviojo baltymo, feritino koncentracijos tyrimai buvo atliekami naudojant „Abbott Architect ci8200 PLIUS“ (*Abbott Laboratories*, JAV) analizatorius. Parathormono koncentracijos tyrimas atliktas „Advia centaur XP“ (*Siemens Healthcare Diagnostics*, JAV) analizatoriumi. Leukocitų, hemoglobino ir trombocitų kiekis kraujyje buvo

nustatytas „SYSMEX XE-5000“ (*Sysmex Corporation*, Japonija) analizatoriumi. Visi laboratoriniai tyrimai atlikti Vilniaus universiteto ligoninės Laboratorinės medicinos centre.

Taip pat skaičiuotos koreguoto kalcio koncentracijos pagal albumino lygį kraujo plazmoje: koreguotas kalcis mmol/l = (0,02 * (normali albumino koncentracija – paciento albumino koncentracija)) + serumo kalcio koncentracija (12, 30). Kalcio ir fosforo produktai apskaičiuoti pagal KDOQI gaires (*KDOQI Clinical Practice Guidelines for Bone Metabolism and Disease in Chronic Kidney Disease, Guideline 6*), t. y. kalcio koncentraciją dauginant iš fosforo koncentracijos plazmoje (12).

3.6. Ar terinio kraujospūdžio matavimas

Patyręs medicinos gydytojas matavo arterinį kraujospūdį žaste pagal *The National Institute for Health and Care Excellence (NICE) 2011* rekomendacijas (31), sėdimoje pozicijoje, po 10–15 minučių poilsio. Arterinio kraujo spaudimo matavimas buvo kartojamas tris kartus kas 2 minutes ir apskaičiuota vidutinė vertė. Matavimui pasirinkta dominuojanti ranka arba ranka, kurioje nėra fistulės. Naudota aparatūra: kalibruotas rankinis sfigmomanometras (*Riester precisa® N Sphygmomanometer*, Vokietija).

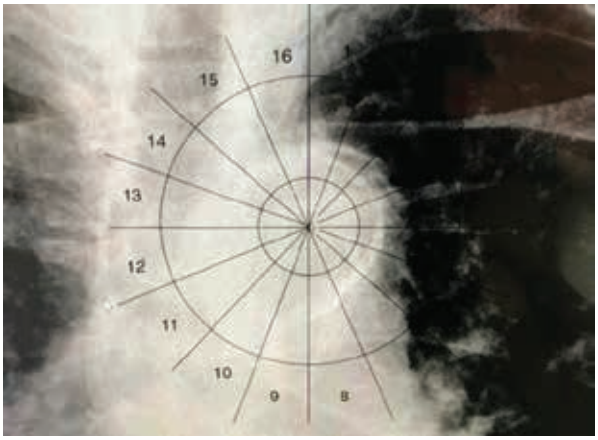
Pulsinis spaudimas apskaičiuotas pagal formulę: pulsinis spaudimas = sistolinis KS – diastolinis KS (mmHdg); vidutinis arterinis spaudimas (vidutinis KS): vidutinis KS = diastolinis KS + 1/3 (sistolinis KS – diastolinis KS) (mmHg).

3.7. Arterijų standumo ir centrinio kraujospūdžio matavimas

Aplanaciniu tonometru (*SphygmoCor, AtCor Medical Pty Ltd*, Sidnėjus, Australija) buvo atlikti centrinio KS, vidutinio KS, širdies susitraukimų dažnio, pulsinio spaudimo, mkPBG, msPBG matavimai taikant anksčiau aprašytą metodiką (32). Atstumas tarp pulsinės bangos registravimo vietų (miego ir kirkšnies bei miego ir stipininės arterijų) buvo padaugintas iš 0,8. Į analizę įtrauktos tik pulsinės bangos formos, turinčios didesnį nei 80 % operatoriaus indeksą, rodantį gebėjimą atkurti bangos formą ir jos signalo stiprumą. PBG santykis buvo apskaičiuotas pagal C. Fortier ir kt. (32) 2015 metais pasiūlytą formulę: PBG santykis = mkPBG / msPBG.

3.8. Aortos lanko kalcifikacijos įvertinimas

2009 metais gydytojas T. Ogawa kartu su savo kolegomis sukūrė unikalią aortos lanko kalcifikacijos (AoLK) vertinimo skalę, kurią galima pritaikyti AoLK priekinėje krūtinės ląstos rentgenogramoje (žr. 2 pav.) (33). Šis AoLK įvertinimo metodas nenusileidžia daugiapjūvės kompiuterinės tomografijos tyrimui (15, 34). Naudoti AoLK skalę gautas raštiškas autorių leidimas.



2 PAV. Aortos lanko kalcifikacijos vertinimo skalė priekinėje 62 m. moters, gydomos hemodialize, krūtinės ląstos rentgenogramoje

Du patyrę Vilniaus universiteto ligoninės Santaros klinikų radiologai uždėdavo AoLK skalę ant krūtinės ląstos rentgenogramos ties aortos lanku ir suskaičiuodavo, kiek sektorių apima kalcifikacija (įvertinama balais nuo 0 iki 16). Atlikus pradinį rentgenogramų įvertinimą Koheno kapos matas (*Cohen's kappa*) tarp dviejų ekspertų buvo 0,81. Nesutampantys rezultatai peržiūrėti pakartotinai ir priimtas bendras sprendimas.

3.9. Išgyvenamumo be kardiovaskulinių įvykių analizė dialize gydomų pacientų populiacijoje

Atliktas perspektyvusis visų mirties įrašų (ligoninėje ar namuose) nuo 2014 m. gruodžio mėn. iki 2017 m. liepos mėn. vertinimas. Patyręs medicinos gydytojas išnagrinėjo visus įrašus, mirties liudijimus ir atrinko tik tuos įrašus, kurie tiesiogiai susiję su KV sistemos patologija. Didieji nepageidaujami kardiovaskuliniai įvykiai (MAcE, angl. *major adverse cardiovascular events*) ir visi KV įvykiai buvo analizuojami atskirai. MAcE buvo priskirta mirtis dėl KV patologijos, miokardo infarktas ir išeminis galvos smegenų insultas. Visi KV įvykiai apėmė prieš tai išvardytus įvykius ir dar papildomai prie jų priskirta koronarų kraujotakos atkūrimas, apatinių galūnių amputacija arba kraujotakos atkūrimas, gydymas stacionare dėl nestabilios krūtinės anginos. Prieširdžių virpėjimas ir širdies nepakankamumas, jei nereikėjo gydymo ligoninėje, nebuvo vertinami kaip KV įvykiai.

3.10. Statistinė analizė

Tolydūs kintamieji buvo išreikšti vidurkiu su standartiniu nuokrypiu, o diskretūs – mediana. Kategoriniai kintamieji aprašomojoje statistikoje pateikti skaitinėmis vertėmis ir skliaustuose – jų procentine išraiška. Prieš taikant Stjudento *t* testą normaliai pasiskirsčiusiems tolydiems duomenims buvo atliktas F testas, kuriuo siekta įvertinti, ar dviejų populiacijų dispersijos lygios. Duomenims, nepasiskirsčiusiems pagal normalųjį skirstinį, taikytas Vilkoksono (Wilkokson) suderintų porų testas. Kategoriniams kintamiesiems taikytas chi (χ^2) kvadratas. Normaliai pasiskirsčiusių duomenų tarpusavio ryšiui nustatyti atliktas Pirsono (Pearson) koreliacijos testas; kitiems duomenims, kur galima, buvo skaičiuojamas Spirmeno rangų koeficientas (Spearman's rank). Ryšys tarp kintamųjų buvo vertinamas pagal koeficientų skaitinę išraišką: stiprus ($-1{,}0 \leq r \leq -0{,}7$ arba $0{,}7 \leq r \leq 1{,}0$), vidutinis ($-0{,}69 \leq r \leq -0{,}4$ arba $0{,}4 \leq r < 0{,}69$), silpnas ($-0{,}39 \leq r \leq -0{,}2$ arba $0{,}2 \leq r < 0{,}39$), labai silpnas arba ryšio nėra ($-0{,}19 \leq r \leq 0$ arba $0 \leq r < 0{,}19$).

Ne pagal normalųjį dėsnį pasiskirstę tolydūs kintamieji buvo logaritmuoti siekiant išgauti duomenų pasiskirstymo normalumą. Vienalypė tiesinė regresija pasitelkta norint nustatyti rodiklius, apibrėžiančius PBG santykį ir arterijų standumo pokytį. Multikolineorumo išvengta įtraukiant tik tarpusavyje nesusijusius kintamuosius į daugialypę tiesinę regresinę analizę.

Pagal AoLK prieš atliekant logistinę regresinę analizę dialize gydomi tiriamieji buvo suskirstyti į dvi grupes: AoLK (ne) – nėra AoLK, 0 balų; AoLK (taip) – matoma AoLK, 1 ir daugiau balų. Daugialype logistine regresija siekta nustatyti veiksnius, susijusius su AoLK. Reikšmingų rizikos veiksnių / modelių jautrumas ir specifiškumas pateiktas braižant ROc kreives. Plotai po kreivėmis palyginti naudojant DeLongo matą (35).

Kaplano–Mejerio (Kaplan-Meier) metodu palygintas išgyvenamumas be KV įvykių. Kokso (cox) regresijos modelis įvertino PBG santykio ir kitų žymenų įtaką KV įvykių pasireiškimui. Dėl mažo MAcE įvykių skaičiaus taikytas tik vienalypės Kokso (cox) regresijos modelis.

Norėdami įvertinti arterijų standėjimo vystymąsi, apskaičiavome koreguotas PBG reikšmes, t. y. PBG reikšmes po tam tikro stebėjimo laikotarpio vertinome kaip nepriklausomus kintamuosius ir jas koregavome pagal bazines PBG reikšmes.

Duomenys laikyti statistiškai patikimais, kai $p < 0{,}05$. Duomenys apdoroti naudojant statistinio paketo *R commander* (*Rcmdr*) 3.3.2 versiją.

4. Dialize gydomų tiriamųjų rezultatai

4.1. Dialize gydomų pacientų charakteristikos

Pagrindinės tiriamųjų charakteristikos nurodytos 1 lentelėje.

Nustatyta reikšminga koreliacija tarp amžiaus ir mkPBG ($r = 0{,}525$, $p < 0{,}001$), PBG santykio ($r = 0{,}556$, $p < 0{,}001$) ir AoLK ($r = 0{,}597$, $p < 0{,}001$). msPBG su amžiumi nekoreliavo ($r = -0{,}141$, $p = 0{,}181$).

1 LENTELĖ. Pagrindinės tiriamųjų charakteristikos

	Iš viso (n = 101)
Amžius (metai)	55,03 ± 15,65
Lytis (vyrai)	50,5 % (51)
Ūgis (cm)	167,91 ± 10,67
Svoris (kg)	72,69 ± 16,63
KMI (kg/m^2)	25,87 ± 5,32
Kūno paviršiaus plotas (m^2)	1,81 ± 0,22
Rūkymas (taip)	13,8 % (14)
Hipertenzija (taip)	94,1 % (95)
Cukrinis diabetas (taip)	21,7 % (22)
Dializių trukmė (dienos)	1035 (93–6556)
Inkstų ligos trukmė (metai)	10,0 (1,0–40,5)
LIL priežastis	
Diabetinė nefropatija	10,89 % (11)
Nediabetinė glomerulopatija	33,66 % (34)
Kraujagyslinė inkstų liga	20,79 % (21)
Tubulointersticinė nefropatija	23,76 % (24)
Paveldima nefropatija	7,92 % (8)
Neaiškios priežasties	2,97 % (3)
Dializės rūšis	
Peritoninė	12,9 % (13)
Hemodializė	87,1 % (88)
Kraujo tyrimai	
Leukocitai (10e9/l)	6,80 ± 2,07
Hemoglobinas (g/l)	114,80 ± 14,19
Trombocitai (10e9/l)	218 (80–547)
Bendrasis baltymas (g/l)	67,71 ± 8,88
Albuminas (g/l)	39,52 ± 4,06

Kraujo tyrimai	
Bendrasis cholesterolis (mmol/l)	5,24 ± 1,43
Kreatininas (μmol/l)	819 (364–1768)
Cistatinas C (mg/l)	5,97 ± 1,29
Šlapalas (mmol/l)	23,41 ± 7,07
C reaktyvusis baltymas (mg/l)	4,87 (0,30–54,50)
β2-mikroglobulinas (mg/l)	35,25 (14,25–100,36)
Feritinas (μg/l)	390 (44–1459)
Šlapimo rūgštis (μmol/l)	359,17 ± 87,81
PTH (pmol/l)	51,4 (0,5–201,4)
Kalcis (mmol/l)	2,23 ± 0,18
Fosforas (mmol/l)	1,88 ± 0,57
Koreguotas kalcis (mmol/l)	2,24 ± 0,17
Kalcio ir fosforo produktai ($mmol^2/l^2$)	4,19 ± 1,25
Hemodinamiką atspindintys ir kardiovaskuliniai žymenys	
Sistolinis KS (mmHg)	146,26 ± 18,76
Diastolinis KS (mmHg)	99,90 ± 12,84
Pulsinis spaudimas (mmHg)	59,76 ± 17,43
Vidutinis KS (mmHg)	105,80 ± 12,61
Pulsas (k./min.)	72,21 ± 11,00
Centrinis sistolinis KS (mmHg)	124,98 ± 15,67
mkPBG (m/s)	11,35 ± 3,54
msPBG (m/s)	10,21 ± 1,79
PBG santykis	1,06 (0,59–3,40)
AoLK (0–16)	2 (0–11)

KMI – kūno masės indeksas, LIL – lėtinė inkstų liga, PTH – parathormonas, KS – arterinis kraujo spaudimas, mkPBG – miego ir kirkšnies arterijų pulsinės bangos greitis, msPBG – miego ir stipininės arterijų pulsinės bangos greitis, PBG – pulsinės bangos greitis, AoLK – aortos lanko kalcifikacija.

4.2. Vartojami vaistai

Nė vienas iš tiriamųjų nevartojo varfarino; tik vienas pacientas vartojo statinus. Keturi pacientai vaistus nuo kraujospūdžio vartojo nereguliariai, 52,47 % (53) vartojo AKF inhibitorius arba sartanus, 60,39 % (61) – kalcio kanalų blokatorius, 64,35 % (65) – beta blokatorius, 27,72 % (28) – alfa blokatorius, 41,58 % (42) – centrinius kraujospūdį mažinančius vaistus, 15,84 % (16) – diuretikus.

4.3. Lyties nulemti požymiai

Vyrų antropometriniai rodikliai buvo aukštesni negu moterų, vyrai daugiau rūkė, turėjo didesnes kreatinino ir β2-mikroglobulino koncentracijas. Moterų cholesterolio vertės buvo aukštesnės, taip pat aukštesnis koreguotas kalcis ir pulsinis spaudimas (žr. 1 priedą).

4.4. Arterijų standumo gradientą atspindintys kintamieji

PBG santykio didėjimas statistiškai reikšmingai koreliavo su amžiumi, cukriniu diabetu, dializių trukme, uždegiminiais rodikliais (β2-mikroglobulinu, feritinu, c reaktyviuoju baltymu) ir su AoLK apimtimi. PBG santykio mažėjimas buvo susijęs su didėjančia bendrojo cholesterolio koncentracija (žr. 2 lentelę). PTH poveikis PBG santykiui tapo reikšmingas tik pakoregavus pagal amžių, cukrinio diabeto buvimą ir AoLK balus.

2 LENTELĖ. PBG santykį apibrėžiantys kintamieji: vienalypė ir daugialypė tiesinė regresinė analizė

Vienalypė tiesinė regresija	**Standartizuotas koef.**	***p***	**R^2**
Amžius (metai)	0,005	< 0,001	0,248
Cukrinis diabetas (taip)	0,115	0,004	0,086
Dializių trukmė † (dienos)	0,140	< 0,001	0,193
β2-mikroglobulinas † (mg/l)	0,240	0,005	0,103
Cistatinas C (mg/l)	0,044	< 0,001	0,173
Feritinas † (μg/l)	0,129	0,011	0,082
C reaktyvusis baltymas † (mg/l)	0,116	< 0,001	0,147
PTH (pmol/l)	0,059	0,095	0,030
Bendrasis cholesterolis (mmol/l)	–0,032	0,002	0,097
AoLK balais †	0,225	< 0,001	0,292
Daugialypė tiesinė regresija			
1 modelis: $p < 0,001$			
Amžius (metai)	0,002	0,019	0,459
Cukrinis diabetas (taip)	0,093	0,004	
Dializių trukmė † (dienos)	0,080	0,004	
AoLK balais †	0,123	0,006	
2 modelis: $p < 0,001$			
Amžius (metai)	0,002	0,009	0,439
Cukrinis diabetas (taip)	0,097	0,003	

Daugialypė tiesinė regresija			
PTH† (pmol/l)	0,064	0,026	
AoLK balais †	0,150	< 0,001	
3 modelis: $p < 0,001$			
Amžius (metai)	0,002	0,023	0,431
Cukrinis diabetas (taip)	0,076	0,023	
Bendrasis cholesterolis † (mmol/l)	–0,235	0,034	
AoLK balais †	0,154	< 0,001	

Vienalypė ir daugialypė tiesinės regresijos.
PTH – parathormonas, AoLK – aortos lanko kalcifikacija.
† log10 logaritmuotos vertės.

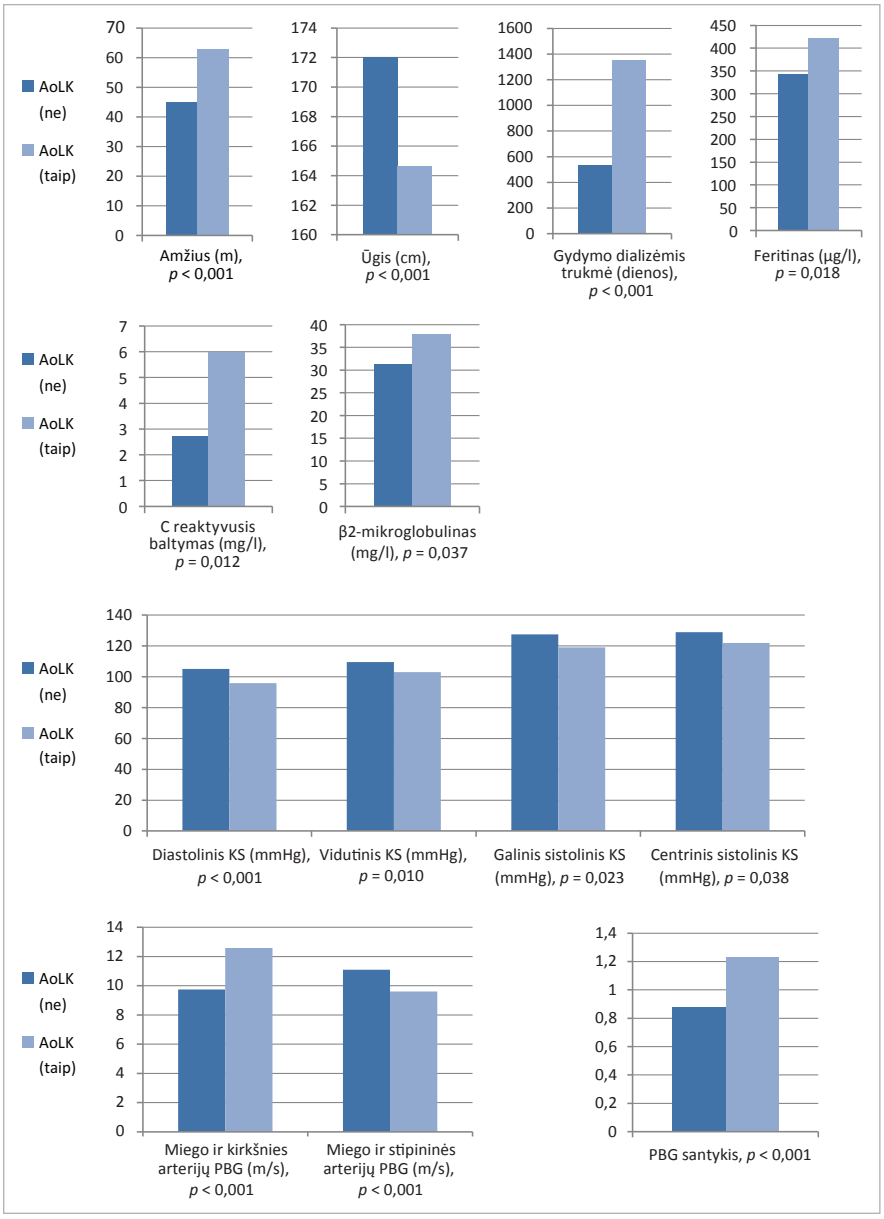
Nustatytas geriausiai PBG santykį apibrėžiantis *1 modelis*, apimantis amžių, cukrinį diabetą, dializių trukmę ir AoLK ($R^2 = 0,459$, $p < 0,001$).

Atsižvelgiant į LIL priežastį buvo palygintos mkPBG, msPBG ir PBG santykio vertės. msPBG nesiskyrė tarp grupių. Diabetinė nefropatija buvo susijusi su aukštesnėmis mkPBG vertėmis nei nežinomos etiologijos LIL ($p = 0,038$). PBG santykio vertės tarp pacientų, sergančių diabetine nefropatija, taip pat buvo didesnės nei kraujagyslinės inkstų ligos ($p = 0,039$), paveldimos nefropatijos ($p = 0,0134$) atvejais ir nereikšmingai didesnės nei nediabetinės glomerulopatijos ($p = 0,0819$) atveju.

4.5. Arterijų standumo gradientas aortos lanko kalcifikacijos kontekste

Prieš taikydami logistinės regresijos modelį, visus tiriamuosius suskirstėme į dvi grupes pagal AoLK pasireiškimą: AoLK (ne) – nėra kalcifikacijos požymių, atitinka AoLK 0 balų; AoLK (taip) – kalcifikacija matoma, atitinka AoLK ≥ 1 balas. 43,56 % (n = 44) tiriamųjų AoLK nebuvo stebima.

Pacientai, kuriems pasireiškė AoLK, buvo vyresnio amžiaus (44,98 ± 14,43 vs. 62,79 ± 11,71 m., $p < 0,001$), žemesnio ūgio (172,04 ± 8,29 vs. 164,66 ± 11,26 cm, $p < 0,001$), turėjo didesnį kūno masės indeksą (24,65 ± 5,47 vs. 26,84 ± 5,03 kg/m^2, $p = 0,042$) ir buvo ilgiau priklausomi nuo nuolatinio gydymo dializėmis (533 (93–5114) vs. 1354 (93–6556) d., $p < 0,001$). Taip pat šiai pacientų grupei buvo būdinga aukštesnė uždegiminių rodiklių, tokių kaip c reaktyvusis baltymas, β2-mikroglobulinas, feritinas, koncentracija (atitinkamai 2,74 (0,3–28,6) vs. 6,00 (0,5–54,5) mg/l, $p = 0,012$; 31,25 (14,25–85,18) vs. 37,99 (15,19–100,36) mg/l, $p = 0,037$; 344,3 (44,0–1024,8) vs. 421,8 (55,1–1459,0) μg/l, $p = 0,018$). Įdomu tai, kad pacientų, kuriems pasireiškė AoLK, diastolinis KS, vidutinis KS, centrinis sisto-

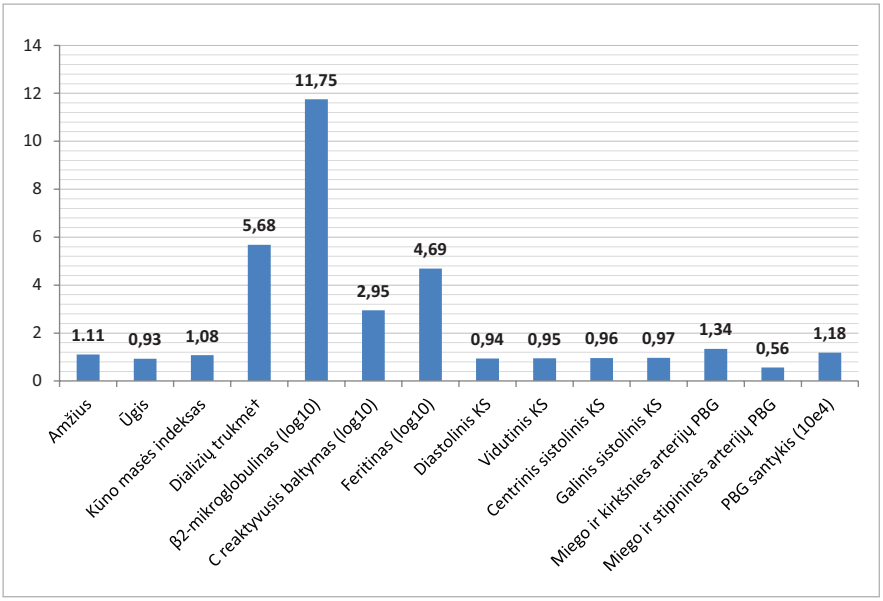


3 PAV. Pagrindinių charakteristikų palyginimas, atsižvelgiant į aortos lanko kalcifikaciją

linis KS ir msPBG buvo žemesni lyginant su pacientais, kuriems nepasireiškė AoLK (atitinkamai 105,02 ± 12,94 vs. 95,85 ± 11,33 mmHg, $p < 0{,}001$; 109,55 ± 12,74 vs. 102,97 ± 12,85 mmHg, $p = 0{,}010$; 128,89 ± 15,29 vs. 121,89 ± 15,42 mmHg, $p = 0{,}038$; 11,10 ± 1,96 vs. 9,61 ± 1,37 m/s, $p < 0{,}001$). Tačiau matuojant mkPBG pacientams, kuriems pasireiškė kraujagyslių kalcifikacija, gauti statistiškai reikšmingai didesni greičiai (9,75 ± 2,40 vs. 12,59 ± 3,79 m/s, $p < 0{,}001$). Ryšys tarp gydymo antihipertenziniais vaistais ir AoLK pasireiškimo nebuvo aptiktas (žr. 3 pav.).

Dvinarės ir daugianarės logistinės regresijos modeliai padėjo nustatyti veiksnius, susijusius su AoLK. Vyresnis amžius (OR 1,11, $p < 0{,}001$), didesnis KMI (OR 1,08, $p < 0{,}001$), ilgesnė priklausomybės nuo nuolatinių dializių trukmė (OR 5,68, $p < 0{,}001$), didesnė c reaktyviojo baltymo koncentracija (OR 2,95, $p = 0{,}012$), didesnė feritino koncentracija (OR 4,69, $p = 0{,}029$), didesnis mkPBG (OR 1,34, $p < 0{,}001$) ir didesnis PBG santykis (OR 1,18e+04, $p < 0{,}001$) buvo susiję su didesne AoLK rizika. Kita vertus, aukštesnis ūgis (OR 0,93, $p < 0{,}001$), didesnis msPBG (OR 0,56, $p < 0{,}001$), padidėjęs diastolinis KS (OR 0,94, $p = 0{,}002$), vidutinis KS (OR 0,95, $p = 0{,}012$) ir centrinis sistolinis KS (0,96, $p = 0{,}043$) buvo susiję su mažesne AoKL rizika (žr. 4 pav.).

Pasitelkdami daugianarę logistinę regresinę analizę atrinkome statistinį modelį (4 modelis), kuris reikšmingiausiai buvo susijęs su AoLK rizika. Šis modelis apėmė amžių, vidutinį KS ir PBG santykį. Apskaičiuotas modelio jautrumas buvo 77 %, o



4 PAV. Aortos lanko kalcifikaciją lemiantys veiksniai

3 LENTELĖ. Plotų po ROC kreivėmis, jautrumo ir specifiškumo palyginimas

Kintamieji	AUC	95 % PI (DeLong)	Jautrumas (%)	Specifiškumas (%)
Amžius	0,833	0,757–0,910	79	71
Ūgis	0,744	0,646–0,842	68	60
KMI	0,592	0,480–0,709	81	61
Dializių trukmė †	0,695	0,586–0,805	74	65
β2-mikroglobulinas †	0,634	0,512–0,757	57	58
C reaktyvusis baltymas †	0,645	0,536–0,755	70	58
Feritinas †	0,675	0,560–0,789	57	56
Diastolinis KS	0,647	0,539–0,755	66	56
Vidutinis KS	0,642	0,532–0,752	68	50
Centrinis sistolinis KS	0,616	0,496–0,737	57	62
Galutinis sistolinis KS	0,655	0,538–0,771	81	61
mkPBG	0,747	0,653–0,842	66	65
msPBG	0,676	0,558–0,793	74	67
PBG santykis †	0,792	0,697–0,887	74	73
4 modelis	0,893	0,826–0,960	77	82

AUC – plotas po ROC kreive; KMI – kūno masės indeksas, mkPBG – miego ir kirkšnies arterijų pulsinės bangos greitis, msPBG – miego ir stipininės arterijų pulsinės bangos greitis, PBG – pulsinės bangos greitis, PI – pasikliautinasis intervalas.
4 modelis: amžius + vidutinis KS + pulsinės bangos greičių santykis.
† log10 logaritmuotos vertės.

specifiškumas 82 %. Nubrėžtos visų statistiškai reikšmingai susijusių su AoLK rizikos veiksnių ROc kreivės ir palyginti plotai po ROc kreivėmis (žr. 3 lentelę).

4.6. Arterijų standumo gradiento ir kitų rizikos veiksnių įtaka kardiovaskulinei išeičiai

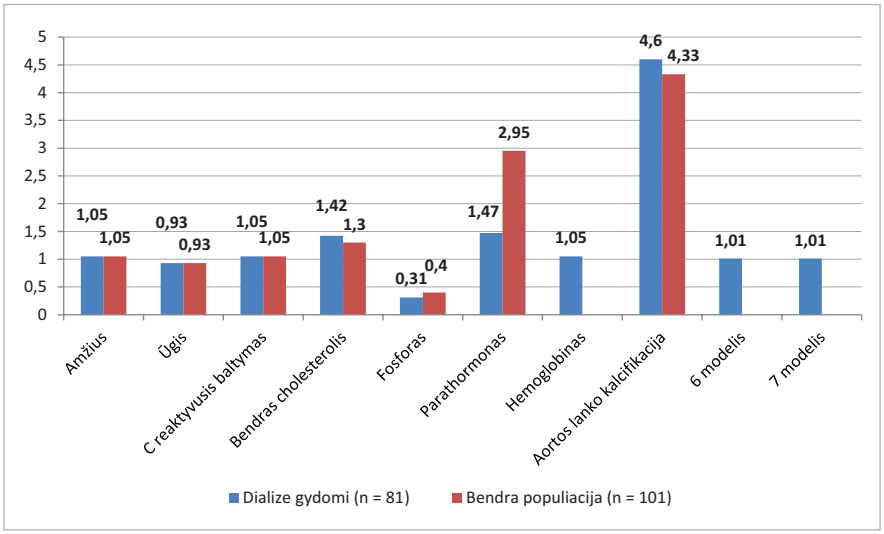
Tiriamieji buvo stebėti vidutiniškai 683 ± 149 dienas. Per šį laikotarpį 20 (19,8 %) pacientų patyrė kardiovaskulinius įvykius. KV įvykiams įtakos turintys veiksniai buvo analizuoti dviem etapais, t. y. bendrai visai tiriamųjų populiacijai (n = 101) (įskaitant ir 20 pacientų, kurie stebėjimo laikotarpiu sulaukė inksto transplantacijos) ir atskirai pacientams, kurie buvo toliau gydomi dializėmis (n = 81).

Dvinarės ir daugianarės Kokso (cox) regresinės analizės rezultatai pateikti Prieduose (žr. 2 priedą). Vertinant visą tiriamųjų populiaciją bendrai pastebėta, kad

vyresnis amžius, žemesnis ūgis, β2-mikroglobulino vertė, aukštesnė už jo medianą (> 35,25 mg/l), didesnė c reaktyviojo baltymo koncentracija, aukštesnė bendrojo cholesterolio ir parathormono koncentracija, žemesnė fosforo vertė ir AoLK vertė ≥ 1 buvo susiję su didesne KV rizika. Įdomu tai, kad nė vienas su hemodinamika susijęs kintamasis nebuvo susijęs su KV rizika bendrai visoje tiriamųjų populiacijoje. Vertinant tik dialize gydomų pacientų, kurie nesulaukė inksto transplantacijos, duomenis buvo stebimos tos pačios tendencijos, tik nenustatyta β2-mikroglobulino įtaka KV įvykiams.

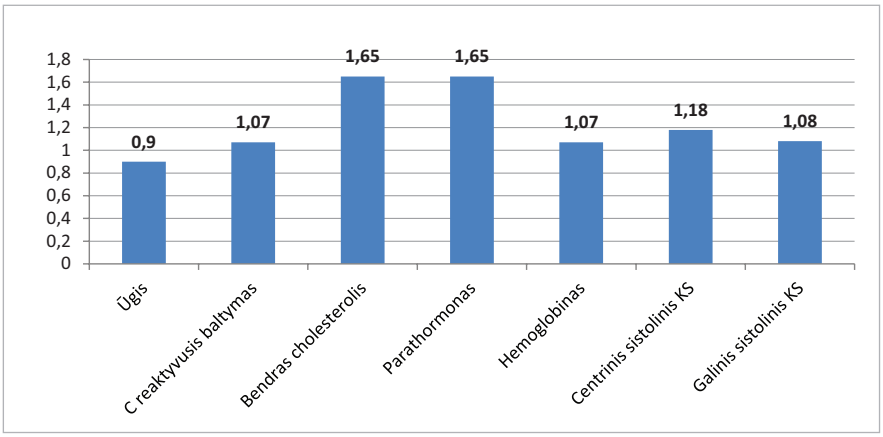
PBG santykio reikšmė vertinant KV riziką buvo pastebėta tik pritaikius daugianarės Kokso (cox) regresijos modelius. 5–7 modeliai, kurie apėmė amžių, AoLK, lytį, buvo statistiškai reikšmingi. cukrinis diabetas nebuvo patikimai reikšmingas nė viename modelyje. Mums nepavyko nustatyti mkPBG vertės prognozuojant KV išeitis, net ir pritaikius skirtingus statistinius modelius (žr. 5 pav.).

Didieji nepageidaujami kardiovaskuliniai įvykiai buvo analizuojami tik toliau dialize gydomų tiriamųjų populiacijoje ir tik taikant dvinarę Kokso (cox) regresinę analizę. Toks pasirinkimas buvo nulemtas nedidelio šio tipo KV įvykių pasireiškimo dažnio – 8,6 % (n = 7). 6 paveiksle pateikti tik reikšmingi su didžiaisiais nepageidaujamais kardiovaskuliniais įvykiais susiję rizikos veiksniai, t. y. žemesnis ūgis, padidėjusi cRB koncentracija, padidėjusi bendrojo cholesterolio koncentracija, padidėjęs



5 PAV. Statistiškai reikšmingų kardiovaskulinės rizikos veiksnių santykinės rizikos vertės.

6 modelis: PBG santykis pakoreguotas pagal amžių, AoLK;
7 modelis: PBG santykis pakoreguotas pagal amžių, AoLK, lytį



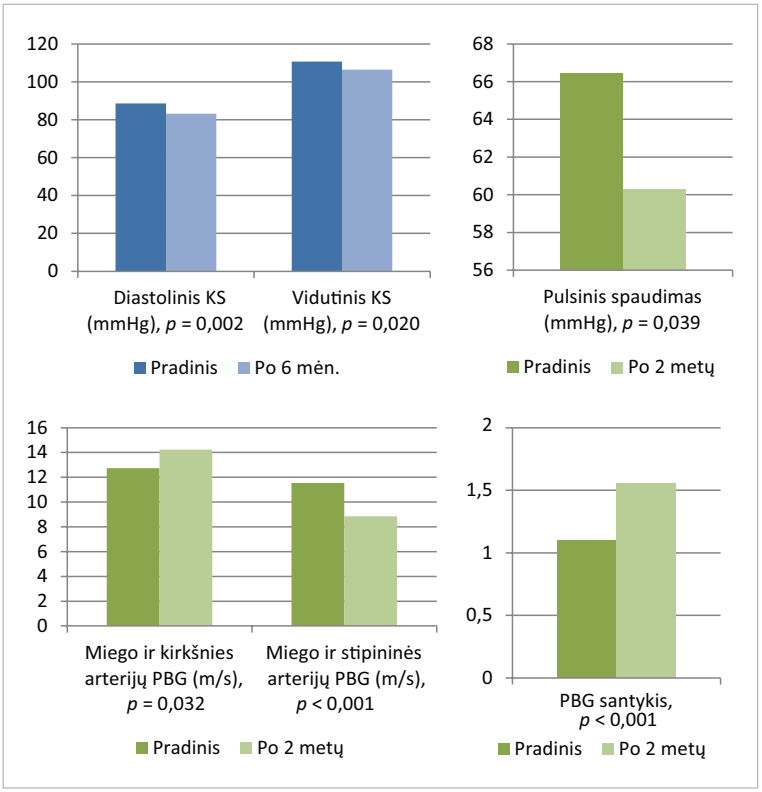
6 PAV. Su didžiaisiais nepageidaujamais kardiovaskuliniais įvykiais susiję rizikos veiksniai

PTH ir hemoglobino kiekis, aukštesnis centrinis sistolinis KS ir galutinis sistolinis KS. Amžius (p = 0,102), mkPBG (p = 0,763) ir PBG santykis (p = 0,796) neturėjo įtakos didiesiems nepageidaujamiems kardiovaskuliniams įvykiams.

4.7. Arterijų standumo vystymasis sergant galutinės stadijos lėtine inkstų liga

Praėjus 6 mėnesių stebėjimo laikotarpiui iš 101 tiriamojo tik 60 pacientų (vidutinis amžius 57,61 ± 13,01 metų) buvo galima pakartotinai įvertinti arterijų standumą atspindinčius rodiklius. 50 % iš jų buvo vyrai, 15,58 % buvo aktyvūs arba buvę rūkaliai, 30,77 % sirgo cukriniu diabetu, 96,15 % sirgo arterine hipertenzija ir visi buvo gydomi lėtinėmis hemodializėmis. Praėjus vidutiniškai 2 metų stebėjimo laikotarpiui tik 46 pacientai galėjo atvykti pakartotinai.

Lyginant kintamųjų vertes tyrimo pradžioje ir praėjus 6 mėnesiams, pastebėtas statistiškai reikšmingas bendrojo baltymo koncentracijos padidėjimas (64,49 ± 12,79 vs. 68,49 ± 5,13 g/l, p = 0,026) ir diastolinio KS (88,56 ± 10,27 vs. 83,21 ± 8,85 mmHg, p = 0,002) ir vidutinio KS sumažėjimas (110,70 ± 10,40 vs. 106,44 ± 9,45 mmHg, p = 0,020). Praėjus dvejų metų stebėjimo laikotarpiui stebėtas reikšmingas kūno masės sumažėjimas (74,34 ± 20,70 vs. 66,00 ± 11,82 kg, p = 0,016), trombocitų skaičiaus sumažėjimas (232,92 ± 75,19 vs. 192,63 ± 63 10e9/l, p = 0,004) bei cistatino c koncentracijos padidėjimas (5,51 ± 1,09 vs. 7,23 ± 1,16 mg/l, p < 0,001), PTH kiekio padidėjimas (44,95 (2,1–201,4) vs. 58,6 (8,8–132,2), p = 0,013), mkPBG padidėjimas (12,73 ± 2,57 vs. 14,24 ± 4,52 m/s) ir PBG santykio padidėjimas (1,10 (0,48–2,05) vs. 1,55 (0,94–2,65), p < 0,001) (žr. 7 pav.).



7 PAV. Skirtingų stebėjimo laikotarpių reikšmingi hemodinamikos pokyčiai

Amžius neturėjo įtakos arterijų standumo pokyčiui per 6 stebėjimo mėnesius, tačiau buvo susijęs su mkPBG padidėjimu (p = 0,037) ir iš dalies susijęs su msPBG sumažėjimu (p = 0,050) per dvejų metų stebėjimo laikotarpį (žr. 4 lentelę). PBG santykio pokytis nebuvo susijęs su senėjimu. c reaktyviojo baltymo koncentracijos kitimas buvo susijęs tiek su mkPBG, tiek su msPBG pokyčiu, o β2-mikroglobulino koncentracijos didėjimas – tik su mkPBG pokyčiu dvejų metų stebėjimo laikotarpiu. Pradinio pacientų vertinimo metu nustatytos AoKL vertės buvo susijusios su arterijų standumo ir PBG santykio pokyčiu stebėjimo laikotarpiu. Bazinės vidutinio KS reikšmės buvo susijusios su msPBG padidėjimu per 6 mėnesius, o vidutinio KS pokytis per 6 mėnesius turėjo ryšį tiek su centriniu, tiek su periferiniu arterijų standėjimu. Daugialypės tiesinės regresijos 8 ir 9 modeliai (žr. 4 lentelę) atskleidė centrinio ir periferinio arterijų standumo ryšį su vidutiniu KS.

4 LENTELĖ. Arterijų standumo pokytį nusakantys veiksniai. Tiesinė regresinė analizė

	Koreguotas $mkPBG_{6\ mėn.}$			Koreguotas $msPBG_{6\ mėn.}$			Koreguotas PBG $santykis_{6\ mėn.}$		
	β	*p*	R^2	Koef.	*p*	R^2	Koef.	*p*	R^2
Amžius †	0,064	0,059	0,158	–0,005	0,883	0,071	0,004	0,240	0,331
Dializės trukmė (log)	–0,214	0,829	0,016	0,281	0,784	0,073	–0,022	0,841	0,281
β2-mikroglobulinas †	–0,014	0,688	0,023	–0,045	0,238	0,137	–0,001	0,700	0,286
△ β2-$mikroglobulinas_{6\ mėn.}$	–0,005	0,931	0,014	–0,011	0,871	0,071	0,001	0,860	0,281
C reaktyvusis baltymas (log) †	0,748	0,489	0,034	–0,731	0,541	0,088	0,125	0,335	0,315
△C reaktyvusis $baltymas_{6\ mėn.}$	–0,010	0,870	0,015	–0,0063	0,323	0,117	0,004	0,511	0,296
Vidutinis KS †	0,079	0,075	0,147	0,114	0,004*	0,402	–0,002	0,651	0,268
△Vidutinis $KS_{6\ mėn.}$	0,081	0,031*	0,245	0,066	0,05*	0,242	0,003	0,342	0,400
AoLK †	0,080	0,646	0,023	–0,297	0,751	0,075	0,014	0,488	0,298
	8 modelis: R2 0,325, *p* = 0,037			*9 modelis:* R2 0,402, *p* = 0,029					
Amžius †	0,071	0,028*		–0,001	0,959		–	–	–
Vidutinis KS †	0,087	0,035*		0,114	0,006*		–	–	–
	Koreguotas $mkPBG_{2\ m.}$			Koreguotas $msPBG_{2\ m.}$			Koreguotas PBG $santykis_{2\ m.}$		
	β	*p*	R^2	β	*p*	R^2	β	*p*	R^2
Amžius †	0,136	0,037*	0,550	–0,036	0,050*	0,441	0,006	0,438	0,571
Dializių trukmė (log)	7,149	0,300	0,502	1,46	0,399	0,136	0,581	0,374	0,625
β2-mikroglobulinas †	0,040	0,675	0,334	0,005	0,799	0,021	–0,001	0,899	0,531
△ β2-$mikroglobulinas_{2\ m.}$	0,219	0,022*	0,568	0,039	0,335	0,142	0,009	0,542	0,556
C reaktyvusis baltymas (log) †	3,977	0,186	0,476	0,528	0,478	0,084	0,346	0,200	0,634
△C reaktyvusis $baltymas_{2\ m.}$	0,331	0,031*	0,599	0,097	0,022*	0,554	0,0247	0,135	0,666
Vidutinis KS †	–0,063	0,613	0,342	–0,007	0,779	0,0232	–0,006	0,558	0,554
△Vidutinis $KS_{2\ m.}$	–0,085	0,471	0,368	–0,002	0,914	0,013	–0,002	0,800	0,573
AoLK †	0,753	0,034*	0,553	0,103	0,384	0,119	0,0717	0,035*	0,710

Koreguotos vertės: PBG praėjus stebėjimo laikotarpiui buvo vertinamas kaip nepriklausomas kintamasis ir koreguotas pagal PBG bazines reikšmes.
△ – kintamųjų verčių pokytis nuo bazinės vertės, 2 m. – dvejų metų laikotarpiu, 6 mėn. – 6 mėnesių laikotarpiu.
β – krypties koeficientas, AoLK – aortos lanko kalcifikacija, mkPBG – miego ir kirkšnies arterijų pulsinės bangos greitis, msPBG – miego ir stipininės arterijų pulsinės bangos greitis, PBG – pulsinės bangos greitis.
† bazinės kintamųjų vertės; * *p* reikšmė ≤ 0,05.

5. Arterijų standėjimo pokyčiai po inkstų transplantacijos

Tyrime dalyvavusių 37 recipientų vidutinis amžius prieš inkstų transplantaciją (IT) buvo 46,94 ± 11,95 metų. 32 pacientams (86,49 %) buvo diagnozuota pirminė arba antrinė arterinė hipertenzija prieš IT, 2 pacientai (11,8 %) sirgo cukriniu diabetu, 7 pacientai (18,92 %) buvo gydomi peritonine dialize, o likusieji – hemodialize prieš IT. Pacientų prieš IT charakteristika pateikta 5 lentelėje.

5 LENTELĖ. Pagrindinės tiriamųjų charakteristikos

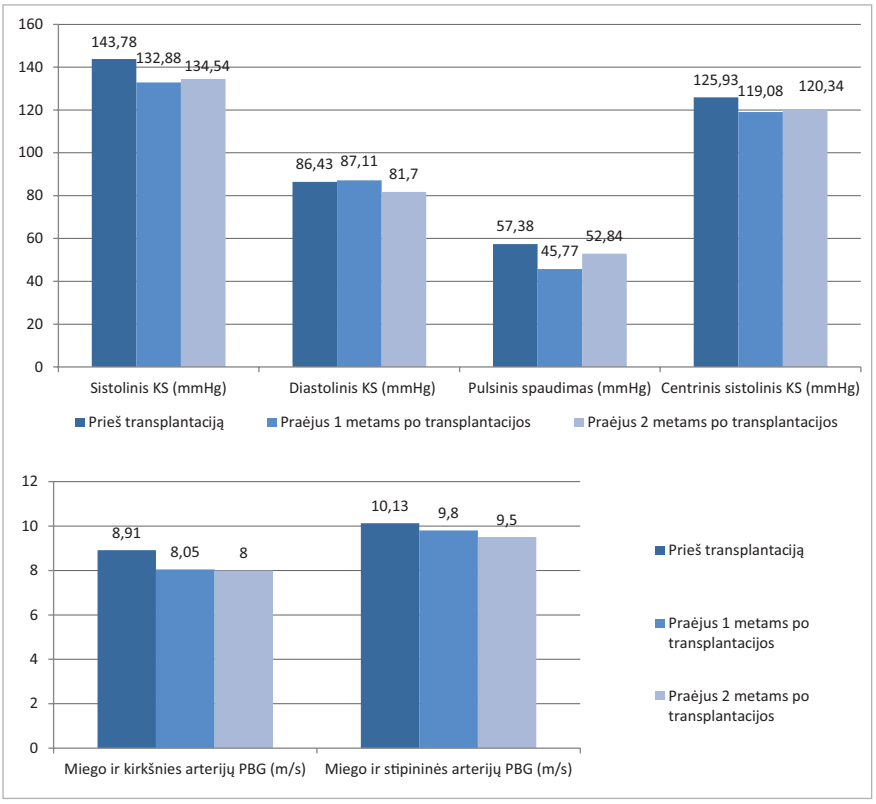
Kintamasis	Vidurkis ± SN (n = 37)
Demografiniai duomenys ir gretutinės ligos	
Recipiento amžius (m.)	46,95 ± 11,96
Recipiento lytis (vyrai)	20 (54,1 %)
KMI (kg/m^2)	24,04 ± 4,57
Hipertenzija	32 (86,5 %)
Cukrinis diabetas	2 (5,4 %)
Donoro amžius	46,33 ± 11,26
Donoro lytis (vyrai)	22 (59,4 %)
Inkstų ligos trukmė (m.)	14,49 ± 11,51
Hemodinaminiai ir kraujagysliniai parametrai	
Sistolinis KS (mmHg)	143,78 ± 16,87
Diastolinis KS (mmHg)	86,43 ± 12,38
Pulsinis spaudimas (mmHg)	57,38 ± 11,23
Širdies susitraukimų dažnis (k./min.)	72,12 ± 11,47
Centrinis sistolinis KS (mmHg)	125,93 ± 15,73
mkPBG (m/s)	8,92 ± 2,12
msPBG (m/s)	10,11 ± 1,35
PBG santykis	0,88 ± 0,27
Laboratoriniai rodikliai	
C reaktyvusis baltymas (mg/l)	2,49 ± 2,66
Mažo tankio cholesterolis (mmol/l)	4,27 ± 1,71
Didelio tankio cholesterolis (mmol/l)	1,24 ± 0,36
Bendrasis cholesterolis (mmol/l)	5,90 ± 1,21
Albuminas (g/l)	44,34 ± 3,48
Kalcis (mmol/l)	2,39 ± 0,15
Jonizuotasis kalcis (mmol/l)	1,14 ± 0,11

Kintamasis	Vidurkis ± SN (n = 37)
Fosforas (mmol/l)	1,67 ± 0,50
PTH (pmol/l)	71,27 ± 57,54
Kreatininas	830,38 ± 215,31
Šlapalas	19,16 ± 7,53
Šlapimo rūgštis	292,74 ± 86,22
Leukocitai (x10e9/l)	6,73 ± 2,00
Hemoglobinas (g/l)	120,79 ± 12,56
Trombocitai (x10e9/l)	219,35 ± 53,52
Dializės tipas	
Hemodializė	30 (81,1 %)
Peritoninė dializė	7 (18,9 %)
Citomegalo viruso serologija	
CMV serologiškai teigiami donorai	33 (89,2 %)
CMV serologiškai teigiami recipientai	30 (81,1 %)
Vartojami vaistai	
Beta adrenoreceptorių blokatoriai	24 (70,6 %)
Kalcio kanalų blokatoriai	21 (61,8 %)
Centrinio veikimo antihipertenziniai vaistai	21 (61,8 %)
Doksazosinas	13 (38,2 %)
Diuretikai	4 (11,8 %)
Angiotenzino receptorių blokatoriai	3 (8,8 %)
Takrolimas	27 (73,0 %)
Ciklosporinas	10 (27,0 %)

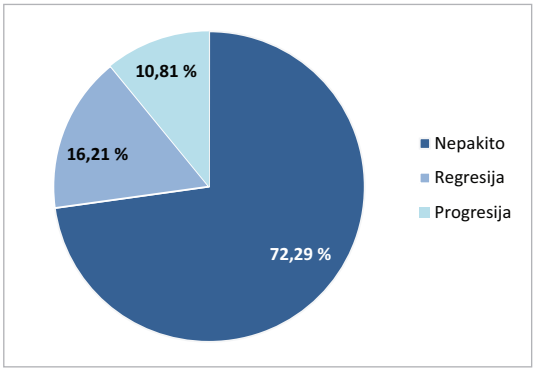
SN – standartinis nuokrypis; KMI– kūno masės indeksas; KS – kraujo spaudimas; mkPBG – miego ir kirkšnies arterijų pulsinės bangos greitis; msPBG – miego ir stipininės arterijų pulsinės bangos greitis; PBG – pulsinis bangos greitis; PTH – parathormonas; CMV – citomegalo viruso infekcija.

Lyginant kraujo tyrimų rezultatus prieš ir po transplantacijos, kaip ir tikėtasi, pastebėtas hemoglobino kiekio didėjimas, fosforo koncentracijos, PTH ir uremi-nių toksinų koncentracijos mažėjimas. Sistolinis KS, pulsinis spaudimas ir centrinis sistolinis KS taip pat sumažėjo, tačiau diastolinis KS ir vidutinis KS reikšmingiau nepakito. Buvo stebimas reikšmingas mkPBG sumažėjimas praėjus 1 metams po IT, tačiau praėjus dvejiems metams šis rodiklis reikšmingiau nekito. msPBG ir AoLK reikšmingiau nekito stebėjimo laikotarpiu po IT.

Lyginant turimus duomenis mkPBG prieš ir po 1 metų IT buvo 8,91 ± 2,11 vs. 8,05 ± 2,00 m/s, p = 0,020, msPBG atitinkamai 10,13 ± 1,24 vs. 9,80 ± 1,37 m/s, p = 0,0680, o PBG santykis – 0,88 ± 0,27 vs. 0,82 ± 0,28, p = 0,354; mkPBG prieš ir po 2 metų IT buvo 8,91 ± 2,11 m/s vs. 8,00 ± 1,90 m/s, p = 0,113, msPBG atitinkamai 10,13 ± 1,24 m/s vs. 9,5 ± 1,80 m/s, p = 0,125, o PBG santykis – 0,88 ± 0,27 vs. 0,84 ± 0,29, p = 0,605 (žr. 8 pav.).



8 PAV. Kraujospūdžio ir pulsinės bangos greičio pokyčiai po inkstų transplantacijos



9 PAV. Aortos lanko kalcifikacijos pokytis dvejų metų laikotarpiu po inkstų transplantacijos

27 (72,29 %) tiriamųjų AoLK nepakito po IT, 6 (16,21 %) pastebėtas AoLK apimties mažėjimas, o 4 (10,81 %) – didėjimas (žr. 9 pav.).

Dvejų metų laikotarpiu po IT buvo dokumentuoti tik penki su širdies ir kraujagyslių sistema susiję įvykiai: 3 miokardo infarktai, 1 plaučių arterijos tromboembolija ir 1 išeminis smegenų infarktas.

Dėl tokio nedidelio kardiovaskulinių įvykių dažnio negalėjome atlikti nei dvinarės, nei daugianarės Kokso (cox) regresinių analizių. Buvo pastebėta, kad pacientai, patyrę širdies ir kraujagyslių įvykius, turėjo prieš transplantaciją didesnę c reaktyviojo baltymo vertę (1,00 vs. 5,25 mg/l, $p = 0{,}012$), rentgenologiškai patvirtintą AoLK ($X^2 = 4{,}36$, $p = 0{,}036$), be to, jiems buvo persodintas vyresnio donoro inkstas ($p = 0{,}045$), tačiau PBG santykis nebuvo statistiškai reikšmingai didesnis (0,80 vs. 1,02, $p = 0{,}352$).

Netikėta buvo tai, kad pacientams, kuriems nepasireiškė AoLK, nustatyta didesnė kreatinino vertė (122,18 vs. 96,00 mkmol/l, $p = 0{,}042$) ir geresnė transplantuoto inksto funkcija pagal apskaičiuotą eGFG (61,15 vs. 47,79 ml/ min/1,73 m^2, $p < 0{,}001$). Taip pat pastebėta, kad pacientų, kurių po IT AoLK išliko nepakitusi, jonizuotojo kalcio vertė reikšmingai mažesnė ($p = 0{,}026$).

6. Diskusija

Šis tyrimas apima pacientus, sergančius GSIL ir gydomus dialize, ir transplantuotus pacientus, neturinčius KV įvykių ir periferinių kraujagyslių ligos prieš IT.

6.1. Pacientai, gydomi dialize

Tarp lyčių stebėti skirtumai buvo tokie, kaip tikėtasi. Moterims būdingos mažesnės kreatinino koncentracijos susijusios su raumenų mase bei baltymų ir energijos eikvojimu (36); padidėjusi koreguoto kalcio koncentracija atspindi su lytimi susijusius mineralinių medžiagų ir kaulų apykaitos ypatumus (37); moterims būdingos žemesnės cholesterolio vertės gali būti susijusios su sisteminiu uždegimu ir mitybos būkle (38). Vyrams nustatytos aukštos β2-mikroglobulino koncentracijos gali būti taip pat susijusios su mitybos nepakankamumu, uždegimu ir ateroskleroze, kurie yra būdingi LIL populiacijai (39). Moterims būdingos aukštesnės pulsinio spaudimo vertės tik atspindi skirtingus lyties nulemtus kraujagyslių senėjimo mechanizmus (40).

Jau nuo 1983 metų kalbama apie amžiaus įtaką arterijų standumui (41). Mūsų tyrimas tik patvirtino reikšmingą ryšį tarp amžiaus bei mkPBG ir PBG santykio, bet

ne msPBG. Tai atskleidžia skirtingas elastinių ir raumeninių kraujagyslių senėjimo tendencijas (42).

2014 m. (43) paminėtas PBG santykio vertinimas iki šiol sukelia daug diskusijų. Elastinėse arterijose, skirtingai nei raumeninėse arterijose, senėjimas sukelia standumo padidėjimą (44). M. Barry ir kt. (45) ištyrė koronarų stipininės arterijos ir kairiosios vidinės krūtininės arterijos transplantatų histologinę sandarą bei aprašė *tunica intima* sluoksnio storėjimą, miocitų migraciją iš *tunica media* sluoksnio, šio sluoksnio hipertrofiją ir fibrozę raumeninėse arterijose (stipininės arterijos transplantatai) bei ryškesnį arterijų standėjimą ir elastino praradimą elastinėse arterijose (krūtininės arterijos transplantatai). Tai gali paaiškinti skirtingas mkPBG ir msPBG vertes tarp pacientų, sergančių GSIL.

Kaip ir kitose studijose (43, 46), mes pastebėjome PBG santykio ryšį su amžiumi, cukriniu diabetu, ilgalaikio gydymo dialize trukme, c reaktyviojo baltymo koncentracija, bendrojo cholesterolio koncentracija ir PTH reikšme. cistatino c ir feritino ryšys su PBG santykiu, kiek mums žinoma, iki šiol dar nebuvo aprašytas. Kai kurių tyrimų, atliktų su pacientais, sergančiais LIL ir koronarine širdies liga, rezultatai rodė cistatino c sąsajas su PBG (47–49), tačiau cistatino c matavimo svarba dializuojamiems pacientams išlieka neaiški, ypač jeigu nėra žinoma liekamoji inkstų funkcija. Nedidelės apimties tyrimas parodė, kad cistatino c koncentracija, ištirta prieš pradedant gydymą dializėmis, yra nepriklausomas KV rizikos žymuo (50).

Lyginant su kitomis GSIL ir PBG santykį analizuojančiomis studijomis (43, 44, 51, 52) galima teigti, kad mes aprašėme žemesnes mkPBG ir didesnes msPBG vertes. Tačiau mūsų tyrime dalyvavę pacientai buvo jaunesni ir neturėjo KV įvykių bei periferinių arterijų okliuzinės ligos istorijos.

Keletas tyrimų (29, 53) koncentravo dėmesį į GSIL priežasties įtaką PBG. Mums nepavyko patvirtinti didesnių mkPBG verčių tarp pacientų, sergančių kraujagyslinės kilmės LIL, tačiau, kaip ir minėti tyrėjai, mes stebėjome aukščiausias mkPBG ir PBG santykio vertes tarp pacientų, sergančių diabetinės nefropatijos sukelta GSIL. Žinoma, cukrinio diabeto žala kraujagyslėms yra neginčijama, ypač poveikis mikrocirkuliacijai (54).

Taip pat mūsų darbe pirmąkart paminėtas PBG santykio ryšys su AoLK. AoLK vertinimo tiesinėje krūtinės ląstos rentgenogramoje (55) metodika yra labai paprasta. Be to, krūtinės ląstos rentgenogramos privalomai atliekamos kiekvienam dialize gydomam pacientui bent vieną kartą per metus. Žinoma, daugiapjūvė kompiuterinė tomografija yra jautresnis kraujagyslių kalcifikacijos vertinimo metodas, tačiau tyrimo kaina ir paciento patiriama apšvita turi būti taip pat įvertintos. K. Nitta ir kt. atliko tyrimą, kuriuo patvirtino AoLK vertinimo tiesinėje krūtinės ląstos rentgenogramoje rezultato stiprią koreliaciją su daugiapjūvės kompiuterinės tomografijos rezultatais (56).

Mes patvirtinome stiprų tarpusavio ryšį tarp AoLK vertės ir PBG santykio. Pastaruoju metu aprašytas žasto ir kirkšnies arterijų PBG ryšys su vainikinių kraujagyslių kalcifikacija, nustatyta kompiuterinės tomografijos metodika, tarp pacientų, sergančių hipertenzija (57). Hemodialize gydomiems pacientams taip pat būdingas PBG ir AoLK ryšys (58, 59), tačiau PBG matavimo metodika labai priklauso nuo tyrėjų. Įdomu tai, kad net pakoregavę duomenis pagal amžių mes stebėjome didesnes mkPBG ir mažesnes msPBG vertes tarp pacientų, kuriems pasireiškė AoLK.

LIL sergantiems pacientams yra būdinga tiek *intima*, tiek *media* arterijų sluoksnių kalcifikacija. *Media* tipo kalcifikacija yra labiau išplitusi, pažeidžianti daugiausia raumenines kraujagysles ir sukelianti kraujagyslių lygiųjų raumenų ląstelių (VSMcs) apoptozę. Ilgainiui vystosi ektopinė kraujagyslių kalcifikacija (60–62). Žinant, kad raumeninės kraujagyslės, lyginant su elastinėmis kraujagyslėmis, turi mažiau elastinių skaidulų ir daugiau VSMcs, anksčiau minėti pokyčiai neigiamai paveikia raumeninių arterijų sugebėjimą susitraukti. Todėl mažėja periferinis pasipriešinimas ir registruojamos žemesnės msPBG vertės. Kita vertus, *intima* tipo kalcifikaciją daugiausia sukelia uždegimas ir aterosklerozė; ji yra labiau lokalizuota, pažeidžia stambias elastines kraujagysles ir sukelia mkPBG padidėjimą. Taip sukeliamas mikrokraujagyslinių struktūrų pažeidimas organuose taikiniuose (54).

Mūsų tiriamieji, kuriems pasireiškė AoLK, pasižymėjo mažesnėmis uždegiminių rodiklių vertėmis (β2-mikroglobulinas, c reaktyvusis baltymas ir feritinas). Deja, mes nerinkome duomenų apie intraveninės geležies skyrimą dializuojamiems pacientams, galinčių iš dalies paaiškinti aukštas feritino reikšmes. Tiesioginis geležies poveikis kraujagyslių sienelei turi būti taip pat aptartas. Tyrimuose *in vitro* pastebėta, kad intraveniškai skiriama geležis virsdama laisva geležimi gali sukelti oksidacinį stresą ir lemti kraujagyslių kalcifikaciją (63). Taip pat pastebėtas geležies kaupimasis aortos vožtuvo kalcifikacijos plotuose; tai leidžia įtarti ryšį tarp geležies preparatų ir kraujagyslių kalcifikacijos sergant GSIL (64).

Ryšys tarp AoLK ir žemesnio ūgio gali būti aiškinamas ir vyresniu pacientų, kuriems pasireiškė AoLK, amžiumi bei galimai labiau pažengusiu mineralinių medžiagų ir kaulų apykaitos sutrikimu.

Kaip ir kiti tyrėjai (58, 59), mes nustatėme žemesnes diastolinio KS ir vidutinio KS vertes pacientams, kuriems pasireiškė AoLK. Vidutinis KS priklauso nuo širdies išmetimo, centrinio veninio spaudimo ir sisteminio kraujagyslinio pasipriešinimo (65) bei yra gana stabilus visame arterijų tinkle (66). Vidutinio KS sumažėjimas leidžia įtarti sumažėjusį kraujagyslių pasipriešinimą esant kraujagyslių kalcifikacijai. Todėl nuo periferinio kraujagyslių pasipriešinimo priklausanti PBG santykio vertė kartu su amžiumi ir vidutiniu KS gali geriausiai atspindėti kraujagyslių kalcifikacijos apimtį. Žemesnės centrinio sistolinio KS vertės taip pat buvo būdingos mūsų tiriamųjų populiacijai, tačiau tai nebuvo susiję su mažesne AoLK rizika (67).

Mūsų tyrimo metu identifikuoti KV rizikos veiksniai, tokie kaip amžius, hipercholesterolemija, padidėjęs c reaktyviojo baltymo kiekis, atitiko nurodytus kituose tyrimuose (68). Tačiau lytis, cukrinis diabetas ir rūkymas neturėjo įtakos KV įvykių pasireiškimui mūsų tiriamųjų populiacijoje.

Ūgio įtaka KV įvykių pasireiškimui yra nevienareikšmė. Vieni tyrimai patvirtina, kad žemesnis ūgis susijęs su didesne KV rizika (69), kiti tyrimai nurodo priešingai, kad aukštesni pacientai, sergantys GSIL, turi didesnę bet kokios priežasties, įskaitant KV, mirties riziką (70). Mūsų studija parodė, kad žemesnis ūgis buvo susijęs su didesne KV rizika. Didesnės apimties tyrimai, vertinantys ūgio, koreguoto pagal amžių ir lytį, sąsajas su KV išeitimis, padėtų patikslinti mūsų gautus rezultatus.

Jau daug metų kalbama apie β2-mikroglobulino kaip KV rizikos (39, 71–73) ir periferinių arterijų okliuzinės ligos (74) žymenį esant skirtingoms LIL stadijoms. Tačiau mums nepavyko patvirtinti šios sąsajos. Tokius rezultatus galėjo nulemti tai, kad β2-mikroglobulino žalingas poveikis širdžiai ir kraujagyslėms pasireiškia gydant ilgą laiką lėtinėmis dializėmis.

Padidėję fosforo ir PTH kiekiai yra svarbūs ne tik KV rizikos veiksniai (75, 76), bet fosforas atlieka svarbų vaidmenį ir kraujagyslių kalcifikacijos procesuose (77). Tačiau yra tyrimų, kurie kaip tik pabrėžia hipofosfatemijos įtaką bet kokios priežasties mirtingumui (75, 78). Mūsų tiriamųjų atveju hipofosfatemija buvo susijusi su didesne KV įvykių rizika. Deja, mes nerinkome duomenų apie fosforo surišiklių vartojimą, vitamino D koncentraciją ir nevertinome mitybos būklės, kuri galėtų lemti mažesnę fosforo koncentraciją kraujyje.

Padidėjusios hemoglobino koncentracijos ir mirtingumo ryšys su KV įvykiais turėtų būti vertinamas eritropoezę stimuliuojančių vaistų ir intraveninių geležies preparatų kontekste. Įrodyta, kad didelės eritropoezę stimuliuojančių vaistų dozės yra susijusios su didesniu mirčių dažniu (79).

Nustatytas kraujagyslių kalcifikacijos ir KV įvykių ryšys atitiko anksčiau publikuotus duomenis (56, 58, 59, 80).

Deja, mums nepavyko patvirtinti mkPBG kaip nepriklausomo KV įvykių rizikos veiksnio. Metaanalizė, apėmusi 17 635 pacientus, neturinčius LIL požymių (81), parodė, kad aortos PBG kartu su tradiciniais rizikos veiksniais palengvina KV rizikos vertinimą. mkPBG kaip nepriklausomas KV sergamumo ir mirtingumo veiksnys yra įtrauktas į keletą gairių (82, 83). Tačiau GSIL populiacijoje mkPBG reikšmė yra dviprasmiška (84, 85). cORD (angl. *the Calcification Outcome in Renal Disease*) kohortinis tyrimas (n = 1 084) (86) atskleidė, kad mkPBG kartu su pilvo aortos kalcifikacijos vertinimu labai tiksliai prognozuoja KV įvykius. Peritonine dialize gydomiems pacientams mkPBG vertė > 9 m/s (87) arba > 10 m/s (43) įvardijama kaip KV rizikos veiksnys. Tyrimai, vertinantys aukštesnių mkPBG reikšmių ir

žemesnių msPBG reikšmių bei šių reikšmių santykio įtaką bendram mirtingumui, parodė, kad tik PBG santykis išliko reikšmingu rizikos veiksniu pakoregavus pagal amžių. Prieš tai įvardyti tyrimo rezultatai tik atskleidžia mkPBG ir PBG santykio prieštarą vertinant klinikines išeitis. Bendrojoje populiacijoje (46) PBG santykis nepasižymėjo pranašumu prieš mkPBG vertinant KV įvykius. Bent 2 tyrimai, atlikti su dialize gydomais pacientais (43, 84), pateikė nevienareikšmius rezultatus. Mūsų tyrime PBG santykis tapo reikšmingu kintamuoju vertinant KV riziką tik pakoregavus pagal amžių, lytį ir AoLK. M. Londonas ir kt. (84) nustatė, kad tiek PBG santykis, tiek mkPBG vienodai svarbūs KV rizikos veiksniai tarp GSIL pacientų, nesergančių cukriniu diabetu. Šis tyrimas taip pat įtraukė ir pacientus, bent 6 mėnesius neturėjusius KV įvykių. O mes įtraukėme ir diabetu sergančius pacientus, tačiau be jokios KV įvykių istorijos. Šie įtraukimo ir išbraukimo kriterijai galėjo nulemti tokį rezultatų netolygumą.

Vertinant pastarųjų metų tyrimus galima teigti, kad arterijų standumo pokytis GSIL populiacijoje yra nevienareikšmis. Daugiausia aprašomas aortos PBG didėjimas (44, 88, 89), atitinkantis ir mūsų tyrimo rezultatus. Tačiau mes atkreipėme dėmesį ir į msPBG lėtėjimą. Deja, pakartotinai netyrėme besimptomių pacientų dėl periferinių arterijų okliuzinės ligos. Taip pat būtų įdomu įvertinti mikrovaskulinius pokyčius mūsų tiriamųjų populiacijoje, nes stebėtas dramatiškas PBG santykio didėjimas ilgainiui gali sukelti organų taikinių pažeidimus.

Arterijų standumo šešių mėnesių pokytis tarp mūsų tiriamųjų buvo priklausomas nuo vidutinio KS pokyčio. Tai tik patvirtina, kad, didėjant elastinių kraujagyslių standumui ir atsirandant periferinių raumeninių kraujagyslių pokyčiams, mažėja sisteminis kraujagyslių pasipriešinimas, todėl didėja vidutinis KS. Tačiau stebėti pokyčiai greičiausiai yra trumpalaikiai, nes nebuvo patvirtinti po dvejų metų stebėjimo.

Tyrimai, kurių metu GSIL sergantys pacientai buvo stebėti 12–36 mėn. (44, 88–91), pateikia skirtingus arterijų standumo pokyčio rezultatus. B. Di Iorio ir kt. (89) arterijų standumo didėjimą nustatė tik pacientams, kuriems būdinga vainikinių kraujagyslių kalcifikacija. Mes taip pat atkreipėme dėmesį, kad bazinė AoLK reikšmė turėjo įtakos arterijų standumo progresavimui ir PBG santykiui dvejų metų laikotarpiu. Kaip ir kiti autoriai (44), mes nurodėme arterijų standumo ir sisteminio uždegimo ryšį bei pirmąkart aprašėme β2-mikroglobulino koncentracijos įtaką PBG. Tai leidžia manyti, kad β2-mikroglobulinas yra svarbus ne tik periferinių (18), bet ir stambiųjų kraujagyslių struktūros pokyčiams.

6.2. Pacientai po inkstų transplantacijos

Tai pirmasis tyrimas Lietuvoje, analizuojantis arterijų standumo gradiento ir AoLK pokyčius tarp inksto recipientų. Taip pat tai viena iš nedaugelio studijų, atskleidusių AoLK įtaką inkstų transplantato veiklos prognozei praėjus 2 metams po IT.

Net 54,05 % pacientų, įtrauktų į mūsų tyrimą, buvo nustatyta AoLK prieš IT. Šis procentas padidėjo iki 67,57 % po IT. Tai atitinka kitų studijų pateikiamus duomenis, kad kraujagyslių kalcifikacijos pasireiškimas prieš ir po IT varijuoja nuo 20 iki 80 % (92–95). Be to, pastebėta, kad kraujagyslių kalcifikacijos apimtis arba stabilizuojasi (96), arba daugeliu atvejų progresuoja (95, 97, 98) po IT.

Įdomu tai, kad mūsų tiriamųjų grupėje AoKL nebuvimas prieš IT buvo susijęs su geresne transplantato veikla praėjus 2 metams po IT. Šis ryšys galėtų būti aiškinamas keliais aspektais. Visų pirma, AoLK prieš IT galimai atspindi kalcifikacijos apimtis visame organizme. Deja, mes nevertinome transplantacijos operacijos metu stebėtų vizualinių kraujagyslių radinių ir neturėjome duomenų apie klubinių kraujagyslių kalcifikaciją prieš IT. Antra, pacientams, kuriems AoLK progresavo po IT, nustatytos didesnės jonizuotojo kalcio reikšmės, kaip ir W. Parko ir kt. (95) studijoje. Padidėjusi kalcio koncentracija gali turėti netiesioginę įtaką transplantuoto inksto veiklai (99), t. y. sukelti glomerulo kraujagyslių spazmą ir ilgainiui kalcio kristalų atsidėjimą tubulointersticiniame audinyje (100, 101). Be to, kalcio koncentracija kraujyje gali atspindėti persistuojančio hiperparatiroidizmo po IT apimtis (102). Literatūros duomenimis, apie 25–66 % (99, 102) pacientų po IT turi padidėjusias kalcio koncentracijas kraujyje.

Vertindami arterijų standumo pokytį praėjus 1 ir 2 metams po IT, stebėjome mkPBG mažėjimą tik 1 metų laikotarpiu. Reikėtų atkreipti dėmesį, kad vidutinė mkPBG vertė mūsų tiriamųjų populiacijoje neviršijo gairėse rekomenduojamos < 10 m/s vertės (82). Tai gali paaiškinti, kodėl po vienų metų mkPBG reikšmė nebemažėjo, be to, atsirado kitų veiksnių (imunosupresiniai vaistai, organizmo imuninės sistemos aktyvumas ir kt.), galinčių turėti įtakos arterijų standumui. Taip pat nepastebėjome msPBG reikšmingo kitimo, o PBG santykis buvo daugiausia žemesnis nei 1,0. Šie pokyčiai turėtų būti vertinami kaip teigiami, nes sumažėja mikrokraujagyslinių struktūrų pažeidimo rizika esant tokiam standumų persitvarkymui (103).

Mūsų tyrimo trūkumai taip pat turi būti įvardyti. Pirma, maža tiriamųjų imtis, nulemta įtraukimo kriterijų pasirinkimo ir kasmetinio transplantacijų skaičiaus mūsų centre. Antra, nedidelis pacientų grįžtamumas pakartotiniam PBG matavimui riboja PBG santykio pokyčio vertinimą po 2 metų.

Mūsų studijos pranašumai yra tęstinis tyrimo dizainas ir kruopščiai atrinkta populiacija, neturinti KV įvykių ir periferinių arterijų ligos, kurie iš esmės yra susiję su didesne pakartotinių KV įvykių rizika. Taip pat mes parodėme, jog paprasčiausias AoLK vertinimas priekinėje krūtinės ląstos rentgenogramoje gali suteikti papildomos informacijos apie būsimas transplantacijos išeitis.

Mes nelyginome transplantuotų pacientų ir dialize gydomų pacientų populiacijų dėl keleto priežasčių: skirtingo amžiaus pasiskirstymo (jaunesni pacientai re-

cipientų grupėje); reikšmingai žemesnių mkPBG reikšmių tyrimo pradžioje tarp inksto recipientų ir skirtingų rizikos veiksnių, galinčių turėti įtakos arterijų standumui ir kraujagyslių kalcifikacijai.

7. Klinikinis pritaikymas

Centrinio ir periferinio arterijų standumo vertinimas turėtų tapti rutininiu tyrimu lėtine dialize gydomiems pacientams bei inksto recipientams ir turi būti atliekamas bent kartą per metus. Šis neinvazinis tyrimas suteikia informacijos apie KV riziką. Šis tyrimas parodė, kad mkPBG nėra pranašesnis už PBG santykį identifikuojant pacientus, kuriems pasireiškė AoLK, ir vertinant KV riziką.

Rutiniškai pakaitine inkstų terapija gydomiems pacientams netiriama β2-mikroglobulino koncentracija yra svarbus elastinių arterijų standumo progresavimo žymuo. β2-mikroglobulino koncentraciją veikiantys gydymo metodai ir liekamosios inkstų veiklos išsaugojimas turi būti vieni iš svarbiausių pacientams, gydomiems pakaitine inkstų terapija.

Dažnai gydytojai neįvertina naudingos informacijos apie AoLK apimtį, kurią gali papildomai suteikti įprastinė tiesinė krūtinės ląstos rentgenograma. Mūsų tyrimas patvirtino AoLK vertinimo, kuris turėtų būti atliekamas vieną kartą per metus, svarbą.

8. Išvados

1. Arterijų standumas ir su juo susiję biožymenys atspindi aortos lanko kalcifikaciją ir dvejų metų kardiovaskulinę riziką tarp pacientų, gydomų pakaitine inkstų terapija:
   1.1. Pulsinės bangos greičių santykis, c reaktyvusis baltymas ir feritino koncentracija atspindi aortos lanko kalcifikaciją tarp dialize gydomų pacientų:
   1.1.1. Pulsinės bangos greičių santykio, pakoreguoto pagal amžių ir vidutinį kraujospūdį modelio jautrumas, diagnozuojant aortos lanko kalcifikaciją, yra 77 %, o specifiškumas 82 %.
   1.2. Padidėjusi c reaktyviojo baltymo koncentracija, padidėjusi bendrojo cholesterolio koncentracija, padidėję parathormono ir hemoglobino kiekiai ir aortos lanko kalcifikacija yra susiję su padidėjusia kardiovaskulinių įvykių rizika tarp dialize gydomų pacientų;

1.3. Pulsinės bangos greičių santykio padidėjimas, pakoreguotas pagal amžių, aortos lanko kalcifikaciją ir lytį, nėra susijęs su kardiovaskuline rizika;
1.4. Miego ir kirkšnies arterijų pulsinės bangos greitis neturi nuspėjamosios vertės kardiovaskulinės rizikos vertinime tiriamųjų populiacijoje.

2. Lėtinė hemodializė sukelia elastinių arterijų standumo padidėjimą ir raumeninių arterijų standumo sumažėjimą:
   2.1. Vidutinio kraujospūdžio padidėjimas sukelia tiek elastinių, tiek raumeninių arterijų standumo padidėjimą šešių mėnesių laikotarpiu;
   2.2. C reaktyviojo baltymo koncentracijos padidėjimas yra susijęs tiek su elastinių, tiek su raumeninių arterijų standumo padidėjimu dvejų metų laikotarpiu;
   2.3. β2-mikroglobulino koncentracijos padidėjimas susijęs su elastinių arterijų standėjimu, bet neturi įtakos raumeninių arterijų standumui dvejų metų laikotarpiu.

3. Inkstų transplantacija pagerina elastinių arterijų standumą, bet neturi įtakos raumeninių arterijų standumui, taip pat tik iš dalies koreguoja aortos lanko kalcifikacijos apimtį:
   3.1. Dvejų metų laikotarpiu po transplantacijos didžiajai daliai inksto recipientų (72,29 %) aortos lanko kalcifikacijos apimtis nepakinta.

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11. Priedai

1 PRIEDAS. Pagrindinių charakteristikų pasiskirstymas pagal lytį

	Moterys (n = 50)	**Vyrai (n = 51)**	***p***
Amžius (metai)	56,51 ± 15,00	53,59 ± 16,29	0,352
Ūgis (cm)	160,44 ± 7,27	175,38 ± 7,95	< 0,001
Svoris (kg)	67,82 ± 13,16	77,56 ± 18,35	0,003
KMI (kg/m^2)	26,57 ± 5,09	25,17 ± 5,50	0,191
Kūno paviršiaus plotas (m^2)	1,70 ± 0,17	1,92 ± 0,22	< 0,001
Rūkymas (taip)	2 % (1)	25,5 % (13)	< 0,001
Hipertenzija (taip)	90 % (45)	98 % (50)	0,112
Cukrinis diabetas (taip)	18,0 % (9)	25,5 % (13)	0,470
Dializių trukmė (dienos)	1106 (93–6556)	878 (93–5114)	0,935
Inkstų ligos trukmė (metai)	11 (1,5–40,5)	10 (1,0–30,0)	0,517
Dializių rūšis			
Peritoninė dializė	18,0 % (9)	7,8 % (4)	0,148
Hemodializė	82,0 % (41)	92,2 % (47)	
Kraujo tyrimai			
Leukocitai (10e9/l)	6,72 ± 2,01	6,87 ± 2,14	0,723
Hemoglobinas (g/l)	114,92 ± 12,15	114,68 ± 16,06	0,934
Trombocitai (10e9/l)	225,46 ± 58,00	225,11 ± 87,41	0,461
Bendrasis baltymas (g/l)	67,74 ± 6,17	67,79 ± 10,97	0,570
Albuminas (g/l)	38,96 ± 3,65	40,07 ± 4,41	0,169
Bendrasis cholesterolis (mmol/l)	5,70 ± 1,49	4,77 ± 1,21	< 0,001
Kreatininas (µmol/l)	765,5 (364–1137)	928 (607–1768)	< 0,001
Cistatinas C (mg/l)	5,67 ± 1,44	6,27 ± 1,04	0,086
Šlapalas (mmol/l)	22,53 ± 6,76	24,26 ± 7,33	0,224
C reaktyvusis baltymas (mg/l)	5,10 (0,30–54,50)	3,81 (0,50–45,10)	0,749
β2-mikroglobulinas (mg/l)	27,68 (14,12–100,36)	35,72 15,19–85,18)	0,013

	Moterys (n = 50)	Vyrai (n = 51)	*p*
Kraujo tyrimai			
Feritinas (μg/l)	420,05 (44,00–1459,00)	380,50 (49,90–1040,40)	0,802
Šlapimo rūgštis (μmol/l)	356,16 ± 78,88	362,11 ± 96,48	0,734
PTH (pmol/l)	62,3 (2,1–201,4)	49,2 (0,5–201,4)	0,230
Kalcis (mmol/l)	2,68 ± 0,19	2,19 ± 0,17	0,058
Fosforas (mmol/l)	1,80 ± 0,49	1,96 ± 0,64	0,148
Koreguotas kalcis (mmol/l)	2,28 ± 0,17	2,19 ± 0,14	0,004
Kalcio ir fosforo produktai ($mmol^2/l^2$)	4,10 ± 1,21	4,28 ± 1,30	0,476
Hemodinamiką atspindintys ir kardiovaskuliniai žymenys			
Sistolinis KS (mmHg)	147,92 ± 18,66	144,60 ± 18,91	0,379
Diastolinis KS (mmHg)	97,76 ± 12,02	102,04 ± 13,42	0,123
Pulsinis spaudimas (mmHg)	63,28 ± 18,77	56,31 ± 15,43	0,044
Vidutinis KS (mmHg)	104,83 ± 12,26	106,76 ± 13,00	0,446
Pulsas (k./min.)	72,32 ± 11,34	72,10 ± 10,77	0,920
Centrinis sistolinis KS (mmHg)	124,55 ± 15,63	125,42 ± 15,88	0,800
mkPBG (m/s)	10,32 ± 1,80	10,09 ± 1,80	0,538
msPBG (m/s)	11,57 ± 3,54	11,14 ± 3,56	0,536
PBG santykis	1,06 (0,60–2,65)	1,07 (0,59–3,40)	0,958
AoLK (0–16)	2 (0–11)	1 (0–8)	0,419

KMI – kūno masės indeksas, LIL – lėtinė inkstų liga, PTH – parathormonas, KS – kraujo spaudimas, mkPBG – miego ir kirkšnies arterijų pulsinės bangos greitis, msPBG – miego ir stipininės arterijų pulsinės bangos greitis, PBG – pulsinės bangos greitis, AoLK – aortos lanko kalcifikacija.

2 PRIEDAS. Su kardiovaskuliniais įvykiais susiję kintamieji: bendroje tiriamųjų populiacijoje ir tarp pacientų, kurie nesulaukė inksto transplantacijos stebėjimo laikotarpiu. Dvinarė ir daugianarė Kokso (Cox) regresinė analizė

	Dializuojami pacientai (n = 81), įvykiai n = 16			**Bendra populiacija (n = 101), įvykiai n = 20**		
Dvinarė	**HR**	**95 % PI**	***p***	**HR**	**95 % PI**	***p***
Amžius	1,05	1,01–1,09	0,012	1,05	1,01–1,08	0,006
Lytis (vyrai)	0,56	0,20–1,56	0,271	0,79	0,32–1,91	0,604
Cukrinis diabetas	0,17	0,02–1,30	0,088	0,17	0,02–1,27	0,084
Rūkymas	0,30	0,04–2,30	0,250	0,31	0,04–2,34	0,26
Ūgis	0,93	0,88–0,98	0,013	0,93	0,89–0,98	0,009
KMI	0,99	0,91–1,08	0,980	1,02	0,94–1,11	0,569
Kūno paviršiaus plotas	0,10	0,01–1,18	0,068	0,16	0,02–1,50	0,11
Dializių trukmė	1,95	0,64–5,94	0,237	1,80	0,67–4,87	0,245
Albuminas	0,95	0,81–1,12	0,549	0,98	0,87–1,10	0,769
β2-mikroglobulinas	1,02	0,99–1,05	0,136	1,02	0,99–1,05	0,084
C reaktyvusis baltymas	1,05	1,01–1,09	0,005	1,05	1,02–1,09	0,001
Feritinas	1,002	0,99–1,003	0,059	1,001	0,99–1,003	0,076
Bendrasis cholesterolis	1,42	1,11–1,83	0,005	1,30	1,01–1,67	0,039
Koreguotas kalcis	11,81	0,42–3,25e+02	0,114	6,61	0,35–124	0,207
Fosforas	0,31	0,12–0,81	0,017	0,40	0,17–0,94	0,036
PTH	1,47	1,14–2,75	< 0,001	2,95	1,65–9,77	0,047
Hemoglobinas	1,05	1,01–1,09	0,016	1,03	0,99–1,06	0,082
AoLK (≥ 1)	4,60	1,30–16,17	0,017	4,33	1,44–12,98	0,008
Sistolinis KS	1,008	0,98–1,03	0,58	1,01	0,98–1,03	0,427
Diastolinis KS	0,98	0,94–1,03	0,539	0,98	0,94–1,02	0,430
Pulsinis spaudimas	1,01	0,98–1,04	0,28	1,01	0,98–1,04	0,242
Vidutinis KS	0,99	0,95–1,04	0,922	1,01	0,97–1,04	0,756
Centrinis sistolinis KS	1,005	0,97–1,04	0,808	1,01	0,97–1,04	0,615
Galutinis sistolinis KS	1,013	0,98–1,04	0,435	1,01	0,98–1,04	0,413

	Dializuojami pacientai (n = 81), įvykiai n = 16			Bendra populiacija (n = 101), įvykiai n = 20		
Dvinarė	**HR**	**95 % PI**	***p***	**HR**	**95 % PI**	***p***
mkPBG	1,04	0,91–1,19	0,581	1,04	0,93–1,17	0,442
msPBG	1,16	0,91–1,47	0,229	1,14	0,90–1,45	0,429
PBG santykis	0,69	0,21–2,25	0,543	0,90	0,33–2,41	0,945
Daugianarė regresija su pulsinės bangos greičių santykiu						
5 modelis	1,02	1,0001–18,11	0,062	1,07	1,001–73,79	0,100
6 modelis	1,01	1,009–12,30	0,038	1,05	1,0009–20,23	0,067
7 modelis	1,01	1,008–9,52	0,049	1,07	1,001–47,42	0,081

KMI – kūno masės indeksas, PTH – parathormonas, KS – kraujo spaudimas, mkPBG – miego ir kirkšnies arterijų pulsinės bangos greitis, msPBG – miego ir stipininės arterijų pulsinės bangos greitis, PBG – pulsinės bangos greitis, AoLK – aortos lanko kalcifikacija, HR – rizikos santykis, PI – pasikliautinasis intervalas.
5 modelis: PBG santykis pakoreguotas pagal amžių.
6 modelis: PBG santykis pakoreguotas pagal amžių, AoLK.
7 modelis: PBG santykis pakoreguotas pagal amžių, AoLK, lytį.

Edita Lycholip

NUOTOLINĖS ŠIRDIES NEPAKANKAMUMO PACIENTŲ STEBĖSENOS TECHNOLOGIJOS: SPECIALISTŲ NUOMONĖ, PACIENTŲ VERTINAMOSIOS BAIGTYS IR PLAUČIŲ IMPEDANSO MATAVIMAS

TELEMONITORING TECHNOLOGIES FOR HEART FAILURE PATIENTS: PROFESSIONALS' OPINION, PATIENTREPORTED OUTCOMES AND MEASUREMENT OF LUNG IMPEDANCE

Biomedical Sciences, Medicine (06B)
Biomedicinos mokslai, medicina (06B)

Santrumpos

AEM – apsaugos nuo edemos monitorius (angl. *Edema Guard Monitor*)

BPI – bazinis plaučių impedansas

ČGLI – Čarlsono gretutinių ligų indeksas

DFA – didelis fizinis aktyvumas

EKD – Europos kardiologų draugija

EŠNSEs – Europos širdies nepakankamumo savirūpos elgesio skalė

IKT valdoma LPS – informacinių ir kompiuterinių technologijų valdoma ligos priežiūros sistema

IKR – interkvartilinės ribos

IN TOUCH tyrimas – ambulatorinių širdies nepakankamumo pacientų inovatyvios IKT valdomos ligos priežiūros kartu su telemonitoringu tyrimas (angl. *INnovative ICT-guided-DMS combined with Telemonitoring in OUtpatient clinics for Chronic HF patients*)

KSIF – kairiojo skilvelio išstūmio frakcija

LFA – lengvas fizinis aktyvumas

LNDs – ligoninės nerimo ir depresijos skalė

LNDs_D – ligoninės nerimo ir depresijos skalės depresijos poskalė

LNDs_N – ligoninės nerimo ir depresijos skalės nerimo poskalė LPS – ligos priežiūros sistema

MGŠNK – Minesotos gyvenimo esant širdies nepakankamumui klausimynas

MET – metabolinis ekvivalentas

NYHA – Niujorko širdies asociacija (angl. *New York Heart Association*)

NT-proBNP – N-terminalinis pro-B tipo natriurezinis peptidas

P.I. – 95 proc. pasikliautinasis intervalas

PI – plaučių impedansas

ΔPIP – plaučių impedanso procentinis pokytis nuo pradinės PI reikšmės

ΔPIB – plaučių impedanso procentinis pokytis nuo bazinės PI reikšmės

PPI – pradinis plaučių impedansas

PVB – pacientų vertinamosios baigtys

SED – sėdimasis fizinis aktyvumas

SPS – sveikatos priežiūros specialistai

SN – standartinis nuokrypis

ŠN – širdies nepakankamumas

ŠS – šansų santykis

TM – telemonitoringas

VFA – vidutinis fizinis aktyvumas

VDFA – vidutinis ir didelis fizinis aktyvumas

1. Įvadas

Nepaisant medikamentinio ir invazinio širdies nepakankamumo (ŠN) gydymo pažangos kas antras pacientas miršta per 5 metus po ŠN diagnozės nustatymo [1]. Hospitalizavimas yra vienas svarbiausių prognozinių mirštamumo veiksnių [2] ŠN pacientams. Ankstyvuoju laikotarpiu po išrašymo, trunkančiu iki 3 mėn., nepalankių baigčių rizika yra ypač didelė, todėl šis laikotarpis buvo pavadintas pažeidžiamumo faze; daugiausia įvykių nutinka per pirmąsias 30 dienų [3, 4]. Ką tik iš ligoninės išrašytiems pacientams medikamentinis gydymas dažniausiai keičiamas, todėl būtina kruopšti priežiūra siekiant greito ir optimalaus vaistų titravimo pereinant į ambulatorinę fazę; be to, pacientai turi iš naujo prisitaikyti prie namų sąlygų.

Savirūpa yra kertinė lėtinio ŠN gydymo dalis, jos pagrindiniai tikslai – pagerinti paciento savijautą ir gyvensenos kokybę, sumažinti hospitalizavimų dažnį ir mirštamumą [5, 6]. Savirūpos koncepcija apima tris pagrindinius komponentus: savikontrolę (pvz., vaistų vartojimas, kaip nurodyta), savistabą (pvz., reguliarus svėrimasis) ir savireguliaciją (pvz., diuretikų dozės keitimas atsižvelgiant į simptomus) [7]. Vienas modernesnių daug vilčių teikiančių lėtinės ligos valdymo būdų yra telemedicina, kai naudojamos telekomunikacijos ir informacinių technologijų priemonės sveikatos priežiūros paslaugoms teikti per atstumą. Dviejų „Cochrane" metaanalizių rezultatai rodo, kad, palyginti su įprastine priežiūra, telemonitoringas (TM) gali sumažinti bendrą mirštamumą ir su ŠN susijusių hospitalizavimų dažnį [8, 9].

Nors kardiologijos bendruomenė vis labiau domisi TM, daug klausimų lieka neatsakytų. Iki šiol neaišku, kuriems pacientams ši strategija būtų naudingiausia, kokia optimali TM taikymo trukmė ir kaip dažnai turi būti siunčiama informacija. Be to, diskutuojama, kuriuos klinikinius parametrus reikėtų stebėti ir kokie turėtų būti sveikatos priežiūros specialistų (SPS) veiksmai reaguojant į gautą informaciją [10]. TM poveikis pacientų savirūpai nebuvo išsamiai tiriamas. Išnagrinėjus 14 literatūros šaltinių, galima daryti išvadą, kad TM naudojimas pagerina ŠN sergančių pacientų savirūpą, tačiau išvados svarumą riboja mažos studijų imtys, skirtinga tyrimų struktūra ir tai, kad ne visada buvo naudojami ŠN specifiniai klausimynai [11].

TM taikymas daugelyje šalių vis dar nėra įprastas, ir žinių apie jo praktinį taikymą trūksta. Tokioje technologijos vystymo fazėje svarbu sveikatos specialistų nuomonė dėl TM naudojimo ŠN sergantiems pacientams ir su tuo susiję lūkesčiai.

Didelis hospitalizavimo ir pakartotinio hospitalizavimo dažnis dėl ŠN yra susijęs su ribotomis plaučių stazės klinikinio įvertinimo galimybėmis. Patikima stazės stebėsena pažeidžiamuoju laikotarpiu yra svarbus ŠN priežiūros tikslas. Ilgam laikui implantuojami intrakardiniai davikliai leidžia stebėti širdies kamerų prisipildymo slėgius ir krūtinės ląstos varžą siekiant anksti nustatyti gresiantį ŠN paūmėjimą [12]. Tačiau toks invazinis TM gali būti taikomas tik nedidelei ŠN pacientų populiacijos daliai. Todėl neinvaziniai intratorakalinio impedanso matavimo būdai yra patraukli alternatyva siekiant anksti aptikti paciento būklės blogėjimą. Vienas tokių prietaisų – apsaugos nuo edemos monitorius (AEM, angl. *Edema Guard Monitor*) – ištirtas ambulatorinių ŠN pacientų atsitiktinių imčių studijoje IMPEDANCE-HF; gauti reikšmingi prognozės gerinimo rezultatai naudojant reguliarų plaučių impedanso (PI) stebėjimą apsilankius klinikoje [13].

Galimybė kasdien stebėti skysčio kiekį krūtinės ląstoje namuose pažeidžiamuoju laikotarpiu iki šiol nebuvo tirta.

1.1 Tyrimo hipotezė

Telemonitoringas gali pagerinti ŠN pacientų vertinamąsias baigtis (PVB) ir teigiamai vertinamas sveikatos priežiūros specialistų. Neinvazinis multimodalinis monitoringas namuose leidžia patikimai įvertinti skysčių kaupimąsi ir ŠN pacientų būklės blogėjimą.

1.2 Tyrimo tikslas

Įvertinti TM poveikį ŠN pacientų vertinamosioms baigtims, SPS nuomonę apie TM ir multimodalinio monitoringo namuose galimybę ankstyvuoju laikotarpiu po išrašymo.

1.3 Tyrimo uždaviniai

1. Atlikti daugiacentrio atsitiktinių imčių IN TOUCH tyrimo duomenų *post-hoc* analizę įvertinant savirūpos dinamiką bei jai įtaką darančius veiksnius, kartu ir TM poveikį.
2. Remiantis IN TOUCH tyrimo duomenimis nustatyti ŠN pacientų, turinčių didžiausią potencialą gerinti savirūpą, profilį.
3. Ištirti ir palyginti Lietuvos, Norvegijos ir Švedijos SPS patirtį, nuomonę ir lūkesčius, susijusius su nuotoline stebėsena.
4. Nustatyti SPS, turinčių didžiausius TM taikymo lūkesčius, charakteristikas.
5. Atlikti multimodalinio PI, fizinio aktyvumo ir svorio monitoringo namuose po hospitalizavimo dėl ŠN galimybių studiją.
6. Įvertinti koreliaciją tarp PI, fizinio aktyvumo ir svorio kitimo 30-ies dienų po hospitalizacijos dėl ŠN laikotarpiu.

1.4 Tyrimo naujumas

Pirmą kartą ŠN pacientų savirūpos analizė remiantis IN TOUCH tyrimo duomenimis parodė, kad ši pacientų vertinamoji baigtis yra itin kintama, nes maždaug pusei tiriamųjų studijos laikotarpiu savirūpa pagerėjo, o kitai pusei pablogėjo. TM neturėjo reikšmingos įtakos savirūpos dinamikai. Pritaikę inovatyvų neparametrinės statistikos metodą – regresijos medžio klasifikaciją, nustatėme pacientų, turinčių didžiausią potencialą gerinti savirūpą, profilį. Paaiškėjo, kad tai pacientai, turintys žemą pradinį savirūpos lygį arba vidutinį pradinį savirūpos lygį, kai kartu nėra depresijos.

Pirmą kartą atlikta reprezentatyvi trijų Šiaurės ir Baltijos šalių kardiologų ir slaugytojų, dirbančių su ŠN pacientais, apklausa dėl TM naudojimo patirties, suvokimo ir lūkesčių. Nors nustatyta, kad TM dar nėra taikomas klinikinėje praktikoje,

apklausa atskleidė bendrą teigiamą SPS nuomonę apie jo potencialią reikšmę, esant tam tikrų nacionalinių skirtumų. Be to, taikant regresijos medžio statistinę analizę, apibrėžtos SPS charakteristikos, susijusios su didžiausiais TM taikymo lūkesčiais. Jaunesni, turintys nedidelį darbo stažą ir gerus kompiuterinius įgūdžius SPS sudaro ankstyvųjų TM diegėjų grupę.

Atlikta galimybių studiją taikant multimodalinį pažeidžiamumo fazės ŠN pacientų monitoringą namuose. Matavimus per 30 dienų po hospitalizavimo sudarė PI vertinimas neinvaziniu didelio jautrumo AEM prietaisu, fizinio aktyvumo registravimas naudojant akcelerometrą „ActiGraph" ir svėrimasis. Pirmą kartą AEM buvo naudojamas plaučių skysčių kiekiui kasdien matuoti namuose po hospitalizavimo. Stebėjimai parodė gaunamų rezultatų patikimumą ir didelį jautrumą reaguojant į diuretikų vartojimą bei kitokią gydymo korekciją. Unikali kryžminės koreliacijos tarp kasdienių PI, fizinio aktyvumo ir svorio verčių analizė neatskleidė patikimo ryšio tarp besikaupiančio skysčio plaučiuose pagal impedanso reikšmes, svorio ir fizinio aktyvumo pokyčių.

1.5 Ginamieji teiginiai

1.5.1 Telemonitoringas pagerina ŠN pacientų savirūpą.

1.5.2 Sveikatos priežiūros specialistai teigiamai vertina ŠN sergančių pacientų TM.

1.5.3 Pacientų, sergančių ŠN, telemonitoringas yra gerai žinomas SPS Šiaurės Europoje.

1.5.4 Telemonitoringo diegimas didesnes pajamas turinčiose Šiaurės šalyse yra labiau pažengęs nei Baltijos šalyse.

1.5.5 Neinvazinis PI matavimas namuose naudojant didelio jautrumo prietaisą leidžia tiksliai įvertinti skysčių kaupimąsi ir ŠN pacientų būklės blogėjimą.

1.5.6 Plaučių impedanso reikšmės koreliuoja su fizinio aktyvumo ir svorio kitimais per 30 dienų po hospitalizavimo dėl ŠN.

2. Metodai

2.1 Daugiacentrio atsitiktinių imčių IN TOUCH tyrimo duomenų post-hoc analizė

2.1.1 IN TOUCH tyrimo dizainas ir pacientų bazinės charakteristikos

IN TOUCH daugiacentris atsitiktinių imčių tyrimas buvo atliktas dešimtyje Olandijos ligoninių [14, 15]. Pacientai buvo įtraukiami dviejų savaičių po hospi-

talizavimo laikotarpiu arba jiems apsilankius poliklinikoje dėl ŠN pablogėjimo ir stebimi 9 mėnesius.

Įtraukimo kriterijai: hospitalizavimas į skubios/intervencinės ar kardiologijos skyrius; 18 metų sulaukę asmenys, kuriems per apsilankymą ambulatoriniame ŠN skyriuje reikėjo optimizuoti geriamųjų ar intraveninių diuretikų dozę; turintys struktūrinę širdies ligą ir dokumentuotą kairiojo skilvelio išstūmio frakciją (KSIF) ≤45 proc. Nuo 2009 metų gruodžio iki 2012 metų sausio mėnesio į tyrimą buvo įtraukti 177 pacientai. Atsitiktiniu būdu 83 pacientai buvo atrinkti į informacinių ir kompiuterinių technologijų valdomą ligos priežiūros sistemos (IKT valdomą LPS) grupę, o 94 – į grupę, kurioje papildomai taikytas ir TM (TM + IKT valdomą LPS).

Nei pirminė baigtis (sudėtinis svertinis balas, susidedantis iš mirčių, hospitalizavimų dėl ŠN dažnio ir gyvenimo kokybės balo), nei antrinės baigtys (atskiri pirminės baigties elementai bei kartotiniai hospitalizavimai ir jų trukmė) tarp grupių reikšmingai nesiskyrė [14]. Į mūsų analizę buvo įtraukti 118 pacientų, kurie užpildė Europos širdies nepakankamumo savirūpos elgesio (EŠNSEs), ligoninės nerimo ir depresijos (LNDs) ir Minesotos gyvenimo esant širdies nepakankamumui (MGŠNK) klausimynus.

Į IKT valdomą ligos priežiūros sistemą apsilankymo klinikoje metu buvo įkelti tiriamųjų anamnezės, fizinio ištyrimo, laboratorinių tyrimų ir klausimynų bei slaugytojo vertinimo duomenys, kurių pagrindu LPS automatiškai sukūrė gydymo korekcijos rekomendacijas. TM + IKT valdomos LPS grupės pacientai namuose papildomai turėjo atlikti svorio, kraujo spaudimo matavimus ir registruoti elektrokardiogramą (EKG) du kartus per savaitę, jeigu buvo didinama beta adrenoblokatorių dozė. Namuose atliktų matavimų duomenys buvo tiesiogiai įkeliami į LPS. Tuo atveju, kai gauti rezultatai viršijo iš anksto numatytas ribas, interaktyvi LPS generuodavo klausimus, susijusius su paciento būkle, ir pateikdavo nemedikamentinio gydymo rekomendacijas, o slaugytojas gaudavo pranešimą į elektroninį paštą arba žinutę telefonu ir per 2 valandas susisiekęs su pacientu aptardavo jo simptomus bei medikamentinį gydymą.

2.1.2 *Post-hoc* analizės duomenų rinkimas

NYHA funkcinės klasės nustatymas, fizinės būklės įvertinimas, EKG ir laboratoriniai tyrimai (iš jų ir N-terminalinio pro-B tipo natriurezinio peptido [NT-proBNP]) buvo atlikti tyrimo pradžioje, po 2 savaičių ir 9 mėnesių. Savirūpos, depresijos ir nerimo bei su liga susijusios gyvenimo kokybės klausimynai buvo užpildyti tyrimo pradžioje ir pabaigoje.

Savirūpos klausimynas

EŠNSEs [16] yra devynių punktų klausimynas apie ŠN pacientų veiksmus, susijusius su savirūpa: farmakologinio ir nefarmakologinio gydymo laikymąsi, ŠN simptomų ir požymių stebėseną ir priemones, kurių imasi, jei jo / jos būklė blogėja. Kiekvienam atsakymui pasirenkamas elgesio variantas nuo vieno (visiškai sutinku) iki penkių (visiškai nesutinku) balų. Aukščiausias savirūpos lygis atitinka 9 balų, žemiausias – 45 balų sumą. Savirūpa buvo laikoma pablogėjusia ar pagerėjusia, jeigu EŠNSEs balas atitinkamai padidėjo ar sumažėjo bent vienu punktu, ir nepakitusia, jeigu balų skirtumas buvo lygus nuliui.

Nerimo ir depresijos vertinimas

LNDs susideda iš 14 klausimų, kiekvienas turi keturis atsakymų variantus nuo nulio (nėra simptomo) iki trijų (maksimaliai išreikštas simptomas). Septyni klausimai skirti depresijos simptomams – depresijos poskalė (LNDs_D), kiti septyni – nerimo simptomams (LNDs_N). Balų vertės kiekvienoje poskalėje gali svyruoti nuo 0 iki 21: nuo 0 iki 7 – nerimo ar depresijos nėra, nuo 8 iki 10 – yra lengvas, nuo 11 iki 14 – vidutinis, nuo 15 iki 21 balo – sunkus nerimas ar depresija [17, 18].

Gyvenimo kokybės vertinimas

MGŠNK [17, 19] yra 21 klausimo su sveikata susijusios gyvenimo kokybės vertinimo instrumentas. Kiekvienas klausimas atspindi ŠN poveikį įprastam gyvenimui per paskutinį mėnesį, išskiriamos fizinė ir emocinė poskalės. Atsakymų reikšmės svyruoja nuo „netrukdė“ (0 balų), „labai mažai“ (1 balas) iki „labai trukdė“ (5 balai). Bendras balas gali siekti nuo 0 iki 105, aukštesnis MGŠNK balas reiškia blogesnę su sveikata susijusią gyvenimo kokybę.

2.1.3 *Post-hoc* analizės statistiniai metodai

Pirmiausia, naudojant Mano, Vitnio ir Vilkoksono rangų sumų kriterijų ir chi kvadrato kriterijų, atlikti bazinių charakteristikų ir pacientų vertinamųjų baigčių (PVB) palyginimai grupių viduje bei tarp grupių. Porinis Stjudento t kriterijus ir Vilkoksono ranginis kriterijus buvo naudoti pokyčiams vertinti bendroje grupėje ir atskirai abiejose studijų grupėse.

Antra, ryšiams tarp studijos kovariančių ir savirūpos pagerėjimo nustatyti buvo naudotas logistinės regresijos metodas. Nepriklausomais kintamaisiais laikyti intervencijos grupė, amžius, lytis, MGŠNK balai, NYHA funkcinė klasė, NT-pro-BNP lygis, KSIF ir LNDs depresijos bei nerimo balai. Kintamieji, kurie buvo statistiškai reikšmingi vienanarėje analizėje, įtraukti į daugianarę žingsninę logistinę regresiją.

Trečia, didžiausią savirūpos gerėjimo potencialą turinčiam pogrupiui identifikuoti buvo naudotas regresijos medis su savirūpos balo pokyčiu kaip tolydžiu tiksli-

niu kintamuoju ir su rodikliais, naudotais logistinėje regresijoje kaip įvesties kintamaisiais. Galiausiai, remiantis regresijos medžio pasiskirstymu, depresijos veiksnio poveikis buvo ištirtas atskirai naudojant vienanarę regresinę analizę trijuose skirtingų pradinių savirūpos lygių pogrupiuose. Analizei naudotas statistinis paketas SPSS (versija 20) ir programos R paketas „party".

2.2 Sveikatos priežiūros specialistų telemonitoringo taikymo patirtis ir lūkesčiai

2.2.1 Telemonitoringmo apibrėžimas ir apklausos dizainas

Šiame tyrime nuotolinė stebėsena buvo apibrėžta kaip neinvazinė internetu vykdoma svorio, kraujo spaudimo, širdies susitraukimų dažnio, dusulio ir kitų požymių bei simptomų, atspindinčių sergančių ŠN pacientų skysčių kaupimąsi, stebėsena. Daugiacentris trijų Šiaurės ir Baltijos šalių skerspjūvio tyrimas – anketinė kardiologų ir slaugytojų apklausa dėl ŠN pacientų TM – buvo atliktas 41-oje Lietuvos, 53-ose Norvegijos ir 61-oje Švedijos ligoninėse ir poliklinikose. Lietuvoje ir Norvegijoje duomenys buvo renkami 2016 metų rugsėjo–gruodžio mėnesiais, o Švedijoje – nuo 2013 metų lapkričio iki 2014 metų gegužės mėnesio. Paštu buvo išsiustos 1004 anketos į 205 valstybines ir privačias gydymo įstaigas.

Keturiasdešimt trijų klausimų Lietuvos ir Norvegijos bei 40 klausimų Švedijos anketos buvo parengtos remiantis de Vries ir bendraautorių sukurta pirmine klausimyno versija (2013) [20]. Anketą sudarė uždarieji ir atvirieji klausimai, susiję su SPS charakteristikomis, patirtimi ir žiniomis apie TM. Pirmoje klausimyno dalyje yra pateikti klausimai apie SPS charakteristikas (amžius, lytis, darbo patirtis, gebėjimai naudoti kompiuterines ir komunikacijos priemones), patirtį ir žinias apie TM, galimus stebėsenos būdus (pvz., nuotolinė stebėsena naudojant internetą, vaizdo ryšį, vizitai poliklinikoje), TM įgyvendinimo galimybes ir tikslines grupes. Antroje ir trečioje klausimyno dalyse yra pateikti klausimai naudojantiems arba iki šiol nenaudojusiems TM specialistams. Šiose dalyse yra klausiama apie priežastis, tikslus, stebėjimo trukmę, pacientų atrankos nuotolinei stebėsenai kriterijus bei kliūtis ją diegiant.

Klausimas „Kas turėtų finansuoti nuotolinę stebėseną?" buvo atviras Lietuvos ir Norvegijos klausimynuose, tačiau Švedijos klausimyne buvo pateikti galimi atsakymų variantai (sveikatos draudimo bendrovė, ligoninė, kardiologijos skyrius, kt.). Atsižvelgiant į kalbos ir kultūrinius skirtumus galutinė klausimyno versija buvo parengta pagal geros vertimo ir kultūrinės adaptacijos praktikos principus [21].

2.2.2 Apklausos duomenų analizė

Apibendrinamoji kokybinių duomenų analizė

Du nepriklausomi tyrėjai išvertė atsakymus į atvirus klausimus iš lietuvių ir norvegų į anglų kalbą (I.T.A ir E.L.) ir suformulavo juos kaip teiginius. Keturi tyrėjai (I.T.A., E.L., J.C., I.L) suskirstė atsakymus į kategorijas ar pogrupius. Hsieh ir Shannon metodu buvo atlikta apibendrinamojo turinio analizė suskirstant atsakymus į pogrupius ir apskaičiuojant procentais [22].

Apklausos statistinė analizė

Aprašomosios statistikos rezultatai pateikti kaip vidurkiai ir standartiniai nuokrypiai arba dažniai, išreikšti absoliučia reikšme ir procentais. Palyginimui tarp grupių naudotas Kruskalo ir Voliso arba chi kvadrato kriterijus analizuojant klausimynų atsakymus ir kitas charakteristikas, atitinkamai duomenų tipui.

Taikant regresijos medžio statistinę analizę, nustatytos didžiausius TM įdiegimo lūkesčius turinčių SPS charakteristikos. Lūkesčiai buvo vertinami pagal skalės balus nuo 0 iki 10, pažymėtus renkantis TM priežastis (aukštesni balai atitiko didžiausius lūkesčius). Įvesties kintamieji (specialybė [kardiologas/slaugytojas], amžius, lytis, šalis, žinojimas apie TM, darbo su kompiuterinėmis programomis patirtis) buvo panaudoti regresijos medyje. Analizei naudotas statistinis paketas SPSS (versija 20) ir programos R paketas „party“.

2.3 Multimodalinio monitoringo namuose galimybių studija

2.3.1 Galimybių studijos dizainas ir duomenų rinkimas

Atlikome multimodalinio monitoringo namuose galimybių studiją siekdami nustatyti, ar tokios priežiūros modelis galėtų būti rekomenduojamas tolesniems efektyvumo vertinimo tyrimams. Vertinome svorio, PI ir fizinio aktyvumo monitoringo 30 dienų po hospitalizavimo dėl ŠN pablogėjimo įgyvendinamumą, praktiškumą, patikimumą, variabilumą, jautrumą ir galimą klinikinę naudą. Duomenys buvo renkami 2017 metų gegužės–rugsėjo mėnesiais, naudojant inovatyvų neinvazinį impedanso matavimo prietaisą – apsaugos nuo edemos monitorių (AEM, Cardioset, Izraelis), akcelerometrą „ActiGraph GT3X“ (AcuMed AS, Norvegija) ir svarstykles.

Į tyrimą buvo įtraukiami suaugę pacientai po hospitalizavimo dėl ŠN pablogėjimo Lietuvoje ir Norvegijoje. Tyrimo pradžioje surinkti demografiniai duomenys, Charlsono gretutinių ligų indeksas (ČGLI) [23], pradžioje ir pabaigoje atlikta fizinė apžiūra, laboratoriniai tyrimai ir echokardiografija.

Plaučių impedanso matavimas

PI matavimui namuose naudojant AEM būtina artimųjų pagalba. Išrašant iš ligoninės pacientai ir jų artimieji (dažniausiai sutuoktiniai ar vaikai) buvo išmokyti, kaip atlikti plaučių varžos matavimus sėdimoje padėtyje, pritvirtinant elektrodus ant paciento krūtinės ir nugaros tiksliai toje pačioje vietoje, maždaug tuo pačiu metu, vieną kartą per dieną, 30 dienų. Trys elektrodai klijuojami vertikaliai iš dešinės krūtinės pusės (1 paveikslas, A). Ant nugaros elektrodai tvirtinami horizontalioje linijoje, einančioje ties apatiniu dešiniosios mentės kampu link stuburo (1 paveikslas, B). Impedanso matavimo reikšmė atsiranda prietaiso ekrane (1 paveikslas, C) [24].

1 PAVEIKSLAS. Plaučių impedanso matavimo atlikimo technika

Kiekvienas dienos matavimas buvo atliekamas tris kartus. Matavimo reikšmės kasdien buvo siunčiamos slaugytojai trumpąja žinute arba pranešamos telefonu.

Svorio matavimas

Paciento buvo prašoma svertis tomis pačiomis svarstyklėmis kasdien ryte, prieš pusryčius, nusišlapinus, dėvint lengvus drabužius, be avalynės, ir užrašyti rezultatą į dienyną.

Fizinio aktyvumo registravimas

„ActiGraph“ prietaisas buvo fiksuojamas diržu ant paciento liemens. Jis buvo prašomas nepertraukiamai 24 val. per parą 30 dienų nešioti prietaisą (taip pat maudantis duše). „ActiGraph“ automatiškai registravo fizinio aktyvumo parametrus, miego ir budrumo laiką.

2.3.2 Galimybių studijos duomenų analizė ir statistiniai metodai

Plaučių impedanso matavimų analizė

Analizuojant PI apskaičiuota vidutinė trijų kasdien kartojamų matavimų reikšmė. Matavimas buvo laikomas netiksliu ir neįtraukiamas į analizę tuo atveju, jeigu skirtumas tarp trijų matavimų buvo didesnis nei 3 Ω. PI mažėjimas buvo interpretuojamas kaip skysčio kiekio plaučiuose didėjimas [25].

Bazinis PI (BPI) buvo nustatomas naudojant anksčiau publikuotą algoritmą [24], kuris apskaičiuojamas atsižvelgiant į paciento amžių, lytį, svorį ir NYHA funkcinę klasę. BPI atitinka visiškai kompensuotą būklę, t. y. „sausų plaučių“ varžą.

Plaučių impedanso dinamika buvo įvertinta dviem būdais:

1. ΔPIB – plaučių impedanso procentinis pokytis nuo bazinės PI reikšmės: ΔPIB = [(Momentinis PI / Bazinis PI) – 1] × 100 proc.
2. ΔPIP – plaučių impedanso procentinis pokytis nuo pradinės PI reikšmės.

Fizinio aktyvumo parametrų analizė

Fizinio aktyvumo duomenys apdoroti „ActiLife“ programine įranga, suskirstyti 60 sekundžių epochomis, išreikšti judesio vienetais per minutę. Fizinis aktyvumas buvo vertintas dviem būdais: pagal laiko trukmę tokių skirtingų fizinio aktyvumo lygių kaip sėdimasis (SED), lengvas fizinis aktyvumas (LFA), vidutinis ir didelis fizinis aktyvumas (VDFA), išreikštas minutėmis per dieną, arba žingsniais per dieną.

Galimybių studijos aspektų analizė

Pagrindiniai nagrinėti galimybių studijos aspektai, iškelti klausimai ir vertinamieji parametrai pateikiami 1 lentelėje. Įgyvendinamumo vertinimas buvo pagrįstas atliktų per 30 dienų 10 ligonių matavimų procentais nuo visų galimų matavimų minėtu laikotarpiu (kitaip, per 300 paciento dienų).

1 LENTELĖ. Galimybių studijų pagrindiniai aspektai ir jų atskleidimo priemonės

Pagrindiniai aspektai	Tyrimo klausimai	Vertinamieji rodikliai
Įgyvendinamumas	Paciento gebėjimas atlikti ir registruoti multimodalinio monitoringo duomenis	Atliekamų matavimų procentas nuo visų galimų matavimų stebėjimo laikotarpiu
Variabilumas	Išilginiai svorio, PI ir fizinio aktyvumo laiko svyravimai	PI, svorio ir fizinio aktyvumo ispersija
Patikimumas	PI matavimų atkartojamumas	Skirtumas tarp trijų dienos matavimų

Pagrindiniai aspektai	Tyrimo klausimai	Vertinamieji rodikliai
Praktiškumas	Koreliacija tarp PI, svorio ir fizinio aktyvumo parametrų Saugumas Monitoringo poveikis tiriamiesiems	Kryžminė PI, svorio kitimo ir fizinio aktyvumo koreliacija Nepageidaujami įvykiai Savistaba, žinios apie stebimus rodiklius
Jautrumas	PI pokytis priklausomai nuo vaistų vartojimo Kliniškai reikšmingų nuokrypių dažnis	Reakcija į diuretiko vartojimą ir jo dozę Didžiausi monitoringo rodiklių pokyčiai
Klinikinė nauda	Įtaka gydymo korekcijai	Stimulų gydymo korekcijai dažnis

Galimybių studijos statistiniai metodai

Poriniam kiekybinių kintamųjų palyginimui naudotas Vilkoksono ranginis ženklų kriterijus, poriniam kategorinių kintamųjų palyginimui – Maknemaro kriterijus. Parametrų (svorio, sėdimojo ir lengvo fizinio aktyvumo, žingsnių) laikinis pokytis pateiktas procentine išraiška nuo pradinės (pirmos stebėtos) reikšmės. PI pokytis pateiktas procentine išraiška nuo jo bazinės reikšmės (ΔPIB) arba pradinės (išrašymo) reikšmės (ΔPIP). Įvertinta parametrų dispersija laiko atžvilgiu. Maksimalus periodas buvo imtas 20 dienų. Naudojantis R paketu „t-series“ tarp PI ir kitų veiksnių laiko atžvilgiu skaičiuota kryžminė koreliacija (angl. *cross-correlation*). Kryžminė koreliacija įvertinta analizuojant PI ir kitų veiksnių skirtingus laiko poslinkius.

3. Rezultatai

3.1 IN TOUCH tyrimo *post-hoc* analizės rezultatai

Į analizę įtraukti 118 pacientų, užpildžiusių savirūpos, gyvenimo kokybės bei nerimo ir depresijos klausimynus, duomenys. Atsitiktiniu būdu 60 pacientų pateko į IKT valdomą LPS, o 58 – į TM + IKT valdomą LPS grupes. Lyginant su visų 177-ių IN TOUCH tiriamųjų charakteristikomis, 118 pacientų grupė buvo panašaus amžiaus, joje buvo panašus skaičius vyrų (70 proc. lyginant su 72 proc.), NT-proBNP lygis reikšmingai nesiskyrė (P = 0,749).

3.1.1 Tiriamųjų charakteristikos

Pacientų amžiaus vidurkis buvo 69 ± 11,5 metai, 70 proc. sudarė vyrai, 81 proc. priskirti NYHA III/IV funkcinei klasei, KSIF vidurkis buvo 27 ± 9,9 proc. (2 lentelė).

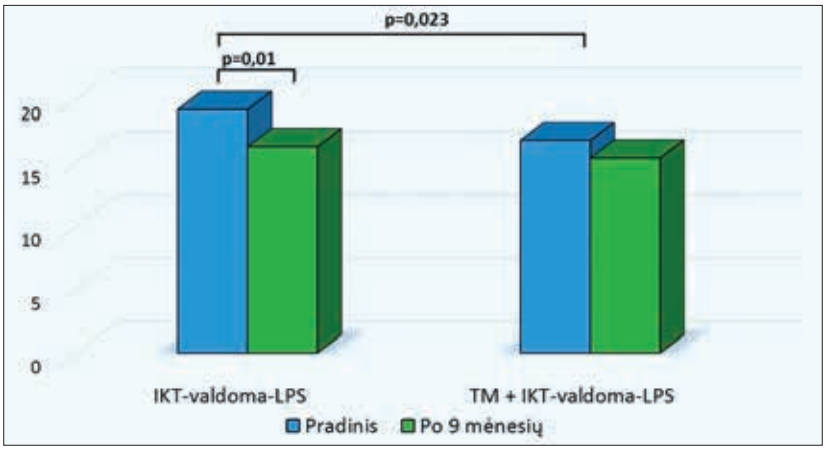
3.1.2 Telemonitoringo įtaka savirūpai

Nors tiriamųjų paskirstymas į studijos grupes buvo atsitiktinis, mes aptikome skirtingą pradinį savirūpos lygį grupėse: TM + IKT valdomoje LPS grupėje EŠNSEs balas buvo reikšmingai mažesnis (16,3 ± 7,5, kas reiškė geresnę savirūpą) nei IKT valdomos LPS grupės (19,2 ± 7,7; blogesnė savirūpa, *P*=0,023). Tyrimo metu savirūpa reikšmingai pagerėjo IKT valdomoje LPS grupėje (nuo 19,2 ± 7,7 iki 16,8 ± 6,9; *P*<0,01), bet ne TM + IKT valdomoje LPS grupėje (nuo 16,3 ± 7,5 iki 15,4 ± 6,7; *P*=0,77) (2 paveikslas). Tyrimo pabaigoje savirūpos balas dviejose grupėse nesiskyrė (2 paveikslas).

2 LENTELĖ. Pradinės IN TOUCH pacientų, įtrauktų į post-hoc analizę, charakteristikos

	IKT valdoma LPS, n=60	TM + IKT valdoma LPS, n=58	*P* reikšmė
Demografiniai duomenys			
Amžius (metai)	69,5 ± 10,4	68,5 ± 12,7	0,94
Vyrai	46 (76,7 proc.)	37 (63,8 proc.)	0,126
NYHA klasės			
II	15 (25,4 proc.)	16 (28,1 proc.)	0,862
III	36 (61,0 proc.)	35 (61,4 proc.)	
IV	8 (13,6 proc.)	6 (10,5 proc.)	
KSIF proc.	28,4 ± 9,1	25,9 ± 10,6	0,155
Laboratoriniai tyrimai			
NT-proBNP (ng/l) mediana (IKR)	824 (268; 1596)	1862 (553; 18224)	0,268
Klausimynai			
MGŠNK_Bendras	46,3 ± 25,1	47,2 ± 20,6	0,840
MGŠNK_Fizinis	23,8 ± 11,9	23,7 ± 10,5	0,899
MGŠNK_Emocinis	8,1 ± 7,1	8,4 ± 6,0	0,553
LNDs_Depresija	5,8 ± 4,3	5,8 ± 3,9	0,886
LNDs_Nerimas	6,0 ± 4,6	5,1 ± 4,2	0,358

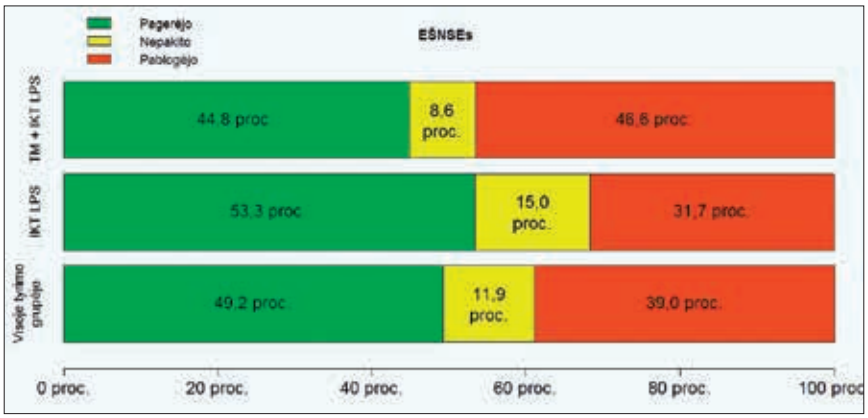
Rezultatai pateikti kaip vidurkiai ir standartiniai nuokrypiai arba dažniai, išreikšti absoliučia reikšme ir procentais.
Santrumpos: IKT valdoma LPS – informacinių ir kompiuterinių technologijų valdoma ligos priežiūros sistema; TM + IKT valdoma LPS – telemonitoringas su informacinių ir kompiuterinių technologijų valdoma ligos priežiūros sistema; KSIF – kairiojo skilvelio išstūmio frakcija; NYHA – Niujorko širdies asociacija; NT-proBNP – N-terminalinis B tipo natriurezinis peptidas; MGŠNK – Minesotos gyvenimo esant širdies nepakankamumui klausimynas; LNDs – ligoninės nerimo ir depresijos skalė; IKR – interkvartilinės ribos.



2 PAVEIKSLAS. EŠNSEs balas tyrimo pradžioje ir po 9 mėnesių dvejose priežiūros grupėse

3.1.3 Savirūpos dinamika ir veiksniai, susiję su savirūpos blogėjimu

Apibendrinus visų tiriamųjų duomenis nustatyta, kad tik 12 proc. tyrime dalyvavusių pacientų savirūpos lygis išliko stabilus, 49 proc. pacientų – pagerėjo, o 39 proc. – pablogėjo. Tolesnei analizei geros / vidutinės pradinės ir nepakitusios

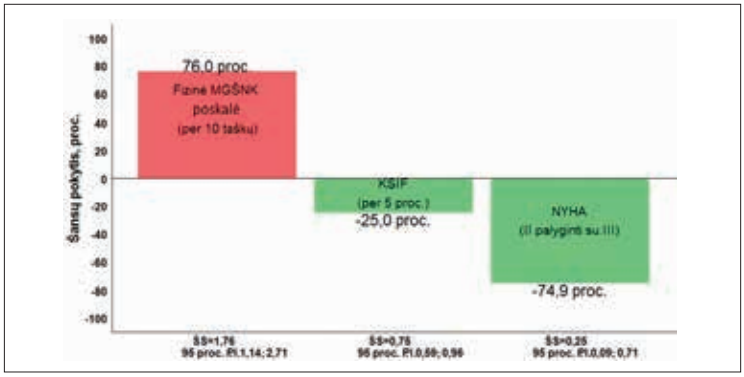


3 PAVEIKSLAS. Savirūpos dinamika (žalia spalva – pagerėjo, geltona – nepakito, raudona – pablogėjo) dviejose grupėse ir visoje tyrimo populiacijoje

Santrumpos: IKT LPS – informacinių ir kompiuterinių technologijų valdomos ligos priežiūros sistemos grupė; TM + IKT LPS – telemonitoringo su informacinių ir kompiuterinių technologijų valdoma ligos priežiūros sistema grupė; EŠNSEs – Europos širdies nepakankamumo savirūpos elgesio skalė.

savirūpos pacientai (jų balas 15,6 ± 7,8) buvo sujungti su pacientais, kurių savirūpa tyrimo metu pagerėjo. Savirūpos pokyčio pasiskirstymas abiejose tyrimo grupėse (*P*=0,210 tarp grupių) ir visoje tyrimo populiacijoje vaizduojamas 3 paveiksle.

Pritaikę logistinės regresijos analizę radome, kad savirūpos elgesio blogėjimui darė įtaką aukštesnis pradinis MGŠNK fizinės poskalės balas, t. y. blogesnis fizinis gyvenimo kokybės aspektas (ŠS 1,8 per 10 poskalės taškų, 95 proc. P.I. 1,14; 2,71; *P*<0,05), mažesnė KSIF (ŠS 0,75 per 5 proc., 95 proc. P.I. 0,59; 0,96; *P*<0,05) ir žemesnė NYHA funkcinė klasė (ŠS 0,3 II palyginti su III klase; 95 proc. P.I. 0,09; 0,71; *P*<0,05) (4 paveikslas).



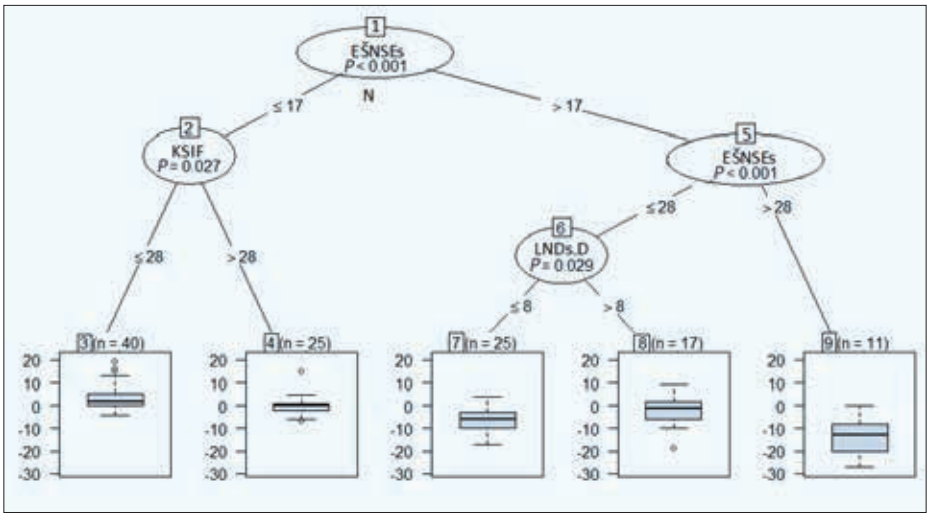
4 PAVEIKSLAS. Veiksniai, susiję su savirūpos pablogėjimu

Santrumpos: MGŠNK – Minesotos gyvenimo esant širdies nepakankamumui klausimynas; KSIF – kairiojo skilvelio išstūmio frakcija, NYHA – Niujorko širdies asociacija.

3.1.4 Pagerėjusios savirūpos pacientų profilis

Regresijos medžio analizė parodė, kad savirūpa labiausiai pagerėjo pacientams, turintiems žemą pradinį savirūpos lygį (EŠNSEs balas >28, blokas 9 paveiksle 5, neigiamas pokytis rodo savirūpos gerėjimą) arba vidutinį pradinį savirūpos lygį (balai nuo 17 iki 28), kai kartu nėra depresijos (LNDs_D ≤ 8, 7 blokas 5 paveiksle). Stebėtina, kad tarp pacientų, turinčių aukštą pradinį savirūpos lygį (EŠNSEs balas ≤17) buvo tokių (3 blokas 5 paveiksle), kurie net ją blogino, jeigu jų KSIF buvo ≤28 proc. Nei TM, Nei NT-proBNP neturėjo įtakos savirūpos pokyčiui.

Depresijos įtaka (LNDs_D>8) buvo papildomai įvertinta pogrupiuose su skirtingais pradiniais savirūpos lygiais. Ji buvo reikšmingai susijusi su savirūpos blogėjimu ne visoje tiriamųjų grupėje, o pogrupyje, kuriame pacientai turėjo vidutinį pradinį savirūpos lygį (EŠNSEs balas nuo 17 iki 28). Šioje grupėje depresija didino šansus bloginti savirūpą 10,2 kartų (95 proc. P.I. 1,8; 57,7) (3 lentelė).



5 PAVEIKSLAS. Regresijos medis, identifikuojantis savirūpą gerinančių pacientų profilį

Apatiniuose blokuose centrinė stačiakampio diagramos linija vaizduoja medianą, o stačiakampio kraštinės – 25 ir 75 procentilius (arba 1 ir 3 kvartilius). Stačiakampio aukštis lygus tarpkvartiliniam pločiui (K3K1). Ūsai parodo žemiausią tašką tarp 1,5 tarpkvartilinio pločio žemiau apatinio kvartilio [K11,5*IKR; K1] ir aukščiausią tašką tarp 1,5 tarpkvartilinio pločio aukščiau viršutinio kvartilio, taškai – ekstremalias reikšmes.

Įvesties kintamieji: intervencijos grupė, lytis, kairiojo skilvelio išstūmio frakcija, Minesotos gyvenimo esant širdies nepakankamumui bendras, fizinis, emocinis aspektai, ligoninės nerimo ir depresijos skalės depresijos ir nerimo poskalių balai; funkcinė klasė pagal Niujorko širdies asociacijos klasifikaciją; Nterminalinio B tipo natriurezinio peptido koncentracija.
Santrumpos: EŠNSEs – Europos širdies nepakankamumo savirūpos elgesio skalė; KSIF – kairiojo skilvelio išstūmio frakcija; LNDs_D – ligoninės nerimo ir depresijos skalės depresijos poskalė.

3 LENTELĖ. Depresijos ryšys su savirūpos blogėjimu

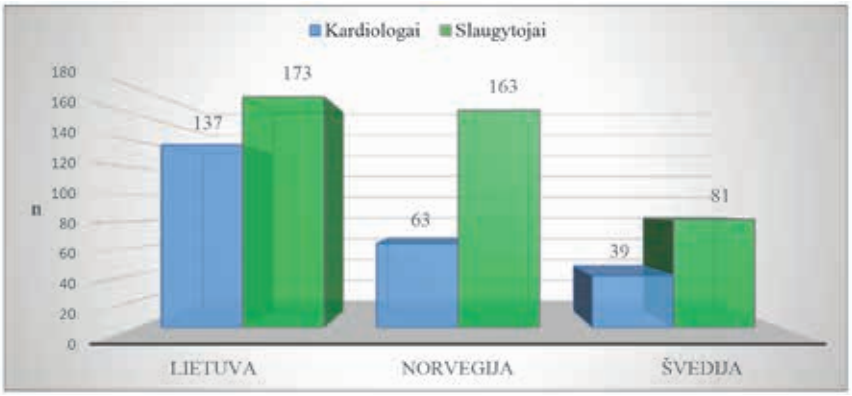
Pradinis savirūpos lygis	Savirūpos blogėjimo šansai			
	Šansų santykis	95 proc. P.I.		*P*
		Apatinis kvartilis	Viršutinis kvartilis	
EŠNSEs_bendras >28 (n=11)	1,9	0,8	4,3	0,137
EŠNSEs ≤ 17 (n=65)	1,4	0,4	4,8	0,619
17 < EŠNSEs ≤ 28 (n=42)	10,2	1,8	57,7	0,008

Santrumpos: EŠNSEs – Europos širdies nepakankamumo savirūpos elgesio skalė, P.I. – pasikliautinasis intervalas.

3.2 Sveikatos priežiūros specialistų apklausos dėl telemonitoringo rezultatai

3.2.1 Apklausos dalyvių charakteristikos

Sulaukėme 68 proc., 69 proc. ir 54 proc. atsakymų į išsiųstus klausimynus atitinkamai iš 47 Lietuvos, 53 Norvegijos ir 61 Švedijos sveikatos priežiūros įstaigų. Dalyvavusių apklausoje specialistų skaičius pateikiamas 6 paveiksle.



6 PAVEIKSLAS. Kardiologai ir slaugytojai, dalyvavę apklausoje trijose šalyse

Trijų šalių respondentai skyrėsi pagal amžių, lytį, darbo stažą, išsilavinimą ir dirbančių universiteto ligoninėje skaičių. Lietuvos kardiologai ir slaugytojai turėjo trumpesnę darbo su kompiuteriu ir kompiuterinėmis programomis patirtį. Daugiau Norvegijos nei Lietuvos ar Švedijos slaugytojų naudojosi internetu savo mobiliajame telefone. Nė vienoje šalyje nebuvo nustatyta skirtumo tarp kardiologų ir slaugytojų naudojimosi internetu ar elektroniniu paštu, taip pat darbo su kompiuteriu bei operacinėmis sistemomis (4 lentelė).

4 LENTELĖ. Sveikatos priežiūros specialistų charakteristikos

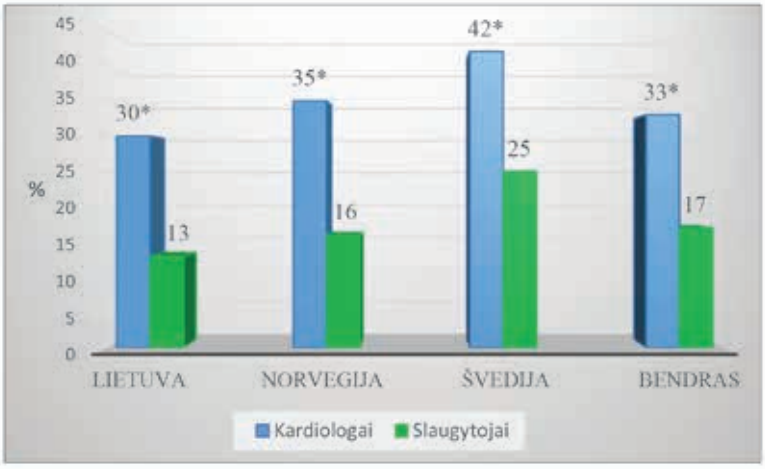
	Lietuva		Norvegija		Švedija	
	Slaugytojai n=173	Kardiologai n=135	Slaugytojai n=157	Kardiologai n=62	Slaugytojai n=81	Kardiologai n=39
Amžius, metai	46,2 ± 9,1	51,2 ± 12,4	44,9 ± 11,0#	48,1 ± 11,5	50,8 ± 8,9&	48,8 ± 8,9
Vyrai, n (proc.)	2 (1,2)*	30 (22)*	10 (6.8)	46 (74,6)	6 (7,5)&	22 (56,4)&
Darbo stažas, metai	24,8 ± 10,0*	24,9 ± 13,0*	17,6 ± 10,2	20,0 ± 11,1	14,9 ± 9,6&	16,5 ± 9,3&
Dirbantys universiteto ligoninėje, n (proc.)	88 (50,9)*	47 (34,3)	25 (15,3)	11 (17,5)	4 (5,2)&	5 (12,8)&

	Lietuva		Norvegija		Švedija	
	Slaugytojai n=173	Kardiologai n=135	Slaugytojai n=157	Kardiologai n=62	Slaugytojai n=81	Kardiologai n=39
Išsilavinimas:						
Mokslų dr., n (proc.)	0 (0)	36 (27)	0 (0)	19 (32,2)#	2 (2,5)	26 (68,4)&
Magistrai, n (proc.)	14 (8,1)	99 (73,3)*	11 (7,1)	12 (20,7)	3 (3,8)	2 (5,3)&
Bakalaurai, n (proc.)	64 (37,0)*	0 (0)	148 (91,9)#	0 (0)	24 (30,4)	0 (0)
Darbo su kompiuteriu patirtis, metai	13,1 ± 5,6*	14,7 ± 6,5*	20,3 ± 5,4#	25,6 ± 6,0#	17,9 ± 5,2&	19,4 ± 5,1&
Dirbantys su kompiuterinėmis programomis, n (proc.)	124 (71,7)*	107 (79,3)*	148 (94,3)	60 (96,8)	73 (90,1)&	38 (97,4)&
Naudojantys el. paštą mob. tel., n (proc.)	118 (68,2)*	106 (78,5)	149 (94,9)#	56 (90,3)	49 (60,5)	29 (74,4)

3.2.2 Specialistų žinios, patirtis ir nuomonė, susijusios su telemonitoringu

3.2.2.1 Žinios apie telemonitoringą

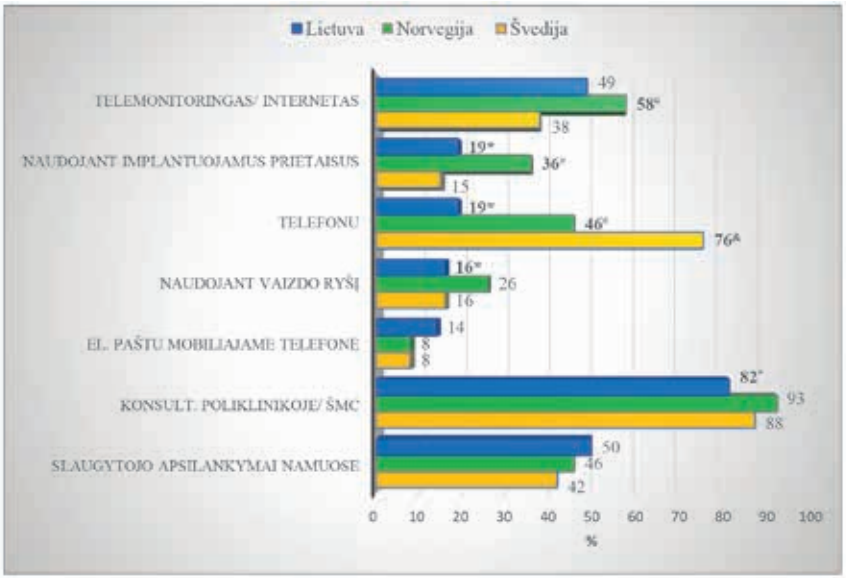
Nė vienoje iš trijų šalių (161 įstaiga) TM nebuvo naudojamas kasdienėje klinikinėje praktikoje ŠN pacientams. Iš viso 23 proc. SPS (20 proc. Lietuvoje, 21 proc. Norvegijoje ir 30 proc. Švedijoje) buvo susipažinę su ŠN pacientų TM (7 paveikslas).



7 PAVEIKSLAS. Sveikatos priežiūros specialistai, susipažinę su TM.
* Statistiškai reikšmingas skirtumas tarp kardiologų ir slaugytojų (P<0,05)

3.2.2.2 Priimtini stabilių ŠN pacientų stebėsenos būdai

Visose trijose šalyse nuotolinė stebėsena (naudojant internetinį monitoringą) buvo pasirinkta kaip tinkamas būdas stabiliems ŠN pacientams stebėti (8 paveikslas).



8 PAVEIKSLAS. Tinkami stabilių ŠN pacientų stebėsenos būdai, pasirinkti Lietuvos (n=310), Norvegijos (n=226) ir Švedijos (n=120) SPS

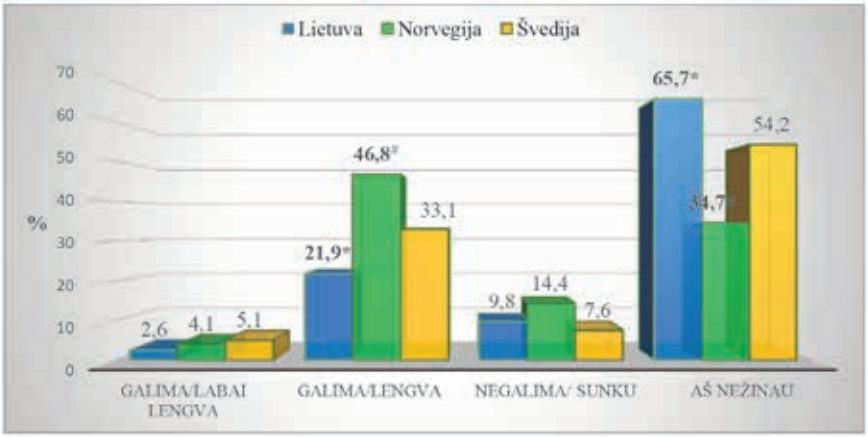
Statistiškai reikšmingas skirtumas tarp šalių (*P* <0,05): * – Lietuva lyginant su Norvegija; # – Norvegija lyginant su Švedija; & – Švedija lyginant su Lietuva.

Santrumpos: ŠMC – šeimos medicinos centras.

3.2.2.3 Galimybė kasdien reaguoti į telemonitoringo duomenis

Norvegijos SPS daug dažniau nei Lietuvos ar Švedijos pažymėjo, kad kasdienis reagavimas į TM yra galimas / lengvas (9 paveikslas).

Tik apie 16 proc. slaugytojų Lietuvoje ir 29 proc. Švedijoje, palyginti su dideliu Norvegijos slaugytojų skaičiumi (50 proc.), buvo tos nuomonės, kad kasdienis reagavimas į TM duomenis yra galimas / lengvas.



9 PAVEIKSLAS. Galimybė kasdien reaguoti į telemonitoringą

Statistiškai reikšmingas skirtumas tarp šalių (*P* <0,05): * – Lietuva lyginant su Norvegija; # – Norvegija lyginant su Švedija; & – Švedija lyginant su Lietuva.

3.2.2.4 Pacientų atrankos nuotolinei stebėsenai kriterijai

Trijų šalių kardiologų ir slaugytojų pasirenkamų kriterijų TM dažniai pateikiami 5 lentelėje.

5 LENTELĖ. Kriterijai, laikomi naudingais pasirenkant pacientus nuotolinei stebėsenai

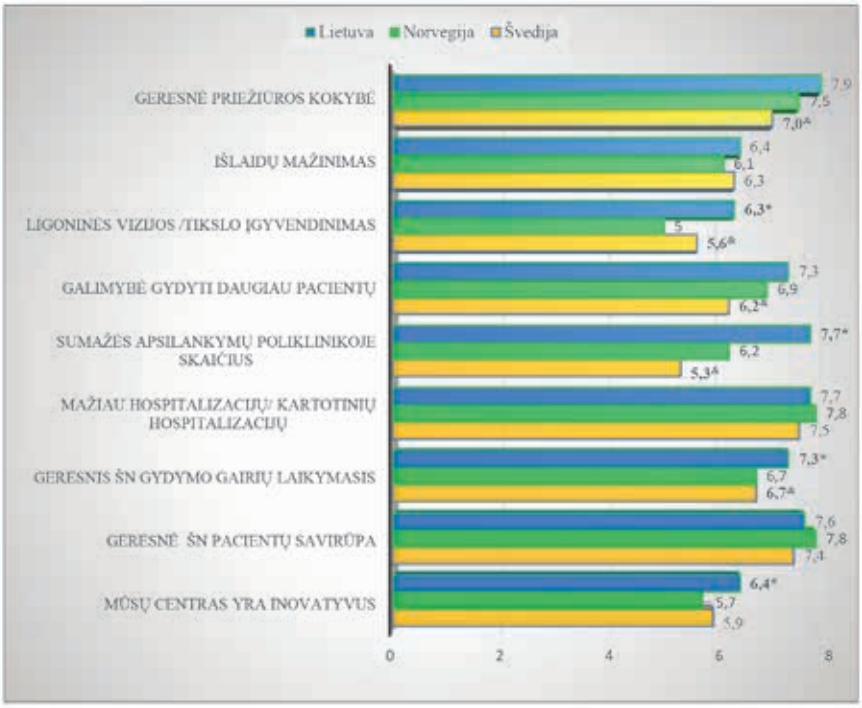
	Lietuva		Norvegija		Švedija	
	Slaugytojai n=173	Kardiologai n=137	Slaugytojai n=163	Kardiologai n=63	Slaugytojai n=81	Kardiologai n=39
Dažnai hospitalizuojami, n (proc.)	111 (64,2)§	106 (77,4)*	98 (60,1)	38 (60,3)#	53 (67,1)§	31 (86,1)
Aukštesnė NYHA klasė, n (proc.)	79 (45,7)§	112 (81,8)*	78 (47,9)	34 (54,0)	48 (60,8)	19 (52,8)&
Medikamentinio gydymo nesilaikymas, n (proc.)	88 (50,9)	81 (59,1)	69 (42,3)	29 (46,0)	43 (54,4)	21 (58,3)
Reikalingas paciento mokymas, n (proc.)	80 (46,2)	65 (47,4)	88 (54,0)	28 (44,4)	30 (38,0)	11 (30,6)
Reikalinga parama ir patarimai, n (proc.)	42 (24,3)*	26 (19,0)*	71 (43,6)	22 (34,9)	37 (47,4)&	14 (38,9)&

Statistiškai reikšmingas skirtumas tarp šalių (P <0,05): * – Lietuva lyginant su Norvegija; # – Norvegija lyginant su Švedija; & – Švedija lyginant su Lietuva; § – slaugytojai lyginant su kardiologais.

3.2.3 Sveikatos priežiūros specialistų lūkesčiai, susiję su telemonitoringu

3.2.3.1 Priežastys diegti ŠN pacientų telemonitoringą

Pasirinktos keturios svarbiausios TM diegimo ŠN pacientams priežastys: „Mažiau hospitalizacijų / kartotinių hospitalizacijų“, „Geresnė ŠN pacientų savirūpa“, „Geresnė priežiūros kokybė“ ir „Sumažėjęs apsilankymų poliklinikoje skaičius“ (10 paveikslas).



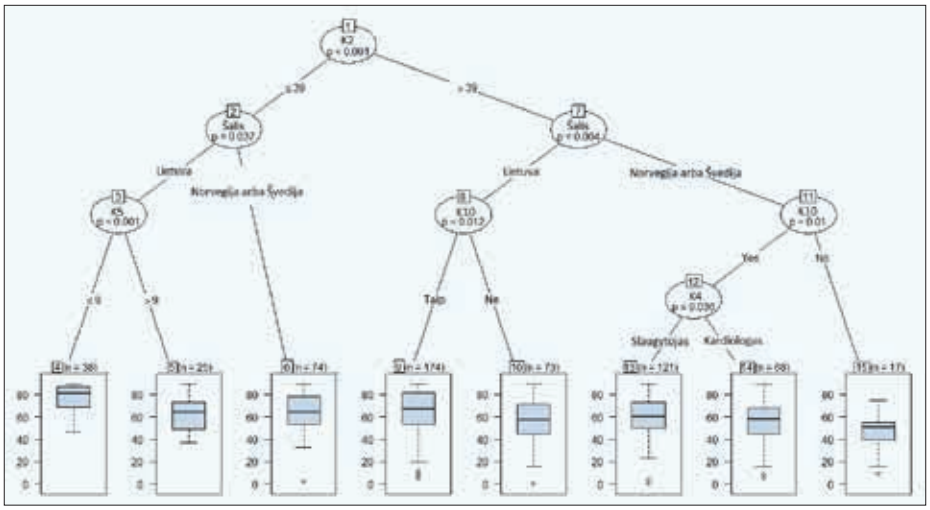
10 PAVEIKSLAS. TM diegimo priežastys, nurodytos Lietuvos (n=310), Norvegijos (n=226) ir Švedijos (n=120) sveikatos priežiūros specialistų

Statistiškai reikšmingas skirtumas tarp šalių (*P* <0,05): * – Lietuva lyginant su Norvegija; # – Norvegija lyginant su Švedija; & – Švedija lyginant su Lietuva.

3.2.3.2 Specialistų, turinčių didžiausius telemonitoringo lūkesčius, charakteristikos

TM diegimo lūkesčiai buvo vertinami skalės balais nuo 0 iki 10, pažymėtais renkantis TM priežastis; aukštesni balai atitiko didžiausius lūkesčius (4, 5, 6, 9 blokai 11 paveiksle). Regresijos medžio analizė (11 paveikslas) parodė, kad didžiausius lūkesčius turėjo jaunesni (iki 39 metų) specialistai, o tarp jų – ypač Lietuvos kardio-

logai ir slaugytojai, turintys ne ilgesnį nei 9 metų darbo stažą. Vyresnių Lietuvos SPS grupėje didelius lūkesčius išreiškė respondentai, turėję didesnę darbo su kompiuterinėmis programomis patirtį. Norvegijos ir Švedijos slaugytojos palankiau vertino TM diegimą nei šių šalių gydytojai.



11 PAVEIKSLAS. Regresijos medis, rodantis didžiausius TM lūkesčius turinčių SPS charakteristikas

Apatiniuose blokuose centrinė stačiakampio diagramos linija vaizduoja medianą, o stačiakampio kraštinės – 25 ir 75 procentilius (arba 1 ir 3 kvartilius). Stačiakampio aukštis lygus tarpkvartiliniam pločiui (K3–K1). Ūsai parodo žemiausią tašką tarp 1,5 tarpkvartilinio pločio žemiau apatinio kvartilio [K1–1,5*IKR; K1] ir aukščiausią tašką tarp 1,5 tarpkvartilinio pločio aukščiau viršutinio kvartilio, taškai – ekstremalias reikšmes.

Įvesties kintamieji: Šalis; K1 – klausimas apie lytį, K2 – klausimas apie amžių; K4 – klausimas apie specialybę (kardiologas / slaugytojas); K5 – klausimas apie darbo stažą; K10 – klausimas, ar teko dirbti su programomis „Word“, „PowerPoint“, „Excel“; K15 – klausimas „Ar žinote apie ŠN pacientų nuotolinę stebėseną?“

3.2.4 Telemonitoringo diegimo kliūtys ir poreikiai

Švedijos SPS manė, kad didžiausios TM diegimo kliūtys yra netinkamas finansavimas ir prioritetų nustatymas (35 proc.), medicinos personalo ir laiko trūkumas (32 proc.) bei skepticizmas dėl telemonitoringo / informacinių technologijų efektyvumo (15 proc.). Atsakymą „Medicinos personalo ir laiko trūkumas“ taip pat rinkosi Lietuvos ir Norvegijos apklausos dalyviai. Lietuvos ir Norvegijos kardiologai ir slaugytojai skirtingai nustatė prioritetus renkantis poreikius diegti TM. Finansavimo (90 proc. vs 69 proc., p<0,001), prietaisų (81 proc. *vs* 55 proc., p<0,001), žinių (73 proc. *vs* 39 proc., p<0,001) ar gairių (61 proc. *vs* 17 proc.; p<0,0001) trūkumas buvo aktualesni Lietuvos SPS nei Norvegijos (šio klausimo nebuvo Švedijos klausi-

myne). Taip pat tokios fizinės ir psichinės būklės kaip vyresnis amžius, daugybinės gretutinės ligos, demencija, psichikos sutrikimai, neraštingumas, technologijų žinių trūkumas buvo pažymėti kaip veiksniai, ribojantys nuotolinės stebėsenos taikymą. Be to, Lietuvos SPS papildomai nurodė nepakankamą interneto paplitimą, o Švedijos – didelius atstumus ir prastą mobilųjį ryšį kaimo vietovėse kaip galimas kliūtis diegti TM.

3.3 Multimodalinio monitoringo namuose galimybių tyrimo rezultatai

3.3.1 Tiriamųjų populiacijos apibūdinimas

Nuo 2017 m. gegužės iki spalio mėnesio buvo įtraukta 10 pacientų iš dviejų šalių (Lietuvoje n=5 ir Norvegijoje n=5). Vidutinis pacientų amžius buvo 65 metai, 90 proc. pacientų buvo vyrai. Visų pacientų KSIF buvo sumažėjusi (<40 proc.). Tiriamųjų demografinės charakteristikos, klinikiniai rodikliai ir vartojami vaistai pateikiami 6 ir 7 lentelėse.

6 LENTELĖ. Tiriamųjų demografinės charakteristikos ir anamnezė

Demografiniai ir antropometriniai duomenys	n=10
Lytis, vyras / moteris, n	9/1
Amžius, metai, mediana (IKR)	65 (50; 83)
Svoris, kg, mediana (IKR)	90 (76; 106)
Ūgis, cm, mediana (IKR)	179 (169; 182)
Šeiminė padėtis	
Vienišas, n	3
Implantuojami prietaisai: IKD, ŠRG, ŠRG-D, n	6
Gretutinės ligos	
Lėtinė inkstų liga, n	2
Cukrinis diabetas, n	2
Miokardo infarktas, n	7
Periferinių kraujagyslių liga, n	4
Hipertenzija, n	6
Reumatinė liga, n	1
Skrandžio opaligė, n	4
Depresija, n	1
Bet kokia piktybinė liga, įskaitant ir leukemiją bei limfomą, n	1
Charlson gretutinių ligų indeksas, mediana (min.; maks.)	5 (2; 13)

Tolydūs kintamieji pateikti kaip mediana su IKR.
Santrumpos: IKR – interkvartilinės ribos; IKD – implantuojamasis kardioverteris defibriliatorius; ŠRG – širdies resinchronizuojamasis gydymas; ŠRG-D – širdies darbą resinchronizuojantis prietaisas defibriliatorius.

7 LENTELĖ. Tiriamųjų klinikiniai ir laboratoriniai tyrimai bei gydymas

Klinikiniai rodikliai ir vartojami vaistai	**Išrašant iš ligoninės** Mediana (IKR)	**Po 30 dienų** Mediana (IKR)
Apžiūra		
Sistolinis kraujo spaudimas, mmHg (n=9)	110 (94; 121)	122 (99; 139)
Diastolinis kraujo spaudimas, mmHg (n=9)	70 (67; 83)	80 (65; 87)
Širdies susitraukimo dažnis (n=9)	77 (64; 85)	73 (64; 90)
Periferinės edemos, n	5	2
Jungo venų išsiplėtimas, n	1	1
NYHA funkcinė klasė, n		
II	-	3
III	8	6
IV	2	1
Laboratoriniai tyrimai		
NT-proBNP, pg/ml	2440 (893; 3230)	2910 (1097; 3493)
Kreatininas, mkmol/l	104 (82; 128)	104 (89; 130)
Troponinas, ng/l	21 (12; 34)	18 (14; 22)
Hemoglobinas, g/dl	15 (14; 16)	15 (14; 16)
Natris, mmol/l	139 (137; 139)	140 (138; 141)
Šlapalas, mmol/l	10.3 (6,8; 16,4)	12.1 (7,8; 108,2)
ALAT, U/L (n=8)	37 (22; 48)	25 (17; 35)
Kalis, mmol/l	4.7 (4,3; 5,1)	4.5 (4,1; 4,8)
Gliukozė, mmol/l	6.2 (5,6; 8,1)	5.3 (5,1; 7,6)
Vaistai		
AKF inhibitoriai, n	8	8
ARB, n	1	2
Beta adrenoblokatoriai, n	9	9
I_f kanalų blokatoriai, n	1	2
Diuretikai, n	10	10
MRA, n	7	5
Digoksinas, n	4	3
Amiodaronas, n	4	4
Nitratai, n	1	1

Tolydūs kintamieji pateikti kaip mediana su IKR.

Santrumpos: IKR – interkvartilinės ribos; NT-proBNP – N-terminalinis pro-B tipo natriurezinis peptidas; AKF – angiotenziną konvertuojantis fermentas; ARB – angiotenzino II receptorių blokatorius; MRA – mineralokortikoidų antagonistai.

3.3.2 Pagrindiniai tiriamųjų duomenys ir multimodalinio monitoringo rodikliai

3.3.2.1 Plaučių impedanso matavimo vertės

Visų pacientų PI mediana buvo 81,6 Ω (IKR 60,0; 92,5), individualių pacientų matavimai pateikiami 9 lentelėje. PI rodiklių sumažėjimas vertintas kaip skysčių kaupimasis plaučiuose, ΔPIP sumažėjimas ≥ 10 proc. ir ΔPIB sumažėjimas ≥ 30 proc. buvo laikomi kliniškai reikšmingais remiantis publikuotais išilginio ambulatorinių pacientų stebėjimo tyrimais [24, 26]. Net penkių pacientų ΔPIB išrašymo iš ligoninės metu buvo sumažėjęs > 30 proc., kas rodo galbūt nepakankamą gydymo poveikį ir išliekančią stazę (8 lentelė). Matyti, kad nė vienas pacientas per 30 dienų nepasiekia bazinio impedanso (t. y. visiško stazės nebuvimo) reikšmės. Kai kurių pacientų plaučių stazė per pirmąjį mėnesį mažėjo (jų ΔPIP turėjo teigiamą ženklą), o kai kurių – didėjo (neigiamas ΔPIP ženklas).

8 LENTELĖ. Tiriamųjų PI vertės išrašant ir jų pokyčiai per 30 dienų

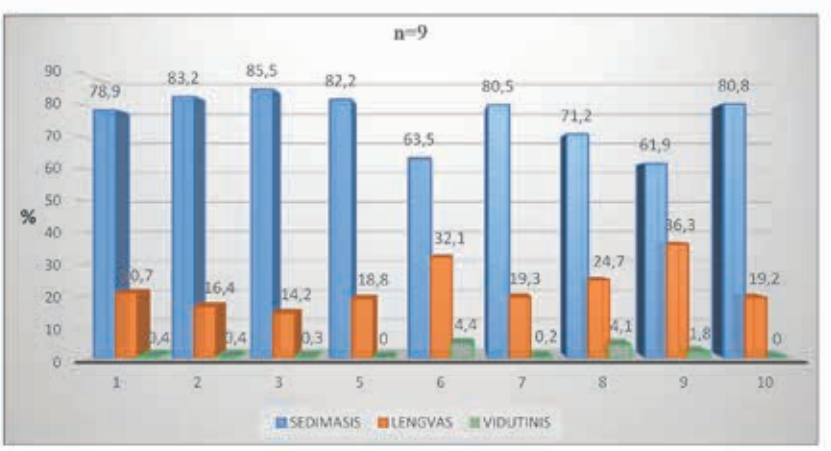
Paciento ID/ (dienų skaičius)	Plaučių impedansas (PI, Ω), 30 d. mediana (IKR)	Apskaičiuo- tas bazinis plaučių impedansas (BPI, Ω)	Pokytis nuo apskaičiuotos bazinės vertės (ΔPIB, proc.) išrašant iš ligoninės	Pokytis nuo pradinės vertės (ΔPIP, proc.), 30 d. mediana (min.; maks.)	Pokytis nuo apskaičiuotos bazinės vertės (ΔPIB, proc.), 30 d. mediana (min.; maks.)
P.1 (30 d.)	53,0 (50,6; 54,7)	63	32,4	24,4 (0; 35,2)	–15,9 (–32,4; –8,6)
P.2 (24 d.)	59,1 (57,3; 60,4)	94	–34,6	–4 (–17,2; 3,4)	–37,2 (–45,9; –33,7)
P.3 (17 d.)	90,1 (88,3; 91,2)	145	–36,9	–2,2 (–35,6; 7,7)	–37,8 (–59,4; –32,1)
P.4 (18 d.)	85,7 (74,3; 88,9)	132	–30,8	–7,2 (–23,2; 6,5)	–35,1 (–46,8; –26,3)
P.5 (30 d.)	80,6 (79,2; 81,7)	121	–37,7	7,1 (–2,2; 18,4)	–33,4 (–39,1; –26,3)
P.6 (30 d.)	59,4 (57,5; 63,4)	80	–25,6	–0,3 (–13,5; 14,1)	–25,6 (–35,6; –15,1)
P.7 (30 d.)	100,9 (97,3; 103,9)	138	–21,8	–6,5 (–17,6; 0)	–26,9 (–35,6; –21,8)
P.8 (30 d.)	79,5 (76,7; 83,3)	108	–26,4	–0,1 (–7,4; 14,2)	–26,5 (–31,9; –15,9)
P.9 (30 d.)	92,2 (88,2; 93,3)	112	–20,9	4,0 (–6,8; 10,4)	–17,8 (–26,3; –12,7)
P.10 (29 d.)	96,8 (89,8; 102,1)	140	–27,3	–4,9 (–14,5; 15,7)	–30,9 (–37,9; –15,9)
Iš viso	81,6 (60,0; 92,5)	116,5 (97,5; 136,5)	–29,1 (–34,1; –25,8)	–0,5 (–35,6; 35,2)	–27,6 (–59,4; –8,6)

Tolydūs kintamieji pateikti kaip mediana su IKR arba mediana su minimalia ir maksimalia reikšme.
Santrumpos: IKR – interkvartilinės ribos.

3.3.2.2 Kasdienio fizinio aktyvumo registravimas

Daugiausia laiko pacientai būdavo mažai judrūs (vidutiniškai beveik 16 val. per dieną registruotas sėdimasis aktyvumas). Net šeši iš devynių pacientų daugiau nei 80 proc. budrumo laiko praleisdavo mažai judėdami (12 paveikslas). Maždaug 5 val.

per dieną pacientai būdavę šiek tiek fiziškai aktyvūs (lengvas fizinis aktyvumas). Tik du pacientai buvo vidutiniškai aktyvūs – maždaug 4 proc. viso budrumo laiko.



12 PAVEIKSLAS. Pacientų fizinio aktyvumo pasiskirstymas pagal intensyvumo lygį n=9*, pašalinus vieną Norvegijos pacientą (nepakankamai nešiojo „ActiGraph“ prietaisą – 7,3 proc. viso nešiojimo laiko per mėn.).

3.3.2.3 Multimodalinio monitoringo rodiklių suvestinė

Tiriamųjų pagrindinių duomenų ir apibendrintų multimodalinio monitoringo rodiklių suvestinė pateikiama 9 lentelėje.

9 LENTELĖ. Pagrindiniai tiriamųjų duomenys ir apibendrinti multimodalinio monitoringo rodikliai

Paciento ID	Amžius (metai)	NT- proBNP išrašant (pg/ml)	Kreatininas išrašant (mkmol/l)	Svorio pokytis * nuo pradinio (proc.)	ΔPIB Mediana (min.; maks.) (proc.)	Žingsniai per dieną Mediana (min.; maks.)
P.1	85	2368	80	–3,23	–15,9 (–32,4; –8,6)	4006 (1724; 7517)
P.2	66	4592	91	2,88	–37,2 (–45,9; –33,7)	4304 (287; 6680)
P.3	64	803	70	0	–37,8 (–59,4; –32,1)	2093 (284; 4215)
P.4#	52	2512	118	0,96	–35,1 (–46,8; –26,3)	–
P.5	84	3146	136	–2,22	–33,4 (–39,1; –26,3)	1183 (390; 3025)
P.6	45	1076	82	0	–25,6 (–35,6; –15,1)	9550 (4634; 21439)
P.7	62	3485	96	0,96	–26,9 (–35,6; –21,8)	4100 (1827; 6747)
P.8	37	923	126	2.7	–26,5 (–31,9; –15,9)	8534 (944; 17506)
P.9	67	179	133	–0,25	–17,8 (–26,3; –12,7)	14199 (8591; 20150)
P.10#	83	2927	111	3,0	–30,9 (–37,9; –15,9)	1976 (321; 3120)
Iš viso	65 (37; 85)	2440 (179; 4592)	103,5 (70; 136)	0,48 (–3,23; 3,0)	–27,6 (–59,4; –8,6)	4119 (284; 21439)

* Svorio pokytis 30-ą stebėjimo dieną; # – pacientas buvo kartotinai hospitalizuotas mėnesio laikotarpiu.

Santrumpos: ΔPIB – PI procentinis pokytis nuo nustatytos bazinės vertės; NTproBNP – N-terminalinis pro-B tipo natriurezinis peptidas.

3.3.3 Multimodalinio monitravimo namuose įgyvendinamumas

Per monitoringo laikotarpį (iš viso buvo stebima 10 pacientų 30 dienų = 300 paciento dienų) PI duomenys buvo perduoti 268 iš 300 paciento dienų (89 proc.), o duomenys apie svorį – 265 iš 300 paciento dienų (88 proc.). Fizinio aktyvumo duomenys, matuojami akcelerometru, buvo registruoti 249 iš 300 paciento dienų (83 proc.). „ActiGraph" nešiojimo laiko mediana buvo beveik 22 val. per parą. PI rodikliai, svorio ir fizinio aktyvumo duomenys, registruoti dviejuose centruose, pateikiami 10 lentelėje. Vieno Norvegijos paciento fizinio aktyvumo duomenys neįtraukti į analizę, nes jis nepakankamai nešiojo „ActiGraph" prietaisą (7,3 proc. per mėn.).

10 LENTELĖ. Multimodalinio monitoringo namuose įvykdomumas

Monitoringo rodikliai	Įvykdyti matavimai ir perdavimai (n=10) dienomis Mediana (min.; maks.)	Įvykdyti matavimai ir perdavimai (n=10) procentais Mediana (min.; maks.)
PI matavimai	29,5 (17,0; 30,0)	98,3 (56,7; 100,0)
„ActiGraph" nešiojimas*	30 (16,0; 30,0)	100 (53,3; 100)
„ActiGraph" nešiojimas, val./dieną*	22,1 (13,7; 23,6)	92,2 (57,0; 98,3)
Svorio registravimas	30 (18,0; 30,0)	100,0 (60,0; 100,0)

SN – standartinis nuokrypis; PI – plaučių impedansas; * – pašalinus vieną Norvegijos pacientą.

3.3.4 Kasdienių matavimų išilginis variabilumas ir patikimumas

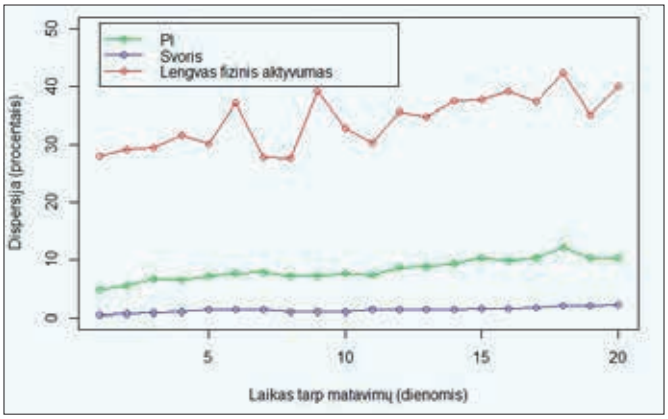
Individualioji pacientų plaučių impedanso, svorio ir lengvo fizinio aktyvumo dispersija (skirtumas tarp to paties paciento matavimų) buvo nagrinėjama laikotarpiais nuo 1 iki 20 dienų (11 lentelė, 13 paveikslas).

11 LENTELĖ. Plaučių impedanso, svorio ir lengvo fizinio aktyvumo dispersija

Periodas	Matavimų variabilumas (%)		
	Svoris	Plaučių impedansas	Lengvas fizinis aktyvumas
1 diena	0,36	4,8	28,3
10 dienų	1,0	7,7	37,6
20 dienų	2,2	12,2	45,9

Tyrimo metu iš viso gauti 804 pavieniai PI matavimai. Jie laikyti patikimais, jei skirtumas tarp trijų tos pačios dienos matavimų buvo mažesnis nei 3 omai. Šią sąlygą patenkino 263 iš 268-ių paciento dienų (98 proc.) gautų PI matavimų. Tik 5 iš 268-ių paciento dienų matavimai buvo netinkami galbūt dėl to, kad elektrodai buvo

uždėti nepakankamai glaudžiai arba ne toje pačioje vietoje. Aptikusi didelį skirtumą tarp gretimų dienų matavimų, vieną kartą slaugytoja apsilankė paciento namuose ir patikrino matavimo techniką, kuri pasirodė esanti taisyklinga.



13 PAVEIKSLAS. PI, svorio ir lengvo fizinio aktyvumo dispersija nuo 1 iki 20 dienų

3.3.5 Multimodalinio monitoringo namuose praktiškumas

3.3.5.1 Plaučių impedanso, svorio kitimo ir fizinio aktyvumo koreliacija

Kryžminė koreliacija tarp PI, svorio ir dviejų serijų fizinio aktyvumo matavimų (žingsnių skaičiaus ir aktyvumo lygio) tirta naudojant įvairius laiko tarpus. Vieno paciento duomenys nevertinti, nes per trumpai buvo dėvimas „ActiGraph“ prietaisas. Keturiems iš devynių pacientų nustatyta kliniškai reikšminga atvirkštinė svorio ir PI koreliacija (mažėjant PI, svoris didėjo). Dviem pacientams PI sumažėjo dieną prieš tai, kai padidėjo svoris – koreliacijos koeficientas –0,738 ($p<0,001$) ir –0,809 ($p<0,001$), trečiam pacientui PI sumažėjo trimis dienomis anksčiau (koeficientas -0,444, $p<0.015$). Ketvirtam pacientui PI ir svorio kryžminė koreliacija buvo reikšminga tą pačią dieną su koeficientu –0,830 ($p<0,001$). Kliniškai svarbių koreliacijų tarp PI ir fizinio aktyvumo rodiklių nenustatyta.

3.3.5.2 Monitoringo saugumas

Atliekant PI matavimus ar nešiojant akcelerometrą „ActiGraph“ nepageidaujamas poveikis nepasireiškė nė vienam iš tiriamųjų.

3.3.5.3 Monitoringo poveikis tiriamiesiems

Bendraujant su pacientais telefonu pastebėta, kad kasdienis PI matavimas ir svėrimasis gerino pacientų savistabą ir savireguliaciją. Tiriamieji atidžiai stebėjo matavimų pokyčius ir jų ryšį su simptomais (dusuliu) ir požymiais (kojų edema) bei

informuodavo apie juos tyrėją. Be to, pacientai ir jų artimieji domėjosi PI svyravimo ribomis, jų reikšme sveikatos būklei bei tinkamu reagavimu koreguojant gydymą (pvz., diuretikų dozių keitimu).

3.3.6 Monitoringo jautrumas klinikinės būklės pokyčiams

3.3.6.1 Kliniškai reikšmingi stebimų rodiklių pokyčiai

Kliniškai svarbus PI sumažėjimas ≥ 10 proc., palyginti su pradiniu, buvo išmatuojamas 10,4 proc. paciento dienų, o didesnis kaip 20 proc. – 1,9 proc. paciento dienų per 268 paciento dienas. Vienas pacientas, kuriam ΔPIP sumažėjimas buvo didesnis kaip 20 proc., hospitalizuotas dėl dekompensacijos. Kita pacientė buvo hospitalizuota dėl sutrikusios inkstų funkcijos ir elektrolitų disbalanso PI padidėjus 15 proc. nuo pradinio.

Svorio padidėjimas

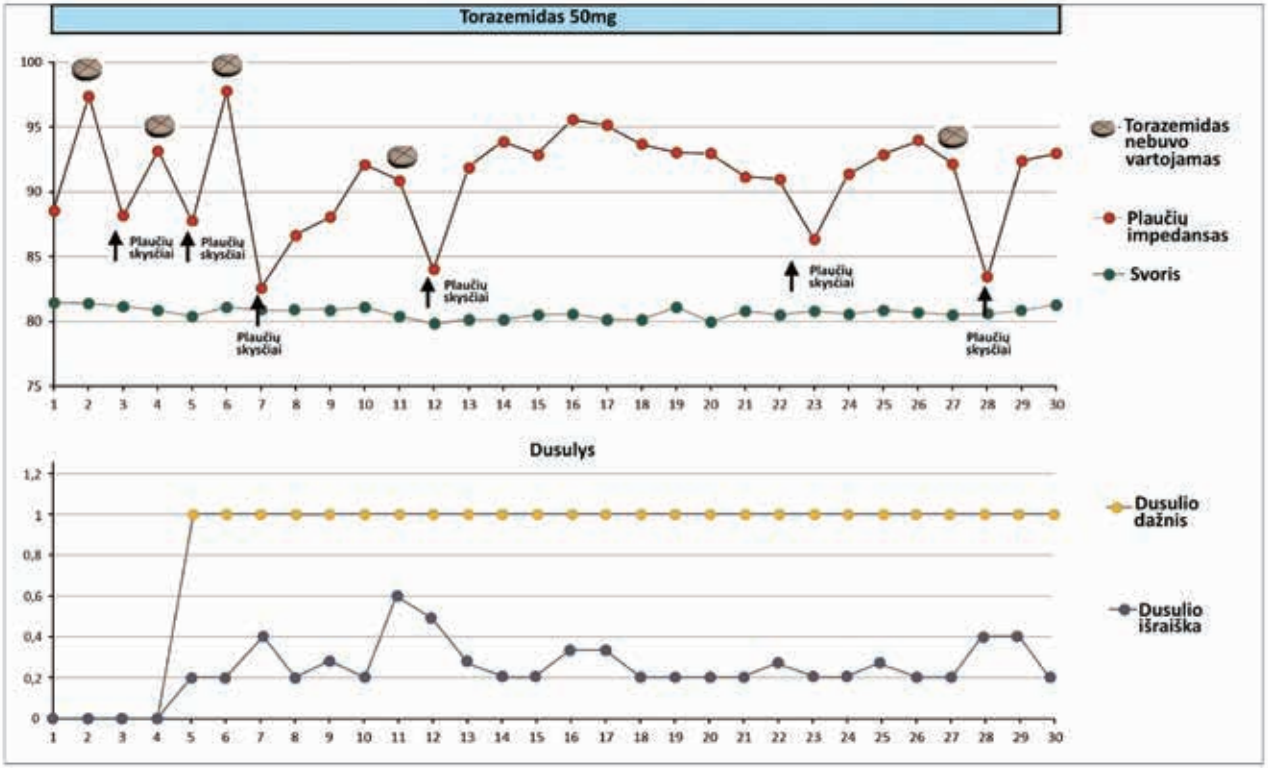
Stebėjimo metu svorio padidėjimas daugiau nei 2 kg po išrašymo per 3 dienas buvo pastebėtas 4,2 proc. paciento dienų (penkiems pacientams, 11 kartų). Beveik pusė (5 kartai iš 11) visų svorio padidėjimų įvyko pacientui, kuris buvo hospitalizuotas dėl dekompensacijos.

Fizinio aktyvumo pokyčiai

Didelis fizinio aktyvumo rodiklių kintamumas neleido pastebėti jų dėsningo ryšio su klinikine būkle mūsų imties ir trukmės tyrime.

3.3.6.2 PI ir svorio reakcija į diuretikų vartojimą – klinikinis atvejis

Vyras, 66 metų, sergantis išeminės kilmės ŠN, po šešių gydymo stacionare dienų išliko III NYHA funkcinės klasės. Išrašant iš ligoninės torazemidas buvo paskirtas gerti kas antrą dieną iki 7-os stebėjimo dienos. Kaip matyti iš 14 paveikslo, kitą dieną po diuretiko nevartojimo PI smarkiai krisdavo: PI vertė vidutiniškai sumažėdavo 9 punktais, arba 9,5 proc. (didžiausias PI buvo 97,8 Ω, mažiausias – 82,6 Ω). Per 30 stebėjimo dienų po hospitalizavimo pacientas dar dvi dienas nevartojo torazemido, PI dydis taip pat reaguodavo reikšmingu sumažėjimu (14 paveikslas). Paciento svoris tuo pačiu metu kito mažai (nuo 0 iki 500 g, dažniausiai mažėjo), o dusulio intensyvumas sustiprėdavo lygiagrečiai su PI svyravimu. Įdomu, kad tomis dienomis, kai pagal PI ir dusulio dinamiką plaučių stazė didėjo, paciento fizinis aktyvumas išliko pakankamas: jis nueidavo vidutiniškai apie 16 tūkstančių žingsnių, lyginant su 14,5 tūkstančio viso mėnesio nueitų žingsnių vidurkiu.



14 paveikslas. Plaučių skysčių, svorio ir dusulio dinamika priklausomai nuo diuretikų dozės [27]

Kreivė *Dusulio dažnis* rodo, ar pacientui pasireiškė dusulys ar ne, o kreivė *Dusulio išraiška* – kaip dažnai, kokio intensyvumo ir kaip stipriai (skalės balai nuo 1 iki 5) dusulys vargino pacientą. [27].

3.3.7 Klinikinė monitoringo nauda

Tyrimo metu atsižvelgdami į kliniškai reikšmingus būklės pokyčius trims pacientams koregavome gydymą nuotoliniu būdu ir kartais kviesdami juos neplaninio vizito (iš viso 4 neplaniniai vizitai, iš kurių vienas pasibaigė hospitalizavimu). Koreguojant gydymą taip pat buvo atsižvelgta į kraujospūdžio ir širdies susitraukimo dažnio kitimus – apie juos buvo klausiama susisiekus su pacientais telefonu arba jiems apsilankius poliklinikoje. Išskyrėme, kokie klinikiniai ir monitoringo rodikliai privertė keisti gydymą (12 lentelė). Matyti, kad tarp kitų klinikinių parametrų PI vertės paskatino daugiausia gydymo keitimų, ypač diuretikų dozavimo.

12 LENTELĖ. Gydymo korekcija atsižvelgiant į klinikinius ir monitoringo rodiklius

Rodikliai	Gydymo korekcija	Korekcijos dažnis, kartai
Simptomai (silpnumas)	Hospitalizavimas dėl sutrikusios inkstų funkcijos, hiperkalemijos	1
Didelis ŠSD	Beta adrenoblokatorių dozės didinimas Prokoralano skyrimas	3 1
Nekoreguotas AKS	AKF inhibitorių dozės didinimas	1
Elektrolitų disbalansas	Spironolaktono dozės didinimas ir torazemido dozės mažinimas	1
PI kritimas	Beta adrenoblokatorių ir spironolaktono dozių didinimas AKF inhibitoriaus ir beta adrenoblokatoriaus dozių didinimas Torazemido dozės didinimas	1 1 5
Svorio didėjimas	–	0

Santrumpos: ŠSD – širdies susitraukimo dažnis; AKS – arterinis kraujo spaudimas; PI – plaučių impedansas.

4. Išvados

1. Daugumos IN TOUCH studijos dalyvių savirūpos elgesys tyrimo metu gerokai keitėsi: beveik pusės pacientų savirūpa gerėjo, kitos pusės – blogėjo.
2. Svorio, kraujospūdžio ir EKG telemonitoringas, kuriuo papildyta informacinių ir kompiuterinių technologijų valdoma ligos priežiūros sistema, neturėjo įtakos savirūpai.
3. Nepatenkinamas gyvenimo kokybės fizinis aspektas, didesnio laipsnio sistolinė disfunkcija ir žemesnė NYHA funkcinė klasė blogino savirūpą.
4. Didžiausią potencialą gerinti savirūpą turėjo tie pacientai, kurių pradinis savirūpos lygis buvo žemas, taip pat vidutinio savirūpos lygio pacientai, jeigu kartu nesirgo depresija.

5. Telemonitoringas nėra rutininės širdies nepakankamumo priežiūros dalis nei Lietuvoje, nei Norvegijoje ar Švedijoje; mažiau nei pusė minėtų šalių sveikatos priežiūros specialistų yra susipažinusių su šiuo priežiūros būdu.
6. Sveikatos priežiūros specialistai teigiamai vertina nuotolinės stebėsenos vaidmenį gerinant priežiūros kokybę, didinant gydomų pacientų ir mažinant hospitalizacijų skaičių.
7. Didžiausius ŠN pacientų telemonitoringo lūkesčius turėjo jaunesni sveikatos priežiūros specialistai, turintys nedidelį darbo stažą, bet darbo su kompiuterinėmis programomis patirtį, taip pat specialistai, dirbantys Lietuvoje.
8. Multimodalinis pacientų monitoringas 30 dienų po hospitalizavimo dėl širdies nepakankamumo pablogėjimo, atliekant plaučių impedanso ir svorio matavimus bei nešiojant „ActiGraph" fizinio aktyvumo registravimo prietaisą, yra nesunkiai įvykdomas ir gana patikimas.
9. Plaučių impedanso mažėjimas koreliavo su svorio didėjimu tik nedidelei daliai pacientų.
   Daugumai pacientų koreliacijos tarp plaučių impedanso, svorio ir fizinio aktyvumo parametrų per 30 dienų po išrašymo iš ligoninės nerasta.
10. Plaučių impedanso matavimas namuose jautriu neinvaziniu prietaisu gali tapti naudinga didėjančios plaučių stazės nustatymo priemone.

5. Praktinės rekomendacijos

1. Prieš taikant ŠN pacientams intervencijas savirūpos elgesiui gerinti, rekomenduojama įvertinti savirūpos lygį pagal Europos širdies nepakankamumo savirūpos elgesio skalę.
2. Įtraukiant pacientus į savirūpos gerinimo programas rekomenduojame įvertinti jų nerimą ir depresiją pagal ligoninės nerimo ir depresijos skalę ir pirmiausia koreguoti esamą depresiją.
3. Kadangi savirūpos elgesys ligos metu keičiasi, gali būti naudinga pakartotinai įvertinti savirūpos lygį.
4. Šiaurės ir Baltijos šalių sveikatos priežiūros specialistai tiki nuotolinės stebėsenos veiksmingumu, todėl racionalu diegti nuotolinės stebėsenos paslaugą.
5. Mokymai, gerinantys sveikatos priežiūros specialistų kompiuterinius gebėjimus, gali palengvinti telemonitoringo įgyvendinimą klinikinėje praktikoje.
6. Mūsų gauti duomenys pagrindžia reikalingumą atlikti bandomąjį atsitiktinių imčių tyrimą, kad būtų galima įvertinti plaučių impedanso matavimo namuose įtaką kartotinei hospitalizacijai.

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Arūnas Strumila

THE EVALUATION OF TREATMENT EFFECTIVENESS AND PROGNOSIS OF INFANTILE HEMANGIOMA WITH INFRARED THERMOGRAPHY

VAIKŲ HEMANGIOMŲ PROGNOZĖS IR GYDYMO VEIKSMINGUMO VERTINIMAS INFRARAUDONŲJŲ SPINDULIŲ TERMOGRAFIJA

Biomedical sciences, medicine (06 B)
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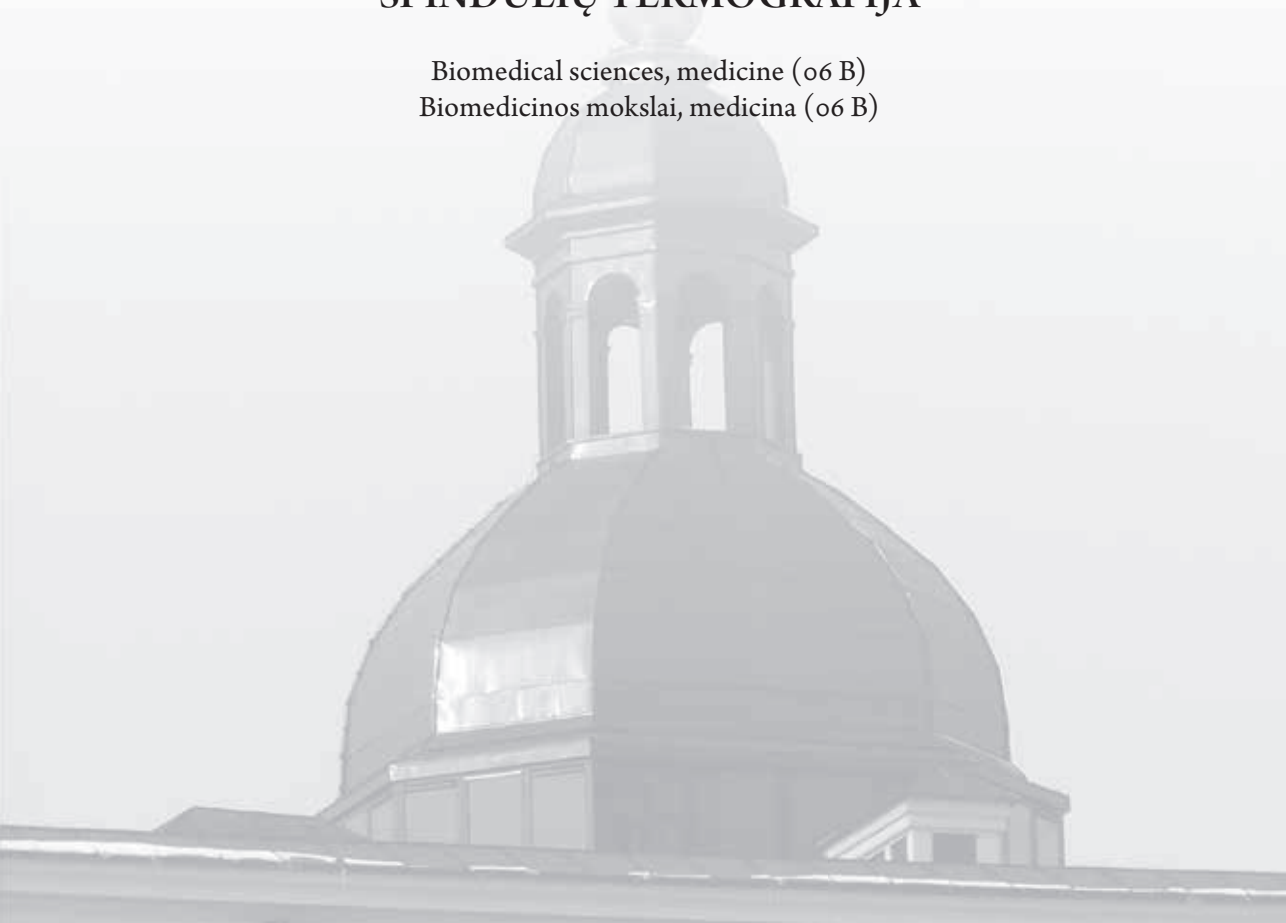


Relevance

The current treatment tactics of infantile haemangiomas are well-defined; however, many discussions still arise concerning the indications for treatment. The literature suggests that treatment of infantile haemangiomas should be initiated as early as possible in order to prevent complications; however, no objective indications, based on which treatment options could be initiated, have been defined. Because of this and the large heterogeneity of haemangiomas, it has been recommended that treatment should be started based on the treating doctor's personal clinical experience. However, there are some absolute indications for the treatment of infantile haemangiomas that have been defined: an ulceration of the haemangioma, bleeding, a large surface area, loss of function due to the haemangioma (eye, eyelid, lip, nose, or throat haemangiomas) and heart failure. These conditions result as a complication of infantile haemangiomas and are harder to treat than uncomplicated haemangiomas. Treatment results would be more favorable if treatment was to be initiated before these serious adverse effects brought about by infantile haemangiomas.

After a thorough review of the literature, we can conclude that no objective test to diagnose an infantile haemangioma, which can lead to complications and, therefore, requires early treatment, currently exists. That is why many studies are now focused on identifying new methods for the detection of the proliferative qualities of an infantile haemangioma.

The aim and objectives of the study

The Aim of the Study is to identify and analyze the prognostic success and failure criteria of the observation and treatment of infantile haemangioma using infrared thermography. *The Objectives of the Study*:

1. To determine the diagnostic value of surface temperature measurements of infantile haemangiomas using infrared thermography;
2. To determine and analyze the peak and average surface temperature values of infantile haemangiomas using infrared thermography;
3. To identify the proliferation risk factors of infantile haemangiomas attributed to the observed group of the research and to determine the diagnostic value of such risk factors;
4. To determine the value of infrared thermography in the assessment of treatment effectiveness for infantile haemangiomas.

Statement to be Defended

Infrared thermography can be used to predict the growth potential of infantile haemangiomas and to evaluate their treatment effectiveness.

Material and methods of the study

A clinical trial was conducted at the Children's Hospital, an affiliate of Vilnius University Santaros Klinikos, during the years 2014–2017. The trial was issued an improvement statement No. 158200-15-798-314 by The Lithuanian Bioethics Committee, and it was authorized by The Lithuanian State Data Protection Inspectorate (authorization No. 2R-6027(2.6-1)) to conduct the processing of personal data. The clinical trial included patients referred for infantile haemangiomas to the outpatient department of the Children's Hospital, affiliate of Vilnius University Santaros Klinikos. Parents or legal guardians of the children were asked to participate in a biomedical research trial after having all of the relevant information about the conducted trial explained to them. Researchers informed the parents or legal guardians of the patients that the decision to not participate in the clinical trial would not affect the further treatment and observation of the children. All parents or legal guardians of the patients signed an informed consent to participate in the clinical trial.

Patient Inclusion and Exclusion Criteria:

A. Inclusion criteria:

1. The patient was diagnosed with an infantile haemangioma;
2. The diameter of the infantile haemangioma is equal to or more than 10 mm;
3. Age at the time of inclusion into the trial was 1 week to 6 months;
4. Parents or legal guardians signed an informed consent form to participate in the clinical trial.

B. Exclusion criteria:

1. The patient is diagnosed with chronic haematological, immunological or oncological diseases;
2. Patient is afflicted by an infantile haemangioma of the limbs;
3. Patient is afflicted by an infantile haemangioma of the airways or parenchymal organs;
4. The patient previously received treatment for his/her infantile haemangioma.

If the patient matched all of the inclusion criteria, his/her parents or legal guardians were asked to participate in the clinical trial. The Informed Patient and

Informed Consent Form was explained, and they were asked to sign it prior to further diagnostic methods. After gaining an informed consent form, each patient was provided with an identification number, which would be used to update data on the database of the clinical trial. According to the protocol of this clinical trial, five consultations were scheduled with the patient every 4±2 weeks. Additional consultations were scheduled if the proliferation of the haemangioma could still be observed during the fifth consultation or if no signs of regression of the haemangioma had appeared. Consultations were scheduled to end after observing signs of stabilization or regression of the haemangioma in two consequent consultations.

The following procedures were performed during each outpatient consultation:

1. The gathering of demographic data

These data would be compiled during the first visit, uploaded into an electronic database and used during further analysis.

2. A clinical assessment of the infantile haemangioma

The color and consistency of the infantile haemangioma was recorded during the clinical assessment. Findings were compared to those of the previous visit. Proliferating haemangiomas are bright red and hard during palpation. A less intense red coloration with a medium consistency during palpation is common for infantile haemangiomas in the plateau phase of growth. Regression is defined by a whitish appearance of the haemangioma and a soft consistency during palpation.

3. Standardized photography of the infantile haemangioma

Pictures of the infantile haemangioma were taken during each consultation; the process was established and standardized. The patient was held in a supine position by the parents sitting on a chair at the centre of a 2-meter diameter plastic rug. The rug was graded at 15- degree angles. A specific site for the placement of the tripod and photo camera was marked. Each time, the pictures were taken at the same angle, distance and flash mode. After each visit, the pictures were compared to previously taken pictures. Frontal pictures were evaluated for surface coloration differences, whereas lateral pictures were evaluated for proliferation of the haemangioma above the skin surface. The visual analogue scale (VAS) was used to assess proliferations above the skin surface (VAS-D) and coloration differences (VAS-S). A 100mm scale was used for VAS, where -100 is defined as a doubling of the haemangioma size, 0 denotes no difference in comparison to the previous picture of the haemangioma, and 100 indicates a total regression of the haemangioma. A 5mm difference in the VAS scale corresponds to a 10% difference.

4. A surface temperature measurement of the infantile haemangioma

The surface temperature of the haemangioma was measured using a MICROLIFE NC120 infrared thermometer and an FLIR C2 infrared thermographer. Measurements were made according to the manufacturer's recommendations. All measurements were performed in the same draught-free room with a fixed temperature of 20–22°C. The patients' skin was exposed in the room for 10-15 minutes. All patients undergoing the consultation had to be healthy and feverless for no less than 36 hours prior to the consultation. A measurement of the surface temperature of the haemangioma using an infrared thermometer was performed until the same value was obtained in three consequent measurements.

Data obtained during the surface temperature measurement using the infrared thermographer were analyzed further with FLIR Tools (version 2.1), which is the specific software application of the FLIR C2 camera manufacturer.

5. An ultrasonography of the infantile haemangioma

An ultrasonography was performed to determine the depth and circulation intensity of the infantile haemangioma during each visit. The sonography was performed in all cases with the same SonoSite Edge ultrasonograph linear probe with a frequency of 6–15 MHz.

6. Blood tests

Complete blood count and C reactive protein tests were performed during each visit in order to identify any underlying inflammatory processes.

7. Treatment

Treatment was initiated with systemic or laser therapy if the haemangioma exhibited fast proliferation and the risk factors of complications were seen, skin abnormalities after involution of the haemangioma persisted or a loss of function appeared as a direct cause of the haemangioma.

Assignation to Test Groups

The performed clinical trial was a prospective cohort study. Two test groups were formed: the observed (untreated) group and the treated infantile haemangioma group. During the first visit, all patients would be assigned to the first (untreated) group. If indications for treatment arose during the trial, such patients would then be reassigned to the treated infantile haemangioma group and treatment would be initiated. During subsequent consultations, all diagnostic procedures would be performed according to the described protocol of the trial.

Patient data of any cohort group was compared to the same patient data obtained during the previous visit. Standardized pictures, a clinical assessment and ultrasonography data comparisons were used to evaluate the clinical stage of the infantile haemangioma. If a haemangioma had become a brighter red, if the haemangioma was bulging in lateral pictures or if its depth, assessed during ultrasonography, increased, the haemangioma was considered to be in the proliferative stage during the first visit. If the red coloration of the haemangioma remained unaltered, and if the bulging of the haemangioma in lateral pictures and ultrasound data matched those of the first visit, the haemangioma was suspected to have been in the plateau stage during the first visit. A haemangioma was considered to be in the involution stage during the first visit if it turned paler during the second visit, the bulging in the lateral pictures remained unchanged or decreased in size, and the depth of the haemangioma during ultrasound remained the same or decreased in comparison to the data of the first visit. The clinical stage of the haemangioma was not determined during the last consultation, as there were no subsequent visits to which researchers could compare the haemangioma to. Thus, the clinical stage of a haemangioma was determined during all up to the last visit. According to the clinical stage of the haemangioma, the patients were also distributed into three subgroups: those associated with either a proliferating haemangioma, a plateau-stage haemangioma and an involuting haemangioma. This sub-classification according to growth of the haemangioma was most convenient in order to achieve the goals of the study, as the common morphological classification of infantile haemangiomas (according to their depth and distribution) did not allow for the assessment of growing infantile haemangiomas.

Cohorts of the researched infantile haemangioma patients:

1. Observed (untreated) infantile haemangioma group;
2. Treated infantile haemangioma group.

Observed and treated infantile haemangioma group subgroups:

1. Proliferating haemangiomas;
2. Plateau phase haemangiomas;
3. Involuting haemangiomas.

Statistical Data Analysis Methods

Statistical data analysis was performed using IBM SPSS 23 for Mac, Microsoft Excel and MedCalc software. Qualitative variables were expressed as categorical variables (n) and their percentages (percent). Quantitative variables were described by mean, median, standard deviation (SD), standard error (SE) and confidence interval (CI). The regularity of the continuous variable and the quantitative variables

distribution was verified by Kolmogorov and Smirnov tests. The Student t criterion was used for comparing the two groups with a normal distribution, while in more groups, the two-phase dispersion analysis (ANOVA) was used. If the distribution was abnormal, the nonparametric Kruskal-Wallis test was used to compare the quantitative data of more than two groups.

A one-dimensional and multivariate binary logistic regression was used to determine the relationship between the variables. An ROC curve analysis was used to determine the diagnostic value of the risk factors for the proliferation of an infantile haemangioma by calculating the area under the ROC curve as well as the sensitivity, specificity, the Youden index and threshold values. The statistical significance level of this clinical trial was a=0.05. A p value $p<0.05$ was accepted as statistically significant.

Sample Size Calculation

The sample size of the trial was determined according to the population size sample error. (S.Bekešienė. Duomenų analizės SPSS pagrindai. Vilnius, Generolo Jono Žemaičio Lietuvos karo akademija, 2015). The sample size of the trial was calculated by the following formula:

$$n = \frac{N \cdot 1.96^2 \cdot p \cdot q}{\varepsilon^2 \cdot (N-1) + 1.96^2 \cdot p \cdot q},$$

where

- N is the population size = 30 000 (child birth rate);
- 1.96 is the value of standard normal distribution, when a=0.05;
- p is the expected prevalence of a variable = 0.05 (the incidence of infantile haemangiomas in a Lithuanian population is 4–5%; therefore, 5% was used for the purpose of this trial);
- q is the expected rate of a variable not occurring, q=1-p=0.95;
- ε is the expected precision rate = 0.05.

The calculated sample size consisted of 74 patients.

Results

General Data of the Patients

In total, 102 patients were included in this prospective biomedical trial conducted in the Children's Hospital, an affiliate of Vilnius University Santaros Klinikos, for the years 2013–2017. Both the parents or legal guardians of each of the 102 patients signed an informed consent to participate in the trial. The parents of one patient had refused further involvement in the trial after the first consultation. Infrared thermography was poorly conducted on three patients, who were consequently eliminated from the study. As a result, a total of 98 patients were included in the trial.

Of the 98 patients included, 73 were girls and 25 were boys. Three of the patients exhibited two haemangiomas that fit the inclusion criteria, resulting in 101 haemangiomas examined during the trial. Tables 1 and 2 describe the prevalence of infantile haemangiomas in the study groups according to the morphological classification of haemangiomas by their depth and distribution patterns.

TABLE 1. The incidence of infantile haemangiomas according to the classification of infantile haemangiomas by the depth of a haemangioma.

Depth	No. of patients (n)	Percent (%)
Superficial	54	53.5
Mixed	47	46.5
Subcutaneous	0	0
Total	101	100

TABLE 2. The incidence of haemangiomas according to the classification of infantile haemangiomas by the distribution pattern of a haemangioma.

Distribution patterns	No. of patients (n)	Percent (%)
Focal	72	71.3
Segmental	8	7.9
Unidentified	21	20.8
Total	101	100

In the trial cohort, infantile haemangiomas were most commonly identified in the dorsal region (28 cases); the lowest incidence was observed in the sacral region (5 cases). The distribution of the evaluated infantile haemangiomas according to anatomical regions is described in Table 3.

TABLE 3. The distribution of infantile haemangiomas according to anatomical localization.

Anatomical region	No. of patients (n)	Percent (%)
Dorsal	28	27.7
Abdominal	24	23.8
Thoracic	18	17.8
Cranial	18	17.8
Facial	8	7.9
Sacral	5	5
Total	101	100

Patients of the trial underwent a total of 513 consultations with an average of 5 visits per patient. Consultations during which infrared thermography was not performed according to protocol were rejected. The last consultation of each patient was not included in the final statistical analysis, as the patients could not be stratified into a subgroup of the study without subsequent visits. Therefore, a total of 366 consultations were evaluated in the final analysis. The age median during the time of the first consultation was 1.6 months (median 2 months). The frequency of consultations according to the patient's age is summarized in Figure 4.

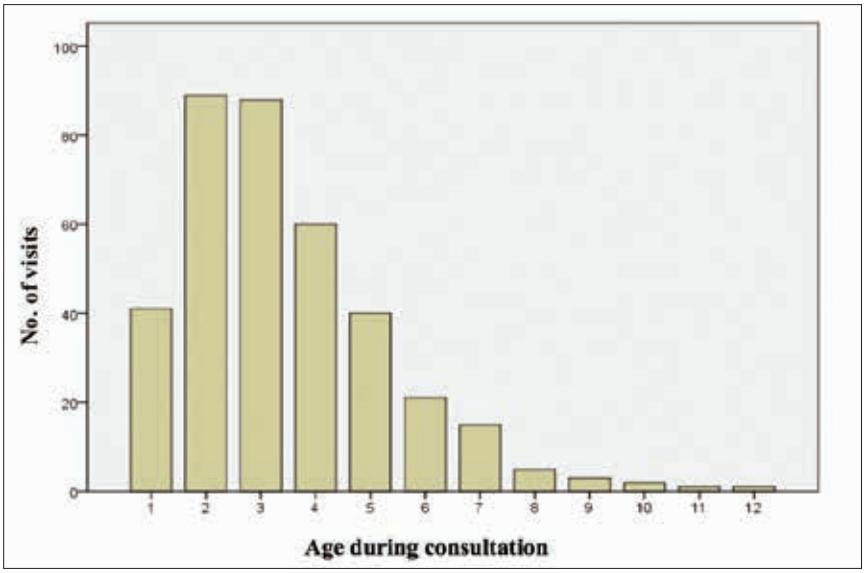


FIGURE 4. The frequency of consultations according to the patient's age (in months).

Overall, 231 consultations were evaluated in the observed patient group. Involution was observed in 42, the plateau stage in 108 and proliferation in 81 consultations. As a result, 32 patients were re-assigned to the treated haemangioma group. These patients were evaluated during a total of 135 consultations. Involution was observed in 44, the plateau stage in 49 and proliferation in 42 consultations of the treated group patients. These data are summarized in Tables 5 and 6.

TABLE 5. The frequency of visits during which the infantile haemangioma was assigned to a subgroup of the observed group.

Observed group subgroups	No. of visits (n)	Percent (%)
Proliferating haemangiomas	81	35.1
Plateau stage haemangiomas	108	46.7
Involuting haemangiomas	42	18.2
Total	231	100

TABLE 6. The frequency of visits during which the infantile haemangioma was assigned to a subgroup of the treated group.

Treated group subgroups	No. of visits (n)	Percent (%)
Proliferating haemangiomas	42	31.1
Plateau stage haemangiomas	49	36.3
Involuting haemangiomas	44	32.6
Total	135	100

TABLE 7. The depth of infantile haemangiomas in the observed and treated groups.

Trial group	Average (cm)	±SD (cm)	95 % ± PI (cm)		P
Observed group	0.61	0.42	0.55	0.66	<0.001
Treated group	1.04	0.53	0.95	1.14	

As mentioned before, during each visit, an ultrasonography of each haemangioma was performed to determine the depth of the haemangioma. The average depth of the observed haemangiomas was 0.61 cm, whilst it almost doubled up to 1.04 cm in the treated group. The data of ultrasonography met the normality conditions (Kolmogorov and Smirnov tests) when $p>0.05$. The Student t test was used to compare the medians of the two test groups. Differences between the group were statistically significant ($p<0.05$). The data are summarized in Table 7.

Proliferation was observed during the first consultation in 64 cases, the plateau stage in 28 cases and involution in 9 cases. Proliferation was most frequently diagnosed during the first and second months of life, in 26 and 29 cases respectively. The plateau stage was most frequently observed in the second and third months of life. The relationship between age and the growth stage of a haemangioma is shown in Table 8.

TABLE 8. The growth stage of an infantile haemangioma during the first visit in relationship to the age of the patient.

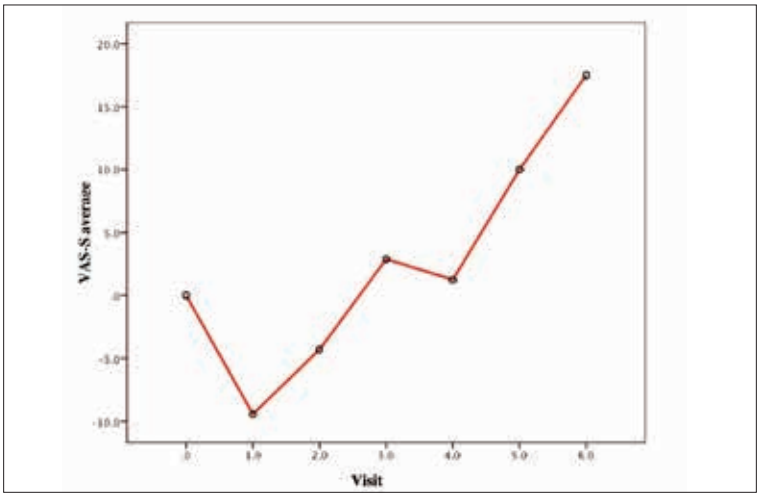
Age (months)	Growth stage			Total
	Involution	Plateau	Proliferation	
<1	0	0	2	2
1	4	13	26	43
2	3	11	29	43
3	1	4	6	11
4	1	0	1	2
Total	9	28	64	101

Observed Infantile Haemangioma Group

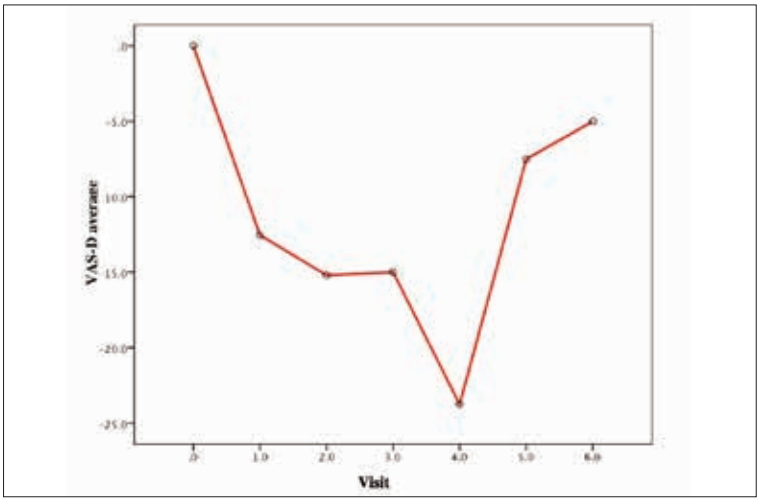
Patients with uncomplicated and small growth potential haemangiomas were included in this group. Patients whose haemangiomas exhibited the plateau stage of development during the first consultation were also included in the observed infantile haemangioma group. The average age of the proliferating haemangioma subgroup was 2.33 months, of the plateau stage – 2.73 months, and 3.81 months of the involuting haemangioma subgroup. No aggressive growth spurts were observed in this group, thus contributing to the young average age of both the plateau stage and involuting haemangioma subgroups. Only two cases of proliferation were observed in older patients (4 months of age) in the observed infantile haemangioma group.

According to literature, infantile haemangiomas reach 80% of their maximal size by the third month of the patient's life. The observation of VAS differences between consultations in our trial concur with the literature data. Starting from the fourth consultation, which matched the 4th–5th month of life of the patient, VAS-S

and VAS-D differences between consultations decreased, the haemangioma entered the plateau stage of growth and would soon start exhibiting signs of involution. During the sixth visit, a VAS-D average of -5 and a VAS-S of 17.5 was observed (the infantile haemangioma remained slightly larger in size in comparison to the first consultation, but the surface had begun to turn paler). Differences of VAS-S and VAS-D averages during each visit are shown summarized in graphs 9 and 10.



GRAPH 9. The differences of VAS-S averages during each visit.



GRAPH 10. The differences of VAS-D averages during each visit.

The proliferating infantile haemangioma subgroup surface temperature, measured by an infrared thermometer, was 36.81°C on average (95% ± CI, 36.73–36.89). Plateau stage haemangiomas exhibited an average surface temperature of 36.71°C (95% ± CI, 36.63–36.78), and involuting haemangiomas – a temperature of 36.63°C (95% ± CI, 36.49–36.76). Differences in average surface temperatures between the subgroups were statistically significant: p=0.03. However, clinical application was limited due to the small variance in temperature: the difference between proliferating and involuting haemangioma surface temperature averages was as little as 0.18°C. Differentiating between a proliferating and involuting haemangioma based solely on the surface temperature of the haemangioma, measured by an infrared thermometer, would be impossible with such a small degree of variance.

Infrared thermography allowed for a more detailed assessment of surface temperature of infantile haemangiomas. First, peak surface temperatures of the haemangiomas were recorded. The proliferating infantile haemangioma subgroup exhibited an average peak surface temperature of 36.62°C (95% ± CI, 36.51–36.75), plateau stage haemangiomas –36.16°C (95% ± CI, 36.05–36.28), involuting haemangiomas – 36.03°C (95% ± CI, 35.83–36.23). Differences in peak surface temperature averages between the subgroups were statistically significant: p<0.001. A wider range in temperature difference was observed with infrared thermography than infrared thermometers. The difference between the peak surface temperature of an infantile haemangioma in its proliferating and involuting stages was 0.59°C. This method allows for a rapid assessment of differences in peak surface temperatures of infantile haemangiomas in two consecutive visits and, by means of the FLIR Tools application, evaluation of the need for treatment.

The FLIR Tools software application was used to calculate the average surface temperature of infantile haemangiomas. The proliferating infantile haemangioma subgroup exhibited an average surface temperature of 36.12°C (95% ± CI, 36.00–36.24), plateau stage haemangiomas – 35.57°C (95% ± CI, 35.45–35.69), and involuting haemangiomas – 35.49°C (95% ± CI, 35.31–35.67). Differences in surface temperature averages between the subgroups were statistically significant: p<0.001. The difference between the average surface temperature of an infantile haemangioma in its proliferating and involuting stages was similar to the difference between their peak surface temperature values: 0.63°C (in comparison to 0.59°C). In conclusion, an infrared thermography measurement of the peak and average surface temperatures of a haemangioma produces similar results; however, the measurement of average temperatures requires additional programming tools and time.

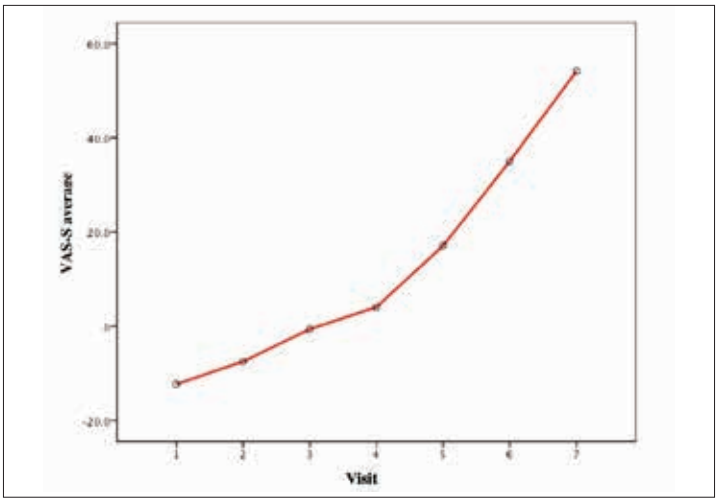
The largest differences between the subgroups were observed while measuring the difference between the average surface temperature of a haemangioma and the average surface temperature of the surrounding unaffected skin. This difference in

the proliferating haemangioma subgroup was an average of 1.65 (95% ± CI, 1.52–1.79), in the plateau stage haemangioma subgroup – 0.87 (95% ± CI, 0.79–0.95), and in the involuting haemangioma subgroup – 0.87 (95% ± CI, 0.75–0.99). Differences between the subgroups were statistically significant: $p<0.001$. The difference between proliferating and involuting haemangiomas was 0.78; the average of proliferating haemangiomas was almost double that of involuting haemangiomas. Having evaluated the different methods of surface temperature measurement, we can conclude that the measurement of the difference in surface temperature between an infantile haemangioma and surrounding unaffected skin allows for the most accurate differentiation between a proliferating and an involuting infantile haemangioma.

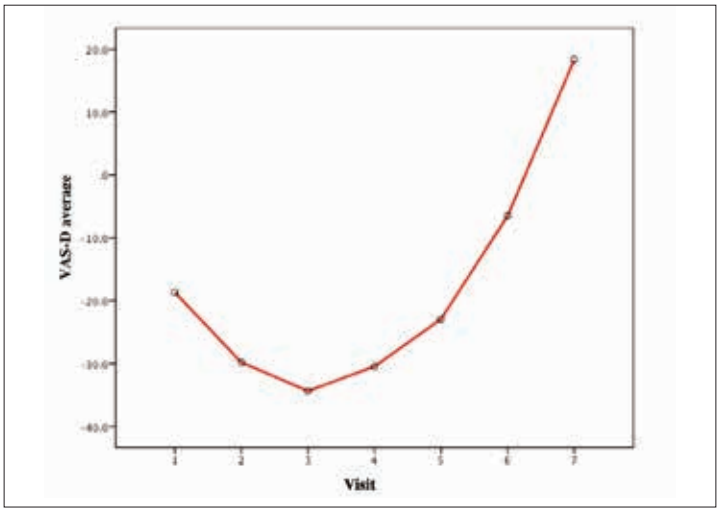
Treated Infantile Haemangioma Group

Treatment had to be initiated for 32 patients in the observed group and they were consequently attributed to the treated infantile haemangioma group. Infrared thermography was poorly performed for two of the patients, so a total of 30 patients were included in this group; these patients underwent a total of 135 outpatient consultations. The average age of the patients during the first consultation was older in comparison to that of the untreated group: 4.6 months (average age in months in the proliferating haemangioma subgroup was 3.78, in the plateau stage – 4.98, and in the involuting haemangioma group – 4.91). The depth observed during the ultrasonographies of infantile haemangiomas was also greater than in the untreated group: an average of 1.05 cm (the average depth in the proliferating haemangioma subgroup was 1.21 cm, in the plateau stage subgroup – 1.0 cm, and in the involuting haemangioma subgroup – 0.95 cm). Treatment progress was evaluated by comparing VAS-S and VAS-D differences between two consecutive consultations. VAS-S and VAS-D are subjective means of infantile haemangioma evaluation, but they remain one of the main methods described in the literature.

When treatment was initiated, the acceleration in the rise of the VAS-S curve was observed. The average VAS-S during the 6th consultation was 35, whereas during the 7th, it was 54.2. The average VAS-D did not change as rapidly during the first consultations: during the 1st and 2nd visits, according to VAS-D, the size of the haemangioma kept increasing. However, after the 3rd consultation, an increase in the VAS-D curve began to be observed. The average VAS-D during the 6th consultation remained at -6.5, whilst in the 7th visit, it shot up to 18.3. The treated group haemangiomas were larger and exhibited a brighter red coloration during the first consultation in comparison to the VAS-S and VAS- D of the untreated group; however, after the initiation of treatment, the differences between consultations in the groups accelerated in the treated infantile haemangioma group. The differences of VAS-S and VAS-D averages between each consultation are summarized by graphs 11 and 12.



GRAPH 11. The differences of VAS-S averages during each visit.



GRAPH 12. The differences of VAS-D averages during each visit.

Within the treated group, the proliferating infantile haemangioma subgroup's haemangioma surface temperature, measured by an infrared thermometer, averaged to 37.13°C (95% ± CI, 37.00–37.24); the plateau stage subgroup averaged to 37.06°C (95% ± CI, 36.95–37.16), while the involuting haemangioma subgroup averaged to 36.92°C (95% ± CI, 36.76–37.11). There was no statistically significant difference between the subgroups: p=0.066. The range of surface temperatures had overlapped,

and the subgroups could not be differentiated between one another based solely on this measurement.

The average peak surface temperatures in the proliferating subgroup, measured by means of infrared thermography, was 37.05°C (95% ± CI, 36.93–37.16), in the plateau stage subgroup – 36.79°C (95% ± CI, 36.62–36.96), and in the involuting haemangioma subgroup – 36.52°C (95% ± CI, 36.25–36.79). Differences between the subgroups in peak surface temperature were statistically significant: p=0.006. By using this method, it was possible to differentiate an infantile haemangioma in its proliferative and involution stages. The difference between peak surface temperature averages in the proliferating and involuting subgroups was 0.53°C, which was a slightly smaller difference than in the observed group.

The average surface temperature of haemangiomas, measured by infrared thermography and FLIR tools software, is distributed as follows: in the proliferating haemangioma subgroup, it was 36.52°C (95% ± CI, 36.37–36.67), in the plateau stage subgroup – 36.26°C (95% ± CI, 36.08–36.45), and in the involuting haemangioma subgroup – 35.93°C (95% ± CI, 35.62–36.23). Differences between the average subgroup surface temperature of infantile haemangiomas was statistically significant: p=0.007. As with the previous method, the differentiation into subgroups based solely on this method was also possible. The difference between surface temperature averages in the proliferating and involuting infantile haemangioma subgroups equalled 0.59°C, which is similar to the difference in the observed group.

The difference between the average surface temperature of a haemangioma and the surrounding unaffected skin in the proliferating haemangioma subgroup was 1.85 (95% ± CI, 1.66–2.04), in the plateau stage haemangioma subgroup – 1.46 (95% ± CI, 1.28–1.65), and in the involuting haemangioma subgroup – 1.39 (95% ± CI, 1.15–1.64). Differences between the subgroups were statistically significant: p=0.006. The difference between proliferating and involuting haemangiomas was 0.46, which was less than the difference found in the observed group.

The differences in the surface temperature between the treated group subgroups were less than the differences seen in the observed group, and no difference was observed after measuring the temperature with an infrared thermometer. A measurement of temperature differences between an infantile haemangioma and the unaffected surrounding skin with infrared thermography remained the most precise method of differentiating between the subgroups. This method provided the greatest precision in detecting a shift toward the plateau stage of the growth cycle and, thus, evaluating the efficacy of treatment.

Based on our findings, the most effective observational method of infantile haemangiomas proved to be a measurement of the surface temperatures of a haemangioma and a comparison of the findings to those of previous consultations. As

an infantile haemangioma turned paler, the surface temperature decreased. The differences between surface temperature averages in the groups during each visit were statistically significant, regardless of the method used for the measurement of surface temperature. In order to ensure that the differences are due to the treatment, a linear correlation between VAS-S, VAS-D and surface temperatures was calculated. A statistically significant, negative average correlation was observed for all variables. Correlation coefficients varied from -

0.44 to -0.60, confirming that with a decrease in surface temperature of an infantile haemangioma, morphological signs of involution do appear.

Based on the findings of the trial, the effectiveness of an infantile haemangioma treatment can be verified by measuring the surface temperature of a haemangioma by any of the three described infrared thermography methods. An infrared thermometer cannot be used for the assessment of treatment effectiveness, as no statistically significant differences between surface temperatures in the subgroups were observed with this method.

Observed Infantile Haemangioma Group Proliferation Risk Factors and Their Diagnostic Value

Certain probable risk factors of haemangioma proliferation were identified before the initiation of the trial. The risk factors were the diameter and depth of a haemangioma, the patient's age at presentation and the surface temperature of the haemangioma. Differences in their averages were statistically significant between the subgroups of the observed patient group. An ROC curve analysis and the threshold values for every risk factor were determined. The difference of >1.3 in the surface temperatures of a haemangioma and the surrounding unaffected skin had the highest correlation to the proliferation of a haemangioma (Youden=0.61, AUC 0.86, p<0.001), a surface temperature measurement with infrared thermography of a haemangioma >35.8 (Youden=0.39, AUC 0.76, p<0.001), the peak surface temperature of a haemangioma >36.4 (Youden=0.38, AUC 0.74, p<0.001), the depth of a haemangioma >0.75 cm (Youden=0.27, AUC 0.63, p=0.001), the diameter of a haemangioma >2.5 cm (Youden=0.32, AUC 0.64, p<0.001) and the patient's age 2 months after the onset of the disease (Youden=0.29 AUC 0.66, p<0.001). Measured by an infrared thermometer, the surface temperature of a haemangioma of >36.7°C had the lowest correlation to the proliferation of a haemangioma (Youden=0.17, AUC 0.6, p=0.009).

Separate regression models were formed for infrared thermography and infrared thermometry. The correlation of infantile haemangioma surface temperature (measured by an infrared thermometer) to the proliferation of a haemangioma had an odds ratio (OR) of 2.9 (95% CI 1.250–6.749, p=0.013, Nagelkerke R^2=0.313), the

peak surface temperature of a haemangioma had an OR of 5.8 (95% CI 2.820–11.947, $p<0.001$, Nagelkerke $R^2=0.414$), the average surface temperature (measured by infrared thermography) had an OR of 6.05 (95% CI 2.997–12.227, $p<0.001$, Nagelkerke $R^2=0.427$), difference between the surface temperature of a haemangioma and the surrounding unaffected skin had an OR of 54.4 (95% CI 17.716–167.149, $p<0.001$, Nagelkerke $R^2=0.644$), the diameter of a haemangioma had an OR of 1.3 (95% CI 1.072–1.606, $p=0.008$, Nagelkerke $R^2=0.284$), the age of the patient had an OR of 0.65 (95% CI 0.365–0.653, $p<0.001$, Nagelkerke $R^2=0.284$) and the depth of a haemangioma had an OR of 1.9 (95% CI 3.080–15.568, $p<0.001$, Nagelkerke $R^2=0.284$).

The correlation between the proliferation risk factors themselves was examined. Only infrared thermography and the depth of a haemangioma exhibited an average correlation (Pearson's correlation coefficient was 0.4–0.53, $p<0.001$). The correlation between the remaining factors was weak. Linear regression models were analyzed in order to identify a possible influence of the depth, the diameter of a haemangioma or the patient's age on the measurements of infrared thermometry and thermography. Only two models were applicable: the influence of the patient's age, haemangioma depth and diameter on the peak surface temperature measurements by infrared thermography ($R^2=0.208$) and the difference in surface temperatures between a haemangioma and the surrounding unaffected skin ($R^2=0.305$). In both models, the depth of a haemangioma had the statistically significantly largest influence on the increase in peak surface temperature ($b=0.595$, $p<0.001$) and the difference in surface temperatures between a haemangioma and the surrounding unaffected skin ($b=0.7$, $p<0.001$). For the remaining linear regression models – $r^2<0.2$; furthermore, they were not further assessed.

In conclusion, an increase in the peak surface temperature measurements and in the difference in surface temperatures between a haemangioma and the surrounding unaffected skin by infrared thermography were the most accurate prognostic factors of proliferation.

Conclusions

1. The highest surface temperature, measured by an infrared thermometer, was seen in the proliferating haemangioma subgroup. Differences between surface temperatures (measured by an infrared thermometer) in the subgroups were statistically significant; however, due to a small variance between the subgroups, a clinical application of infrared thermometry would be complicated. Infrared thermography allowed for an easier differentiation of subgroups, and it is, therefore, favored by the researchers in this trial.

2. Differences between average surface temperatures in the subgroups, measured with an infrared thermographer, were statistically significant.
3. A higher surface temperature, measured with an infrared thermographer, increased the risk of a haemangioma proliferation. Differences between the subgroups were statistically significant. An infantile haemangioma proliferation can best be predicted by measuring the difference in surface temperature between a haemangioma and the surrounding unaffected skin.
4. The transition of treated proliferating haemangiomas into the plateau stage of growth could be concluding after the measurement of surface temperatures with infrared thermography, as infrared thermometry was statistically insignificant. The differences in surface temperature between visits, measured by infrared thermometry and thermography, were statistically significant, and a decrease in temperature was observed with a positive clinical effect of the treatment.

Methodical recommendations

1. Infrared thermography measurements of peak surface temperature should be performed for every patient with an infantile haemangioma. If the peak surface temperature is >36.4°C, the difference between the surface temperature of the haemangioma and the surrounding unaffected skin should be calculated. If the difference in temperatures is >1.3 and the haemangioma is localized on the face or scrotal region, treatment should be initiated with the consent of the parents. If the parents decide to continue with the observation of the haemangioma, a follow-up consultation is recommended after two weeks.
2. If the peak surface temperature of an infantile haemangioma is between 36°C and 36.4°C, the patient should be followed-up every four weeks. Once the peak temperature decreases to <36°C, a follow-up can be done every 6 months.
3. After the initiation of treatment, infrared thermography should be performed during each consultation. If no difference is seen either in the surface temperature, coloration or size of the haemangioma between visits, treatment tactics should be reconsidered.

SANTRAUKA

Aktualumas

Nors dėl gydymo šiandien diskusijų labai nedaug, tačiau gydymo indikacijos išlieka gana subjektyvios. Daugelyje straipsnių pažymima, kad gydymą reikia pradėti kuo anksčiau siekiant išvengti komplikacijų, tačiau objektyvių indikacijų, kuriomis remiantis gydymą būtų galima pradėti labai anksti, dar nėra. Literatūroje minima, kad dėl didelio hemangiomų heterogeniškumo dėl gydymo reikalingumo turi nuspręsti konsultuojantis daktaras, remdamasis savo ir klinikos praktika. Absoliučios gydymo indikacijos yra šios: hemangiomos išopėjimas, kraujavimas, didelis dydis, funkcijų sutrikimas (akių, vokų, lūpų, nosies, gerklų hemangiomos), širdies nepakankamumas. Šios būklės atsiranda, kai hemangioma sukelia komplikacijų. Gydymo rezultatas būtų geriausias, jeigu gydymą pradėtume anksčiau, nei atsiranda minėtos komplikacijos.

Atlikus literatūros analizę, galima daryti išvadą, kad šiuo metu nėra objektyvaus tyrimo, kuris leistų nustatyti, kuri hemangioma sparčiai didės ir komplikuosis. Todėl tyrėjai vis ieško naujų metodų, kurie leistų numatyti infantilinės hemangiomos (IH) proliferacijos galimybes.

Darbo tiklas ir uždaviniai

Tyrimo tikslas- nustatyti ir įvertinti vaikų infantilinių hemangiomų stebėjimo ir gydymo veiksmingumo prognozinius kriterijus naudojant infraraudonųjų spindulių termografijos metodą.

Tyrimo uždaviniai

1. Nustatyti vaikų infantilinių hemangiomų paviršiaus temperatūrų diagnostines reikšmes infraraudonųjų spindulių termometrijos metodu.
2. Nustatyti ir įvertinti infantilinės hemangiomos paviršiaus aukščiausias ir vidutines temperatūrų diagnostines reikšmes infraraudonųjų spindulių termografijos metodu.
3. Išsiaiškinti stebėtos grupės vaikų infantilinių hemangiomų proliferacijos rizikos veiksnius ir įvertinti jų diagnostinę reikšmę.
4. Nustatyti infraraudonųjų spindulių termografijos metodo galimybes vaikų infantilinių hemangiomų gydymo efektyvumui įvertinti.

Ginamieji teiginiai

Infraraudonųjų spindulių termografijos metodas leidžia prognozuoti infantilinių hemangiomų eigą ir vertinti gydymo veiksmingumą.

Tyrimo metodika

Klinikinis tyrimas buvo atliktas 2014-2017 metais Vaikų ligoninėje, VšĮ Vilniaus universiteto ligoninės Santaros klinikų filiale. Tyrimui atlikti gautas Vilniaus regioninio biomedicininio tyrimų etikos komiteto leidimas Nr. 158200-15-798-314. Valstybinė duomenų apsaugos inspekcija išdavė leidimą (Nr. 2R-6027(2.6-1)) atlikti asmens duomenų tvarkymo veiksmus.

Tyrime dalyvavo vaikai, kurie atvyko konsultuotis dėl infantilinės hemangiomos į Vaikų ligoninės VšĮ Vilniaus universiteto ligoninės Santaros klinikų filialo konsultacijų polikliniką. Tėvams pasiūlyta dalyvauti biomedicininiame tyrime, suteikta visa informacija apie tyrimą. Tyrėjai paaiškindavo, kad tėvų apsisprendimas nedalyvauti tyrime nedaro įtakos vaiko medicinos priežiūrai ir gydymo kokybei. Visų pacientų tėvai ar globėjai pasirašė informuoto asmens sutikimą dalyvauti tyrime.

Ligonių įtraukimo ir atmetimo kriterijai buvo šie:

A. Įtraukimo į tyrimą kriterijai:

1. Vaikui nustatyta infantilinė hemangioma.
2. Infantilinės hemangiomos skersmuo ne mažesnis nei 10 mm.
3. Amžius atrankos dieną – nuo 1 savaitės iki 6 mėnesių.
4. Tėvai ar globėjai pasirašė informuoto asmens sutikimą dalyvauti tyrime.

B. Atmetimo kriterijai:

1. Vaikas serga lėtinėmis kraujo, onkologinėmis, imuninės sistemos ligomis.
2. Infantilinė hemangioma yra rankų ar kojų.
3. Infantilinės hemangiomos yra kvėpavimo takų ar parenchiminių organų.
4. Vaikui jau buvo skirtas gydymas.

Jei pacientas atitiko visus įtraukimo kriterijus, jo tėvams buvo pasiūloma dalyvauti tyrime. Tėvai buvo supažindinti su „Asmens informavimo ir informuoto asmens sutikimo forma“, kurią jie pasirašydavo prieš atliekant tyrimus. Tėvams sutikus ir pasirašius sutikimą, pacientams suteikiamas identifikacijos numeris, kuris naudotas įkeliant duomenis į duomenų bazę.

Pagal tyrimo protokolą buvo numatomi 5 tiriamieji vizitai, kas 4±2 savaites. Vizitų skaičius buvo didinamas, jeigu penkto ir paskesnių vizitų metu išlieka hemangiomos augimas arba nebuvo regresijos požymių. Vizitai nutraukiami anksčiau, jeigu du vizitus iš eilės hemangioma neaugo ir atsirado regresijos požymių.

Kiekvieno vizito buvo atliekamos šios procedūros:

1. Demografinių duomenų rinkimas.

Šie duomenys pirmo vizito metu buvo įkelti į elektroninę duomenų bazę ir naudojami tolesnei analizei.

2. Klinikinis hemangiomos vertinimas.

Klinikinis vertinimas buvo atliekamas remiantis hemangiomų spalva, konsistencija ir lyginant su ankstesnių vizitų fotografijomis. Proliferuojančių hemangiomų konsistencija kieta, spalva ryškiai raudona. Stabilių hemangiomų raudona spalva nebe tokia intensyvi, konsistencija minkšta. Regresuojančios hemangiomos paviršiaus spalva yra su balkšvais plotais, konsistencija minkšta.

3. Standartizuotos fotografijos.

Jos buvo atliekamos per kiekvieną apsilankymą. Naudojamas plastikinis 2 metrų skersmens kilimas, kurio centre statoma kėdė asmeniui, laikančiam ant rankų tiriamąjį pacientą. Kilimas sužymėtas kas 15 laipsnių, pažymėta vieta trikojui, ant kurio tvirtinamas fotoaparatas. Fotografijos atliktos vienodu kampu, vienodu atstumu nuo fotoaparato, vienodu fotoa-

parato ir blykstės režimu. Po kiekvieno vizito visos paciento nuotraukos lyginamos su pirmo vizito nuotrauka. Žiūrima, ar hemangioma kilo virš odos (vertinama šoninė infantilinės hemangiomos nuotrauka), ir vertinami hemangiomos paviršiaus spalvos pokyčiai. Iškilimo virš odos (VAS-D) ir spalvos pokyčiams (VAS-S) registruoti buvo naudojama vaizdinė analogijų skalė (VAS). VAS naudojome 100 mm skalę, kurioje -100 žymi, kad IH padvigubėjo, 0 – IH nepasikeitė, 100 parodo, kad IH visiškai išnyko. VAS skalėje 5 mm pokytis atitiko 10 procentų pokyčius.

4. Hemangiomos paviršiaus temperatūros matavimas

Paviršiaus temperatūra buvo matuojama infraraudonųjų spindulių termometru „MICROLIFE NC120“ ir infraraudonųjų spindulių termografu „FLIR C2“. Matavimai buvo atlikti pagal gamintojų rekomendacijas. Tyrimo kabinete buvo pastovi 20–22 °C temperatūra, nebuvo skersvėjų, paciento tyrimo vieta buvo apnuoginta nuo 10 iki 15 minučių, kad oda ir hemangioma prisitaikytų prie kambario temperatūros. Pacientai buvo sveiki, nekarščiuojantys ne mažiau nei 36 valandos iki tyrimo. Infantilinės hemangiomos paviršiaus temperatūros matavimai infraraudonųjų spindulių termometru buvo atliekami tol, kol tris kartus iš eilės buvo išmatuojama tokia pati temperatūrą.

Duomenys, gauti infantilinių hemangiomų paviršiaus temperatūrą matuojant infraraudonųjų spindulių termografu, buvo apdoroti kameros gamintojo programos „FLIR Tools“ 2.1 versija.

5. Ultragarsinis tyrimas

Per visus apsilankymus atlikti hemangiomų ultragarsiniai tyrimai „SonoSite Edge“ aparatu su linijiniu (6–15 Mhz dažnio) davikliu. Tyrimo metu buvo įvertinamas hemangiomos gylis ir kraujotakos intensyvumas.

6. Kraujo tyrimas

Pacientui per kiekvieną apsilankymą buvo atliekamas bendras kraujo tyrimas ir nustatomas C reaktyviojo baltymo kiekis kraujuje. Tyrimai buvo atliekami siekiant įvertinti, ar nėra uždegimo procesų.

7. Gydymas

Jei hemangioma sparčiai proliferuoja ir jei yra tikimybė atsirasti negrįžtamoms deformacijoms, odos pakitimams po involiucijos, funkcijos sutrikimams, buvo skiriamas gydymas medicininiais lazeriais ir (ar) sisteminė terapija.

Tiriamųjų grupių sudarymas

Šis tyrimas yra kohortinis perspektyvusis. Tyrimo metu sudarytos dvi pagrindinės kohortos: stebėtų (negydytų) ir gydytų infantilinių hemangiomų. Pirmo vizito metu pacientai buvo priskiriami stebėjimo kohortai, o jei per kitus apsilankymus paaiškėdavo, kad infantilinę hemangiomą reikia gydyti, šie pacientai po gydymo skyrimo patekdavo į gydytų infantilinių hemangiomų kohortą ir jų stebėjimas vykdomas toliau pagal numatytą tyrimo procedūrų planą.

Abiejų kohortų pacientų per kiekvieną vizitą gauti duomenys buvo lyginami su paskesnio vizito duomenimis, norint išsiaiškinti hemangiomos klinikinę stadiją. Buvo lyginamos standartizuotos fotografijos, klinikinio hemangiomos vertinimo (spalva, konsistencija) ir

echoskopijos duomenys (infantilinės hemangiomos gylis). Buvo lyginama pirmas vizitas su antru, antras su trečiu ir taip toliau. Jei, lyginant pirmą vizitą su antru vizitu, matoma, kad infantilinės hemangiomos raudonumas ryškėja, fotografijose virš odos esanti dalis didėja ir echoskopijos duomenys rodo, kad IH gilėja, pirmo vizito infantilinė hemangioma priskiriama proliferacijos stadijai. Jeigu infantilinės hemangiomos raudonumas išlieka nepakitęs, fotografijose virš odos esanti dalis nedidėja ir echoskopijos duomenys rodo, kad IH gylis nekinta, pirmo vizito infantilinė hemangioma priskiriama plato stadijai. Jei infantilinės hemangiomos raudonumas blykšta, ji šviesėja, fotografijose virš odos esanti dalis išlieka nepakitusi ar mažėja ir echoskopijos duomenys rodo, kad IH negilėja arba mažėja, pirmo vizito infantilinė hemangioma priskiriama involiucijos stadijai. Paskutinio vizito duomenys nevertinami, nes mes nežinome, kaip infantilinė hemangioma toliau keisis, todėl šie duomenys naudojami tik priešpaskutiniam vizitui įvertinti. Tokiu būdu hemangiomos buvo suskirstytos į tris pogrupius: proliferuojančios, plato ir involiuojančios infantilinės hemangiomos. Šis skirstymas būdas pagal augimo stadiją labiausiai tiko tyrimo tikslams įgyvendinti. Morfologinė klasifikacija (pagal gylį ir pasiskirstymą paviršiuje) neleidžia išskirti augančių infantilinių hemangiomų.

Tiriamųjų infantilinių hemangiomų kohortos:

1. stebėtų hemangiomų grupė;
2. gydytų hemangiomų grupė.

Stebėtų ir gydytų hemangiomų grupių pogrupiai:

1. proliferuojančių hemangiomų;
2. plato hemangiomų;
3. involiuojančių hemangiomų.

Statistinės duomenų analizės metodai

Statistinė duomenų analizė atlikta „IBM SPSS 23 for Mac“, „Microsoft Excel“ ir „MedCalc“. Kokybiniams kintamiesiems aprašyti naudojami absoliutūs duomenų skaičiai (n) ir jų procentinė išraiška (proc.). Kiekybiniams kintamiesiems aprašyti naudojamas vidurkis, mediana, standartinis nuokrypis (SD), standartinė paklaida (SE) ir pasikliautinasis intervalas (PI). Tolydaus kintamojo ir kiekybinių kintamųjų skirstinio normalumas tikrintas Kolmogorovo ir Smirnovo testu. Dviejų grupių, kurių skirstinys buvo normalusis, vidurkiams palyginti taikytas Studento t kriterijus, esant daugiau grupių taikyta dvifaktorė dispersinė analizė (ANOVA). Jeigu skirstinys nebuvo normalusis, daugiau nei dviejų grupių kiekybiniams duomenims palyginti naudojome neparametrinį Kruskal–Wallis testą.

Kintamųjų ryšiui įvertinti naudojome vienmatę ir daugiamatė dvinarę logistinę regresiją. Prognostiniams faktoriams diagnostinis efektyvumas nustatytas naudojant ROC kreivių analizę – apskaičiuojant plotą po ROC kreive, jautrumą, specifiškumą, Youden indeksą, slenkstines reikšmes. Visame tyrime statistinio reikšmingumo lygmuo $a=0,05$, skirtumas statistiškai reikšmingas, kai $p<0,05$.

Imties dydžio skaičiavimas

Numatomo tyrimo imtis paskaičiuota pagal populiacijos dydį imties paklaidą (S.Bekešienė. Duomenų analizės SPSS pagrindai. Vilnius, Generolo Jono Žemaičio Lietuvos karo akademija, 2015). Imties dydis skaičiuotas pagal formulę

$$n = \frac{N \cdot 1.96^2 \cdot p \cdot q}{\varepsilon^2 \cdot (N-1) + 1.96^2 \cdot p \cdot q}$$

čia:

- N – populiacijos dydis, jis lygus 30 000 (vaikų gimstamumas);
- skaičius 1,96 yra standartizuoto normaliojo skirtinio reikšmė, kai a=0,0;5
- p yra numatoma įvykio tikimybė, kad požymis pasireikš tiriamojoje populiacijoje, jis lygus 0,05 (IH atsiradimo dažnis populiacijoje 4–5 procentai, mes imame didesnę reikšmę – 5 procentus);
- q yra numatoma įvykio tikimybė, kad požymis nepasireikš populiacijoje, q=1- p=0,95;
- ε yra pageidautinas tikslumas, jis lygus 0,05. Apskaičiuotas imties dydis n=74 pacientai.

Tyrimo rezultatai

Bendri tiriamųjų duomenys

Nuo 2013 iki 2017 metų Vaikų ligoninės, VšĮ Vilniaus universiteto ligoninės Santaros klinikų filialo, Vaikų chirurgijos centre į perspektyvųjį biomedicininį tyrimą įtraukta 102 pacientai. Šių pacientų abu tėvai ar globėjai susipažino su asmens informavimo ir informuoto asmens sutikimo forma ir ją pasirašė. Vieno paciento tėvai po pirmo vizito atsisakė dalyvauti tyrime. Trims pacientams buvo netinkamai atliktos termografijos, todėl jų duomenys nebuvo panaudoti. Tyrimo pabaigoje liko 98 pacientai.

Tyrime dalyvavo 73 mergaitės ir 25 berniukai. Iš viso vertinta 101 hemangioma (trys pacientai turėjo po dvi hemangiomas). Tiriamųjų pacientų hemangiomų klasifikacija pagal jų gylį ir pasiskirstymą paviršiuje pateikiama 1 ir 2 lentelėse.

1 LENTELĖ. Infantilinių hemangiomų klasifikacija pagal gylį

Gylis	Tiriamųjų skaičius	Procentai
Paviršinė	54	53,5
Mišri	47	46,5
Poodinė	0	0
Iš viso	101	100

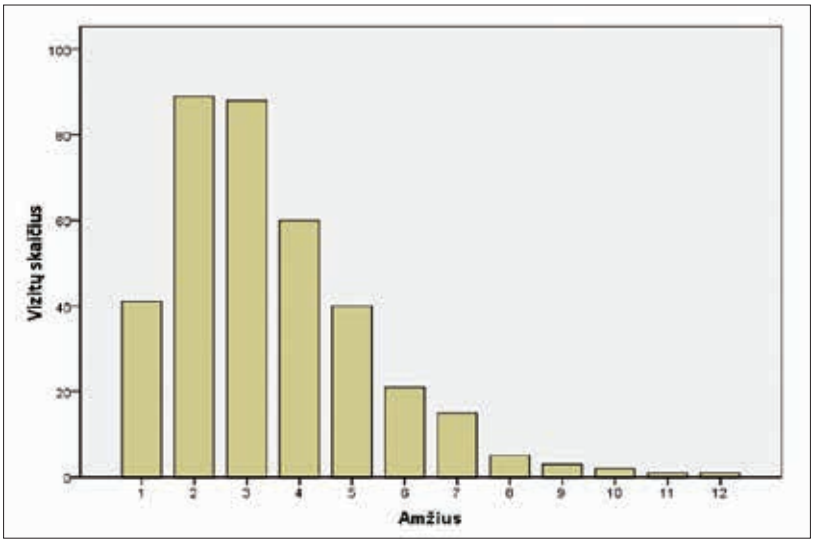
2 LENTELĖ. Infantilinių hemangiomų klasifikacija pagal pasiskirstymą paviršiuje

Pasiskirstymas paviršiuje	Tiriamųjų skaičius	Procentai
Fokalinė	72	71,3
Segmentinė	8	7,9
Neaiskių ribų	21	20,8
Iš viso	101	100

Tiriamųjų pacientų infantilinės hemangiomos dažniausiai rastos nugaroje (28 atvejais), rečiausiai – sėdmenyse (5 atvejais). Infantilinių hemangiomų anatominė lokalizacija pateikiama 3 lentelėje.

3 LENTELĖ. Infantilinių hemangiomų anatominė lokalizacija

Lokalizacija	Tiriamųjų skaičius	Procentai
Nugara	28	27,7
Pilvas	24	23,8
Krūtinė	18	17,8
Galva (plaukuotoji dalis)	18	17,8
Veidas	8	7,9
Sėdmenys	5	5
Iš viso	101	100



4 PAV. Vizitų skaičius pagal tiriamojo paciento amžių (mėnesiais)

Pacientai atvyko 513 vizitų, vidutiniškai kiekvienam pacientui teko 5 tiriamieji vizitai. Atmesti tie vizitai, kurių metu blogai atlikta infraraudonųjų spindulių termografija, ir atmestas kiekvieno paciento paskutinis vizitas (nes nesant dar vieno vizito, negalima buvo priskirti pogrupiui). Galutinai buvo vertinti 366 vizitai. Pirmo vizito metu amžiaus vidurkis buvo 1,6 mėnesio (mediana 2 mėnesiai). Vizitų skaičius kiekvieno amžiaus laikotarpiu pateikiamas 4 paveiksle.

Stebėtų pacientų grupėje vertintas 231 stebėjimų vizitas. Involiucija nustatyta 42 vizitų, plato stadija – 108 vizitų ir proliferacija – 81 vizito metu. Į gydytų infantilinių hemangiomų grupę įtraukti 32 pacientai, jie atvyko 135 vizitų. Proliferacija nustatyta 42 vizitų, plato – 49 vizitų ir involiucija – 44 vizitų metu. Duomenys pateikiami 5 ir 6 lentelėse.

5 LENTELĖ. Stebėtų infantilinių hemangiomų grupės pacientų vizitų skaičius

Stebėtų IH pogrupiai	Vizitų skaičius	Procentai
Proliferacija	81	35,1
Plato	108	46,7
Involiucija	42	18,2
Iš viso	231	100

6 LENTELĖ. Gydytų infantilinių hemangiomų grupės pacientų vizitų skaičius

Gydytų IH pogrupiai	Vizitų skaičius	Procentai
Proliferacija	42	31,1
Plato	49	36,3
Involiucija	44	32,6
Iš viso	135	100

7 LENTELĖ. Stebėtų ir gydytų infantilinių hemangiomų gyliai centimetrais

IH grupės	Vidurkis	±SD	95 % ± PI		P
Stebėti pacientai	0,61	0,42	0,55	0,66	<0,001
Gydyti pacientai	1,04	0,53	0,95	1,14	

Kiekvieno vizito metu pacientams buvo atlikta echoskopija ir išmatuotas IH gylis. Stebėtų pacientų grupėje gylių vidurkis buvo 0,61 cm, gydytų pacientų grupėje – 1,04 cm. Echoskopojos duomenys tenkino normalumo sąlygas (Kolmogorovo ir Smirnovo testas), kai p>0,05. Abiejų grupių vidurkiams palyginti taikytas Studento t kriterijus. Vidurkiai tarp grupių sta-

tistiškai reikšmingai skyrėsi (p>0,05). Duomenys 7 lentelėje pateikti vidurkiu, standartiniu nuokrypiu (±SD) ir pasikliautinuoju intervalu (95 proc. ± PI).

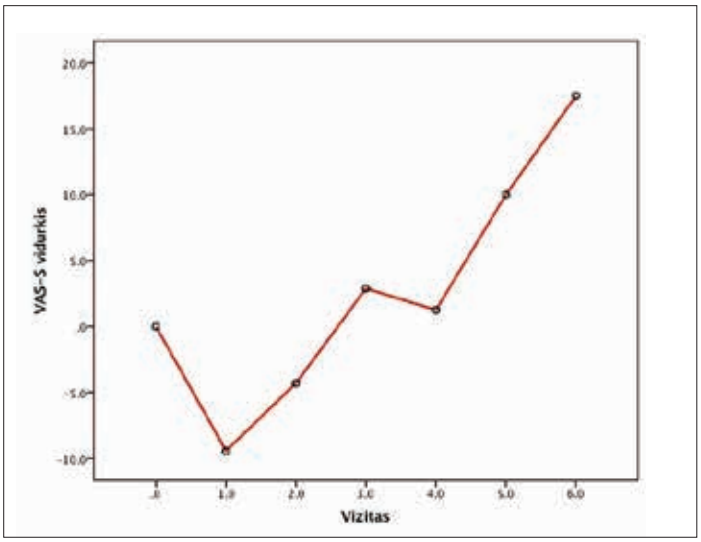
Pirmo vizito metu proliferacijos stadijos buvo 64 IH, plato stadijos – 28 IH, o involiucijos stadijos – 9 IH. Proliferacija dažniausiai nustatyta paciento pirmą ir antrą gyvenimo mėnesį – atitinkamai 26 ir 29 atvejais. Plato stadija pirmo vizito metu taip pat dažniausiai nustatyta antrą ir trečią mėnesį. Duomenys pateikiami 8 lentelėje.

8 LENTELĖ. Augimo stadija pirmo vizito metu

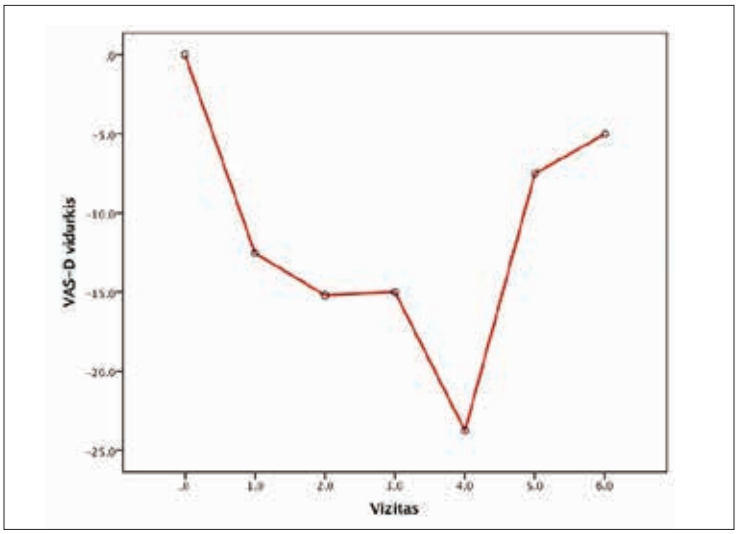
Amžius	Augimo stadija			Iš viso
	Involiucija	Plato	Proliferacija	
Iki 1 mėn.	0	0	2	2
1 mėn.	4	13	26	43
2 mėn.	3	11	29	43
3 mėn.	1	4	6	11
4 mėn.	1	0	1	2
Iš viso	9	28	64	101

Stebėtų IH grupė

Šią grupę sudarė pacientai, kurių IH proliferacija nebuvo labai aktyvi, hemangiomos augo mažai ir skirti gydymo neprireikė. Taip pat šioje grupėje buvo dalis pacientų, kurių IH atvykus pirmo vizito jau buvo plato stadijos. Proliferuojančių IH pogrupio pacientų amžiaus vidurkis buvo 2,33 mėnesio, plato – 2,73 ir involiuojančių IH – 3,81. Kadangi stebėtų infan-



9 PAV. VAS-S vidurkių kitimas per kiekvieną vizitą



10 PAV. VAS-D vidurkių kitimas per kiekvieną vizitą

tilinių hemangiomų grupėje nebuvo labai ilgo ir agresyvaus augimo, tai plato ir involiucijos stadijų amžiaus vidurkis nedidelis. Šioje grupėje keturių mėnesių amžiaus pacientams užregistruoti tik du atvejai, kai nustatyta proliferacija, vyresniame amžiuje proliferacijos nebuvo.

VAS pokyčiai stebėtų IH grupėje atitiko literatūros duomenis, kad IH iki 3-io mėnesio pasiekia 80 procentų savo dydžio. VAS-S ir VAS-D maždaug nuo ketvirto vizito (tai atitiktų ketvirtą penktą paciento gyvenimo mėnesį) pradeda aiškiai gerėti, tuo metu IH hemangioma pereina į plato stadija ir netrukus pradeda nykti. Šešto vizito metu VAS-D vidurkis yra -5 (IH dar išlieka didesnė, nei buvo pirmo vizito metu), o VAS-S pasiekė 17,5 (paviršius yra šviesesnis ir raudonio yra mažiau nei pirmo vizito metu). VAS-S ir VAS-D vidurkių pokyčiai per kiekvieną vizitą pateikti 9 ir 10 pav.

Analizuojant IH paviršiaus temperatūras, išmatuotas infraraudonųjų spindulių termometru (IS), nustatyta, kad proliferuojančių IH pogrupyje jų vidurkis buvo 36,81 °C (95 % ± PI, 36,73–36,89). Plato stadijos hemangiomų nustatytas temperatūrų vidurkis buvo 36,71 °C (95 % ± PI, 36,63–36,78), o involiucijos stadijos – 36,63 °C (95 % ± PI, 36,49–36,76). Tarp pogrupių temperatūrų vidurkiai statistiškai reikšmingai skyrėsi p=0,03. Tačiau temperatūros skyrėsi labai mažai, klinikinėje praktikoje tai būtų labai sudėtinga pritaikyti. Proliferacijos ir involiucijos stadijų temperatūrų vidurkių skirtumas tik 0,18 °C. Esant tokiam mažam skirtumui, atskirti proliferuojančią IH nuo involiuojančios, žinant tik paviršiaus temperatūrą, yra neįmanoma.

Matuojant infantilinių hemangiomų paviršiaus aukščiausias temperatūras infraraudonųjų spindulių termografu, šių temperatūrų vidurkiai skiriasi labiau. Proliferacijos stadijos hemangiomų paviršiaus aukščiausių temperatūrų vidurkis buvo 36,62 °C (95 % ± PI, 36,51–36,75), plato stadijos – 36,16 °C (95 % ± PI, 36,05–36,28) ir involiucijos stadijos – 36,03 °C

(95 % ± PI, 35,83–36,23). Tarp pogrupių temperatūrų vidurkiai statistiškai patikimai skyrėsi, p<0,001. Aukščiausių temperatūrų vidurkių skirtumai tarp pogrupių didesni nei matuojant IS termometru. Matuojant termografu IH aukščiausios paviršiaus temperatūros (PT) tarp proliferacijos ir involiucijos stadijų skyrėsi 0,59 °C. Šiuo būdu matuojant nereikalinga papildoma programinė įranga matavimo reikšmėms sužinoti, tai leidžia labai greitai įvertinti paviršiaus temperatūrą ir pagal gautus duomenis nuspręsti, ar reikia tolesnio ištyrimo duomenų jau naudojant „FLIR Tools" programą.

Paskesnė duomenų analizė atlikta su „FLIR Tools" programa. Buvo išmatuota IH paviršiaus vidutinė temperatūra ir apskaičiuoti jų vidurkiai. Proliferacijos stadijos hemangiomų temperatūrų vidurkis buvo 36,12 °C (95 % ± PI, 36,00–36,24), plato stadijos – 35,57 °C (95 % ± PI, 35,45–35,69) ir involiucijos stadijos – 35,49 °C (95 % ± PI, 35,31–35,67). Tarp pogrupių temperatūrų vidurkiai skyrėsi statistiškai patikimai, p<0,001. Skirtumas tarp proliferuojančių ir involiuojančių IH temperatūrų vidurkių panašus kaip ir aukščiausių temperatūrų vidurkių – 0,63 °C. Šie matavimo metodai panašūs, tik matuojant aukščiausias IH temperatūras nereikia papildomos programinės įrangos ir matavimo duomenys gaunami greičiau.

Labiausiai pogrupių duomenys skyrėsi, kai buvo skaičiuojamas IH paviršiaus temperatūros vidurkio ir aplinkinės odos paviršiaus temperatūros vidurkio skirtumas.

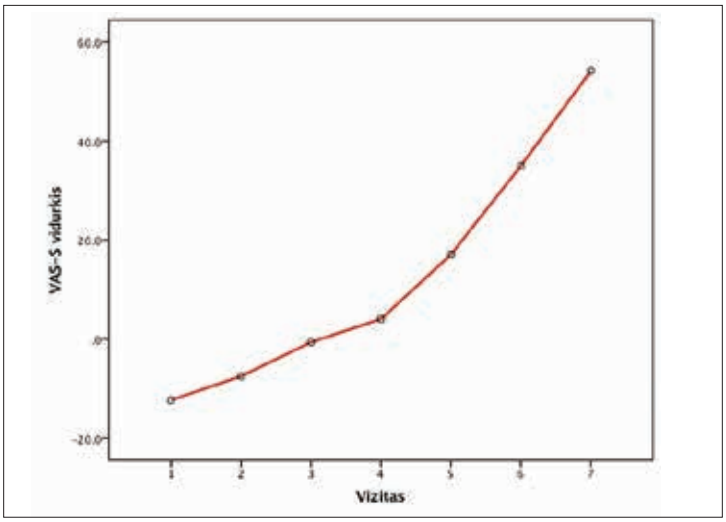
Proliferacijos stadijos hemangiomų šis temperatūrų skirtumas buvo 1,65 (95 % ± PI, 1,52–1,79), plato stadijos – 0,87 (95 % ± PI, 0,79–0,95) ir involiucijos stadijos – 0,87 (95 % ± PI, 0,75–0,99). Tarp pogrupių temperatūrų vidurkiai skyrėsi statistiškai patikimai, p<0,001. Proliferacijos ir involiucijos stadijų IH temperatūrų vidurkių skirtumas buvo 0,78, t. y. duomenys skyrėsi vos ne dvigubai. Įvertinę mūsų gautus stebėtų IH grupės duomenis, galime teigti, kad ši metodika leido tiksliausiai išskirti proliferuojančias IH.

Gydytų IH grupė

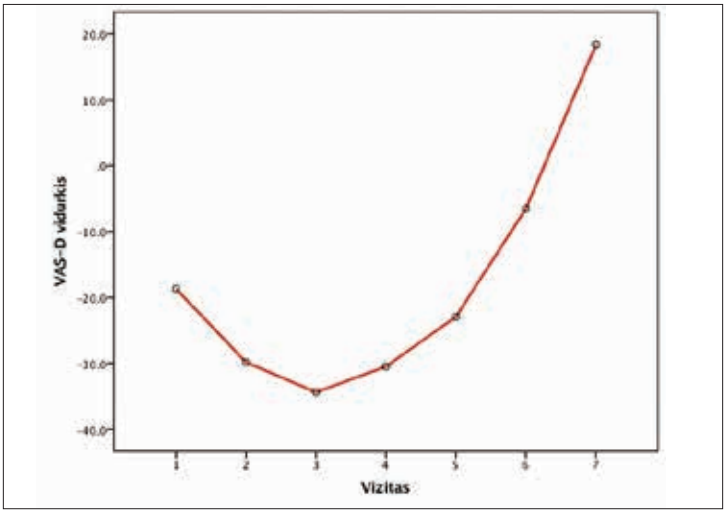
Iš stebėtų IH grupės 32 pacientai tyrimo metu buvo skirtas gydymas. Šie pacientai priskirti gydytų IH grupei. Atmetus du pacientus, kuriems blogai atlikta termografija, galutinai vertinta 30 pacientų. Jie atliko 135 vizitus. Amžiaus vidurkis šioje grupėje daugi didesnis – 4,6 mėnesio (proliferacijos pogrupio – 3,78, plato – 4,98 ir involiucijos – 4,91). IH gylis buvo daug didesnis nei stebėtų pacientų grupėje – vidutiniškai 1,05 cm (proliferacijos pogrupyje – 1,21 cm, plato – 1,0 cm ir involiucijos – 0,95 cm).

Gydymo progresą parodė VAS-S ir VAS-D vidurkių pokyčiai kiekvieno vizito metu. Šie abu matavimai yra subjektyvūs, labai priklausomi nuo tyrėjo patirties, tačiau literatūroje jis išlieka vienas iš pagrindinių IH pokyčių rodiklių.

Skyrus gydymą VAS-S kreivė vis kilo į viršų, VAS-S vidurkis 6 vizito metu buvo 35, o 7 vizito metu – jau 54,2. VAS-D duomenų vidurkiai pirmų vizitų metu taip greitai nekito, netgi 1 ir 2 vizitų metu ir IH dydis padidėjo, tačiau nuo 3 vizito kreivė kyla į viršų. Šešto vizito metu IH dydžio vidurkis dar -6,5, o 7 vizito metu – 18,3. Lyginant su stebėtų ir negydytų IH VAS-S ir VAS-D, gydytų IH pirmais vizitais buvo ir didesnės ir intensyvesnės spalvos, pradėjus gydymą gydytų IH grupėje gerėjimas buvo akivaizdžiai greitesnis. VAS-S ir VAS-D vidurkių pokyčiai per kiekvieną vizitą pateikti 11 ir 12 pav.



11 PAV. VAS-S vidurkių kitimas kiekvieno vizito metu



12 PAV. VAS-D vidurkių kitimas per kiekvieną vizitą

Išmatavus IH paviršiaus temperatūras IS termometru, nustatyta, kad proliferacijos pogrupio IH paviršiaus temperatūros vidurkis 37,13 °C (95 % ± PI, 37,00–37,24). Plato grupėje temperatūra kiek mažesnė – 37,06 °C (95 % ± PI, 36,95–37,16). Involiuojančių IH pogrupyje, termometrijos duomenimis, IH paviršiaus temperatūros vidurkis 36,92 °C (95 % ± PI, 36,76–37,11). Tarp grupių statistiškai reikšmingo skirtumo nenustatyta, p=0,066. Šiuo metodu nepavyko išskirti augimo stadijų.

IS termografu išmatavus aukščiausias IH paviršiaus temperatūras, nustatyta, kad proliferacijos pogrupyje temperatūrų vidurkiai buvo 37,05 °C (95 % ± PI, 36,93– 37,16). Plato pogrupyje vidurkiai buvo mažesni – 36,79 °C (95 % ± PI, 36,62–36,96). Involiuojančių IH paviršiaus temperatūrų vidurkiai – 36,52 °C (95 % ± PI, 36,25– 36,79). Temperatūrų vidurkiai tarp pogrupių statistiškai reikšmingai skyrėsi, p=0,006. Šiuo metodu pavyko išskirti pogrupius. Temperatūrų vidurkio skirtumas tarp proliferuojančių ir involiuojančių IH pogrupio yra 0,53 °C, jis šiek tiek mažesnis nei stabilių IH pogrupyje. IH paviršiaus vidutinių temperatūrų vidurkiai pogrupiuose pasiskirstė taip: proliferacijos pogrupyje temperatūros vidurkis – 36,52 °C (95 % ± PI, 36,37–36,67), plato – 36,26 °C (95 % ± PI, 36,08-36,45) ir involiucijos – 35,93 °C (95 % ± PI, 35,62–36,23). Tarp pogrupių duomenys statistiškai patikimai skyrėsi, p=0,007. Kaip ir ankstesniu metodu, galime išskirti pogrupius. Temperatūrų vidurkio skirtumas tarp proliferuojančių ir involiuojančių IH pogrupio yra 0,59 °C, jis panašus kaip ir stabilių IH pogrupyje. Išmatavus IH paviršiaus vidutinės temperatūros ir aplinkinės odos vidutinės temperatūros skirtumą, gauta, kad proliferacijos pogrupyje šis skirtumas buvo 1,85 (95 % ± PI, 1,66–2,04), plato – 1,46 (95 % ± PI, 1,28–1,65) ir involiucijos – 1,39 (95 % ± PI, 1,15–1,64). Pogrupių duomenys statistiškai patikimai skyrėsi, p=0,006. Tokio didelio skirtumo tarp proliferacijos ir involiucijos vidurkių, kaip nustatėme IH grupėje, neradome – čia šitas skirtumas buvo 0,46.

Gydytų IH grupėje temperatūrų skirtumai tarp pogrupių buvo mažesni, o matuojant termometru – net nenustatyti. Tiksliausias išlieka IH vidutinių temperatūrų ir aplinkinės odos vidutinių temperatūrų skirtumas. Šiuo metodu galima tiksliausiai nustatyti, ar jau prasideda plato stadija, kas reikštų, kad paskirtas gydymas yra veiksmingas.

Kaip parodė mūsų tyrimas, geriausias gydymo efektyvumo stebėjimo būdas – kiekvieno vizito metu atliktų temperatūros matavimų lyginimas tarpusavyje. IH mažėjant, šviesėjant kiekvieno vizito metu temperatūra vis mažėja. Ir visų matavimo metodų grupėse IH temperatūrų vidurkiai tarp vizitų statistiškai patikimai skiriasi. Norėdami įsitikinti, kad tai tikrai susiję IH gydymo pokyčiais, apskaičiavome kiekybinių kintamųjų tiesinio ryšio stiprumą tarp VAS-S, VAS-D ir temperatūrų. Visiems kintamiesiems nustatytas statistiškai patikimas vidutinis neigiamas ryšys. Koreliacijos koeficientai buvo nuo -0,44 iki -0,60. Tai rodo, kad mažėjant IH paviršiaus temperatūroms IH atsiranda nykimo požymių.

Atsižvelgiant į mūsų tyrimo duomenis, gydymo efektyvumą galima vertinti ir per kiekvieną vizitą matuojant temperatūrą (tinka visi mūsų naudoti metodai), ir matuojant paviršiaus temperatūras, išskyrus IS termometriją, nes šiuo metodu nebuvo galima išskirti gydytų IH pogrupių.

Stebėtų IH grupės proliferacijos rizikos veiksniai ir jų diagnostinė reikšmė

Šio tyrimo etapo pradžioje išskyrėme pagrindinius veiksnius, kurie gali turėti įtakos proliferacijos atsiradimui. Šiems veiksniams priskyrėme IH skersmenį ir gylį, paciento amžių ir mūsų išmatuotas temperatūras. Visų jų vidurkiai stebėtų pacientų grupėje statistiškai reikšmingai skyrėsi tarp pogrupių.

Atlikome ROC testą ir nustatėme visiems faktoriams slenkstines reikšmes. Proliferacijai atsirasti didžiausią įtaką daro IH paviršiaus vidutinės temperatūros (PVT) ir aplinkinės odos PVT skirtumas >1,3 (Youden=0,61, AUC 0,86, p<0,001), IH PVT (matuojant IS termografu) >35,8 (Youden=0,39, AUC 0,76, p<0,001), IHP aukščiausios temperatūros (matuojant IS termografu) >36,4 (Youden=0,38, AUC 0,74, p<0,001), IH gylis >0,75 cm (Youden=0,27, AUC 0,63, p=0,001), IH skersmuo >2,5 cm (Youden=0,32, AUC 0,64, p<0,001), paciento amžius £2 mėnesiai (Youden=0,29 AUC 0,66, p<0,001). Mažiausią įtaką turėjo IH PT (matuojant IS termometru) >36,7 (Youden=0,17, AUC 0,6, p=0,009). Tyrimo metu sudaryti logistinės regresijos modeliai atskirai termometrijos ir termografijos duomenims. Nustatyta IH temperatūrų įtaka proliferacijai atsirasti: IH PT (matuojant IS termometru) ŠS 2,9 (95 proc. PI 1,250–6,749, p=0,013, Nagelkerke R^2=0,313), IHP aukščiausios temperatūros (matuojant IS termografu) ŠS 5,8 (95 proc. PI 2,820–11,947, p<0,001, Nagelkerke R^2=0,414), IH PVT (matuojant IS termografu) ŠS 6,05 (95 proc. PI 2,997–12,227, p<0,001, Nagelkerke R^2=0,427), IH PVT ir aplinkinės odos PVT skirtumo (matuojant IS termografija) ŠS 54,4 (95 proc. PI 17,716–167,149, p<0,001, Nagelkerke R^2=0,644), IH skersmens ŠS 1,3 (95 proc. PI 1,072–1,606, p=0,008, Nagelkerke R^2=0,284), paciento amžiaus ŠS 0,65 (95 proc. PI 0,365–0,653, p<0,001, Nagelkerke R^2=0,284), IH gylio ŠS 1,9 (95 proc. PI 3,080–15,568, p<0,001, Nagelkerke R^2=0,284). Patikrinus faktorių tarpusavio koreliaciją, tik tarp IS termografijos duomenų ir IH gylio nustatytas vidutinis ryšys, Pearsono koreliacijos koeficientas – nuo 0,4 iki 0,53, p<0,001. Tarp likusių faktorių koreliacija silpna. Siekiant įvertinti galimą IH gylio, skersmens ar paciento amžiaus įtaką termometrijos ir termografijos duomenimis, buvo sudaryti tiesinės regresijos modeliai. Tačiau tinkami buvo tik du modeliai, kuriuose išanalizuota amžiaus, skersmens ir gylio įtaka IH PT aukščiausioms vertėms (R^2=0,208) bei IH PVT ir aplinkinės odos PVT skirtumų vertėms (R^2=0,305). Abiejuose modeliuose temperatūrų didėjimui statistiškai patikimai didžiausią įtaką turėjo gylis, IH PT aukščiausioms vertėms sudarytame modelyje IH gylio b=0,595, p<0,001, IH PVT ir aplinkinės odos PVT skirtumo modelyje b=0,7, p<0,001. Likusių modelių negalima vertinti, nes r^2<0,2. Apibendrinant galima pasakyti, kad tiksliausiai proliferaciją galima numatyti matuojant IH paviršiaus temperatūrą infraraudonųjų spindulių termografu. Patys tiksliausias metodai – apskaičiuoti IH PVT ir aplinkinės odos PVT skirtumą, IH PVT ir IH PT aukščiausias reikšmes.

Išvados

1. Didžiausia IH paviršiaus temperatūra (matuojant termometru) nustatyta proliferuojančių IH pogrupyje. Temperatūros tarp pogrupių skyrėsi statistiškai patikimai, tačiau tarp grupių temperatūrų vidurkių skirtumai labai nedideli, todėl, remiantis tyrimo rezultatais, klinikinėje praktikoje būtų tikslingiau remtis IS termografijos duomenimis.
2. Matuojant IH paviršiaus temperatūrą IS termografu, temperatūrų vidurkiai tarp pogrupių skyrėsi statistiškai patikimai.
3. Aukštesnė IH paviršiaus temperatūra, matuojama IS termografu, didina IH proliferacijos tikimybę. Visų matavimų duomenys skyrėsi tarp pogrupių statistiškai pati-

kimai. Tiksliausiai nustatyti IH proliferaciją galima matuojant IH PVT vidurkių ir aplinkinės odos PVT vidurkių skirtumą.

4. Gydomų IH perėjimą iš proliferuojančių į plato pogrupius galima stebėti IS termografu, nes termometrijos duomenys buvo statistiškai nepatikimi. Stebint temperatūros pokyčius per kiekvieną vizitą, matomas statistiškai patikimas IH temperatūros mažėjimas, kas rodo teigiamą gydymo dinamiką. Duomenys statistiškai patikimi matuojant IS termometru ir IS termografu.

Metodinės rekomendacijos

1. Atvykus IH turinčiam pacientui, reikėtų atlikti IS termografiją, pamatuoti IH PT aukščiausią temperatūrą. Jei temperatūra >36,4 °C, apskaičiuoti IH PVT ir aplinkinės odos PVT skirtumą. Jei šis skirtumas >1,3 ir IH yra veide, lytinių organų srityje, tarpvietėje, pasitarus su tėvais rekomenduojama pradėti gydymą. Jei tėvai nusprendžia stebėti, rekomenduojama kontrolė po dviejų savaičių.

2. Esant IH PT aukščiausiai temperatūrai nuo 36 °C iki 36,4 °C, rekomenduojama stebėti kas keturias savaites. Jei temperatūra mažesnė nei 36 °C, reikia atvykti kontrolės kas šešis mėnesius.

3. Skyrus gydymą, termografiją atlikti per kiekvieną vizitą. Jei temperatūra nemažėja ir nekinta IH spalva, konsistencija ir dydis, rekomenduojama koreguoti gydymą.

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THE IMPORTANCE AND CLINICAL APPLICATION OF MAGNETIC RESONANCE TOMOGRAPHY CONTRAST UNENHANCED SEQUENCES IN DIAGNOSTICS OF AORTA PATHOLOGY

MAGNETINIO REZONANSO TOMOGRAFIJOS BEKONTRASTINIŲ SEKŲ REIKŠMĖ IR KLINIKINIO TAIKYMO GALIMYBĖS AORTOS LIGŲ DIAGNOSTIKAI

Biomedicinos mokslai, Medicina (06 B)
Biomedical sciences, Medicine (06 B)

Abbreviations

3D-MRA three dimensional magnetic resonance angiography
AA ascending aorta
ABP arterial blood pressure
AO aorta
AS sinuses of aorta (Vansalva)
AT applanation tonometry
AVS aortic valve stenosis
b-SSFP balanced steady state free precession sequence
WBS white blood sequence
DA descending aorta
BAOV bicuspidal aortic valve
RA right atrium
RPA right pulmonary artery
RV right ventricle
EF ejection fraction
BBS black-blood sequence
C/M contrast media
BMI body mass index
LA left atrium
LPA left pulmonary artery
BSA body surface area
LV left ventricle
LVH left ventricular hypertrophy
LVOT left ventricular outflow tract
CNRc ontrast to noise ratio
MV mitral valve
MPR multiplanar reconstruction
MRA magnetic resonance angiography
PA pulmonary artery
PWV pulse wave velocity
PH pulmonary hypertension
SD standard deviation
SSFP steady state free precession sequence
STJ sinotubular junction
SNR signal to noise ratio
CT computed tomography
MRI magnetic resonance imaging
HR heart rate
US ultrasound examination (echocardiography)
TAOV tricuspid aortic valve
TEE transosophageal echocardiography
TTE transthoracic echocardiography
LGE late gadolinium enhancement
VUL SK Vilnius University Hospital Santaros Klinikos

1. Relevance of the study

In the last decade, the method of investigation - magnetic resonance imaging of the heart and large blood vessels - has been increasingly used in clinical practice. Because of many advantages, magnetic resonance imaging is widely used in many areas of diagnostics and one of them is the assessment of the aorta. This clinical method is very important for the detection of major vascular diseases, confirmation of clinical diagnosis and further evaluation and follow- up of treatment efficacy. Magnetic Resonance Angiography (MRA) with a contrast enhancement is a technique carried out using 3- dimensional (3D) fast gradient echo sequences after administration of gadolinium contrast media. This method allows to obtain ultra-high- quality vascular images and is considered a “gold standard”. In recent years,

magnetic resonance angiography with intravenous contrast has become one of the standard methods for evaluating the diseases of chest aorta and follow-up in many treatment and diagnostic centers over the world. Because of its high diagnostic accuracy for vascular pathology, rapid acquisition of images and ability to reconstruct images in 3D, magnetic resonance angiography has become an integral part of the research methodology not only in diagnosis, but also in the planning of further surgical or interventional therapies. Nevertheless, injection of intravenous contrast media and the possible undesirable consequences of this lead to a reconsideration of contrast unenhanced magnetic resonance imaging methods and their benefits in assessing cardiovascular diseases. In 2017 the use of gadolinium- containing linear contrasts in the European Union was limited, providing even greater need for alternatives to MRI angiography with contrast media. The doctoral dissertation presents an overview of the possibilities of native (contrast unenhanced) magnetic resonance imaging aorta examination and comparing it to the magnetic resonance angiography with a contrast agent for assessing the chest aorta and its segmental diameters, possibilities, advantages and disadvantages of these research methods, practical application possibilities.

Recent literature encourages the use of contrast unenhanced segmental analysis for measurement of lumen diameters in aorta, but the data are partially controversial and there is no unified contrast unenhanced aortic magnetic resonance imaging protocol in Lithuania. Intravenous contrast agents with gadolinium intravenous injection and the resulting undesirable effects motivate the study of contrast unenhanced magnetic resonance imaging methods and their benefits in assessing aortic diseases, diagnosis and treatment. In addition, there is an importance of an economical factor, as contrast enhanced MRI is more expensive due to the price of the contrast media itself and its supplemental equipment.

Possibility for measurement of aortic diameters without contrast media due to the good tissue contrast is one of the MRI strengths compared to other imaging modalities. However, MRI is also a functional imaging method that, as in the case of US, can be used to measure blood flow parameters in major blood vessels, including aorta, using Doppler technique. Aortic stiffness is the indicator of aging and pathological changes, as well as dependent factor to the central hemodynamics. Recent research has revealed that the MRI method is likely to be as successful as the ultrasound method (applanation tonometry) to be applied for pulsed wave velocity evaluation in aorta.

Studies have shown that, without using intravenous contrast in MRI studies, qualitative and quantitative parameters of thoracic aorta diameters do not underperform comparing to contrast enhanced MRA and other imaging methods or even surpass them in evaluating aortic wall changes. And while contrast enhanced MRA

images can be obtained much faster than in native (unenhanced) MRI examinations, the lower risk to the patient and the lower cost of the test are unquestionably the benefits of contrast unenhanced MRI. So far, most of the comparative studies in the segmental measurements of the chest aorta have been performed with a constant free spin sequence (SSFP) and magnetic resonance imaging angiography (MRA) with contrast media, and this fact should not be astonishing, as the SSFP has an advanced and high probabilistic test method with its high diagnostic value.

2. The aim of the study

To determine the practical application of MRI contrast unenhanced sequences in diagnosis of aortic diseases and to build up protocol for this study.

2.1 Objectives

1. Comparison of aortic contrast unenhanced MRI sequences with aortic contrast MRI angiography for the diagnosis of aortic dilatation in bicuspidal aortic valve (BAOV) and patients with primary arterial hypertension (PAH).
2. Comparison of aortic contrast unenhanced MRI sequences with aortic ultrasound (US) in diagnosing aortic dilatation in BAOV and PAH.
3. To evaluate the correlation of diagnostic accuracy of aortic stiffness in MRI comparing with applanation tonometry ("gold standard").
4. To evaluate the aortic stiffness diagnostic possibilities in the MRI method for patients with aortic pathology (BAOV and PAH) and control group patients.

3. Scientific novelty and practical significance

In cardiology patients, using an echocardiographic (US) method, due to poor echogenicity in patients or for other reasons, sometimes the cardiac and vascular anatomy cannot be reliably estimated; therefore, only two main alternatives - computed tomography (CT) and MRI remain for aortic dilatation diagnosis using imaging. The main disadvantages of the CT method are ionizing radiation and iodine-containing nephrotoxic contrast media. In the case of evaluation of the diameter of the aorta, the side effects of the CT examination are greater than the possible benefits of alternative methods.

MRI for aortic diameters measurement nowadays is also routinely performed using intravenous contrast media with gadolinium containing contrast agents. Following the ban on linear contrast media in 2017, MRI examination of aorta with no contrast media gets even more clinical significance. On the basis of dissertation's goal and results, we see real practical benefits and we suggest, in the case of routine cases in Lithuania, to apply a contrast unenhanced MRI method to the assessment of maximal measurements of aorta according to the methodology described by dissertation. Especially, when cardiovascular diseases in Lithuania and in the world are not decreasing and the ways are sought for the simplest and most economical, but reliable research of aortic pathological changes. The MRI method is particularly relevant as it is a non-invasive and non- ionizing radiation examination.

To date, comparative studies have not been carried out in Lithuania to analyze MRI aortic angiography with and without contrast media. In the world, there are only isolated aortic stiffness and PWV comparative studies between MRI and AT methodologies, and research in Lithuania on these issues is not yet available. In addition, we were one of the first to perform comparative measurements for aortic PWV evaluation not only in the aortic arc but also in the aorta. We believe that due to aging or pathological changes, the varying diameter and elasticity of the aorta (whose decrease leads to an increasing overload and promotes other pathophysiological changes) can also be evaluated using the MRI method without contrast media as an alternative to other methodologies (especially when they cannot be applied or the results are non-informative).

4. Principle statements for defence

1. MRI aortic measurements without contrast enhancement using "white blood" sequences in the axial plane allow statistically reliable and most accurate estimation of the degree of dilatation of aortic aorta and other segments in the case of TAOV compared to the "black blood" sequences.
2. The results of all methods of measurement for aortic sinus and ascending aorta in MRI better correlate in-between that with US measurements for both TAOV and BAOV.
3. The PWV measurements using MRI method are most accurate in the aortic arch and statistically reliably correlate with the AT method (the "golden standard").
4. The MRI methodology for determining the PWV in the arch reflects the age-related and pathological changes in stiffness of the aorta and has statistically significant gender difference in PWV, therefore may be an alternative to the AT technique.

5. Materials and methods

The study was conducted in accordance with the recommendations of the Vilnius Regional Biomedical Research Ethics Committee issued on 2013 February 12 and supplemented on 2014 April 8 (Annexes 1 and 2) with permission No. 158200-13-576-178 (Supplement No. 158200-576-PP1 -14). Study Type: Clinical Prospective Study.

The study included all conscious and willing to participate (with written consent) in the study of adult patients (18-75 years of age) with cardiovascular disease, who had been assigned to perform heart and aorta MRI at the Center for Radiology and Nuclear Medicine of Santaros Klinikos of Vilnius University from April 2014 to April 2017.

Subjects for research:

1. Patients with primary arterial hypertension (including resistant to medical therapy) with TAOV.
2. Patients with bicuspidal AOV.
3. Patients without structural heart disease and with tricuspid AOV served as a control group.

Inclusion criteria:

1. The aforementioned pathology has been diagnosed.
2. A grown-up patient (18-75 years old).

Exclusion criteria:

1. Patient's refusal to conduct an examination.
2. The patient is a minor or unable to express his will.
3. Vulnerable persons.
4. Contraindications for the MRI study.

Control group: In addition to no structural heart disease - heart and aorta in other imaging studies appears to be unchanged.

During the study, MRI examinations were performed for 136 subjects. All subjects were divided into 3 main groups according to the selection criteria:

1. Patients with resistance to treatment for primary arterial hypertension (PAH) who were undergoing MRI testing prior to renal artery sympatic denervation (RASD) and after 6 months post RASD. In the PAH group: 52 patients with a mean age of 53.3 ± 8.1 years, 28 men and 24 women.
2. Patients with a bicuspidal aortic valve (BAOV) who were undergoing studies for the changes of the BAOV and the patients with an average age of 30.8 ± 9.8, 31 males and 11 women.
3. The control group consisted of patients without structural heart disease and a tricuspid aortic valve (TAOV). TAOV group: 42 patients, mean age 36.6 ± 15.8, 26 men and 16 women.

5.1 Magnetic resonance tomography imaging protocol

Prospective study was performed using a 1.5 T Siemens Avanto (Germany, Erlangen) MRI machine using prospective ECG and breath holds (~15-20 s). All patients were examined in supine position. For a segmental and general left ventricle (LV) function evaluation, a special sequence of balanced steady state free precession sequences (b-SSFP) motion was used, characterized by a high signal- to-noise ratio (SNR), very good in blood and myocardial contrast ability and low sensitivity to motion artefacts. With the patient holding the breath, this cine sequence allowed to obtain images of four, three, two chamber views and short cardiac axes in 8 mm thick slices without any gaps (from 7 to 13 short axis slices).

Segmental diameters of the aorta were evaluated by "black blood" (HASTE) and "white blood" (TrueFISP, TRUFI) sequences as well with 3D contrast enhanced T1 isometric sequence.

Characteristics of the HASTE "black blood" sequence: field of view (FOV) - 420 mm, FOV phase - 75%, slice thickness - 6 mm, distance between slices - 0 mm, TR 649 ms, TE 42 ms, flip angle (FA) 160 °, volume element size - 2.2 × 1.3 × 6 mm.

Technical characteristics of TrueFISP "white blood" sequence: FOV - 340 mm, FOV phase - 87.5%, thickness of layer - 6 mm, distance between slices - 0 mm, TR 270 ms, TE 1.2 ms, FA 60 °, volume element (voxel) size - 2.1 × 1.3 × 6 mm.

Using phase velocity mapping sequence, the blood flow in aorta was scanned in 2 steps. The first slice was chosen so that the plane perpendicularly slices proximal ascending aorta (immediately above the sinotubular junction) and also covers the descending part of the aorta in the same plane. The scans in this plane were performed twice, and the result from the two measurements was averaged. The second slice was positioned in the distal part of the infrarenal aorta, perpendicular to the lumen, and immediately above the aortic bifurcation. The scan is also done 2 times. The result obtained from the two measurements was averaged.

Technical characteristics of the phase encoding sequence: field of view (FOV) - 320 mm, FOV phase - 69%, the thickness of slice - 5.5 mm, TR 47 ms, TE 1 ms, FA 30 °, the volume element size - 1.7 × 1.7 × 5.5 mm, 50 phases in 1 s (time resolution 20 ms / section. Note: according to international guidelines, the resolution time for flow analysis should be <30 ms/one slice, therefore our chosen sequence had a sufficient time resolution for recording a good quality flow curve). An analogous sequence was also applied for the determination of antegrade and retrograde flow through the aortic valve.

Aortic 3D angiography was performed using a T1 isometric sequence with 0.15 mmol/kg gadolinium containing gadofenetate dimeglumine or gadodiamide infusion via a brachial vein catheter using a special high-pressure automatic syringe.

The scan time after contrast injection was chosen for each patient individually measuring the hemodynamic curve by injection of 1 ml of contrast media. 3D T1 angiography sequence technical characteristics: field of view (FOV) - 500 mm, FOV phase - 66%, thickness of slice - 1.4 mm, TR 3 ms, TE 1 ms, FA 25°, volume element size - 1.4 × 1.3 × 1.4 mm.

10-15 minutes after injection of gadolinium gadofenetate dimeglumine or gadodiamide (both containing gadolinium) via a special high-pressure automatic syringe, late gadolinium enhancement (LGE) sequence was performed to exclude structural myocardial pathology. A special gradient-echo sequence with an inversion recovery was used to determine delayed accumulation of contrast 3.2 ms, FA 25°; field of view size (FOV) 400 mm; volume element size 2.1 × 1.6 × 8 mm, scanned by inhalation of the patient. In order to suppress the myocardial signal, the optimal time was chosen between a premature inverse 180° pulse and a 90° radio pulse (typically from 240 ms to 330 ms). Images were obtained in the same four, two, three chamber view and short LV axis planes, as well as motion images. The planes of both long and short cardiac axes were identical in both the cine and the LGE images. Total duration of MRI study according to the protocol was 45-60 min.

5.2 Magnetic Resonance Imaging post-processing analysis

The analysis of MRI images was carried out in accordance with the recommendations of the Working Group on Image Reconstruction. An independent analysis of MRI for cardiac morphology and function was performed in each case using Siemens Argus software (version 4.01, Germany). The advantage of our work is that the images of MRI were evaluated by two experienced researchers (>10 years of practice with MRI, blinded to other available clinical data). Both researchers are accredited at EuroCMR II level at the London UCL Hospital with prof. James Moon. The fact that the performance of the research and the results of the assessment are influenced by the experience of the specialist is proved by other research studies.

LV volumes and functional indicators are estimated from LV short axes (basis-to-apex images) using the Argus program (Siemens). The endocardium and epicardial margin of the systole and the end of the diastole were defined manually by the usual method. LV wall thickness was measured at the end of the diastole perpendicular to the wall. The gender and body surface area (BSA) of each patient was adapted to normalize left ventricular final systolic volume (LVESV), final diastolic volume (LVEDV), and LV mass index (LVMI). The area of the left atrium (LA) was evaluated in the four-chamber view using the most appropriate plane with the best coverage of the LA (selecting one of 3 slices in 4-chamber view). The contours of the LA plot area were defined manually during the final systolic phase of the LV.

For the analysis of blood flow in the aorta, dedicated software "ART-FUN" (version 1.0, Laboratoire d'Imagerie Fonctionnelle, France) was used to measure aortic flow measurements as well as to determine the segmental PWV. This software is validated by other clinical studies. The aortic flow areas were defined in a semi- automated manner.

The length of the aorta was evaluated from T1 isometric images after intravenous contrast using the Lumen track software (Siemens, VesselView). Meanwhile, the AorteGeo software package was used to evaluate the length of the aortic arch as the initial and final measuring points were placed in the ascending and descending aortic flow central positions in the phase contrast sequence, while the remaining points were localized in the longitudinal aortic phase contrast image (the latter was obtained using semi-automatic triangulation, when in the axial images 3 main points of the plane were localized - in the ascending and descending aorta and aortic arch, with the maximum choice of position close to the center of the lumen).

The segmental diameters of the aorta were evaluated from the 3D T1 contrast enhanced sequence from the reconstructed transverse planes using MPR, measuring the maximum diameter 2 times and choosing a larger measured result. We used aortic segmental system by T. Kaiser et al., measuring 5 main aortic segments: 1) at the level of the aortic sinus, 2) ascending aorta at the right pulmonary artery (RPA) level, 3) aortic arch in the T2 segment, or between the first two branches (if there were only two branches of the aortic arch), 4) descending aorta at the left pulmonary artery (LPA) level. In the same manner, the same segments were measured in "black blood" sequences and in "white blood" sequences measuring the largest dimension in the axial plane of the body. Measurements of the maximum segmental diameter of the aorta in the true axial plane of the body are easier for the inexperienced researcher and is suitable for a wide range of routine applications, especially for researchers who have not been able to apply multiplanar reconstruction (which also takes up additional time).

A delayed gadolinium enhancement (LGE) sequence was used 10-15 minutes after administration of the contrast agent, using a 0.15 mmol/kg dose of gadolinium DTPA and using an inverted gradient echo sequence. The LGE sequence was performed on the planes of both long and short cardiac axes as in the preceding cine sequences in order to exclude a possible previously unknown myocardial pathology (myocardial infarction, chronic changes after previous myocarditis, etc.). The pre-pulse delay (time before inversion - TI) was individually evaluated with a pre-pulse sequence. The contrast agent was contraindicated if the glomerular filtration rate (GFR) was <30 ml/min. Therefore, two patients were not included in the sample and pulse rate (PWV) in the whole aorta was also not evaluated. Two radiologist technologists who have >10 years of experience of performing MRI performed all MRI examinations.

After 6 months, the MRI was repeated in PAH patients using the same protocol.

5.3 Arterial stiffness studies using applanation tonometry

Aortic stiffness and central blood pressure are evaluated using the Sphygmocor (AtCor Medical, Australia) applanation tonometry system with high precision piezoelectric crystal micromanometer (Millar®, Millar Instruments, USA). Finding the pulse of carotid, radial and femoral arteries where at the sites where they are closest to the body surface and based on solid anatomical structures - in the wrists, neck and groin areas. In these places, a special piezoelectric transducer of the transverse tonometry is sequentially applied during the study. The special software recorded patient data: height, weight, age, peripheral arterial pressure and measured distances. Sequential recording of the pulse wave velocity curves of the proximal (i.e., common carotid artery) and distal arteries (i.e., radial and femoral arteries). The pressure and pulse volume curves were recorded for 20 seconds in each measurement series (each curve is synchronized with an electrocardiogram R peak) and the results of each measurement are averaged. The goal is to have a calculated researcher's index of software >85%. By recording these curves, data are analyzed, arterial stiffness parameters are calculated: pulse wave rate, augmentation index and central arterial blood pressure. The pulse wave velocity between carotid and femoral arteries, the "gold standard" for the evaluation of arterial stiffness, is calculated automatically by dividing the distance (measured by using a flexible ruler) from time (meters per second) and multiplying it by 0.8, as recommended by the European expert guidelines. Estimates are based on literature and age-related and sex-dependent normal values in the software of the device.

5.4 Ultrasound protocol

A two-dimensional (2D) cardiac ultrasound examination (US) was performed using a color Doppler ultrasound system using a 1.0 to 5.0 MHz sensor (GE Vivid 7; General Motors Corporation, New York, USA). The following morphological parameters were evaluated: the final diastolic thickness (IVSd) of the mediastinal partition, the diastolic thickness (LVPWd) of the left ventricular (LV) and left diastolic diastolic diameter (LVd) of the left ventricle (LV). Following formulas have been used for calculations: body surface area (BSA) = 0.0061 × height (cm) + 0.128 × weight (kg) - 0.1529 (m^2), left ventricle mass (LVM) = 0.8 × 1.04 × [(IVSd + LVPWd + LVd)*3-LVd*3]+0.6 (g), left ventricle mass index (LVMI) = LVM / BSA (g/m^2) and relative wall thickness (RWT) = (IVSd + LVPWd) / LVd * 6.7.

The LV ejection fraction (LVEF) was calculated using the Simpson BP method. The LV diastolic function evaluated the use of Pulse Doppler for assessing the maximum early diastolic flow velocity (E) and the maximum rate of atrial contraction

(A), E / A ratio, mitral valve (MV) A wavelength (Adur), E wave deceleration time (DTE), as well as tissue Doppler parameters with early (E ‘) and late (A’) mitral valve speeds (MV rings). The E / E ratio was calculated as the LV filling pressure index. The isovolemic relaxation time (IVRT) was measured from the end of the flow of the aorta to the start of the flow through the mitral valve with a parallel representation of the AOV and MV flow.

The aortic measurements were performed in a 3-chamber view. The maximum diameters of the aortic sinus and the proximal part of the ascending aorta were measured.

Circular images of 4 and 2 chambers were obtained by evaluating the atrial length and area evaluation (from the middle of the MV plane to the back wall). The maximum area and length of the left atrium (LA) was prior to the mitral valve opening, excluding LA auriculum and pulmonary veins. The LA area was calculated and indexed to BSA. The original images were saved and the blind data was later analyzed using EchoPAC software (GE Medical Systems).

5.5 Statistical analysis

Statistical computations were performed using STATISTICA 64 statistical package. All results are presented with 95% confidence intervals.

The results of the different pulse wave rate measurement methods (AT and MRI comparison were performed using the Bland-Altman method).

$$S(x,y) = \left(\frac{S_1 + S_2}{2}, S_1 - S_2\right)$$

The PWV measurements AT (S_1) and MRI (S_2) were performed independently and the resulting in 2n data array of data points. Each of the n samples is shown in the graph, assigning the average of both measurements as the abscissa (x-axis) value and the difference between the two values as the ordinate (y-axis) value.

The Pearson correlation coefficient (R) is calculated using the following method:

$$r = \frac{\sum_{i=1}^{n}(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^{n}(x_i - \bar{x})^2}\sqrt{\sum_{i=1}^{n}(y_i - \bar{y})^2}}$$

when n is the sample size, x_i and y_i are the sample individual sample sizes in the position, and the sample meanings are calculated according to the following formula:

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i$$

If the correlation between the individual tests was high (R = 0.7 or higher) then measurements were considered as having a good level of testing and repeatability.

Independent and unequal group t-test (Welch) method was applied for the statistically significant difference between the PWV and aortic diameters for the determination of the groups. The t-test for groups whose averages and variations are different were calculated using the formula:

$$t = \frac{\bar{X}_1 - \bar{X}_2}{s_{\bar{\Delta}}} \quad \text{, where} \quad s_{\bar{\Delta}} = \sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}$$

4. Results

After measurements of the aortic segments before and after 6 months, the PAH group revealed a very high repeatability of the measurements, both with contrast enhanced and contrast unenhanced methods. Although the measurements of all segments strongly correlated with the statistical significance ($p < 0.05$) in the contrast enhanced 3D-MRA method (in the aortic sinus region R = 0.92, in the ascending aorta R = 0.92, in the aortic arch R = 0.93, in the descending aorta R = 0.83 and in the diaphragmatic aortic part R = 0.91), but the strongest correlation was determined using the method of "white blood" sequences (in the aortic sinus region R = 0.94, in the ascending aorta R = 0.94, in the aortic arch R = 0.94, in the descending aorta R = 0.93 and diaphragm aortic part R = 0.94). These findings correspond to Krishnam and co. performed studies with 50 patients, when no significant difference was observed in the measurements of aortic segments between MRA with contrast media and "white blood" sequences. Although the Koktzoglou study found that the precision of measurement in the aortic sinus region in the "white blood" sequence was lower than in MRA with contrast, but using our "white blood" sequences, we obtained good reproducibility results in all segments. Meanwhile, using the measurements of "black blood" sequences, the worst correlation was found in the aortic sinuses (as in the Koktzoglou study) and diaphragmatic segments: in the aortic sinus region R = 0.88, in the ascending aorta R = 0.95, in the aortic arch R = 0.96, in the descending aorta R = 0.93 and in the diaphragmatic aortic part R = 0.87 ($p < 0.05$). It should also be noted that measurements of the maximum segmental diameters of aorta in the normal axial body planes (as we suggest in our methodology) are simpler for an inexperienced researcher and suitable for a wide range of routine applications, and are statistically insignificantly different from the "golden standard" (MRA with contrasting media) and also good reproducibility results have been achieved in the TAOV group. From the results obtained, it can be seen that

although the "black blood" and "white blood" sequences are statistically reliably and strongly correlated with each other, but somewhat better reproducibility is observed in "white blood" sequences, therefore, when measuring aortic segmental diameters in the case of TAOV, without the contrast media, we recommend use these sequences. From 2017 July 20

Gadolinium containing linear MRI contrast media in the European Union and in Lithuania are no longer used. Since deposition of linear contrasts in the brain has been established since 2017, aortic MRI study (as in other body areas) without contrast media has gained even more clinical significance. Based on our work results, we see real practical benefits, and we suggest in Lithuania, in the case of routine cases, to apply a contrast unenhanced MRI method using "white blood" MRI sequences to evaluate the maximum measurements of aortic segments. The contrast unenhanced MRI 3D-SSFP with ECG synchronization during the study could not be applied due to technical limitations. This sequence is increasingly being applied in the world due to the availability of new MRI methods due to the updating of the hardware base, but it is not yet applicable in Lithuania. Therefore, we believe that our study will help reduce the number of aortic angiography in Lithuania with contrast media, until the next decade, it is likely in Lithuania that we will be able to fully switch to contrast unenhanced 3D angiography for aortic diameters and PWV measurements.

In the BAOV group, aortic contrast enhanced and contrast unenhanced measurements had the weakest correlations in the aortic sinus area. This can be explained by both the physiological pulsation of the aorta root (especially if ECG synchronization is not applicable) and a significant discrepancy between US and MRI measurements due to the asymmetry of the BAOV sinus. A significant discrepancy in the measurement of the size of the BAOV sinus with MRI and US methods was found during the initial study data collection. After analyzing the reasons, it was found that the difference is most likely due to the difference in measurement methodology. The MRI minimum and maximum diameters of aorta sinuses (AS) were determined by evaluating the image of a short-axis reconstructed by MPR, while the US plane is routed to AS from a 3-chamber image whose plane generally coincides with the smaller AS-dimension. Having detected the disadvantages of US methodology, VUL SK specialists in congenital and acquired heart disease took into account these differences in methodologies and started (in the case of BAOV and normal TAOV) measuring US diameters of the AS in a short axis image corresponding to the MRI reconstruction plane.

After analysis of aortic measurements in US and MRI studies, the following results were obtained: in the PAH group, US aortic sinus measurements correlated most closely with "white blood" sequences ($R = 0.9$, $p < 0.05$), weaker with 3D MRA with c/m ($R = 0.88$, $p < 0.05$), and the worst result was in "black blood" sequence

(R = 0.83, p<0.05). The "black blood" sequence is usually used as an additional assessment of the morphology of the aortic wall, but with pulsation of the wall, we have found that it is more difficult to estimate it's true wall position than in "white blood" sequences - this could be one of the main reasons, why in our results, in most cases, better results were obtained with "white blood" sequences. The US measurements of the ascending aorta strongly correlated with the "white blood" sequences and with the 3D MRA with c/m (R = 0.86, p <0.05). Minimal, but stronger correlation was observed in measurements with "black blood" sequences (R = 0.85, p <0.05). From the results obtained, it is seen that the strongest correlation in the PAH group was found between the US and "white blood" sequences. Since this trend has been established both between different MRI sequences (comparing sequences with and without c/m) and between different methods (US vs. MRI), therefore, in the measurement of aortic segmental diameters in TAOV, we recommend using "white blood" sequences without contrast media.

In the BAOV group, aortic sinus US measurements were most strongly correlated with "black blood" sequences (R = 0.86, p <0.05), weaker with 3D MRA with c/m (R = 0.80, p <0.05) and the worst result was with "white blood" sequences (R = 0.77, p <0.05). In the BAOV group, the ascending aorta US measurements correlated strongly with the 3D MRA with c/m (R = 0.70, p <0.05), much weaker with "black blood" sequences (R = 0, 68, p <0.05), and the worst result was with "white blood" sequences (R = 0.60, p <0.05). From the results, it is seen that the lowest correlation in the BAOV group (unlike PAH) was found between the US and "white blood" sequences for both aortic sinus and ascending aortic measurements. According to some authors, the aortic diameters are measured most accurate in "white blood" sequences because of the excellent contrast between the aortic wall and the lungs, but this trend was not observed only in the BAOV group.

Analyzing the results, we found that the results of the US measurements were more varied than in the MRI. We believe that this is mainly due to the fact that the US measurements were performed by different researchers and is more researcher-dependent, which results in a greater variation between the research methodology, the researchers' experience and the results of the measurements.

Measurements of applanation tonometry and MRI for the PWV in the aorta statistically reliably, but weakly correlated (R = 0.35, P <0.05). According to the linear regression equation, we have found that PWV_AT = (PWV_MRI – 3.58) / 0.49 (R = 0.354, P<0.05). A statistically significant correlation between PWV in the whole aorta and AT was not obtained (R = 0.225961, P> 0.05), but the determination of PWV by MRI method in the aortic arch and in the whole aorta was statistically reliably correlated (R = 0.306, P <0. 05). The determination of PWV in the aortic arch is performed in one scan, so, as we have found, this measurement

method is more precise than setting the PWV throughout the aorta. The method for determining the PWV in the whole aorta determines the time interval according to the blood flow curves recorded from two separate sequential scans, which is likely to significantly increase the error comparing to the one-time scan flow curves, and therefore the resulting measurement has sufficiently significant higher hardware and/or measurement errors. The AT PWV measurements statistically reliably correlate with the age of patients ($R = 0.354$, $P < 0.05$) as also has been determined by Doupis and co-authors. Bland Altman's analysis showed that the mean total PWV measurements between the AT and MRI in aortic arch were 1.46 m/s higher in the AT methodology. Jehill D. Parikh research have published similar data as our analysis. Their measured PWV values were measured using the MRI method and were found to be lower than in the AT method. The values of our MRI PWV were on average 1.46 m / s lower compared to 1.6 m / s in D. Parikh's studio, which indicates a similar difference between AT and MRI. The PWV measurements by the MRI method in the PAH group, the BAOV and the control group have found statistically significant difference for the PWV ($p<0.01$) between PAH and control groups, as well as between PAH and BAOV groups. There was no statistically significant difference between the BAOV and the control group. This could be explained by the fact that changes in PWV depend more on age than on aortic and pathological changes in AOV at younger age. In addition, in all 3 groups, PWV was statistically significantly higher in males ($p < 0.01$) than in women as in previously published healthy population studies.

In our study, accurate anthropometric measurements of the total length of the aorta were also measured using MRI. The total length of the aorta had statistically significant difference ($p < 0.01$) in all 3 groups. Longer aortic length of the PAH group may be explained by the loss of elastic properties of the aorta with age due to the aging process and PAH (106). Meanwhile, the statistically significant difference between the lengths of the aortic BAOV and the control group is likely due to the fact that patients with BAOV loose elasticity in aortic wall at a younger age than those with TAOV.

When measuring aortic flow velocity in different aortic segments, an accidental finding was detected - in all 3 groups, the average flow rate of the distal abdominal aorta was practically identical (~1.97-1.99 m/s) and had a significantly lowest standard deviations than in the remaining aortic segments. This interesting finding predisposes the hypothesis that, irrespectively of aortic stiffness and other indicators of hemodynamics, the aortic flow in the distal part of aorta is bound up to ~ 2 m/s. In literature, we did not find work (in vivo or in vitro) that would explain the convergence of such blood flow rates, which could be the subject of new scientific studies.

5. Conclusions

1. Using contrast unenhanced magnetic resonance imaging sequences, the most reliable of all aortic segments can be measured the diameters of an ascending aorta and the choice of priority is to apply "white blood" sequences for better accuracy of results compared to the "gold standard" - magnetic resonance angiography with contrast media.
2. The results of the measurements of magnetic resonance imaging of the aortic sinuses and the ascending part of aorta were more strongly correlated in-between than with the ultrasound measurements with both tricuspid and the bicuspid aortic valve. Therefore, we determined that ultrasound measurements of the aortic sinus and the ascending part have lower diagnostic accuracy than in magnetic resonance angiography (both in contrast enhanced and unenhanced sequences).
3. Pulse wave velocity measurements using the magnetic resonance tomography technique are the most accurate in aortic arch and statistically reliably correlate with measurements using the applanation tonometry method with a 1.46 m/s difference between the methods. Therefore, the method of measuring pulse wave velocity with the magnetic resonance imaging in aorta is considered as an alternative to applanation tonometry study.
4. The method of magnetic resonance imaging has statistically reliably determined pulse wave rate differences in the test groups that reflect the age-related, sex-related and pathological changes in stiffness in the aorta; therefore, it is proposed that the methodology should be used to determine aortic pulse wave velocity in both healthy population and patients with aortic pathology.

6. Practical recommendations

1. It is recommended to apply a magnetic resonance imaging in accordance with the methodology we have developed and proposed (see Magnetic Resonance Imaging Protocol), with the priority of "white blood" instead of computer tomography or in the absence of cardiac ultrasound, for the detection and follow-up of maximum diameters of aorta using contrast unenhanced methodology.
2. It is recommended to use the contrast unenhanced method of magnetic resonance imaging to apply for pulsed wave velocity determination in the aortic arch as an alternative to the applanation tonometry study.
3. It is recommended to evaluate the diameters of the bicuspidal aortic valve in short-axis images in all imaging techniques, in order to avoid measurement inaccuracy due to congenital aortic sinus asymmetry.

List of author publications, summaries and notifications

ISI Publications

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Abstracts

1. Renal sympathetic denervation effectiveness evaluation using magnetic resonance imaging. Darius Palionis, Andrius Berukstis, Nerijus Misonis, Ruta Kliokyte, Kamile Cerlinskaite, Diana Zakarkaite, Ligita Ryliskyte, Jelena Celutkiene, Nomeda Valeviciene, Aleksandras Laucevicius. 61st Annual Conference of the Israel Heart Society in Association with the Israel Society of Cardiothoracic Surgery. 2014. Tel Aviv, Israel.

2. ECR 2017. Routine unenhanced MRI for maximal diameters of thoracic aorta?

Oral presentations

1. Baltic Congress of Radiology 2014. Congenital vascular malformations using MRI. Parnu, Estonia.

2. Radiology 2015. Vilnius, Lithuania. Changes in morphology and hemodynamics of left ventricle and aorta after sympathic denervation of renal arteries.

3. Multimodality Imaging in Cardiology 2016. Cardiac CT: principles, role and future perspectives.

4. Ultrasound 2016. Should we make MRI immediately?

5. Evolutionaty medicine: prie-existing mechanisms and patterns of current health issues 2016. Cardiovascular Magnetic Resonance (CMR) of Left Ventricle (LV) and Aorta in Patients prior and after Renal Artery Sympatic Denervation (RASD). Best phD student oral presentation award.

6. Baltic Congress of Radiology 2016. Measurement of segmental diameters and pulse wave velocity using contrast unenhanced MRI.

Andrius Berūkštis

AN EVALUATION OF THE EFFICACY OF CATHETER-BASED RENAL SYMPATHETIC DENERVATION FOR TREATING RESISTANT ARTERIAL HYPERTENSION

REZISTENTIŠKOS ARTERINĖS HIPERTENZIJOS GYDYMO, TAIKANT PERKATETERINĘ SIMPATINĘ INKSTŲ ARTERIJŲ DENERVACIJĄ, EFEKTYVUMO VERTINIMO TYRIMAS

Biomedical Sciences, Medicine (06B)
Biomedicinos mokslai, medicina (06 B)

Abbreviations

ACEI – angiotensin converting enzyme inhibitor
AH – arterial hypertension
BP – blood pressure
ARA – angiotensin receptor antagonists
ARB – angiotensin II receptor blockers
AlB – Alpha blockers
BB– Beta blockers
CAD – centrally acting drugs
H/M – heart-to-mediastinum ratio
RSD – renal sympathetic denervation
MIBG – metaiodobenzylguanidine
PWV – pulse wave velocity
PWVc-f – carotid-femoral pulse wave velocity
RF – radiofrequency ablation
RAAS – renin-angiotensin-aldosterone system
ROC – receiver operating characteristics
SNS – sympathetic nervous system
HF – heart failure
CCB – calcium channel blockers
BMI – body mass index
LV – left ventricle
LVH – left ventricular hypertrophy
MRI – magnetic resonance imaging
WR – washout rate

1. Introduction

1.1 The Problem and Relevance of the Study

Arterial hypertension (AH) is a very common and dangerous disease. Without treatment, it leads to a target-organ damage and can cause death. Arterial hypertension is one of the most common risk factors for mortality worldwide. The prevalence of AH has reached a pandemic level. In 2010, 1.39 billion (or 31.1%) of the world's adults had hypertension. From 2000 to 2010, the prevalence of hypertension increased by 5.2% (1). If in 2004 hypertension had been estimated to cause 7.5 million deaths worldwide (2), this number reached 9.4 million in 2010 (3). Since arterial hypertension is the disease of civilization, determined by genetic and environmental factors (obesity, high salt intake and alcohol consumption, sedentary lifestyles, emotional stress etc.), the numbers above are probably going to grow even more due to population ageing, the sedentary lifestyle and malnutrition.

Blood pressure (BP) is a continuous, strong, independent and etiologically directly related risk factor for cardiovascular and renal diseases (4). In 2001, about 54% of strokes and 47% of ischemic heart disease events worldwide were attributed to elevated BP (5). Each difference of 20/10 mm Hg usual BP results in a twofold difference in the death rates from cardiovascular diseases (6).

There is a wide choice of drug therapies for AH. A number of different antihypertensive medication groups have been developed to treat AH since the introduc-

tion of diuretics in the 1950s. Usually, one antihypertensive medicine or a combination of several antihypertensive agents is used in treating AH. Many research studies have found that effective medication-based treatment for AH involves lowering blood pressure and reducing the risk of target-organ damage and cardiovascular diseases. However, in real life, it means a long-term treatment requiring good control and continuous adjustments. Findings have shown that only 32.5% of patients treated with medications for AH reach good BP control (BP lower than 140/90 mm Hg) (7).

The failure to reach blood pressure goals with at least three anti-hypertensive medications, including a diuretic, is defined as resistant arterial hypertension (RAH). Complicate diagnostics of RAH make it difficult to measure the exact prevalence of RAH among patients with diagnosed AH. In observational studies and trials, this number ranges somewhere between 12 and 15% (8). Patients with RAH belong to a group known to be at a very high risk. A hazard ratio for stroke, ischemic heart disease and all-cause mortality in patients with RAH versus ordinary AH is 1.25, 1.69 and 1.29, respectively (9).

In 2009, a new interventional treatment method – catheter-based renal (artery) sympathetic denervation (RSD) – emerged as a potential treatment for RAH. This form of treatment is used in addition to drug therapy. Studies on the efficacy of RSD have yielded different findings. It is not clear to date what type of patients benefit most from the treatment with catheter-based RSD and why some patients have no changes in their blood pressures after the procedure. One of the largest trials on catheter-based RSD, the Symplicity HTN-3 trial, did not confirm findings in favor of catheter-based RSD for BP lowering as compared to the sham-control group (10). This trial has generated considerable criticism for patients' enrollment criteria, the correctness of the antihypertensive regimen and the possibly insufficient quality of catheter-based RSD. As patients with secondary arterial hypertensions have very similar symptoms to those with RAH, the criteria of patients' enrollment are very important in order to ensure the efficacy of catheter-based RSD. In addition, there is no method allowing an assessment of the performed procedures in day-to-day clinical practice. Accordingly, it is impossible to assess the effectiveness of the destruction of renal nerves during or after the catheter-based RSD procedure.

Patients with RAH are at very serious risk in terms of target-organ damage. The observed reduction in the left ventricular hypertrophy after the catheter-based RSD procedure (11, 12) is an important prognostic marker. The activation of the SNS plays an important role in the development of LVH. Research is necessary to investigate the effects of catheter-based RSD on cardiac sympathetic innervation.

To answer the questions above, this research must cover a wide range of post-catheter-based RSD studies.

1.2 Research Hypothesis

With strict selection criteria, catheter-based renal sympathetic denervation considerably reduces sympathetic nervous system activity and aortic stiffness in patients with resistant arterial hypertension, hence lowering blood pressure and reducing target-organ damage.

1.3 Research objective

To evaluate early and late changes in the sympathetic nervous system by measuring pulse wave velocity, cardiac 123-I MIBG and their effects on blood pressure and target-organ damage after catheter-based renal sympathetic denervation in patients with resistant arterial hypertension.

1.4 Research tasks

To analyze the effects of catheter-based renal sympathetic denervation on blood pressure in patients with resistant arterial hypertension at 3, 6, 12 and 24 months after the procedure on the basis of office blood pressure readings and 24-hour ambulatory blood pressure monitoring.

To compare changes in blood pressure in a catheter-based renal sympathetic denervation group versus a medication-based control group after 6 months on the basis of office blood pressure readings and 24-hour ambulatory blood pressure monitoring.

To assess aortic and peripheral arterial stiffness by measuring pulse wave velocity changes in the early post-intervention period (24–48h) and at 3, 6, 12 and 24 months after the procedure.

To assess changes in cardiac sympathetic innervation with cardiac 123-I MIBG at 6 months after the catheter-based renal sympathetic denervation procedure.

To assess changes in target organs in the late post-intervention period by echocardiography, cardiac magnetic resonance tomography and by measuring pulse wave velocity.

1.5 Statements to be Defended

1. With a strict selection protocol, catheter-based renal sympathetic denervation considerably lowers blood pressure in patients with resistant arterial hypertension.
2. The effects of catheter-based renal sympathetic denervation manifest already in the early post-intervention period.

3. Catheter-based renal sympathetic denervation leads to a reduction in cardiac sympathetic innervation.
4. Catheter-based renal sympathetic denervation reduces target-organ damage.

1.6 Novelty of the Study

To date, there is no test available in clinical practice to assess changes in SNS in the early post-intervention period. Arterial stiffness, assessed by measuring the PWV, has been found to be dependent on SNS activity and to decrease considerably at 1, 3 and 6 months after the procedure (13). However, there is no test that would assess early changes in the PWV (after 0–48 hours).

Many studies have assessed changes in BP after catheter-based RSD, but human-based trials involving the direct assessment of sympathetic innervation changes are very scarce. A reduction in SNS activity after catheter-based RSD has been found on the basis of local noradrenaline release (14) and microneurographic measurements (15–17). However, there is a lack of data on changes in cardiac sympathetic innervation after renal artery denervation. We assessed changes in cardiac sympathetic innervation on the basis of cardiac scintigraphy with 123I-metaiodobenzylguanidine (MIBG).

This study also differs from other studies on RSD by patients' enrollment. In order to ensure the efficacy of catheter-based RSD, a detailed randomization protocol was designed (including laboratory hormone analysis, CT, MRI, adrenal vein sampling, polysomnography etc.) not only to examine patients for any evidence of secondary AH but also to adjust and control their drug therapies.

2. Methodology

This prospective cohort study was carried out at the Vilnius University Hospital Santaros Klinikos (VUL SK). The study was authorized by the Vilnius Regional Biomedical Research Ethics Committee (No. 158200-13-641205). The study enrolled patients with resistant hypertension who failed to achieve a BP target at least one month after treatment adjustment and who were scheduled for the catheter-based renal denervation unless contraindicated to the procedure. Prior to formal enrollment in the study, all interested patients signed an informed consent form.

Enrolled patients were interviewed, and the anonymized data on their health records were collected in a database specially designed for the study. The collected data included epidemiological information (age, sex, smoking history, the presence of diabetes, AH, dyslipidemia, renal failure, coronary heart disease) and instrumental analysis data.

2.1 Enrollment protocol

Patients with diagnosed or suspected RAH were referred for a cardiologist's assessment at the Vilnius University Hospital Santaros Klinikos. During the initial visit, patients were asked questions to determine risk factors and obtain information on their medical history information, current and past drug use and any adverse reactions to drugs (intolerances or allergies). Based on the patient's anamnesis, objective data and available laboratory analysis, a further test design for secondary hypertension was chosen.

During the initial visit, patients were recommended to follow certain lifestyle changes (low-salt diet, low alcohol consumption, weight loss, regular physical activity, quitting smoking) and drug therapies were adjusted in accordance with the 2013 ESH/ESC Guidelines for the management of arterial hypertension (18).

The second visit was appointed one month after the initial one. Office and 24-hour ambulatory BP was measured in patients who followed the prescribed medical regimens. Eligible patients with office BP >140/90 mm Hg and/or 24-hour ambulatory BP > 130/80 mm Hg during the second visit were explained the possible benefits and risks of catheter-based RSD intervention. Patients who agreed to participate in the study signed an informed consent form. Those who refused the catheter-based RSD procedure were offered participation in a control group.

Out of the 537 study candidates, 73 patients were enrolled in the main intervention group, and 21 patients were enrolled in the control group. The most frequent reason for exclusion was pseudo-resistance (24-hour BP < 130/80 mm Hg one month after treatment adjustment). Patients with diagnosed secondary AH and those who refused to participate were also excluded from the study.

2.2 Patient inclusion criteria

1. Female and male patients aged > 18;
2. Patients with RAH whose office BP was > 140/90 mm Hg and/or 24-hour ambulatory BP was >130/80 mm Hg one month after treatment adjustment;
3. Patients having signed a Patient Informed Consent Form and agreed with the intervention procedures.

2.3 Patient exclusion criteria

1. A diagnosis of secondary AH for which other forms of treatment are available;

2. A post-renal transplant or patients on dialysis;
3. Hypotensive episodes in anamnesis (systolic BP < 100 mm Hg);
4. A severe heart-valve problem when low BP can be life-threatening;
5. An acute coronary syndrome occurring less than a month ago;
6. A severe concomitant disease, e.g., malignancy (with survival up to 1 year);
7. Pregnancy or breastfeeding;
8. Participation in other studies of medical devices or medicinal products at the same time.

2.4 Follow-Up Protocol

Patients who met the inclusion criteria and signed the informed consent form underwent the following additional medical examination before catheter-based RSD: 24-hour BP monitoring and office BP measurement, echocardiography, applanation tonometry for the measurement of arterial stiffness and central hemodynamics, MRI and cardiac scintigraphy with 123I-MIBG. Post-catheter-based RSD patients were studied at 24–48 hours, 3, 6, 12 and 24 months after the procedure. Patients were examined and questioned during each visit. Additional examinations were conducted when appropriate. Patients' compliance with an antihypertensive regimen was also verified during each visit. All enrolled patients were asked not to modify the medication regimen, whether arbitrarily or with the family physician's consultation and without any serious reason (adverse reactions, intolerance etc.). When necessary, medication regimens were modified during study visits. This was particularly important in the cardiac 123I-MIBG group, where patients were excluded from further participation if modifications in their medication regimen were necessary. In 24–48 hours after the procedure, patients were assessed only for arterial stiffness and central hemodynamics. Afterward, 24-hour BP monitoring, office BP and arterial stiffness and central hemodynamics were measured in patients at 3, 6, 12 and 24 months after the procedure. Echocardiographic and subgroup analyses (cardiac MRI and cardiac scintigraphy with 123I-MIBG) were carried out in 6 months after the procedure. The scheduling of study procedures is presented in Table 1.

TABLE 1. Procedure schedule.

	- 4 weeks to - 1 week	CATHETER-BASED RSD	24–48 hrs	3 months	6 months	12 months	24 months
Informed consent form	X						
Inclusion/exclusion criteria	X						
Anamnesis and concomitant illnesses	X						
Use of anti-hypertensive drugs	X			X	X	X	X
Examination	X		X	X	X	X	X
Office BP	X		X	X	X	X	X
24-hour BP	X			X	X	X	X
AS and CH measurement	X		X	X	X	X	X
Echocardiography	X				X		
Cardiac 123I-MIBG							
MRI scan	X				X		

AS – arterial stiffness, CH – central hemodynamics, MRI – magnetic resonance imaging.

2.5 General Characteristics of Study Participants

2.5.1 Characteristics of the Catheter-Based Renal Sympathetic Denervation Group

A total of 73 patients were originally enrolled in the catheter-based RSD group, of whom 34 were males (46.58%) and 39 (53.42%) were females. The youngest patient was aged 33, and the oldest patient was aged 72. The mean age of the patients was 56.03 ± 7.76. There was no statistically significant difference in age between women and men (p=0.2987, t-test). However, even though there was no significant difference in the mean age between women and men, Figure 1 shows a greater age concentration within 50 to 55 in men, while the age distribution in women was broader, with no marked age peak as in the case with men.

Almost all participants in the catheter-based RSD group were obese or overweight (body mass index (BMI) calculation: < 18.5 kg/m^2 – underweight, 18.5 – 24.9 kg/m^2 – normal weight, 25 – 29.9 kg/m^2 – overweight, > 30 kg/m^2 – obesity (19,20)). The average BMI among participants was 34.29 ± 5.64 kg/m^2, showing no statistically significant difference in the average BMI between men and wom-

en (p=0.9031, t-test). Like with age, BMI for male patients was more concentrated around the average, while the female BMI had a broader distribution. There were only two BMI values below 25 at baseline; both had referred to women.

An analysis of the anamnesis data of the catheter-based RSD group revealed that chronic HF was diagnosed for 61 patients (83.6 %), only 5 patients disclosed that they were current smokers (6.8 %), 5 patients had strokes (6.8 %), atrial fibrillation was diagnosed in 5 patients (6.8 %), 29 patients had type 2 diabetes (39.7%), coronary heart disease was diagnosed in 16 patients (21.9 %), and 32 patients had dyslipidemia (43.84%).

2.5.2 Characteristics of the Control Group

The control group had 21 patients, of whom 9 were males and 12 were females. There was no difference in the proportions of men and women in the intervention and control groups (p=0.9578, chisq test). The table below shows that men and women accounted for 42.86% and 57.14%, respectively, in the control group, and, very similarly, 46.58% and 53.42%, respectively, in the intervention group (Table 2). Likewise, there was no statistically significance difference in age between women and men in the control and intervention groups (p=0.642) (Table 3). The average BMI in the control group was 32.67 ± 5.28 kg/m^2, showing no statistically significant difference versus the intervention group (p=0.22). The intervention group had more cases of concomitant diseases compared with the control group (83.56% vs. 52.38%) (p=0.007).

TABLE 2. Group distribution by gender.

Group	Men	Women	Total
Control group	9 (42.86%, 20.93%)	12 (57.14%, 23.53%)	21 (100%, 22.34%)
Intervention group	34 (46.58%, 79.07%)	39 (53.42%, 76.47%)	73 (100%, 77.66%)
Total	43 (45.74%, 100%)	51 (54.26%, 100%)	94 (100%, 100%)

TABLE 3. Group distribution by age.

Group	Min	Median	Mean	Max	SD
Control group	40	53	55.10	74	8.09
Intervention group	33	55	56.03	72	7.76

2.5.3 Characteristics of the Cardiac 123I-Metaiodobenzylguanidine Group

A total of 16 participants underwent cardiac 123I-MIBG, of whom 9 were males (56.25 %) and 7 were females (43.75 %). The mean age of the participants was 54.9 ± 7.9. The average BMI was 34.16 ± 4.02 kg/m^2. Concomitant diseases: 5 patients (31.25 %) had coronary heart disease, 5 patients (31.25 %) had type 2 diabetes, 11 patients (68.75 %) had dyslipidemia diagnosis, and 1 patient (6.25 %) was smoking. The average use of antihypertensive drugs was 6.44 ± 0.96. Pre-catheter-based RSD patients receiving this treatment had mean ambulatory BP 185.63/109.06 ± 21.52/14.05 mm Hg, HR – 67.5 ± 7.6 bpm. 24-hour BP was 161.07/97.6 ± 20.12/16.25 mm Hg, HR – 64.47 ± 7.87 bpm.

2.5.4 Characteristics of the Magnetic Resonance Imaging Group

A total of 27 participants were enrolled in the MRI group, of whom 12 were females (44.44 %) and 15 were males (55.56 %). Their mean age was 51.89 ± 10.03. The average BMI was 32.65 ± 4.66 kg/m^2. 21 patients (77.77%) had concomitant diseases. The average use of antihypertensive drugs was 6.13 ± 0.91. Pre-catheter-based RSD patients receiving this treatment had mean ambulatory BP 189.80/110.20 ± 22.77/11.60 mm Hg, HR – 70.77 ± 7.90 bpm. 24-hour BP was 159.70/99.71 ± 16.42/14.52 mm Hg, HR – 70.25 ± 9.56 bpm.

2.6 Catheter-Based Renal Sympathetic Denervation

Patients are required to abstain from food and full liquids for at least 6 hours before the scheduled procedure. Antihypertensive medications have had to be taken in accordance with earlier recommendations. Patients receive adequate intravenous hydration prior to the procedure. Hydration is also continued after the procedure.

Anaesthesiologist-administered intravenous sedation and analgesia are provided, as the procedure is painful. To avoid long-term central nervous system effects, patients receive intravenous propofol infusion that is delivered automatically from the infusion pump for sedation during the procedure. Dosage is adjusted as needed. Intravenous paracetamol is used as an analgesic during the procedure.

An arterial access catheter is inserted by puncturing the common femoral artery using the modified Seldinger technique (21). An F-6 to 8-F hydrophilic sheath is usually used for arterial access. After the cannulation of the artery, 5000 heparin units are administered to manage anticoagulation. A guiding catheter is advanced

through the arterial access catheter, over a 0.035-inch wire, into the abdominal aorta. Renal artery ostia are cannulated using the guiding catheter (the type and shape of the guide catheter depends on the anatomy and derivation of renal arteries) and an angiography of the renal arteries is performed with manual contrast-media injection. An RSD catheter is then positioned distally in the renal artery so that to come into contact with the inner wall of the artery distally. If the contact with the arterial wall is proper, a flat impedance curve is displayed on the generator. Then, a radiofrequency ablation is initiated by pressing the button on the catheter. The catheter is rotated about 90 degrees after the RF ablation, pulled back proximally toward the aorta and then readvanced to repeat the radiofrequency ablation. The number of RF ablations depends on the vascular anatomy. A control angiography is performed after the final RF ablation to exclude complications (artery perforation or dissection). The complete procedure is repeated with the other renal artery. If angiography shows accessory renal arteries of ≥ 3 mm in diameter, the radiofrequency ablation procedure is repeated on these arteries following the same methodology.

RSD is performed using point-by-point RF ablation. The Symplicity Flex™ catheter powered by the Symplicity G2™ generator or Symplicity Spyral™ catheter powered by the Symplicity G3™ generator (manufacturer: Medtronic Plc, Ireland) are used for the radiofrequency ablation procedure.

The Symplicity Flex™ catheter/Symplicity G2™ generator is used for single 2-min. ablations. The temperature and effect on electrical impedance are monitored continuously in the ablation zone during the procedure. The radiofrequency ablation is performed automatically, with the generator using a preset algorithm of up to 8W power and 70°C temperature.

The Symplicity Spyral™ catheter is powered by the Symplicity G3™ generator and delivers FR energy for 1-min. ablation for all 4 points (electrodes) simultaneously. Temperature and electrical impedance are monitored continuously in the ablation zone during the procedure. The radiofrequency ablation is performed automatically, with the generator using a pre-set algorithm of up to 7W power and 70 °C temperature. When this type of catheter is used, the methodology of the procedure is slightly different. First, a 0.014- inch wire is directed through the guiding catheter to the renal artery, and a denervation catheter is placed on the wire, pushing four sequential electrodes. Once the wire is withdrawn from the area of electrodes, the ablation catheter takes on a spiral shape and sticks to the inner wall of the artery. After having controlled that there is proper contact between the electrodes and the arterial wall (i.e., no marked fluctuations of the impedance trace), a radiofrequency ablation is performed, and the procedure is further continued in accordance with the aforementioned methodology.

After the radiofrequency applications, the RSD catheter is removed, and a control angiography is performed to exclude complications. Once the procedure is completed, the guides are removed, and the puncture sites are closed by applying mechanical pressure or using closure devises to suture arterial access sites.

As a histological examination has revealed no significant difference in ablation-caused damage to the arterial media between the first-generation Symplicity Flex and the second-generation Symplicity Spyral (22), patients were not classified into groups by this parameter. In our study, we assessed the number of ablations irrespective of the generation of the denervation electrode used for catheter-based RSD.

3. Results

3.1 Consumption of Antihypertensive Drugs

In the overall consumption of medications at baseline (immediately before the procedure), diuretics, beta-blockers, CCBs and CADs were the most frequently used antihypertensive drugs, accounting for 94.37%, 92.96%, 88.73% and 80.28%, respectively. ARBs, aldosterone receptor agonists and alpha-blockers were slightly less common (77.46%, 67.61% and 63.38%, respectively), and ACE inhibitors were the least common antihypertensive drug (32.39%). The mean number of antihypertensive medications was 5.97, i.e., the patients had received an average of six medications. Given that the treatment protocol provided for a stable medication-based treatment for at least six months (and preferably throughout the entire study), the total number of consumed medications was not significantly modified during the study. The total number of medications consumed at baseline and in dynamics is shown in Table 4.

TABLE 4. The number of medications consumed at baseline and in dynamics.

Time	min	median	mean	max	SD	n	p
Baseline	2	6	5,97	8	1,10	71	-
3 months	2	6	5,63	8	1,37	63	0,371
6 months	2	6	5,65	8	1,36	66	0,568
12 months	2	6	5,72	8	1,37	58	0,176
24 months	1	6	5,37	7	1,28	52	0,721

3.2 Characteristics of Catheter-Based Renal Sympathetic Dener vation

The number of radiofrequency ablations (RA) was analyzed as the main performance indicator for the catheter-based RSD procedure. The mean number of RAs per patient was 16.21 ± 8.95. The mean number of RAs per left and right renal artery was 7.85 ± 4.36 and 7.87 ± 4.65, respectively. The mean number of RAs per left and right renal accessory artery was 6.33± 2.93 and 6.88 ± 3.14, respectively. The minimum number of RAs per patient was 7 and the maximum number was 46 (25 per left renal artery and 21 per right renal artery).

3.3 Office Blood Pressure Changes after Catheter-Based Renal Sympathetic Dener vation

Office BP measured at 3 months after catheter-based RSD showed a significant reduction in BP from office BP 190/107 ± 21.59/13.17 mm Hg at baseline to 160/91 ± 21.18/12.58 mm Hg ($p<0.001$). The reduction in office BP was observed throughout the study: 156/90 ±24.95/12.10 mm Hg after 6 months, 162/94 ± 25.56/16.74 mm Hg after 12 months, and 164/94 ± 25.36/15.82 mm Hg after 24 months ($p<0.001$, at all points). The dynamics of office systolic and diastolic BP per patient is illustrated in Figure 1.

After 6 months, a reduction in office systolic and diastolic BP was apparent in 89% and 85% of the patients, respectively. The mean achieved reduction in office systolic BP was 39.24 mm Hg, and the mean achieved reduction in office diastolic BP was 19.83 mm Hg.

3.4 24-Hour Blood Pressure Monitoring Changes following Catheter-Based Dener vation of Renal Arteries

In 3 months after the procedure, the 24-hour systolic and diastolic BP values showed a significant reduction from 163/97 ± 17.51/13.48 mm Hg to 151/89 ± 22.39/12.18 mm Hg ($p<0.001$). The reduction in the 24-hour BP was observed throughout the study: 149/90 ± 19.53/9.61 mm Hg after 6 months, 152/91 ± 17.34/9.74 mm Hg after 12 months, and 148/91 ± 18.32/12.28 mm Hg after 24 months ($p<0.005$). No significant changes were found in dipping (daytime/night-time systolic BP) at 3 months after the procedure ($p=0.286$) and in the late post-procedural periods at 6, 12 and 24 months after the procedure ($p=0.88$, $p=0.926$ and $p=0.157$, respectively). The distribution of the 24-hour systolic and diastolic BP values in dynamics is shown in Figure 2.

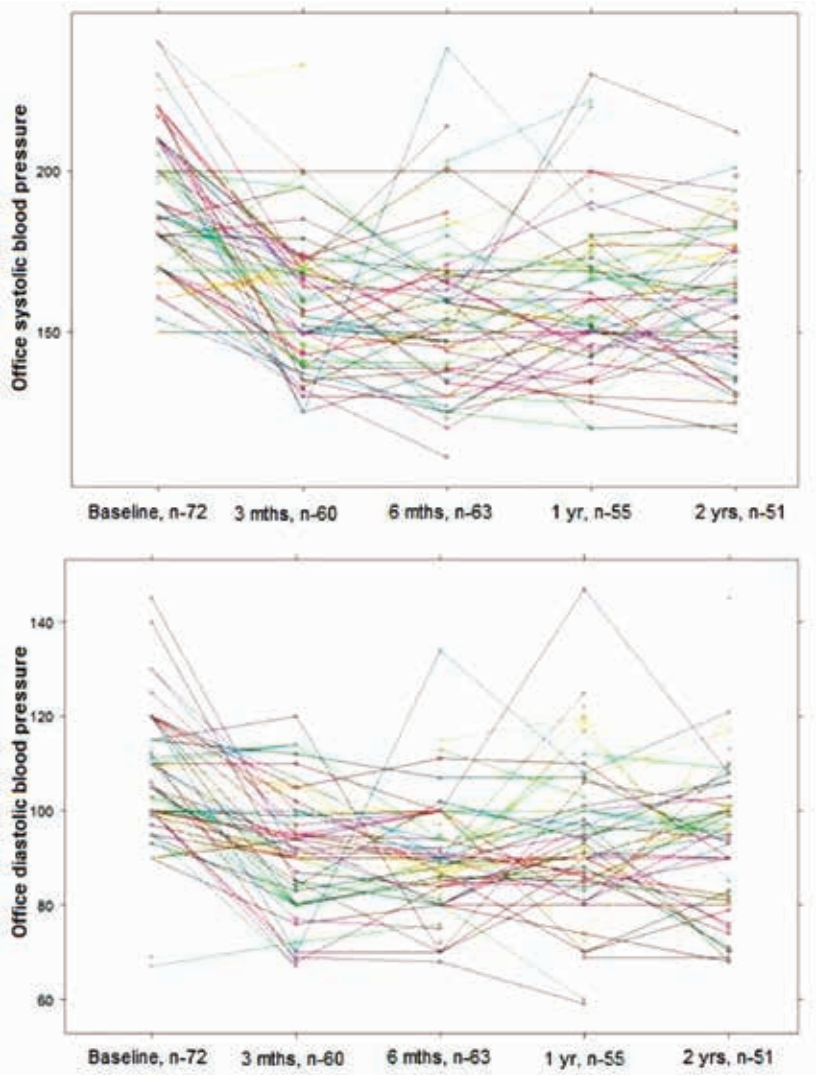

FIGURE 1. The dynamics of office systolic and diastolic blood pressure values per patient.

After 6 months, the assessment of the 24-hour BP changes showed a reduction in 24-hour systolic and diastolic BP in 81% and 74% of patients, respectively. The mean achieved reduction in 24-hour systolic BP was 21.28 mm Hg and the mean achieved reduction in 24-hour diastolic BP was 13.56 mm Hg.

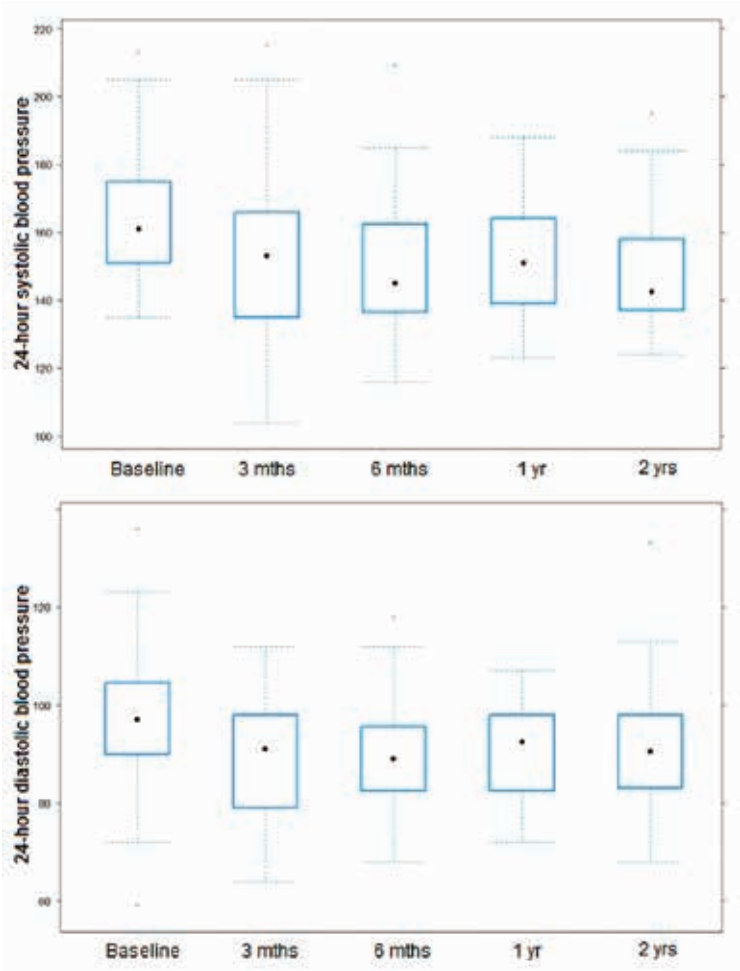

FIGURE 2. The distribution of the 24-hour systolic and diastolic blood pressure values in dynamics.

3.5 Predictors for office Systolic Blood Pressure Variations at 6 Months.

The linear mixed model, which had incorporated all the grouping factors (age, sex, BMI, numbers of RAs and office systolic BP at baseline) as fixed independent effects, showed no difference in office systolic BP variations at 6 months after the procedure among patients with the different number of RAs. The statistical significance of the parameter for comparing the office systolic BP change after 6 months between

patients with 9 to 16 RAs with those who had less than 9 RAs was p=0.7948. The statistical significance of the parameter for comparing the office systolic BP change after 6 months between patients with more than 16 RAs with those who underwent less than 9 RAs was p=0.4532; the statistical significance of the parameter for comparing the office systolic BP change between patients with more than 16 RAs with those who had 9 to 16 RAs was p=0.098. Figure 3 displays the absence of a difference in office systolic BP variations among patients with the different number of RAs.

The model also showed no difference in office systolic BP variations at 6 months after the procedure among the patients whose MBI was below or above 35 kg/m^2 (p=0.6983); neither there was any difference in BP changes over time between women and men (p=0.3014).

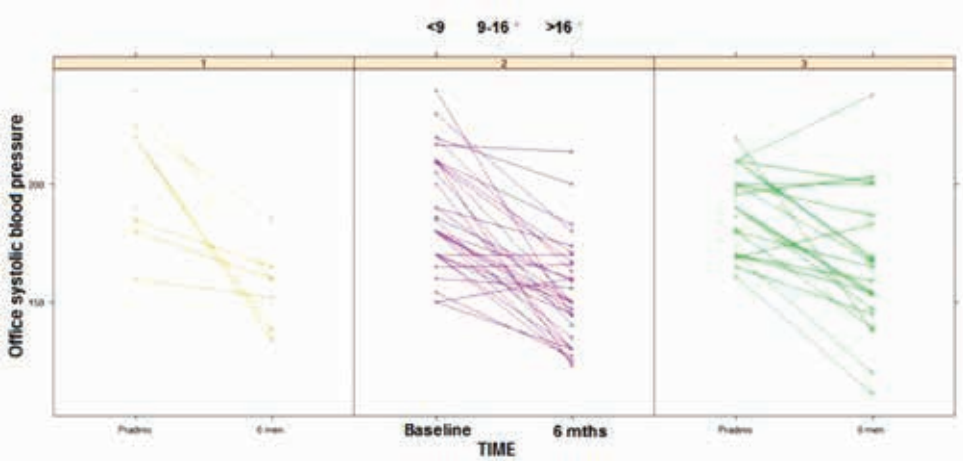

FIGURE 3. Office systolic blood pressure change in relation to the number of radiofrequency ablations.

The comparison of office systolic BP variations over time in patients of different age revealed an average difference of -21.95 mm Hg in BP variations between patients whose age was less than 61 and those aged above 61, i.e., patients aged < 61 experienced a more rapid drop in ambulatory systolic BP 6 months after the procedure than those aged >61 (p=0.0008). The change in office systolic BP by age groups can be seen in Figure 4.

Likewise, the results showed a correlation between office systolic BP over time and ambulatory systolic BP at baseline, i.e., the higher the baseline BP was (the higher the group was), the more rapid was the downward change over time. The comparison of the BP values at 6 months after the procedure between patients whose BP was > 210 mm Hg at baseline (group 3) with patients whose baseline BP was

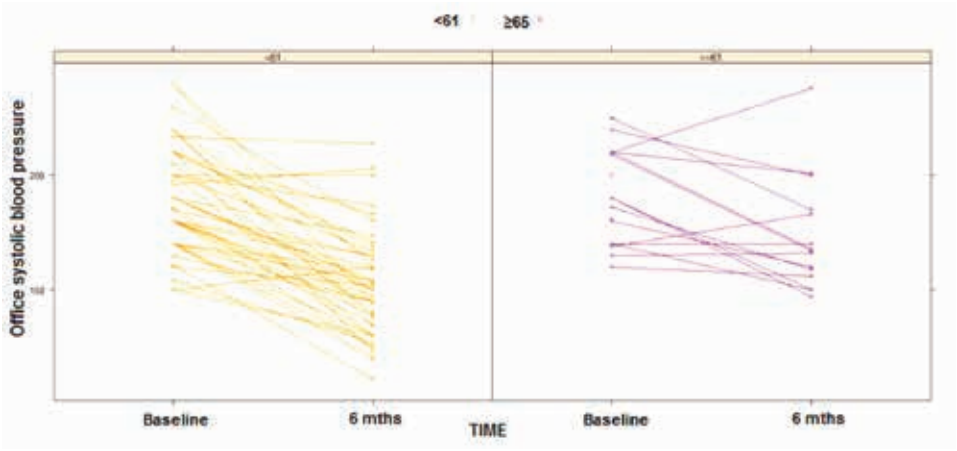

FIGURE 4. Office systolic blood pressure change by age groups.

<=170 (group 1) confirmed a more rapid change (downward) over time in group 3, i.e., the average difference over time between group 3 and group 1 was -34,11 mm Hg (p=0.0005), and the aver age difference over time between group 3 and group 2 was -22.97 mm Hg (p=0.0079). The change in office systolic BP by the baseline office systolic BP values is shown in Figure 5.

It was thus determined that office systolic BP was dependent on office systolic BP at baseline and on age. Younger patients and those with higher office systolic BP before the catheter-based RSD procedure experienced a more significant drop in office systolic BP at 6 months after the procedure.

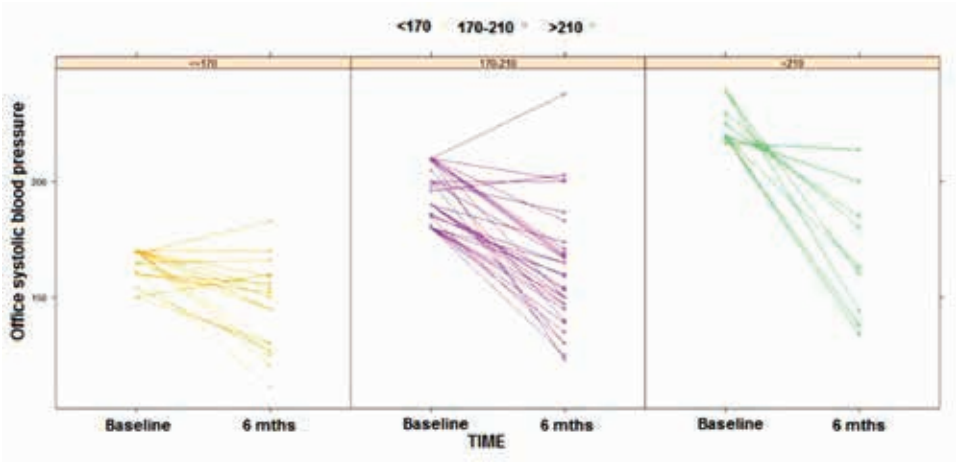

FIGURE 5. The change in office systolic blood pressure by the baseline office systolic blood pressure values.

3.6 Predictors for office Diastolic Blood Pressure Variations at 6 Months

The same grouping was applied in respect of the office diastolic BP indicator, except for the grouping of the baseline values of office diastolic BP, which were divided into three subgroups (≤ 100 mm Hg; > 100 and ≤115 mm Hg; >115 mmHg).

The linear mixed model for the diastolic BP indicator, which had incorporated all the grouping factors (age, sex, BMI, the number of RAs and diastolic BP at baseline) as fixed independent effects, showed that a statistically significant difference in office diastolic BP variations at 6 months after the procedure was related only to the ambulatory diastolic BP values at baseline: there was a -17.15 mm Hg difference in change over time between group 3 patients, whose diastolic BP was >115 mm Hg at baseline, and group 1 patients, whose diastolic BP at baseline was <=100 mm Hg (p=0.0001); the average difference at 6 months between group 2 patients with diastolic BP 100-115 mm Hg at baseline and group 1 was -7.53 mm Hg (p=0.057). In other words, the higher the office diastolic BP at baseline was, the higher the reduction after 6 months turned out to be. Office diastolic BP variations by the baseline office diastolic BP values are shown in Figure 6.

It was thus determined that office diastolic BP was dependent only on diastolic BP at baseline. Patients with higher office diastolic BP before the catheter-based RSD procedure experienced a more significant reduction in office diastolic BP at 6 months after the procedure.

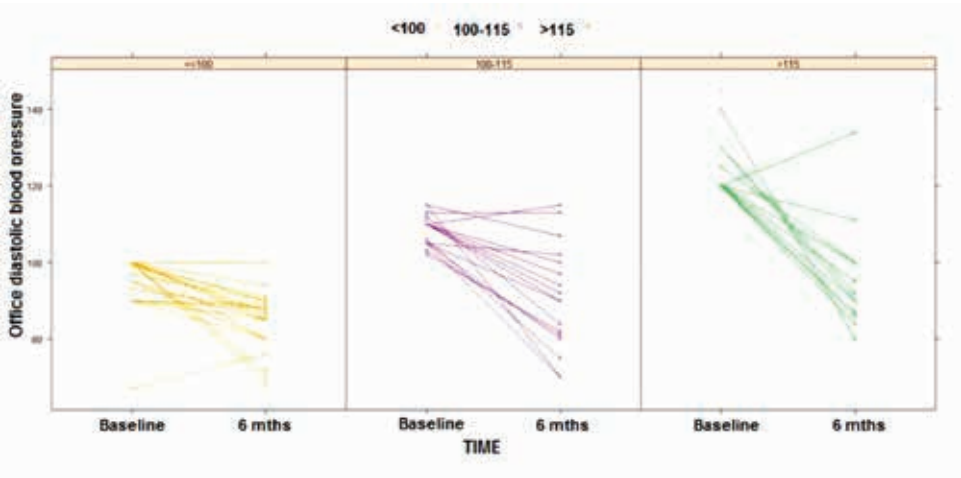

FIGURE 6. The change in office diastolic blood pressure by the baseline office diastolic blood pressure values.

3.7 Predictors for 24-Hour Systolic Blood Pressure Variations at 6 Months

The baseline 24-hour systolic BP values were inappropriate to form the same three groups as in the case of office systolic BP due to a few values of the 24-hour systolic BP being higher than 210 mm Hg. Therefore, the 24-hour systolic BP values were divided into two groups (≤170 mm Hg and >170 mm Hg).

The linear mixed model for the 24-hour systolic BP indicator, which had incorporated all the grouping factors (age, sex, BMI, the number of RAs and 24-hour

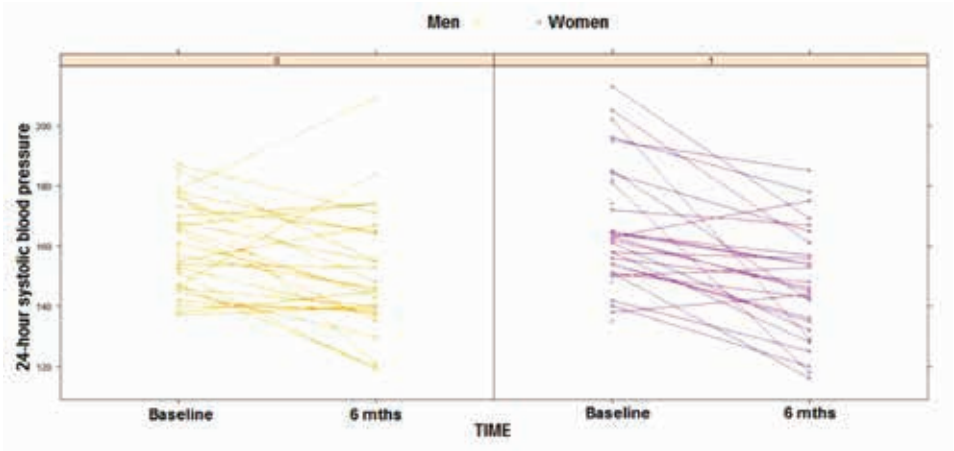

FIGURE 7. The change in 24-hour systolic blood pressure by gender.

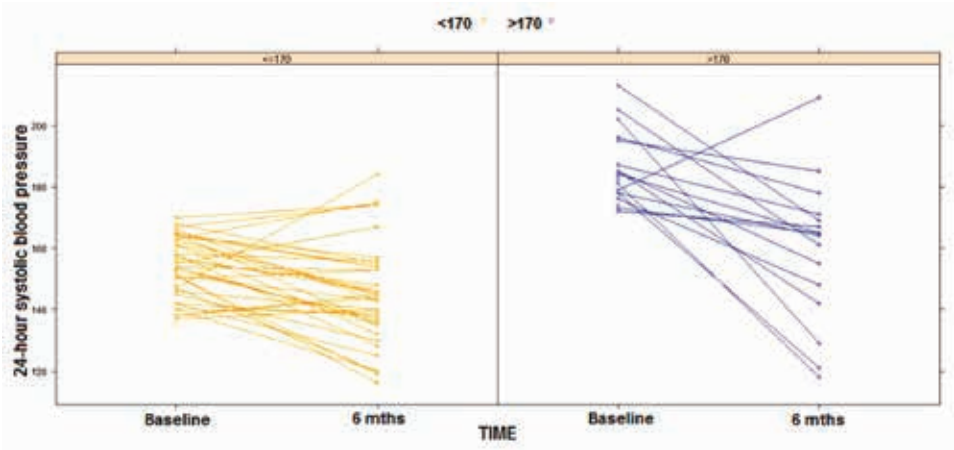

FIGURE 8. The change in 24-hour systolic blood pressure by the baseline values of 24-hour systolic blood pressure.

systolic BP at baseline) as fixed independent effects, showed a statistically significant difference in 24-hour systolic BP variations after 6 months by gender: the difference in 24-hour systolic BP change after 6 months between women and men was -11.34 mm Hg for women (p=0.0128), i.e., a reduction in 24-hour systolic BP at 6 months was more marked for women than for men. The change in 24-hour systolic BP by gender is illustrated in Figure 7. Statistically significant changes in 24-hour systolic BP were also observed by the BP baseline values: patients with higher 24-hour systolic BP at baseline (>170 mm Hg) exhibited an average difference of -16.33 mm Hg after 6 months as compared to the patients with ≤170 mm Hg baseline 24-hour systolic BP (p=0.0026); i.e., patients with higher 24-hour BP at baseline experienced a higher reduction at 6 months. The change in 24-hour systolic BP by the baseline values of 24-hour systolic BP is shown in Figure 8.

The results further revealed a statistically significant difference in 24-hour systolic BP at 6 months by age groups. The difference in the reduction between patients aged <61 and those aged >61 was 20.09 mm Hg on average at 6 months after the procedure. The change in 24-hour systolic BP by age groups can be seen in Figure 9.

Accordingly, we determined correlations between the 24-hour systolic BP variations and baseline systolic BP, age and sex. Younger patients, female patients and those with higher 24-hour systolic BP before catheter-based RSD experienced a more marked reduction in 24-hour systolic BP at 6 months after the procedure. No statistically significant difference in 24-hour systolic BP after 6 months was found between patients with a different number of ablations and different BMIs.

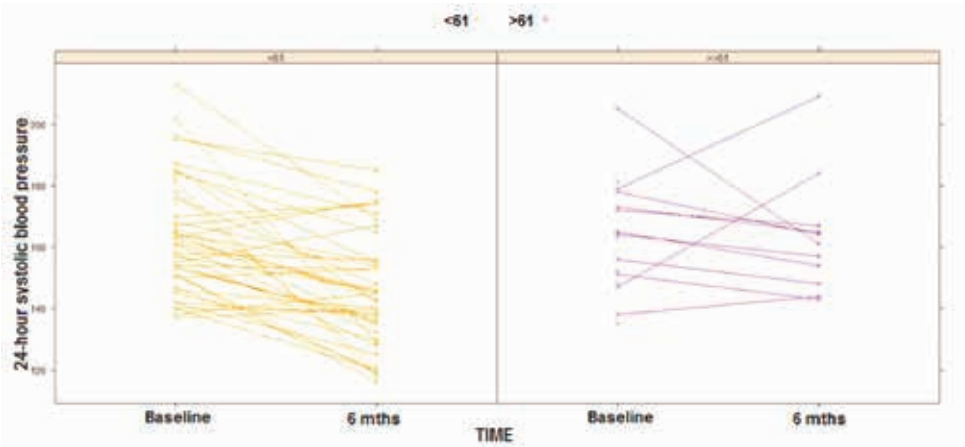

FIGURE 9. The change in 24-hour systolic blood pressure by age groups.

3.8 Predictors for 24-Hour Diastolic Blood Pressure Variations at 6 Months

The linear mixed model for the 24-hour diastolic BP indicator, which had incorporated all the grouping factors (age, sex, BMI, the number of RAs and 24-hour diastolic BP at baseline) as fixed independent effects, showed a statistically significant difference in 24-hour diastolic BP variations after 6 months by the 24-hour diastolic BP baseline values: patients whose 24-hour diastolic BP was >100 mm Hg at baseline exhibited an average difference of -11.78 mm Hg after 6 months versus those patients with ≤100 mm Hg baseline 24-hour diastolic BP (p=0.0008). In other words, the higher the 24-hour diastolic BP at baseline was, the more significant was a reduction in time (at 6 months). The change in 24-hour diastolic BP by the baseline values of 24-hour diastolic BP is shown in Figure 10. The comparison of 24-hour diastolic BP variations in time between different age groups revealed an average difference of -11,91 mmHg in the BP change after 6 months between patients aged <61 and those aged >61, i.e., patients aged < 61 experienced a more rapid drop in 24-hour diastolic BP than those aged >61 (p=0.003). The change in 24-hour diastolic BP by age groups can be seen in Figure 11.

Hence, we determined correlations between 24-hour diastolic BP variations and baseline diastolic BP and age. Younger patients and those with higher 24-hour diastolic BP before catheter-based RSD experienced a more marked reduction in 24-hour diastolic BP at 6 months after the procedure. No statistically significant difference in 24-hour diastolic BP variations after 6 months was found between patients with the different number of ablations and different BMIs, and between different genders.

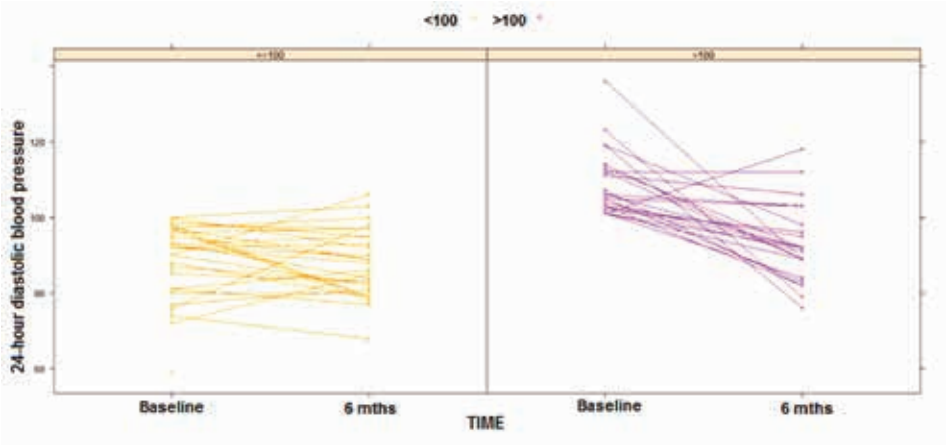

FIGURE 10. The change in 24-hour diastolic blood pressure by the baseline values of 24-hour diastolic blood pressure.

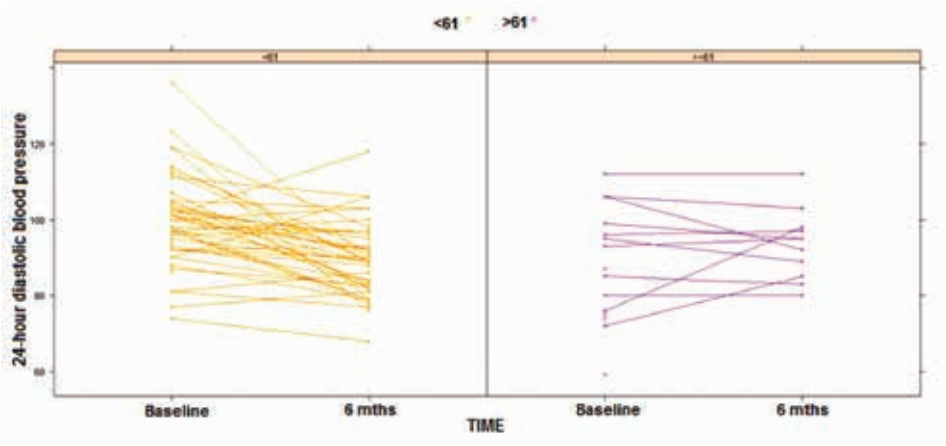

FIGURE 11. The change in 24-hour diastolic blood pressure by age groups.

3.9 A Comparison of Blood Pressure Variations after 6 Months between Study Groups

In the catheter-based RSD group, the change in office systolic BP at 6 months after the procedure was -33.80 mm Hg (± 3.01, p<0.0001). No significant change was observed in the control group (-5.87 mm Hg (±6.04, p=0.334)). The difference in the between-group change was statistically significant (-27.92 mm Hg (±6.75, p=0.001). In the catheter-based RSD group, the change in office diastolic BP at 6 months after the procedure was -16.67 mm Hg (± 1.70, p<0.0001); the control group exhibited no significant change (-3.66 mm Hg (±3.37, p=0.281), and the difference in the between-group change was statistically significant (-13.01 mm Hg (±3.77, p=0.001).

In the catheter-based RSD group, the change in 24-hour systolic BP was -14.82 mm Hg (± 2.47, p<0.0001); the control group exhibited no significant change (3.23 mm Hg (±4.95, p=0.516), and the difference in the between-group change was statistically significant (-18.06 mm Hg (±5.54, p=0.001). In the catheter-based RSD group, the change in 24-hour diastolic BP was -7.28 mm Hg (± 1.66, p<0.0001); the control group exhibited no significant change 2.91 mm Hg (±3.23, p=0.372), and the difference in the between-group change was statistically significant (-10.19 (±3.63, p=0.01).

3.10 Changes in Aortic Pulse Wave Velocity after Catheter-Based Renal Sympathetic Denervation

The analysis of post-catheter-based RSD changes in PWVc-f showed a significant reduction in the early post-operative period (at 24 to 48-hour interval). The PWVc-f lowered from 11.3 m/s to 10.3 m/s (p=0.001). The change remained statistically significant at 3, 6 and 12 months after the procedure; the reduction was no more statistically significant after 24 months, p=0.067 (Table 5). It should be noted that the sample was reduced by half of the patients at this point of time.

TABLE 5. Numerical characteristics of the pulse wave velocity (carotid-femoral) changes after catheter-based renal sympathetic denervation.

Time	N	Mean, m/s	Standard deviation	p, significance
Immediately before the procedure	62	11.3	2.7	
After 24–48 h	58	10.3	2.6	0.001
After 3 months	44	10.3	2.9	0.013
After 6 months	48	10.0	2.6	0.001
After 12 months	45	10.4	2.7	0.013
After 24 months	32	10.4	3.5	0.067

The evaluation of the parameters of central hemodynamics in the early post-procedural period also showed a reduction in the mean central BP by 12.7 mm Hg in the early period (± 19.9, p<0.001). Therefore, a linear regression analysis was performed to determine whether there was a relationship between the PWVc-f change in the early post-procedural period and the reduction in the mean central BP. The PWVc-f change in the early post-procedural period was found to be independent from the reduction in the mean central BP, r=0.165, p=0.214.

To analyze the predictors of PWVc-f changes in the late post-procedural period (after 6 months), a univariate analysis of quartile dispersion was carried out, which showed a correlation between the PWVc-f baseline value and PWVc-f change in the late post-procedural period: the higher the PWVc-f baseline value was, the greater the reduction at 6 months after the procedure turned out to be. The quartile analysis for the relationship between the PWVc-f baseline values and PWVc-f changes at 6 months after the procedure is presented in Table 6; Figure 12 illustrates the change distribution by quartile. No correlation was found between PWVc-f changes in the late period and the BP baseline values, whether office BP (systolic r=0.113, p=0.451, diastolic r=0.146, p=0.328) or 24-hour BP (systolic r=-0.026, p=0.866, diastolic r=0.141, p=0.348).

TABL E 6. Effects of the baseline pulse wave velocity on pulse wave velocity changes after 6 months.

Quartile	Q1	Q2	Q3	Q4
Quartile limits, m/s	<9.5	9.5-10.699	10.7-13.2	>13.2
PWVc-f before, m/s	8.4 ± 1	10.1 ± 0.4	12.2 ± 0.8	15.3 ± 1.7
PWVc-f after 6 months, m/s	8.4 ± 1.7	8.9 ± 1.6	10.3 ± 2.1	13.2 ± 2.1
Reduction, m/s	0.05 ± 1.6	1.1 ± 1.4	1.8 ± 1.7	2.8 ± 2.1
p, significance	0.002			

PWVc-f - pulse wave velocity between carotid and femoral arteries.

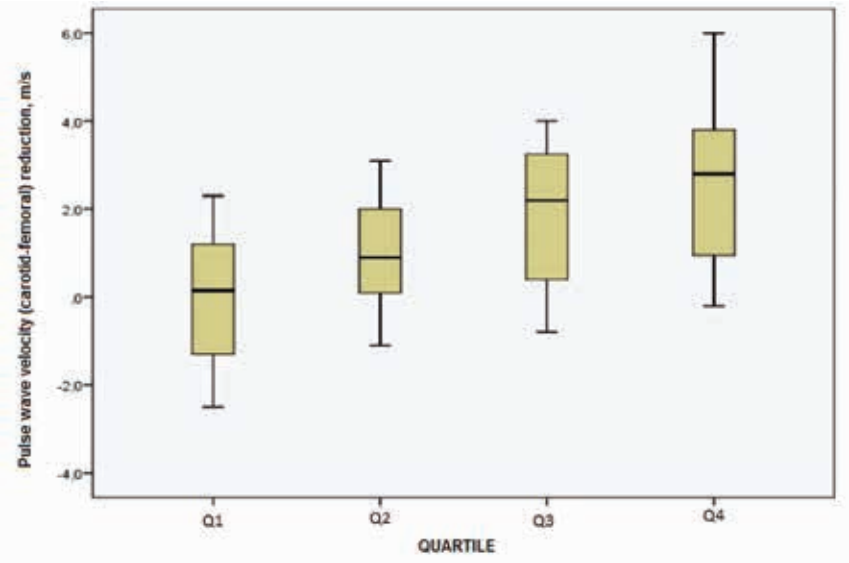

FIGURE 12. The distribution of pulse wave velocity (carotid-femoral) reductions at 6 months by quartile.

In order to determine a PWVc-f threshold giving the greatest prognostic accuracy for patients to whom PWVc-f changes would be more significant at 6 months after catheter-based RSD, an ROC (Receiver operating characteristics) curve analysis was carried out. The determined PWVc-f threshold value at baseline was 9.55 m/s. At this point, sensitivity was 0.81, specificity was 0.58, and the area under the curve was 0.653, p=0.116. The ROC curve is presented in Figure 13.

3.11 Changes in echocardiographic Parameters after Catheter-Based Renal Sympathetic Denervation

An analysis of echocardiographic parameters after catheter-based RSD showed positive dynamics in morphometric indicators of the left ventricle: interventricular septum thickness in diastole decreased from 1.14 ± 0.22 cm to 1.08 ± 0.19 cm ($p<0.001$); LV posterior wall thickness in diastole decreased from 1.08 ± 0.18 cm to 1.02 ± 0.14 cm ($p<0.001$) in 6 months after the catheter-based RSD. A reduction in LV mass index was from 114.40 ± 29.40 g/m^2 to 106.37 ± 24.35 g/m^2 ($p<0.001$). A reduction in the left atrium volume index was also observed in 6 months after the catheter-based RSD, from 2.16 ± 0.24 cm/m^2 at baseline to 2.13 ± 0,25 cm/m^2 ($p=0.05$).

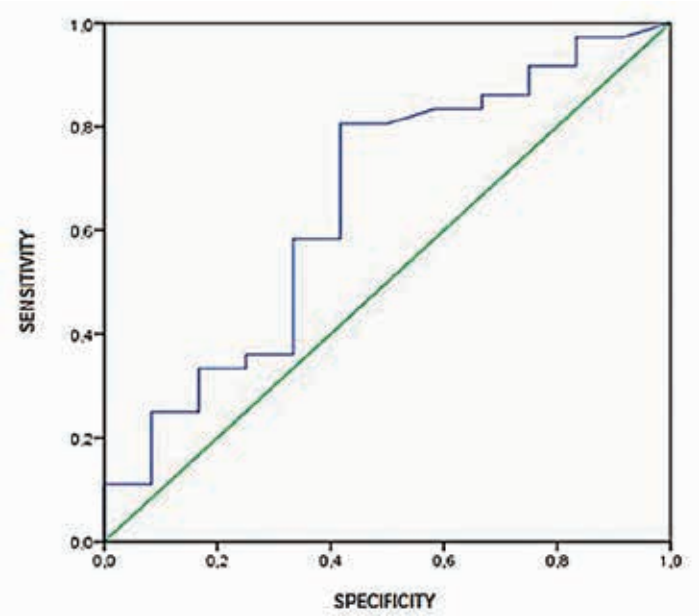

FIGURE 13. The ROC curve for pulse wave velocity (carotid-femoral) threshold values at baseline.

The dispersion graphs we made and computed correlation coefficients showed that the LV mass index change was independent from office systolic BP change ($r=-0.035$), office diastolic BP change ($r=0.121$), 24-hour systolic BP change ($r=-0.164$) and 24-hour diastolic BP change ($r=-0.209$). The relationship between the LV mass index change and office/24-hour BP variations is shown in Figures 14 and 15, respectively.

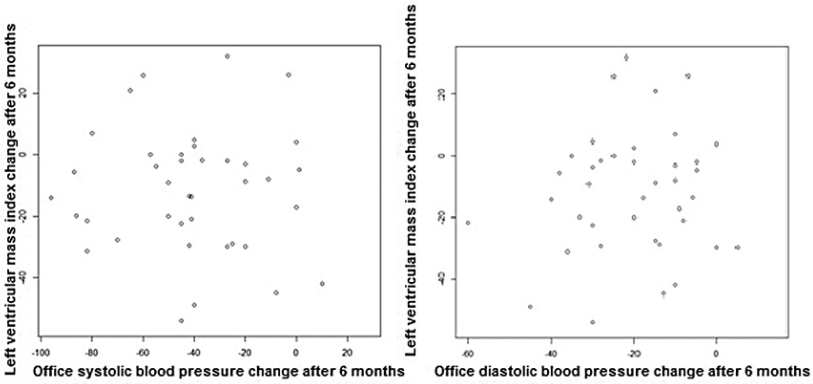

FIGURE 14. A dispersion graph for the left ventricular mass index change dependence on office blood pressure variations.

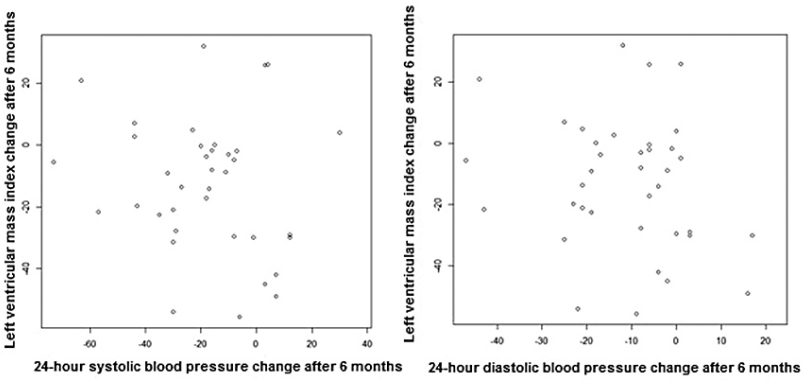

FIGURE 15. A dispersion graph for the left ventricular mass index change dependence on 24-hour blood pressure variations.

3.12 Changes in Magnetic Resonance Imaging Parameters after Catheter-Based Renal Sympathetic Denervation

An analysis of MRI data at 6 months after the catheter-based RSD showed positive dynamics in morphometric indicators of the left ventricle: LV posterior wall thickness (PWT) decreased from 11.56 ± 3.38 mm to 10.61 ± 2.31 mm (p=0.0364), LV mass dropped from 211.33 ± 88.9 g to 196.51 ± 71.75 g (p=0.0047), and a reduction in LV mass index (MI) was from 101.11 ± 36.86 g/ m^2 to 93.53 ± 30.49 (p=0.0067). The dynamics of significant changes in LV parameters and dynamics for each patient are depicted in Figures 16 and 17.

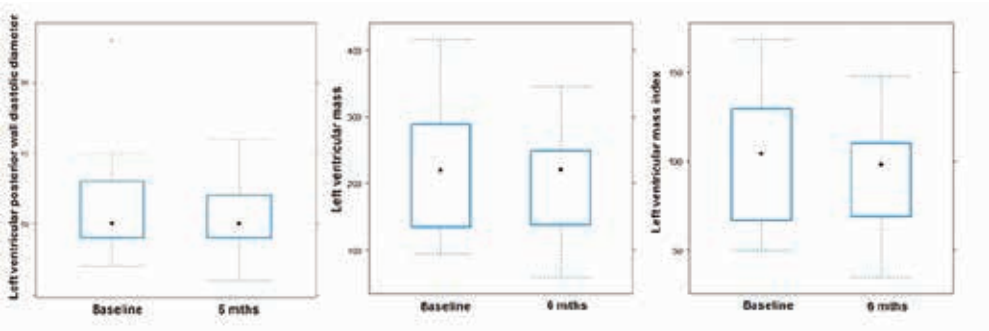

FIGURE 16. Magnetic resonance imaging changes in left ventricular posterior wall, left ventricular mass and left ventricular mass index after catheter-based renal sympathetic denervation.

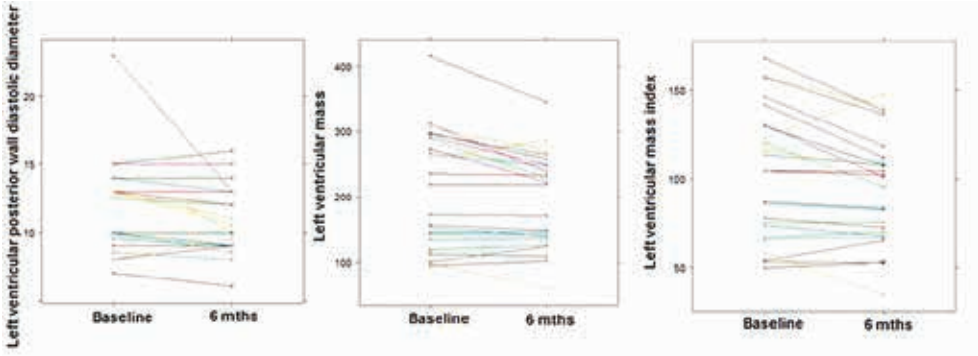

FIGURE 17. Magnetic resonance imaging changes in left ventricular posterior wall, left ventricular mass and left ventricular mass index after catheter-based renal sympathetic denervation per patient.

Based on the dispersion graphs and computed correlation coefficients, we determined that the LV mass index change was not dependent on office systolic BP change (r=0.150), office diastolic BP change (r=0.279), 24-hour systolic BP change (r=0.065), and 24-hour diastolic BP change (r=-0.209). The relationship between the LV mass index change and the office/24-hour BP variations can be seen in Figures 18 and 19, respectively.

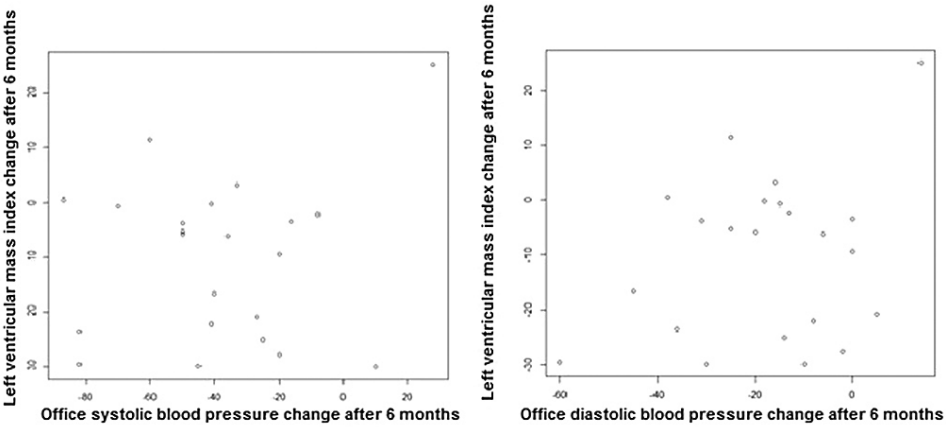

FIGURE 18. Dispersion graph for the left ventricular mass index change's dependence on office blood pressure variations.

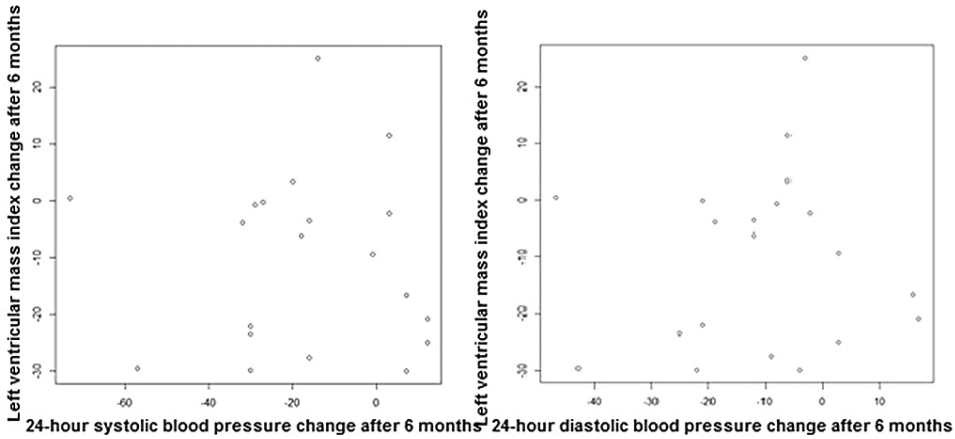

FIGURE 19. Dispersion graph for the left ventricular mass index change's dependence on 24-hour blood pressure variations.

3.13 Cardiac 123I-Metaiodobenzylguanidine Changes after Catheter-Based Renal Sympathetic Denervation

The evaluation of the 123I-MIBG scan showed positive dynamics in the late heart-to-mediastinum ratio with an increase from 2.21 ± 0.47 at baseline to 2.35 ± 0.52 (p=0.02). There was no statistically significant change in the early heart-to-mediastinum ratio and washout rate. Changes in cardiac sympathetic innervations are shown in Table 7.

TABL E 7. Changes in cardiac sympathetic innervation.

Variable	Baseline (mean ± SD)	After 6 months (mean ± SD)	p
Early H/M	2.28 ± 0.42	2.57 ± 0.75	0.163
Late H/M	2.21 ± 0.47	2.35 ± 0.52	0.020
WR	24.32 ± 17.97	21.51 ± 17.24	0.679

H/M - heart-to-mediastinum ratio; WR – washout rate.

4. Discussion

4.1 Catheter-Based Renal Sympathetic Dener vation effects on Blood Pressure

The findings reported by earlier studies are conflicting. While the first studies to investigate the efficacy and safety of catheter-based RSD, Symplicity HTN-1 and Symplicity HTN-2, proved this method to be safe and found a substantial reduction in office BP (14, 23), Symplicity HTN-3, a double-blind, placebo-controlled (sham control) trial, failed to demonstrate any significant change in blood pressure between the groups (10). The abovementioned studies differed not only in their design (only Symplicity HTN-3 was placebo-controlled), but Symplicity HTN-3 used 24-hour BP as the primary efficacy endpoint in contrast to the earlier studies, which assessed in-office BP values. Although Symplicity HTN-3 had many strong aspects, it generated substantial criticism for poor compliance with drug therapy and insufficient quality of the procedure. Only 26 interventionists out of 111 in Symplicity HTN-3 had performed 5 or more denervations and only 9.2 ± 2.2 successful ablations on average. The importance of patient selection was also emphasized in recommendations for device-based therapies for resistant hypertension developed at the European Expert Group's conference in 2016 (24). First of all, secondary hypertension is diagnosed in ~10% of AH patients and is considerably more frequent among patients with RAH (25). Therefore, it is very important to investigate in detail the likely causes of secondary AH. Pre-enrollment treatment adjustment and 24-hour BP monitoring also play an important role. This was confirmed by the DENERHTN trial demonstrating good BP control on 24-hour BP monitoring after 1 month in 10% of patients (12/121) with resistant hypertension receiving the standard three-drug combinations (diuretic, ACEI and CCB) (26).

We used a strict randomization protocol to exclude secondary hypertension in addition to therapy adjustments and 24-hour BP assessments. As a result, only 94 patients out of a total of 537 participants were enrolled for the study. The patients

enrolled in our study differed from patients in all three Symplicity trials by very high baseline BP levels (mean office BP was 190/107 mm Hg) and the number of consumed antihypertensive drugs (patients in our study were receiving one medication more on average compared to patients in all three Symplicity studies). Our patients had better 24-hour BP indicators than Symplicity HTN-3 patients (163/97 mm Hg versus 159/88 mm Hg). Office BP variations observed by us were very similar to those reported in Symplicity HTN-1 and Symplicity HTN-2. Our study showed a substantial reduction in office BP at 3 months, from 190/107 mm Hg at baseline to 160/91 mm Hg after 3 months. The reduction was observed throughout the study: 156/90 mm Hg after 6 months, 162/94 mm Hg after 12 months and 164/94 mm Hg after 24 months ($p<0.001$, at all points), and there was a significant change compared with data in the control group ($p=0.001$). A reduction in 24-hour BP from 163/97 mm Hg to 151/89 mm Hg ($p<0.001$) at 3 months also continued throughout the study: 149/90 mm Hg after 6 months, 152/91 mm Hg after 12 months, and 148/91 mm Hg after 24 months ($p<0.005$, at all points). The data above are consistent with and complimentary to the significant reduction in 24-hour BP reported in Symplicity HTN-3. We further observed a significant difference between intervention and control groups in 24-hour BP variations at 6 months after the procedure: the between-group difference in 24-hour systolic and diastolic BP was -18.06 mm Hg (± 5.54, $p=0.001$) and -10.19 mm Hg (± 3.63, $p=0.01$), respectively. We cannot compare those findings with Symplicity HTN-3 results, where the control group underwent a placebo (sham) procedure. However, a statistically significant reduction in BP for the sham group (−11.74 ± 25.94 mm Hg, $p<0.001$) (10) was the main factor for no change in the results between the groups in Simplicity HTN-3. One explanation of such a large drop in BP in the sham group could be the Hawthorne effects (i.e., patients changing behavior depending on their participation in a trial, in this case, starting to take their drugs stricter, just as prescribed) and a strong, intervention-induced placebo effect. Already in 1961, it was established that, in the treatment of arterial hypertension, parenteral placebo administration caused a more significant decrease in BP than placebo administration per os (27). A meta-analysis of the studies show that the larger the scope of the intervention is, the greater the placebo effect eventually becomes (28). The sensitivity of a large drop of BP in the control group of the Symplicity HTN-3 trial to the Hawthorne effect was also confirmed by the Spyral HTN-OFF MED trial, which reported a significant decrease in office and 24-hour BP for patients with AH (not RAH) absent from antihypertensive medication in the catheter-based RSD group, while no significant changes were seen in the sham-control group (29).

4.2 Predictors of a Reduction in Blood Pressure after Catheter-Based Renal Sympathetic Denervation

Our study found out that the key factors resulting in the reduction in systolic BP at 6 months after the catheter-based RSD procedure were the baseline values of BP and age, regardless of BP measurement type (whether in office or 24-hour BP monitoring). The key predictor for reductions in diastolic BP was the baseline value of BP, regardless of the BP measurement type (whether in office or 24-hour BP monitoring). As for the 24-hour BP monitoring, it revealed a more substantial reduction in 24-hour systolic BP among women versus men. An assessment of the reduction in 24-hour diastolic BP showed a dependence on age, too.

Symplicity HTN-1 and Symplicity HTN-3 also reported a dependence of BP variations on its baseline values; the Symplicity HTN-3 trial observed a dependence on age in addition to the baseline values. These trials are complemented by our finding that a reduction in diastolic BP also depends on its baseline value.

Earlier studies have shown no gender-specific differences in systolic BP variations, but many of the previous catheter-based RSD studies had a small number of female participants who accounted for 23% to 41% (3, 10, 26, 30, 31). The assessment of those data is difficult, because it is known that women have a varying SNS activity, which is dependent on the phase of the menstrual cycle (32). Therefore, we think it is necessary to conduct a meta-analysis of the prospective studies to verify gender-specific effects.

It should be further noted that the analysis of subgroups in the Symplicity HTN-3 trial demonstrated a correlation between the number of radiofrequency ablations and systolic BP variations. Indeed, when ≤9 RAs were delivered, the office systolic BP at 6 months increased by 12.1 mm Hg versus the sham group, but ≥10 RAs already demonstrated a significant reduction in the intervention group (10 RAs -0.4 mm Hg; 11 RAs -5.0 mm Hg; 12 and 13 RAs -4.1; ≥14 RAs -14.1 mmHg, trend p=0.01) (33). Therefore, the average number of 9.2 ± 2.2 of RAs performed during the Symplicity HTN-3 trial could be insufficient. Our study did not show a relationship between systolic BP variations and the number of RAs, but, for the purpose of comparison, it should be noted that we performed a considerably higher total number of successful ablations (an average of 16.21 ± 8.95 RAs per patient), and differences between the groups could be shaded as a result of sufficient denervation.

4.3 Changes in Arterial Stiffness after Catheter-Based Renal Sympathetic Denervation

The stiffness of the aorta, determined by measuring PWVc-f, is known to be an independent prognostic predictor of mortality in hypertensive patients (34). A

reduction in arterial stiffness and central hemodynamic indices at 1, 3 and 6 months after catheter-based RSD has been reported by Brandt MC et al. (13). However, to our best knowledge, there is no study available today that assessed the early changes. Our study showed that PWVc-f, assessed for the first time for the early period (within an interval from 24 to 48 hours), decreased from 11.3 m/s to 10.3 m/s (p=0.001). The change remained statistically significant in the late period, i.e., after 3, 6 and 12 months. These late period data replicate the findings by Brandt MC et al. (13). It is also important to note that Brandt MC et al. failed to determine a clear relationship between BP and PWVc-f variations (13). Our findings reconfirm the complementary effects of catheter-based RSD on the minimization of risks of cardiovascular diseases. Interestingly, the PWVc-f change determined in our study for the early post-procedural period was found to be independent from a reduction in mean central BP, r=0.165, p=0.214. A reduction in arterial stiffness not related to BP has been observed earlier with modification of the sympathetic nervous system through ganglion block therapy or surgical sympathectomy (35, 36). In the clinical parameters of post-catheter-based RSD available from earlier studies, the earliest drop in PWV (13) and 24-hours BP (37) was observed after 1 month. Our data for the early post-procedural period suggest that a catheter-based RSD-induced decrease in sympathetic tone manifests in the very early post-procedural period (in 24–48 hours). Thus, the risk of cardiovascular diseases starts lowering in the early post-procedural period.

In our opinion, the early change in PWVc-f determined by us could serve as an efficacy endpoint for the procedure after an additional trial. So far, there has been no method readily available in day-to-day clinical practice that would allow for an assessment of the success of radiofrequency ablation. The efficacy of renal artery ablation in the post-procedural period was assessed in a small study conducted by Pokushalov E. et al. They applied a high-frequency stimulation (20 Hz, 15 V, 10 ms) of the renal artery before and after the catheter-based RSD procedure, considering the procedure to be effective if the intra-arterial BP did not rise to >15 mm Hg with high-frequency stimulation (38). Unfortunately, the method has many flaws, requires additional equipment, the procedure is lengthy, and the safety of the method has not been established. Therefore, this method is not applied in clinical practice today. The above-described methods for assessing sympathetic nerve activity (local noradrenaline release and muscle microneurography) have not been applied in the early post-procedural period and are difficult to incorporate into day-to-day clinical practice due to their complexity and additional required intervention. Being independent from BP variations, change in PWVc-f in the early post-procedural period could become a marker for assessing the efficacy of the procedure.

The analysis of the likely predictor of PWVc-f changes in the late post-procedural period (after 6 months) showed a correlation between the PWVc-f baseline

value and PWVc-f change in the late post-procedural period. On the other hand, no correlation was found between PWVc-f changes in the late period and the BP baseline values, whether using office BP measurements (systolic r=0.113, p=0.451, diastolic r=0.146, p=0.328) or 24-hour BP monitoring (systolic r=-0.026, p=0.866, diastolic r=0.141, p=0.348). Accordingly, the higher the PWVc-f baseline value was, the greater the reduction was in 6 months after the procedure: Q_1 8.4 ± 1, Δ 0.05 ± 1.6 / Q_2 10.1 ± 0.4, Δ 1.1 ± 1.4 / Q_3 12.2 ± 0.8, Δ 1.8 ± 1.7 / Q_4 15.3 ± 1.7, Δ 2.8 ± 2.1, (p=0.002). In order to determine a PWVc-f threshold value giving the greatest prognostic accuracy for patients to whom PWVc-f changes would be more significant at 6 months after the catheter-based RSD procedure, an ROC curve analysis was carried out. The determined PWVc-f threshold value at baseline was 9.55 m/s. At this point, sensitivity was 0.81 and specificity was 0.58. Basing on the fresh data above, we may conclude that the greater the risk for a patient is (in terms of PWVc-f), the greater is the possible benefit of a catheter-based RSD. A PWVc-f threshold of 9.55 m/s is recommended for patient examination and randomization before conducting catheter-based RSD.

4.4 Changes in Cardiac Sympathetic Inner vation after Catheter-Based Renal Sympathetic Denervation

Experimental trials have determined that SNS is directly associated with the development of LV hypertrophy in hypertensive patients via beta-adrenergic receptor mediated cardiomyocyte stimulation (39). An increased sympathetic nerve activity in HF is indicated by elevated plasma noradrenaline concentrations (40) and the higher number of impulses per minute in nerve terminal microneurography (41). In addition, patients with HF have an inhibited reabsorption of noradrenaline in the nerve terminals (42). Metaiodobenzylguanidine is a guanidine analog stored in the nerve terminals and is believed to have the same uptake and storage mechanisms as noradrenaline (43). Some studies have shown that cardiac 123I-MIBG concentration and distribution can be used in assessing HF prognosis and response to therapy (44–46).

Our study showed, for the first time, a decrease in cardiac sympathetic innervation measured by 123I-MIBG concentration and distribution. A late heart-to-mediastinum ratio and washout rate are sympathetic activity markers. An early heart-to-mediastinum ratio reflect the integrity of the cardiac SNS. We found positive dynamics in the late heart-to-mediastinum ratio with an increase from 2.21 ± 0.47 at baseline to 2.35 ± 0.52 (p=0.02) (the increase is indicative of decreased sympathetic activity). There was no statistically significant change in the early heart-to-mediastinum ratio and washout rate. The changes above reflect the positive dynamics of the HF biochemical markers, which, in turn, have effects on the regression of

LV remodelling (this group also showed a significant decrease in LV hypertrophy and improved diastolic function parameters).

Findings from other two studies on changes in cardiac sympathetic innervation were published in the same year as our results. The findings of the other two studies were contradictory. The study conducted by Brussel PM et al. demonstrated no significant changes in cardiac sympathetic innervation 123I-MIBG indices after catheter-based RSD (47). The study conducted by Donazzan L. et al. showed a decrease in sympathetic innervation, which is in line with our findings. However, unlike us, they observed a significant drop in WR from 24.1 ± 12.7 to 7.9 ± 25.3% ($p = 0.047$) without a significant change in the late H/R (48). The difference in the findings of one study could be determined by the different protocols and calibration of cardiac 123I-MIBG, a small sample of patients and a very high level of heterogeneity in patient groups by the consumed drugs that have effects on cardiac innervation.

4.5 Changes in the Left Ventricle after Catheter-Based Renal Sympathetic Denervation

It is well known that LVH (left ventricular hypertrophy) is a negative factor in hypertensive patients, putting them at a higher risk of cardiovascular diseases, including HF mortality and morbidity, atrial fibrillation and sudden cardiac deaths (49). The role of LVH regression in improving prognosis independent of any BP changes has been confirmed by the HOPE study (50). Therefore, most studies conducted in the area of antihypertensive drugs are aimed not only at BP control and outcomes but also at LVH regression. It should be noted in this context that our catheter-based RSD procedures were performed in patients already receiving a combination of six antihypertensive drugs. Even added to the very intense antihypertensive therapy, catheter-based RSD substantially reduced LVH. In our study, we observed LVH regression by echocardiography and MRI.

Echocardiographic parameters showed positive dynamics in morphometric indicators of the left ventricle: interventricular septum thickness in diastole decreased by 0.08 ± 0.03 cm ($p<0.001$); LV posterior wall thickness in diastole decreased by 0.07 ± 0.02 cm ($p<0.001$) at 6 months after the catheter-based RSD procedure. A reduction in LV mass index was 10.48 ± 2.89 g/m^2 ($p<0.001$). There are quite many similar studies conducted in this area, and their findings with regard to decreases in myocardium mass index (this indicator has been chosen as varying the least after the studies) after catheter-based RSD are in line with our study's findings (11, 51–56). An improvement in the diastolic function has been also observed by some authors (11, 56). Unfortunately, large-sample, randomized studies assessing the LV structure and function are not available. The RDT-PEF study, aimed at assessing the effects of catheter-based RSD on patients with HF and preserved ejection fraction,

was terminated early because of difficulties in recruitment and was underpowered due to the small sample size (134). The meta-analysis of some studies assessing the LV structure and function, including data from our publication *Could careful patient selection for renal denervation warrant a positive effect on arterial stiffness and left ventricular mass reduction?* (58), showed that catheter-based RSD causes LVH regression and improvement in the LV function (59).

Although cardiac MRI is a more accurate and reproducible method than echocardiography (137), studies assessing LV changes after catheter-based RSD by MRI are scarce probably due to the accessibility and complexity of the study. To our best knowledge, apart from our study, there are three other postcatheter-based RSD studies of a similar design using MRI. We observed a significant reduction in the LV mass index from 101.11 ± 36.86 g/m^2 to 93.53 ± 30.49 (p=0.0067), which replicate the findings reported by Mahfoud F. et al. (12) and McLellan A. et al. (53). In contrast to our study and those of the aforementioned authors, the study conducted by Verloop L. et al. was the only one to demonstrate a 3.11% reduction in the LV mass index, which was below statistical the significance, p=0.09 (61).

In our study, the determined decrease in LV was index was not related to BP reduction (both office and 24-hour BP). These data are in line with and complementary to the meta-regression data (59). In addition, a significant decrease in the LV mass index after catheter-based RSD was also observed in patients with no BP reduction (12, 62).

To sum up, our study showed a significant decrease in LV hypertrophy at 6 months after the catheter-based RSD procedure by echocardiography and MRI. This reconfirms and complements the meta-analysis data of many similar studies.

5. Conclusions

1. With a strict selection protocol, catheter-based renal sympathetic denervation does considerably reduce blood pressure in patients with resistant arterial hypertension. The changes were captured at 3 months after the procedure and were maintained during the study period (after 6, 12 and 24 months).

2. Significant changes in the sympathetic nervous system after the catheter-based renal sympathetic denervation manifest in the early post-procedural period (on the second day after the procedure) and are confirmed by a considerable drop in pulse wave velocity (-0.8 ± 3.0 ms p=0.001) independently from blood pressure change.

3. There is a considerable decrease in cardiac sympathetic innervation at 6 months after the catheter-based renal sympathetic denervation procedure. The

123I-MIBG scan shows an increase in the late heart-to-mediastinum ratio from 2.21 ± 0.47 at baseline to 2.35 ± 0.52 (p=0.02).

4. Catheter-based renal sympathetic denervation reduces target-organ damage and arterial stiffness in the early post-procedural period, and it leads to a left ventricular hypertrophy regression at 6 months after the procedure.

7. Recommendations for clinical practice

1. With the use of a strict randomization protocol, an exclusion of secondary hypertension causes and strict adherence to drug therapy, catheter-based RSD is an effective treatment method for patients with resistant AH that fail to reach their blood pressure goals with medications.
2. Catheter-based RSD reduces BP and target-organ damage in patients with resistant AH.
3. Based on patient randomization for the catheter-based RSD procedure, we found out that the greatest benefits of the procedure are expected in:
   - Patients with higher BP values at baseline.
   - Younger patients (in our study, we observed a more considerable reduction in BP among patients under 61).
   - Patients with higher PWVc-f at baseline (in our study, we determined a 9.55 m/s PWVc-f threshold).
4. An early reduction in PWVc-f after the catheter-based RSD procedure can be used as an indicator for the efficacy of the procedure.

8. References

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Annexes

8.1 Authorization Provided by the Vilnius Regional Biomedical Research Ethics Committee

VILNIAUS UNIVERSITETO MEDICINOS FAKULTETAS

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VILNIAUS REGIONINIS BIOMEDICININIŲ TYRIMŲ ETIKOS KOMITETAS

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LEIDIMAS ATLIKTI BIOMEDICININĮ TYRIMĄ

2013-06-11 Nr.158200-13-641-205

Tyrimo pavadinimas:
Naujųjų arterinių žymenų prognostinės vertės, numatant kairiojo skilvelio diastolinės disfunkcijos atsiradimą ir progresavimą bei kardiovaskulinius įvykius, tyrimas.

Protokolo Nr.: VD041
Versija: 02
Data: 2013-06-05

Asmens informavimo ir asmens sutikimo forma (ŠLPPP ir turintiems atsparią gydymui arterinę hipertenziją) (lietuvių kalba):
Versija: 02
Data: 2013-06-05

Asmens informavimo ir asmens sutikimo forma (prieš planuojamą inkstų arterijų denervacijos procedūrą) (lietuvių kalba):
Versija: 02
Data: 2013-06-05

Asmens informavimo ir asmens sutikimo forma (CD rizikos biožymenų paieškos dalis)) (lietuvių kalba):
Versija: 02
Data: 2013-06-05
Pagrindiniai tyrėjai: Aleksandras Laucevičius

Tyrimo centras:
Įstaigos pavadinimas: VšĮ Vilniaus Universiteto Ligoninės Santariškių Klinikos
Įstaigos adresas: Santariškių g. 2, 08661 Vilnius

Leidimas galioja iki: 2019-06-30

Leidimas išduotas Vilniaus regioninio biomedicininių tyrimų etikos komiteto posėdžio (protokolas Nr. 158200-2013/06), vykusio 2013 m. birželio mėn. 11 d., sprendimu.

Vilniaus regioninio biomedicininių tyrimų etikos komiteto ekspertų grupės nariai			
Nr.	Vardas, pavardė	veiklos sritis	dalyvavo posėdyje
1	doc. Dr.Laimutė Jakavonytė	filosofija	ne
2	prof.dr.Jolanta Dadonienė	epidemiologija, medicina	taip
3	doc.dr. Jaunius Gumbis	teisė	ne
4	Genovaitė Bulzgytė	slauga	taip
5	Laura Linkevičienė	odontologija	taip
6	prof.dr. Augustinai Jankauskienė	medicina	taip
7	dr. Laura Malinauskienė	[illegible]	ne
8	Eglė Žubienė	psichologija	taip
9	Ugnė Šakūnienė	pacientų atstovas	taip

Pirmininkė Laura Malinauskienė

Publications

Berukstis A., Vajauskas D., Gargalskaite U., Misonis N., Burneikaite G., Zakarkaite D., Miglinas M., Laucevicius A. Impact of renal sympathetic denervation on cardiac sympathetic nerve activity evaluated by cardiac MIBG imaging. *Eurointervention* 2016, 11(9):1070-1076.

Palionis D., **Berukstis A.,** Misonis N., Ryliskyte L., Celutkiene J., Zakarkaite D., Cerlinskaite K., Valeviciene N., Tamosiunas A., Laucevicius A. Could careful patient selection for renal denervation warrant a positive effect on arterial stiffness and left ventricular mass reduction? *Acta Cardiologica* 2016, 71(2):173-183.

Thesis, posters, oral presentations

Berukstis A., Laucevičius A. Modification of the sympathetic nervous system in treatment of hypertension, one of the biggest evolutionary diseases. *The 4th International Conference of Evolution Medicine*, June 12–17, 2018. Vilnius, Lithuania.

Berūkštis A. Inovatyvus ambulatorinis 24val. arterinio kraujospūdžio stebėjimas. Papildomų parametrų vertinimas. *Didelės kardiovaskulinės rizikos pacientų atrankos ir prevencijos priemonių programos dešimtmetis*. 2016 m. sausio 21 d. Vilnius

Berukstis A., Vajauskas D., Gargalskaite U., Misonis N., Miglinas M., Petrauskiene B., Burneikaite G., Ivaska J., Laucevicius A. Effect of renal sympathetic denervation on cardiac sympathetic nerve activity assessed with iodine 123 metaiodobenzylguanidine scintigraphy. *The 64th American College of Cardiology Annual Scientific Session and Expo*, 14-16 March 2015, San Diego, US (poster). *J Am Coll Cardiol* 2015; 65(10):A1251. DOI: 10.1016/S0735-1097(15)61251-7.

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Berukstis A., Misonis N., Ryliskytė L., Navickas R., Cerlinskaitė K., Laucevicius A. CRT-606 Arterial Stiffness as an Early Marker of Renal Sympathetic Denervation Effectiveness. *Cardiovascular Research and Technologies*, February 24–25, 2014. Washington, US. *JACC: Cardiovascular Interventions* 2014;7(2) Suppl S: S49. DOI:10.1016/j.jcin.2014.01.128

Berūkštis A. Nemedikamentinis arterinės hipertenzijos gydymas. Mokslinė praktinė konferencija *X-oji intensyvios ir invazinės kardiologijos konferencija*. 2013 m. balandžio 19-20 d., Druskininkai.

Berūkštis A., Misonis N., Brazauskaitė B.O., Laucevičius A. Renal artery denervation in a patient with five renal arteries. *Workbook Trends 2013: CSI Congress*, March 1-2, 2013, Frankfurt/Darmstadt, Germany. *Abstracts book*: 44-45.

Berukstis A., N. Misonis, L. Ryliskyte, J. Aganauskiene, R. Navickas, D. Zakarkaite, Laucevicius A. Early changes in arterial stiffness and central hemodynamic after renal sympathetic denervation. *Artery Research* 09/2013; 7(3-4):170. DOI: 10.1016/j.artres.2013.10.030

SANTRAUKA

Įvadas

Arterinė hipertenzija (AH) yra labai dažna ir pavojinga liga, kuri negydant sukelia organų taikinių pažeidimą ir gali būti mirties priežastimi. Arterinė hipertenzija yra vienas iš dažniausiai pasitaikančių mirtingumo rizikos veiksnių pasaulyje. Sergamumas AH yra pasiekęs pandemijos lygį. 2010 metais AH sirgo 1,39 bilijono žmonių ir tai sudarė 31,1 proc. pasaulio suaugusiųjų, sergamumas nuo 2000 iki 2010 metų padidėjo 5,2 proc. Ir jeigu 2004 metais AH lėmė 7,5 milijonų mirčių pasaulyje, tai 2010 metais šis skaičius išaugo iki 9,4 milijonų. Kadangi AH - tai civilizacijos liga, lemiama genetinių ir aplinkos veiksnių (nutukimo, didelio druskos ir alkoholio kiekio suvartojimo, sėslaus gyvenimo būdo, emocinės įtampos ir kt.), tikėtina, jog dėl gyventojų senėjimo, sėslaus gyvenimo būdo ir netinkamos mitybos, šie skaičiai ateityje gali dar labiau didėti.

Medikamentinio AH gydymo pasirinkimo galimybės labai plačios. Nuo 1950-jų, kai AH gydymui pradėti taikyti diuretikai, buvo sukurta daug antihipertenzinių vaistų grupių. AH dažniausiai gydoma skiriant vieną arba kelis antihipertenzinius vaistus. Daugeliu tyrimų nustatyta, jog medikamentinis AH gydymas yra efektyvus mažinant AKS, organų taikinių pažeidimą ir širdies ir kraujagyslių ligų riziką. Tačiau realiame gyvenime, reikia nepamiršti, jog tai ilgalaikis, reikalaujantis geros kontrolės ir nuolatinės korekcijos gydymas. Kai skiriant tris vaistus, iš kurių vienas yra diuretikas, nepavyksta pasiekti AKS kontrolės – tokia AH forma vadinama rezistentiška arterine hipertenzija(RAH). RAH gydyti 2009 metais buvo pasiūlytas naujas intervencinis gydymo metodas – perkateterinė simpatinė inkstų arterijų denervacija (SIAD) . Šis gydymas skiriamas papildomai prie jau vartojamo medikamentinio gydymo. Vertinant šio gydymo efektyvumą, atliktų tyrimų duomenys skiriasi. Šiai dienai nėra aišku, kuriems pacientams perkateterinė SIAD duoda didžiausia naudą ir kodėl kai kurių pacientų AKS po procedūros lieka nepakitęs. Pacientų, sergančiųjų antrine arterine hipertenzija, simptomatika pasireiškia labai panašiai kaip ir RAH, todėl pacientų atrankos kriterijai yra labai svarbūs, siekiant užtikrinti perkateterinės SIAD efektyvumą.

Taip pat nėra metodo, kuris kasdienėje klinikinėje praktikoje leistų įvertinti atliktos procedūros kokybę. Taigi, atlikę perkateterinę SIAD, mes nežinome, ar pasiekta pakankama nervinių jungčių destrukcija.

RAH sergantys pacientai priklauso itin didelės rizikos grupei dėl organų taikinių pažeidimo. Pastebėtas kairiojo skivelio hipertrofijos (KSH) sumažėjimas po perkateterinės SIAD yra geras prognostinis žymuo. SNS aktyvumo padidėjimas yra vienas iš veiksnių, įtakojančių KSH išsivystymą. Reikalingas tyrimas, kuriame būtų ištirtas perkateterinės SIAD poveikis širdies simpatinei inervacijai.

Tyrimo hipotezė

Taikant griežtus atrankos kriterijus, pacientams, sergantiems rezistentiška arterine hipertenzija, perkateterinė simpatinė inkstų arterijų denervacija ženkliai sumažina simpatinės nervų sistemos aktyvumą ir aortos standumą, dėl ko sumažėja AKS ir organų taikinių pažeidimas.

Tyrimo tikslas

Įvertinti ankstyvuosius ir vėlyvuosius simpatinės nervų sistemos pokyčius, vertinant pulsinės bangos greičio matavimus, širdies 123-I MIBG ir jų įtaką arteriniam kraujo spaudimui ir organų taikinių pažeidimui po perkateterinės simpatinės inkstų arterijų denervacijos, atliekamos pacientams. sergantiems rezistentiška arterine hipertenzija.

Ginamieji teiginiai

1. Taikant griežtą atrankos protokolą, perkateterinė simpatinė inkstų arterijų denervacija ženkliai sumažina arterinį kraujo spaudimą pacientams, sergantiems rezistentiška arterine hipertenzija.
2. Perkateterinės simpatinės inkstų arterijų denervacijos poveikis pasireiškia jau ankstyvuoju poprocedūriniu laikotarpiu.
3. Perkateterinė simpatinė inkstų arterijų denervacija sukelia simpatinės širdies inervacijos poveikio sumažėjimą.
4. Perkateterinė simpatinė inkstų arterijų denervacija sumažina organų taikinių pažeidimą.

Tyrimo naujumas

Šiai dienai klinikinėje praktikoje nėra būdo įvertinti simpatinės nervų sistemos (SNS) pokyčius ankstyvuoju poprocedūriniu laikotarpiu. Nustatyta kad arterijų standumas vertinamas matuojant PBG yra priklausomas nuo SNS aktyvumo ir reikšmingai sumažėja praėjus 1, 3 ir 6 mėnesiams po procedūros. Tuo tarpu tyrimo, kuris įvertintu ankstyvuosius (0-48 val. po procedūros) PBG pokyčius nėra.

Daugelyje atliktų tyrimų vertintas AKS pokytis po perkateterinės SIAD, kai tuo tarpu tyrimų su žmonėmis, kurių metu tiesiogiai buvo tiriami simpatinės inervacijos pokyčiai, nedaug. Nustatytas SNS aktyvumo sumažėjimas po perkaterinės SIAD vertinant lokalų noradrenalino išskyrimą ir atliekant raumenų mikroneurografiją, tačiau trūksta duomenų apie širdies simpatinės inervacijos pokyčius po atliktos inkstų arterijų denervacijos.

Taip pat šis tyrimas nuo kitų SIAD tyrimų skiriasi pacientų atranka į tyrimą. Siekiant užtikrinti perkateterinės SIAD efektyvumą, buvo sukurtas detalus atrankos protokolas, kurio metu pacientai buvo ne tik detaliai ištiriami dėl antrinės AH, bet ir jiems atliekama medikamentinio gydymo korekcija ir kontrolė.

Metodika

Šis tyrimas atliktas prospektyviąja metodika. Tyrimas buvo atliktas Vilniaus Universiteto ligoninės Santaros klinikose (VUL SK). Buvo gautas Vilniaus regioninio biomedicininių tyrimų etikos komiteto leidimas atlikti biomedicininį tyrimą (Nr.158200-13-641-205). Į tyrimą buvo įtraukiami pacientai, sergantys rezistentiška AH, kuriems, praėjus ne mažiau kaip 1 mėnesiui po gydymo korekcijos, nepavyko pasiekti tikslinio AKS ir nesant kontraindikacijų buvo planuojama atlikti perkateterinę inkstų arterijų denervaciją. Prieš įtraukiant į tyrimą visi tiriamieji pasirašė asmens informavimo ir asmens sutikimo formą.

Įtraukimo į tyrimą protokolas

Pacientai, sergantys rezistentiška AH arba kuriems įtariama rezistentiška AH, buvo nukreipti kardiologo konsultacijai į Vilniaus Universiteto ligoninės Santaros klinikas. Pirmojo apsilankymo metu buvo atliekama paciento apklausa, sužinomi rizikos veiksniai ir sveikatos istorija, išsiaiškinama kokius vaistus pacientas vartoja, kokius vartojo anksčiau ir kokių vaistų netoleravo arba yra alergiškas. Šio vizito metu, remiantis paciento anamneze, objektyviais duomenimis ir jau atliktais laboratoriniais tyrimais, buvo sudaromas tolesnis tyrimo planas dėl antrinės AH. Pirmojo vizito metu pacientui rekomenduojama gyvenimo būdo korekcija ir atliekama medikamentinio gydymo korekcija remiantis "Europos kardiologų draugijos ir Europos hipertenzijos asociacijos 2013 metų gairėmis".

Antrasis apsilankymas atliekamas po 1 mėnesio. Jeigu pacientai laikėsi paskirto medikamentinio gydymo - atliekamas AKS matavimas gydytojo kabinete ir paros AKS monitoravimas. Pacientams, kuriems antrojo vizito metu nustatomas AKS gydytojo kabinete > 140/90 mm Hg ir/arba paros AKS > 130/80 mm Hg ir jie atitinka įtraukimo į tyrimą kriterijus, paiškinamos intervencinio gydymo - perkateterinės SIAD galima nauda ir rizika. Jeigu pacientas sutinka dalyvauti tyrime, jis pasirašo asmens informavimo ir asmens sutikimo formą. Jeigu pacientas atsisako perkateterinės SIAD, jam siūloma dalyvauti tyrimo kontrolinėje grupėje.

Iš 537 pacientų, dalyvavusių atrankoje, į pagrindinę tyrimo grupę buvo įtraukti 73, o į kontrolinę 21 tiriamasis. Dažniausia neįtraukimo į tyrimą priežastis buvo pseudorezistencija (praėjus 1 mėnesiui po medikamentninio gydymo korekcijos paros AKS < 130/80 mm Hg). Taip pat į tyrimą nebuvo įtraukti pacientai, kuriems buvo nustatyta antrinė AH, bei pacientai atsisakę dalyvauti tyrime.

Pacientų įtraukimo į tyrimą kriterijai:

1. Vyrai ir moterys, kurių amžius > 18 metų.
2. Pacientai, sergantys rezistentiška AH.
3. Pacientai, sergantys rezistentiška AH, kurių AKS gydytojo kabinete > 140/90 mm Hg ir/arba paros AKS 130/80 mm Hg, praėjus 1 mėnesiui po gydymo korekcijos.
4. Asmenys, pasirašę asmens informavimo ir sutikimo formą ir sutinkantys su tyrimo procedūromis.

Pacientų neįtraukimo į tyrimą kriterijai:

1. Diagnozuota antrinė AH, kuri gali būti gydoma kitais gydymo metodais.
2. Pacientai po inkstų transplantacijos ar gydomi hemodializėmis.
3. Hipotenzijos epizodai anamnezėje (sistolinis AKS < 100 mm Hg)
4. Ženklios širdies vožtuvų ydos, kuomet žemas AKS gali būti pavojingas gyvybei.
5. Mažiau negu prieš mėnesį įvykęs ūminis koronarinis sindromas.
6. Sunkus gretutinis susirgimas, pvz. piktybinis (kai išgyvenamumo tikimybė < 1 metai).
7. Nėštumas ar maitinimas krūtimi.
8. Tiriamasis tuo pačiu metu dalyvauja kitame medicinos prietaiso ar vaistinio preparato tyrime.

Stebėsenos protokolas

Pacientai, kurie atitiko įtraukimo kriterijus ir pasirašė sutikimo dalyvauti tyrime formą, prieš atliekant perkateterinę SIAD buvo papildomai ištiriami. Prieš atliekant perkateterinę SIAD pacientams buvo atliktas paros AKS monitoravimas ir AKS matavimai gydytojo kabinete, echokardiografija, arterijų standumo ir centrinio arterinio kraujospūdžio tyrimas aplanacinės tonometrijos metodu, subgrupėse atlikti širdies MRT ir širdies 123I-MIBG scintigrafijos tyrimai. Pacientai, kuriems buvo atlikta perkateterinė SIAD, buvo tiriami praėjus 24 - 48 valandoms, 3, 6, 12 ir 24 mėnesiams po procedūros. Kiekvieno vizito metu buvo atliekama paciento apžiūra, apklausa ir, jeigu reikia, papildomi tyrimai. Praėjus 3, 6, 12 ir 24 mėnesiams po procedūros buvo atliekamas paros AKS monitoravimas, AKS matavimai gydytojo kabinete, arterijų standumo ir centrinio arterinio kraujospūdžio tyrimas. Echokardiografija ir tyrimai subgrupėse (širdies MRT ir širdies 123I-MIBG scintigrafija) buvo atliekami praėjus 6 mėnesiams po procedūros.

Rezultatai

Viso perkateterinės SIAD grupėje tyrimo pradžioje buvo 73 pacientai, iš jų 34 (46,58%) vyrai ir 39 (53,42%) moterys. Vidutinis pacientų amžius 56,03 ± 7,76 m. Kadangi remiantis tyrimo protokolu, buvo siekiama išlaikyti stabilų medikamentinį gydymą bent 6 mėnesius, o pageidautina ir visą tyrimo laikotarpį - bendras vartojamų medikamentų skaičius viso tyrimo metu statistškai reikšmingai nekito. Kaip pagrindinis perkateterinės SIAD procedūros charakteristikos rodiklis vertintas radiodažnuminių abliacijų (RA) skaičius. Nustatytas RA vienam tiriamajam vidurkis 16,21 ± 8,95. Vidutinis RA skaičius kairėje renalinėje arterijoje buvo 7,85, dešinėje renalinėje arterijoje 7,87 abliacijos, kairėje pridėtinėje renalinėje arterijoje 6,33, o dešinėje pridėtinėje renalinėje arterijoje 6,88.

Vertintant AKS gydytojo kabinete po perkateterinės SIAD praėjus 3 mėnesiams stebėtas reikšmingas AKS mažėjimas, AKS gydytojo kabinete nuo pradinio 190/107 mm Hg atitinkamai sumažėjo iki 160/91 mm Hg ($p<0,001$). AKS gydytojo kabinete sumažėjimas išliko visą tyrimo laikotarpį po 6 mėnesių 156/90 mm Hg, po 12 mėnesių 162/94 mm Hg ir po 24 mėnesių 164/94 mm Hg ($p<0,001$).

Stebėtas reikšmingas paros AKS sumažėjimas vertinant diastolinį ir sistolinį AKS: praėjus 3 mėnesiams po procedūros paros AKS sumažėjo nuo 163/97 mm Hg iki 151/89 mm Hg ($p<0,001$). Paros AKS sumažėjimas išliko visą tyrimo laikotarpį: atitinkamai po 6 mėnesių 149/90 mm Hg, po 12 mėnesių 152/91 mm Hg ir po 24 mėnesių 148/91 mm Hg ($p<0,005$).

Vertinant $PBG_{mš}$ pokytį po perkateterinės SIAD stebėtas reikšmingas sumažėjimas ankstyvuoju poprocedūriniu laikotarpiu (laiko intervale tarp 24 ir 48 valandų), $PBG_{mš}$ sumažėjo nuo 11,3 m/s iki 10,3 m/s ($p=0,001$), šis pokytis išliko statistiškai reikšmingai sumažėjęs po 3, 6 ir 12 mėnesių. Po 24 mėnesių sumažėjimas statistiškai nebereikšmingas ($p=0,067$), reikia atreipti dėmesį, kad šiame laiko momente yra perpus sumažėjusi tiriamųjų imtis.

Vertinant MRT duomenis praėjus 6 mėnesiams po perkateterinės SIAD stebėta teigiama kairiojo skilvelio morfometrinių rodiklių dinamika: KS užpakalinės sienelės storis sumažėjo nuo 11,56 ± 3,38 mm iki 10,61 ± 2,31 mm ($p=0,0364$), KS masė sumažėjo nuo 211,33 ± 88,9 g

iki 196,51 ± 71,75 g (p=0,0047), o KS masės indeksas nuo 101,11 ± 36,86 g/m^2 iki 93,53 ± 30,49 (p=0,0067).

Vertinant širdies 123I-MIBG tyrimą nustatyta teigiama vėlyvojo širdies ir tarpuplaučio impulsų santykio dinamika, nuo pradinio 2,21 ± 0,47 jis padidėjo iki 2,35 ± 0,52 (p=0,02). Ankstyvasis širdies ir tarpuplaučio impulsų santykis ir išsiplovimo rodiklis statistiškai reikšmingai nekito.

Išvados

1. Taikant griežtą atrankos protokolą, perkateterinė simpatinė inkstų arterijų denervacija ženkliai sumažina arterinį kraujo spaudimą pacientams, sergantiems rezistentiška arterine hipertenzija: pokyčiai stebėti praėjus 3 mėnesiams po procedūros ir išliko visą tyrimo laikotarpį (praėjus 6, 12 ir 24 mėnesiams).
2. Simpatinės nervų sistemos pokyčiai po perkateterinės simpatinės inkstų arterijų denervacijos pasireiškia ankstyvuoju poprocedūriniu laikotarpiu, tai patvirtina reikšmingas nuo arterinio kraujo spaudimo nepriklausomas pulsinės bangos greičio sumažėjimas (-0,8 ± 3,0 ms p=0,001) antrąją diena po procedūros.
3. Praėjus 6 mėnesiams po perkateterinės simpatinės inkstų arterijų denervacijos ženkliai sumažėja širdies simpatinės inervacijos poveikis: širdies 123I-MIBG tyrime vėlyvasis širdies ir tarpuplaučio santykis padidėjo nuo 2,21 ± 0,47 iki 2,35 ± 0,52, (p=0,02).
4. Perkateterinė simpatinė inkstų arterijų denervacija sumažina organų taikinių pažeidimą: arterijų standumas sumažėja ankstyvuoju poprocedūriniu laikotarpiu, o praėjus 6 mėnesiams po procedūros regresuoja ir kairiojo skilvelio hipertrofija.

Hammad Mohammad Ahmad Aliahmed

AORTIC VALVE REPLACEMENT THROUGH MEDIAN STERNOTOMY AND MINISTERNOTOMY: A COMPARISON OF THE RESULTS

VIDURINĖS STERNOTOMIJOS IR MINISTERNOTOMIJOS AORTOS VOŽTUVO KEITIMO OPERACIJŲ REZULTATŲ PALYGINIMAS

Biomedical Sciences, Medicine (06 B)
Biomedicinos mokslai, medicina (06 B)

Abbreviations

BMI – body mass index
CABG – coronary artery bypass grafting
CI – confidence interval
ECHO – echocardiography
ECMO – extracorporeal membrane oxygenation
FFP – fresh frozen plasma
HFFC – heart failure functional class
INR – international normalized ratio
ISTD – interventricular septum thickness in diastole at rest, cm
LV – left ventricle of the heart
LVDD – diameter of the left ventricle in diastole, cm
LVEF – left ventricular ejection fraction
LVSD – diameter of the left ventricle in systole, cm
MI – minimal invasion
NYHA – New York Heart Association Functional Classification
OR – odds ratio
PG max – maximum gradient on aortic valve
PG mean – mean gradient on aortic valve
VAC – Vacuum-Assisted Closure
VAS – visual analogue scale
WA – weighted average (mean)
ECMO – Extracorporeal membrane oxygenation
IABP – Intra-aortic balloon pump

Introduction

Relevance of the problem

Over the past decade, the frequency of the aortic valve pathology has increased enormously, which has led to a search for sparing methods of surgical treatment. As a result, in 1996, new, minimally invasive approaches appeared in the arsenal of surgeons (upper and lower ministernotomy, V-shaped, Z-shaped, T-shaped, J-shaped and other types of ministernotomies). The development of new methods has allowed for improving the results of treatment and reducing the incidence of complications compared with median sternotomy; however, the question of their advantage over the traditional median sternotomy is still open.

It is known that the "gold standard" of aortic valve replacement by median sternotomy in the conditions of artificial circulation certainly gives better access to the heart, but at the same time, it is associated with greater traumatism, a risk of bleeding and a subsequent development of mediastenitis. In addition, severe pain syndrome in the early postoperative period requires the repeated use of narcotic analgesics even after discharge of patients and may contribute to an onset of respiratory failure due to the violation of breathing mechanics. In contrast, it has been proved that minimally invasive approaches reduce blood loss, decrease the likelihood of infection and the duration of hospitalization, improve the cosmetic effect and accelerate patient recovery.

However, some authors, in addition to the positive aspects of ministernotomy, emphasize its shortcomings – such as the longer operative duration, longer period of aortic cross-clamping with the transverse clamp and artificial circulation. These aspects are particularly relevant for patients at risk, in whom they can significantly affect the results of the operation and the subsequent well-being of the patient.

There is an opinion that with the accumulation of surgical experience, all of the abovementioned problems can be solved, and that ministernotomy results will become better, providing the possibility of expanding the indications for the application of minimally invasive methods. Therefore, the experience of each individual clinic is of interest.

It should be noted that the choice of optimal access is not only medical, but also socioeconomic in nature, since specific complications arising after operations significantly worsen treatment results and often lead to a persistent disability of the patients, and their treatment requires additional financial costs.

Importance and novelty of the research

Despite the fact that there are many studies comparing the results of median sternotomy and minimally invasive techniques to date, all of them are mainly concerned with surgical nuances. At the same time, studies regarding the need for medications, investigations of postoperative course features and the restoration of physiological functions in two groups of patients have not been given due attention. In addition, modern studies focus on short-term results, but the effect of the method of performing the operation on long-term outcomes and patient survival cannot be excluded either, and this requires further study as well. Thus, it is necessary to carry out the detailing of the available results by conducting a detailed comparison of operations by means of ministernotomy and median access, revealing not only features of the course of operation itself, but all the subtleties of the postoperative period and the period of long-time observation.

In addition, the peculiarity of works available to date is that most of them are studying individual aspects of treatment results, such as the course of operation, early postoperative results or long-term results.

At the same time, there is a need for an integrated study that considers all these factors, as it would be a comprehensive study of an issue that would determine the global advantages of a particular technique.

The innovative value of this work lies in that it is a comprehensive study of all possible influences of the type of access on the patient's health.

The results of the work detail the course of operation and the early postoperative period in patients undergoing ministernotomy and median access. We have studied not only the generally accepted characteristics but also the needs in various groups of drugs, the courses of the recovery period and the subjective feelings of the patient.

This study presents the most complete dynamic assessment of the clinical characteristics and echocardiography indicators for a duration of 3 years, which characterizes the functional state of the patients' cardiovascular systems and their overall well-being, and this significantly complements the results of other studies that did not disclose the detailed course of the postoperative period in these patients.

Another important aspect is the need for the correction of indications for performing access, since with the accumulation of technical experience, there is a change in the principles of patient selection for carrying out one or the other type of intervention. This is especially clearly seen in the example of patients at risk. Particularly in the case of patients with obesity, we see that not so long ago, overweight was considered a contraindication for the implementation of minimally invasive interventions. However, recently, a rethinking of these views has occurred, and studies have appeared that show that the use of ministernotomy is associated with a smaller volume of operational trauma, a reduction in the number of postoperative complications and a faster recovery of patients. Thus, it is necessary to develop new principles for the selection of patients for ministernotomy. However, information about the long-term results of surgical interventions also remains very scarce and requires additional research.

As part of the development of such a strategy of selecting patients as a basis, it is important to study the effects of anthropometric, clinical and laboratory indicators before surgery on the course of surgical intervention and on the course of the nearest postoperative period in patients when using various techniques, especially since such studies are single.

By means of a correlation analysis, the effect of preoperative indices on the course of surgical intervention and on the course of the nearest postoperative period in patients after ministernotomy and median access was assessed, which can serve as a basis for new criteria for choosing a particular type of operation depending on the patient's initial characteristics.

Thus, a detailed comparison of operation results, including the short- and long-time results of mini-invasive and traditional methods, is quite relevant for selecting the most prioritized type of access.

The aim of the research

The purpose of this study is to compare the results of aortic valve replacement by means of median sternotomy and minimal invasion (ministernotomy).

The objectives of the research

1. An assessment of the effectiveness and safety of the ministernotomic approach when replacing an aortic valve in patients suffering from aortic valve pathology and its comparison with the median (standard) sternotomic approach.
2. A comparative analysis of the operative peculiarities of aortic valve replacement in patients from groups when applying the ministernotomic and median sternotomic approaches.
3. A comparative analysis of the postoperative indices in patients from groups who have received ministernotomy and sternotomy treatments when assessing the indices during the patients' stays in an intensive care unit.
4. A study of the early postoperative clinical results in patients from comparative groups, including the rate of complications, sensations of pain, the ability to perform simple actions and the length of hospital stays.
5. A study of long-term clinical results in patients from comparative groups.
6. An assessment of the impact of anthropometric, clinical and laboratory indices in patients who underwent preoperative examinations on the process of surgical intervention and the peculiarities of the early postoperative period when applying the minimum and median sternotomic approaches.
7. A comparative analysis of characteristics in patients who are overweight after surgery for aortic valve replacement when applying the minimal and median sternotomic approaches, and an assessment of early and long-term results in patients from both groups.
8. An assessment of what impact does overweight have on the operative and postoperative results in patients from both groups in question.

Scientific novelty

The innovative value of this work lies in that it is a comprehensive study of all possible influences of the type of access on the patient's health.

The results of the work detail the course of operation and the early postoperative period in patients undergoing ministernotomy and median access. We have studied not only the generally accepted characteristics but also the needs in various groups of drugs, the courses of the recovery period and the subjective feelings of the patient.

This study presents the most complete dynamic assessment of the clinical characteristics and echocardiography indicators for a duration of 3 years, which characterizes the functional state of the patients' cardiovascular systems and their overall well-being, and this significantly complements the results of other studies that did not disclose the detailed course of the postoperative period in these patients.

By means of correlation analysis, the effect of preoperative indices on the course of surgical intervention and on the course of the nearest postoperative period in patients after ministernotomy and median access was assessed, which can serve as a basis for new criteria for choosing a particular type of operation depending on the patient's initial characteristics.

Practical significance of the research

The practical significance of this study lies in the possibility of using the results obtained in the work of cardiac and cardiosurgical departments and centers to optimize the treatment of patients with aortic valve pathology, to reduce surgical lethality and to shorten the period of hospitalization.

As a result of the work done, the main problems in the early and distant period were identified in patients who had undergone median sternotomy and ministernotomy. A more sparing effect of ministernotomy is noted, which makes it possible to recommend this access as a priority intervention in the case of aortic valve pathology.

A clarification of the criteria for choosing a particular type of operation, depending on the patient's initial characteristics, makes it possible to expand the indications for ministernotomy.

It is proved that ministernotomy not only accelerates the patient's return to everyday life but also contributes to more favorable clinical results and probably has a higher economic efficiency due to the shorter duration of hospitalization and the use of fewer medicines.

1. Subjects and Methods of the Research

1.1 Research Subjects

This is a retrospective study of patients who underwent isolated aortic valve replacements by means of either median sternotomy or ministernotomy during 2011–2016 on the base of Vilnius University Hospital Santaros Klinikos. In order to conduct the study, Permission No. 158200-14-715-235 was obtained from the Vilnius Regional Committee for the Ethics of Biomedical Research. A total of 426

patients, aged from 18 to 88 years with isolated aortic valve pathologies, were examined. Seventy of the patients underwent mininsternotomies, and 356 patients underwent median sternotomies. Data were collected from medical case histories, surgical and anesthesia protocols and outpatient cards.

At the first stage of the research, two groups, each consisting of 70 patients, matched for age, sex, body mass index, primary diagnosis, disease etiology, NYHA score and echocardiography parameters before surgery, were selected using the method of propensity score matching (PSM). **The first (main) group** included patients who had undergone aortic valve replacement by means of a ministernotomy. **The second group (control group)** consisted of patients who had undergone median sternotomy.

The second phase of the research was devoted to an analysis of the results of interventions to replace the aortic valve in patients who were overweight. Fifty-six patients, matched for age, sex, body mass index, primary diagnosis, disease etiology, NYHA score and echocardiography parameters before surgery, were selected from each group using the method of propensity score matching (PSM).

The criterion for inclusion into research: only an isolated pathology of the aortic valve;

Exclusion criteria:

- Multi-valve damage;
- Acquired heart defects requiring correction in conjunction with CABG.
- Age of patients <18 years.

1.2 Research Methods

All the patients under examination underwent a survey that included a complex of clinical, laboratory-instrumental and functional methods.

During the clinical examination of patients, the presence of indications and contraindications to this or that method of surgical correction of the cardiac pathology was being detected.

To assess the presence of obesity and its degree, a calculation of the Quetelet index was carried out.

Preoperative investigations

- 12-lead electrocardiogram;
- 24-hour ECG monitoring;
- Bicycle stress test, treadmill test, 6-minute walk test (to determine the functional class of heart failure);
- Transthoracic cardiac echocardiography (to exclude structural heart disease and determine inotropic indicators);

- Doppler echocardiography;
- Radiography;
- Complete blood test, creatinine, C-reactive protein, potassium, sodium;
- Other studies performed on individual indications (usually coronary angiography, thyroid hormones, a magnetic resonance imaging of the brain, fibrogastroscopy, an ultrasound examination of the abdominal cavity organs).

Postoperative Investigations

The clinical examinations of patients, laboratory studies, instrumental studies (electrocardiograms, echocardiographies) were performed in 5–10 days after surgery and at discharge.

Echocardiographic examinations performed in 5–10 days after surgery and in the outpatient department after 6 months, 1, 2, 3, 4 and 5 years.

Postoperative follow-up visits were performed in outpatient departments after 6 months, 1, 2, 3, 4 and 5 years.

A patient survey was conducted to analyze treatment results.

To evaluate the intensity of postoperative pain, a visual analogue scale was used. In addition, the survey was conducted to determine the ability of patients to perform simple manipulations, such as coughing, inhaling and exhaling deeply, eating, walking for a short distance and brushing their teeth.

Another survey was devoted to patients' attitudes regarding the appearance of their post- operative wounds (the cosmetic effect).

Postoperative observations, including clinical examinations, ECHO, ECG and laboratory tests were performed in 5–10 days after surgery, at discharge, on the 30th day after surgery as well as 1, 2, 3 and 4 years after surgery.

2. Clinical characteristics of patients

2.1.1. The First Stage of the Study

The main group (N = 70) had a prevalence of men – 60.0%. The mean age was 60.8 ± 11.4 years, the Quetelet index was 27.7 ± 4.4 kg/m^2, and the majority of the patients – 34 (48.6%) – were overweight.

The control group (N = 70) also had a prevalence of men – 42 (60.0%). The mean age was 61.4 ± 11.9 years, while the Quetelet index was 27.9 ± 4.5 kg/m^2.

The clinical characteristics of the patients were similar in comparison (Table 1).

In both groups, patients with the Euroscore II 1–3% had predominated, but the median access group contained a significantly greater number of patients with the Euroscore II >3% (p = 0.049).

An analysis of comorbidities showed that chronic obstructive pulmonary disease was significantly more frequent in patients with minimally invasive access – 4 (5.7%) (p = 0.042), but all of them did not take medicines. Among patients after median sternotomy, this pathology was not diagnosed. Also, those patients who had undergone ministernomy had significantly more often suffered from hypertension (22 versus 10, p = 0.016).

There were no previous strokes before surgery in patients in the ministernotomy group, while in the sternotomy group, 2 patients had a stroke more than 90 days prior to surgery (2.86%, p = 0.154).

A coronary pathology was more common in the ministernotomy group, but the differences were not statistically significant – 4.29% versus 1.43% (p = 0.310).

TABLE 1 – The preoperative clinical characteristics of patients.

Indicator	Median sternotomy (N = 70)	Ministernotomy (N = 70)	p
Diagnosis, n (%)			
Aortic stenosis	45 (64.3%)	51 (72.9%)	0.275
Aortic regurgitation	13 (18.6%)	12 (17.1%)	0.825
Combined	12 (17.1%)	7 (10%)	0.217
Etiology of the disease, n (%)			
Senile degeneration	53 (75.71%)	53 (75.71%)	1.000
Annular expansion	12 (17.14%)	13 (18.6%)	0.825
Mitral valve	0 (0%)	1 (1.4%)	0.316
Infective endocarditis	5 (7.14%)	3 (4.3%)	0.466
EuroSCORE II			
<1%, n (%)	20 (28.6%)	24 (34.3%)	0.466
1-3%, n (%)	42 (60%)	44 (62.9%)	0.728
>3%, n (%)	8 (11.4%)	2 (2.9%)	0.049*
Euroscore_II, mean score %	1.67 ± 0.07	1.48 ± 0.45	0.256
NYHA, n (%)			
II	6 (8.6%)	12 (17.14%)	0.130
III	64 (91.4%)	57 (81.43%)	0.084
IV	0 (0%)	1 (1.43%)	0.316
International normalized ratio (INR), mean±SD	1.04 ± 0.15	1.07 ± 0.04	0.200
Creatinine clearance, (ml/min)			
<50, n (%)	36 (51.4%)	41 (58.6%)	0.396
50–85, n (%)	31 (44.3%)	27 (38.6%)	0.493
>85, n (%)	3 (4.3%)	2 (2.86%)	0.649
Mean creatinine level, μmol/l	80.93 ± 20.60	82.14 ± 15.84	0.425
The highest creatinine level, μmol/l, mean±SD	100.26 ± 60.92	92.65 ± 25.07	0.325

Indicator	Median sternotomy (N = 70)	Ministernotomy (N = 70)	p
Hemoglobin level, g/l	129.43 ± 17.67	134.63 ± 15.85	0.165
The lowest level of hemoglobin, g/l	103.79 ± 10.77	108.71 ± 13.37	0.045*
The lowest platelet level, thousand /µl	198.68 ± 70.83	197.41 ± 70.83	0.784
Total bilirubin, µmol/l	12.19 ± 0.70	10.43 ± 0.34	0.229
Diabetes mellitus, n (%) *Treated with tablets* *Treated with insulin*	5 (7.1%) 2 (2.86%) 3 (4.29%)	11 (15.7%) 7 (10%) 4 (5.7%)	0.111 0.085 0.698
COPD, n (%)	0 (0%)	4 (5.7%)	0.042*
Hypertension, n (%)	10 (16.0%)	22 (31.4%)	0.016*
Peripheral vascular disease, n (%)	1 (1.4%)	0 (0%)	0.316
History of stroke, n (%) *<90 days* *>90 days*	2 (2.9%) 0 (0%) 2 (2.86%)	0 (0%) 0 (0%) 0 (0%)	0.154 --- 0.154
Coronary artery disease, n (%)	1 (1.4%)	3 (4.3%)	0.310
Renal failure, n (%) *Treated with diuretic* *The use of hemofiltration* *The use of hemodialysis*	1 (1.4%) 1 (1.4%) 0 (0%) 0 (0%)	2 (2.9%) 2 (2.9%) 0 (0%) 0 (0%)	.559 0.559 --- ---
Anticoagulation therapy 1 week before surgery *– Aspirin, n (%)* *– Warfarin, n (%)*	 0 (0%) 0 (0%)	 1 (1.43%) 1 (1.43%)	 0.316 0.316
Rhythm disorders before surgery, n (%) *Permanent atrial fibrillation* *Paroxysmal atrial fibrillation* *Pacemakers*	 2 (2.9%) 0 (0%) 2 (2.9%)	 6 (8.6%) 2 (2.9%) 5 (7.1%)	 0.145 0.154 0.245
LVEF %, n (%) *<30%* *30-49%* *≥50%*	 0 (0%) 11 (15.7%) 59 (84.3%)	 0 (0%) 16 (22.9%) 54 (77.1%)	 ---- 0.284 0.284
Mitral valve insufficiency, in total, n (%) *I degree* *II degree*	30 (51.1%) 25 (83.3%) 5 (16.7%)	23 (32.9%) 22 (95.7%) 1 (4.3%)	0.223 0.161 0.161
Tricuspid valve insufficiency, in total, n (%) *I degree* *II degree* *III degree*	22 (31.4%) 22 (100%) 0 (0%) 0 (0%)	4 (5.7%) 4 (100%) 0 (0%) 0 (0%)	<0.001* 1.00 --- ---

Indicator	Median sternotomy (N = 70)	Ministernotomy (N = 70)	p
Aortic valve insufficiency, in total, n (%) *I degree* *II degree* *III degree*	49 (70%) 13 (26.5%) 20 (40.8%) 16 (32.65%)	50 (71.4%) 10 (20%) 20 (40%) 20 (40%)	0.246 0.591 1.000 0.620
ISTD, cm	1.16 ± 0.20	1.18 ± 0.21	0.212
LVDD, cm	5.41 ± 0.70	5.58 ± 0.75	0.398
LVSD, cm	3.66 ± 0.90	3.94 ± 0.69	0.452
PG max, mm Hg	60.62 ± 35.50	64.90 ± 40.80	0.650
PG mean, mm Hg	43.65 ± 22.50	51.79 ± 23.05	0.682

* p<0, 05 – differences between groups are significant.

2.1.2. At the Second Stage of the Study

The main group (N = 56) had a prevalence of men – 38 (67.9%). The mean age was 61.9 ± 14.1 years, the Quetelet index was 30.34 ± 4.15 kg/m^2, and the majority of the patients – 44 (78.6%) – were overweight.

The control group (N = 56) also had a prevalence of men – 36 (64.3%). The mean age was 62.08 ± 10.55 years, while the Quetelet index was 27.9 ± 4.5 kg/m^2.

A detailed assessment of the clinical characteristics showed that the most common pathology requiring surgery was the stenosis of the aortic valve, occurring in about 78.6% of the patients. Primarily, the etiology of the disease, which had led to the surgical intervention, was a senile degeneration of the aortic valve, which was observed in almost 83.9% of all patients.

In both groups, patients with the Euroscore II 1–3% had predominated. According to the NYHA classification, most patients in both groups had NYHA III – almost 85.7% of all patients.

Diabetes mellitus occurred in 11 (19.7%) patients from both groups. In the ministernotomy group, 7 patients took tablets and 4 were treated with insulin. In the median sternotomy group, 8 patients took tablets and 3 were treated with insulin.

Chronic obstructive pulmonary disease was registered in 4 (7.1%) patients of both groups. All patients did not take medicines and did not use bronchodilators.

A coronary pathology was more common in the sternotomy group, but the differences were not statistically significant – 10.7% versus 5.4% (p = 0.410).

The preoperative characteristics of patients are presented in Table 2.

TABLE 2. The preoperative clinical characteristics of patients.

Indicator	Median sternotomy (N = 56)	Ministernotomy (N = 56)	P
Diagnosis, n (%) *Aortic stenosis* *Aortic regurgitation* *Combined*	 41 (73.2%) 9 (16.1%) 6 (10.7%)	 44 (78.6%) 9 (16.1%) 3 (5.4%)	 0.508 1.000 0.297
Etiology of the disease, n (%) *Senile degeneration* *Annular expansion* *Mitral valve*	 47 (83.9%) 9 (16.1%) 0 (0%)	 44 (78.6%) 11 (19.6%) 1 (1.8%)	 0.468 0.622 0.315
EuroSCORE II *<1%, n (%)* *1-3%, n (%)* *>3%, n (%)*	 18 (32.1%) 37 (66.1%) 1 (1.9%)	 11 (19.6%) 43 (76.8%) 2 (3.6%)	 0.675 0.331 0.309
Euroscore II, mean score %	1.35 ± 0.70	1.40 ± 0.69	0.700
NYHA, n (%) *II* *III* *IV*	 7 (12.5%) 47 (83.9%) 2 (3.6%)	 7 (12.5%) 48 (85.7%) 1 (1.8%)	 1.000 0.792 0.558
Creatinine clearance, (ml/min) *<50, n (%)* *50–85, n (%)* *>85, n (%)* Mean creatinine level, μmol/l	 2(3.6%) 22(39.3%) 32(57.1%) 83.93 ± 19.73	 34(60.7%) 21(37.5%) 1(1.8%) 82.96 ± 15.81	 0.701 0.846 0.558 0.776
International normalized ratio (INR), (mean±SD)	1.07 ± 0.09	1.36 ± 0.81	0.110
Diabetes mellitus, n (%)	11 (19.7%)	11 (19.7%)	1.000
COPD, n (%)	4 (7.1%)	4 (7.1%)	1.000
Hypertension, n (%)	12 (21.03%)	12 (20.1%)	0.203
Coronary artery disease, n (%)	6 (10.7%)	3 (5.4%)	0.410
Peripheral vascular disease, n (%)	0 (0%)	0 (0%)	---
Stroke, n (%)	0 (0%)	0 (0%)	---
Rhythm disorders, n (%) *Permanent atrial fibrillation* *Paroxysmal atrial fibrillation* *Pacemakers*	 10 (17.9%) 2 (3.6%) 2 (3.6%)	 5 (8.9%) 1 (1.8%) 3 (5.4%)	 0.557 0.559 0.647
Anticoagulation therapy 1 week before surgery	4 (7.1%)	2 (2.9%)	0.261

Indicator	Median sternotomy (N = 56)	Ministernotomy (N = 56)	P
LVEF %, n (%) <30 %	0 (0%)	0 (0%)	---
30%-50 %	42 (75%)	16 (28.6%)	0.580
≥50%	14 (25%)	40 (71.4%)	0.654
ISTD, cm	1.21 ± 0.23	1.18 ± 0.21	0.482
LVDD, cm	5.55 ± 0.88	5.58 ± 0.75	0.822
LVSD, cm	3.77 ± 0.85	3.94 ± 0.69	0.277
PG max, mm Hg	64.22 ± 30.93	64.90 ± 40.80	0.921
PG mean, mm Hg	50.33 ± 14.06	51.79 ± 23.05	0.744
Aortic valve insufficiency, in total, n (%) *I* *II* *III*	40 (71.4%) 11 (27.5%) 17 (42.5.7%) 12 (30%)	42 (75%) 8 (19.04%) 18 (42.85%) 16 (38.09%)	0.682 0.411 0.260 0.383

*p<0, 05 – differences between groups are significant.

2.2. Operational techniques

2.2.1. Performing operations with minimally invasive accesses

The technique of operation does not differ from the technique with standard sternotomy for intraoperative indices. Tracheal intubation was performed in the usual manner by means of a single-lumen tube. For operations on the aortic valve, the ascending aorta and the right atrial appendage were cannulated and left ventricular drainage was performed through the right superior pulmonary vein.

For a cannulation of the aorta, we used both curved and straight cannulas. A two-tier venous cannula was used for venous drainage.

When performing the operations in accordance with a minimally invasive technique, myocardial protection was obtained by the patient cooling down to 26–28°C. A cardioplegic solution was fed antegradely through the aortic root or directly through the coronary openings after aortotomy. After the aortic valve replacement, the wound was stitched stepwise; a drainage of the pericardium was carried out through the drainage tubes in the epigastric region.

Under a **J-shaped ministernotomy**, a 6–7 cm skin incision was started at the level of the second rib and led to the level of the fourth rib; a sternum cut was started from the jugular fossa and led to a 4 intercostal space and then continued to the right.

Under a **W-ministernotomy**, a skin incision was started 2 cm lower than during the median sternotomy, when the standard skin incision is made along the midline to the second intercostal space. Further in the second intercostal space, the body of the sternum was intersected, starting from the two lateral sides (right and left) to the median line. Then, the body of the sternum was cut from the jugular notch to the apex of the V-type cut at the level of the second rib.

A **partial sternotomy** is not difficult access, since it can be used for repeated operations. In the case of conversion, a partial sternotomy may be extended to a median sternotomy.

During the operation, an incision was started on the jugular notch of the sternum and ended just below presternum synchondrosis (to the level of the VI intercostal space). The skin, fascia and periosteum were incised at the midline along the sternum. After stopping the ventilation of the lungs, the cut was made from top to bottom – to the presternum synchondrosis or slightly below, and then the ventilation of the lungs was resumed. Bleeding from periosteum edges was stopped using electrocoagulation and by using a spongy substance – by rubbing wax with paraffin. When using this method, it is important to perform an accurate widening of the sternum edges, because in the case of a technological violation, a transverse fracture of the sternum near the bottom end of the cutting is inevitable. For these purposes, we used a small Tuffier rib spreader.

Figures 1–3 present the implementation of the minimally invasive operations.

FIGURE 1. The size of the surgical wound under ministernotomy.

FIGURE 2. An operation with artificial circulation.

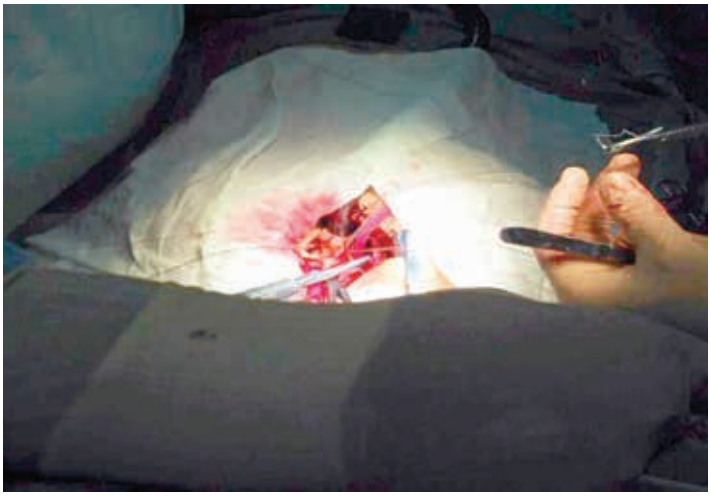

FIGURE 3. The course of surgery.

2.3. Statistical processing

The statistical processing of the data was carried out using the methods of variation statistics and with the application package IBM SPSS 20.0 (IBM Corp., NY, US), Statistica 12" (Stat Soft., Tulsa, OK, US) and the R packages party and rpart.

Data evaluation for normal distribution was carried out using a Lilliefors normality test, whereby it was found that the data are not normally distributed. Accordingly, nonparametric statistical methods were used for processing the results.

A Kruskal-Wallis test was used to test median difference, and the exact significance level of differences between the groups was calculated using the Mann-Whitney method.

To analyze the differences in the frequencies between two independent groups of research subjects and test the null statistical hypothesis, we used a chi-square Mantel-Haenszel and a Fisher's exact tests (for small numbers with a binomial distribution, if the number of observations in one or more cells of 2×2 tables was ≤5).

In processing the survey data, a survival analysis using a Kaplan-Meier and Long-rank test was conducted. In the tables, data are presented as mean values and mean value errors (M ± m).

The results obtained were evaluated with the help of the p-value. This is the probability of the random nature of the effect obtained in the study. The P-value assesses the overall statistical significance of differences between groups. The value of $p \leq 0.05$ was made to be a condition of statistical significance.

Chapter 3. Results of own research

3.1. An assessment of indicators in selected groups of patients

3.1.1 Operative indicators

An estimation of surgery duration in selected groups of patients showed that the duration of the operation in the two groups was comparable regardless of access type ($p = 0.856$).

In the ministernotomy group, a biological valve was installed significantly more often, and in the median sternotomy group, a mechanical valve was installed significantly more often, $p<0.001$. The frequency of the use of valves having different sizes was comparable in the two groups.

The most popular manufacturer of valves in both groups was St. Jude – in 88.57% of the patients; this firm prevailed in patients with median access in 74.29% of all cases. Under median sternotomy, valves manufactured by a company belonging to the Sorin group were used significantly more frequently – 8.57% versus 0.0% in the ministernotomy group ($p = 0.012$).

Ministernotomies required longer Aortic cross-clamping times during the surgeries. On average, in the main group of the patients, the aorta was clamped 8 minutes longer than in the control group of patients ($p = 0.007$).

The use of extracorporeal circulation was longer under ministernotomy. It lasted almost 12 minutes longer than under sternotomy ($p = 0.049$).

During resternotomy, an extracorporeal membrane oxygenation (ECMO) was used in 1 patient (1.4%) undergoing ministernotomy. This procedure was not used among the selected patients of the median access group (p = 0.428). An intra-aortic balloon pump was not used in any patient of the selected groups.

The operation characteristics of the two groups of patients are presented in Table 3.

TABLE 3. The characteristics of the operative performance in groups.

Indicator	Median sternotomy (N = 70)	Ministernotomy (N = 70)	p
Surgery duration (min), (mean±SD)	256.9 ± 79.7	263.5 ± 62.0	0.856
Aortic cross-clamping time (min), (mean±SD)	80.3 ± 24.6	88.7 ± 20.7	0.007*
Cardiopulmonary bypass time (min), (mean±SD)	132.9 ± 44.9	144.0 ± 29.9	0.049*
Number of cardioplegia cycles	4.42 ± 1.40	4.30 ± 1.01	0.779
Cardioplegia duration, (min)	18.23 ± 9.80	17.14 ± 7.94	0.259
The first cardioplegia, n (%) *Aortic root* *Coronary mouth* *Retrograde cardioplegia*	 1 (1.4%) 60 (85.7%) 9 (12.9%)	 12 (17.1%) 58 (82.9%) 0 (0%)	 0.001* 0.642 0.002*
Cardioplegia during surgery, n (%) *Retrograde cardioplegia* *Coronary mouth* *Combined*	 4 (5.7%) 30 (42.9%) 36 (51.4%)	 0 (0%) 70 (100%) 0 (0%)	 0.042* <0.001* <0.001*
Cardioplegic solution, n (%) *Pharmaco-cold cardioplegia* *Cold blood cardioplegia*	 37 (52.9%) 33 (47.1%)	 62 (88.6%) 8 (11.4%)	 <0.001* <0.001*
Repeated cardioplegia, n (%)	3 (4.3%)	0 (0.0%)	0.080
Aortotomy type, n (%) *Transverse aortotomy* *Hockey stick aortotomy*	 6 (8.6%) 64 (91.4%)	 32 (45.7%) 38 (54.3%)	 <0.001* <0.001*
Bleeding during surgery, n (%) *Source – aortotomy* *Other source*	1 (1.4%) 1 (1.4%) 0 (0%)	0 (0%) 0 (0%) 0 (0%)	0.316 0.316 ---
The lowest patient's body temperature during surgery,°C	28.45 ± 2.40	28.05 ± 0.87	0.668
Valve type, n (%) *Biological* *Mechanical*	 24 (34.3%) 46 (65.7%)	 60 (85.7%) 10 (14.3%)	 <0.001* <0.001*

Indicator	Median sternotomy (N = 70)	Ministernotomy (N = 70)	p
Aortic valve size, n (%)			
19 mm	0 (0%)	0 (0%)	---
21 mm	7 (10%)	4 (5.7%)	0.346
23 mm	36 (51.4%)	33 (47.1%)	0.612
25 mm	19 (27.14%)	29 (41.4%)	0.075
27 mm	8 (11.43%)	4 (5.7%)	0.227
29 mm	0 (0%)	0 (0%)	---
Aortic valve manufacturer, n (%)			
St. Jude	52 (74.3%)	62 (88.6%)	0.030*
ATS	0 (0.0%)	2 (2.86%)	0.154
Sorin	6 (8.6%)	0 (0%)	0.012*
Medronic Hall	12 (17.1%)	6 (8.57%)	0.130
Additional procedures, n (%)			
Aortic root widening	1 (1.4%)	0 (0%)	0.316
Left atrial appendage ligation	0 (0%)	6 (8.6%)	0.012*
ECMO, n (%)	0 (0%)	1 (1.4%)	0.428
IABP, n (%)	0 (0%)	0 (0%)	---

*$p<0.05$ – differences between the groups are significant.

3.1.2. An evaluation of postoperative indicators

The duration of artificial lung ventilation under a ministernotomy was 2 hours shorter ($p<0.001$).

The number of patients with more than 1000 ml of blood loss within 24 hours after surgery was not significantly different in both groups. The amount of blood flowing through the drain during 24 hours after surgery in the ministernotomy group was almost 150 ml smaller ($p<0.001$).

The number of patients who underwent a transfusion of platelets, fresh frozen plasma (FFP) or red blood cells in the two groups did not differ significantly.

Postoperative bleeding occurred in 1 patient from the ministernotomy group (1.4%) and in 2 patients (2.9%) from the sternotomy group ($p = 0.559$). In all cases, an aortotomy did not cause any bleeding.

Drug-induced coagulopathy correction was required in 14 patients of the control group (20.0%). Protamine was used for this purpose in all patients. In the ministernotomy group, no patients required a drug correction of coagulopathy ($p<0.001$).

The duration of hospitalization in the ministernotomy group of patients was significantly shorter – almost by 3 days ($p = 0.012$). However, if we take into account the length of the hospital stay after the operation, the differences between the groups become no longer statistically significant ($p = 0.113$). The length of the patients' stays in the ICU also did not differ significantly.

The mortality rate in both groups was comparable – no deaths were recorded during surgery. Postoperative mortality under ministernotomy was equal to 1.4% – 1 patient died. This occurred on the 7th day. In patients after a median sternotomy, no fatal outcomes were recorded in the postoperative.

There were no cases of reintervention in any of the groups.

Postoperative wound infection occurred in two patients of the ministernotomy group – in 1 (1.4%) patient, it was treated with antibiotics alone, and in the other, vacuum-assisted closure therapy was used. In the median access group, this complication occurred in two patients – one was treated with antibiotics, and the other underwent sternum drainage with washing.

A cardiac tamponade was reported in 1 patient from the median sternotomy group and in 1 patient from the minimal access group (p = 1.000). In all cases, the treatment of complications was carried out in the ICU.

Acute renal failure occurred in 2.9% (2 patients) of patients in the ministernotomy group and in 1.4% (1 patient) of patients in the median sternotomy group. All patients were treated with medicines.

The incidence of different variants of arrhythmias in the postoperative period in the two groups did not differ significantly. In the postoperative period, a pacemaker implantation was required in 3 patients from the ministernotomy group and 4 patients from the median access group (p = 0.698).

The features of the postoperative outcomes of patients in both groups are presented in Table 4.

TABLE 4. The characteristics of the postoperative outcomes in groups.

Indicator	Median sternotomy (N = 70)	Ministernotomy (N = 70)	p
Ventilation time (h), (mean±SD)	11.7 ± 1.4	9.7 ± 1.7	<0.001*
Blood loss≥1000 mL/24 h, n (%)	2 (2.9%)	1 (1.4%)	0.559
24h chest tube drainage (mL), (mean±SD)	407.25 ± 40.37	256.2 ± 28.6	<0.001*
Number of patients, who underwent red blood cells transfusion, n (%)	15 (21.4%)	12 (17.1%)	0.520
Red blood cells transfused, in total, ml	572.2 ± 82.1	505.4 ± 79.4	0.215
Number of patients, who underwent platelets transfusion, n (%)	1 (1.4%)	4 (5.7%)	0.172
Platelets transfused, in total, ml	95.0 ± 25.02	435.0 ± 177.4	0.881
Number of patients, who underwent FFP transfusion, n (%)	5 (7.1%)	1 (1.4%)	0.095

Indicator	Median sternotomy (N = 70)	Ministernotomy (N = 70)	p
Fresh frozen plasma transfused, in total, ml	935.80 ± 146.17	1040 ± 423.35	0.898
Drug correction of coagulopathy, n (%)	14 (20%)	0 (0%)	<0.001*
ICU stay (h), (mean±SD)	88.14 ± 20.62	68.97 ± 6.29	0.319
Hospital stay (days), (mean±SD)	21.9 ± 1.9	18.3 ± 1.9	0.012*
Hospital stay, post-surgery (days), (mean±SD)	15.2 ± 1.5	13.0 ± 1.0	0.113
Intraoperative mortality, n (%)	0 (0%)	0 (0%)	---
30-day mortality, n (%)	0 (0%)	1 (1.4%)	0.316
Resternotomy, n (%)	2 (2.9%)	1 (1.4%)	0.559
Bleeding, n (%) Source – aortotomy Other source	2 (2.9%) 0 (0%) 2 (2.9%)	1 (1.4%) 0 (0%) 1 (1.4%)	0.559 --- 0.559
Endocarditis, n (%) *Treated with drugs* *Treated with surgery*	5 (7.1%) 5 (7.1%) 0 (0.0%)	1 (1.4%) 1 (1.4%) 0 (0.0%)	0.095 0.095 ---
Valve thrombosis, n (%)	0 (0%)	0 (0%)	---
wound infection, n (%) *Superficial wound infection - treated with antibiotic only* *Mediastinitis - treated with VAC* *Mediastinitis - treated with sternum drainage* *Mediastinitis - treated with sternum drainage with washing*	2 (2.9%) 1.43% 0(0%) 0(0%) 1.43%	2 (2.9%) 1 (1.4%) 1 (1.4%) 0(0%) 0(0%)	1.000 1.000 0.316 --- 0.316
Cardiac tamponade, n (%)	1 (1.4%)	1 (1.4%)	1.000
Acute renal failure, n (%) *Treated with a diuretic* *Treated with a hemofiltration* *Treated with a hemodialysis*	1 (1.4%) 1 (1.4%) 0 (0%) 0 (0%)	2 (2.9%) 2 (2.9%) 0 (0%) 0 (0%)	0.559 0.559 --- ---
Embolism, n (%), *In the form of a neurological disorder* *In the form of a stroke*	 1 (1.4%) 0 (0%)	 2 (2.9%) 0 (0%)	 0.559 ---
Cardiac rhythm at discharge, n (%) *Sinus rhythm* *Atrial fibrillation* *An implantation of a pacemaker within the period from surgery to discharge*	 63 (90%) 3 (4.3%) 4(5.71%)	 59 (84.3%) 4(5.7%) 3(4.29%)	 0.098 0.698 0.698
An implantation of a pacemaker within the whole postoperative period, n (%)	4(5.71%)	3(4.29%)	0.698

Indicator	Median sternotomy (N = 70)	Ministernotomy (N = 70)	p
LVEF%, n (%), at discharge	(n = 70)	(n= 69)	
<30%	0 (0%)	0 (0%)	---
30-49%	13 (18.6%)	12 (17.4%)	0.856
≥50%	57 (81.4%)	57 (82.6%)	0.856
PG max, (mm Hg), at discharge	28.31 ± 7.30	33.57 ± 3.64	0.006*
PG mean, (mm Hg), at discharge	17.08 ± 6.92	23.41 ± 2.65	0.015*
ISTD, cm	1.20 ± 0.23	1.28 ± 0.21	0.788
LVDD, cm	5.10 ± 0.60	5.11 ± 0.16	0.910
LVSD, cm	3.65 ± 0.85	3.26 ± 0.25	0.065

*p<0.05 – differences between the groups are significant.

An evaluation of drug therapy for the hemodynamic support showed that adrenalin was significantly more often used in the median access group – 30.0% against 5.71% in the ministernotomy group (p<0.001). No significant differences neither in the duration of medicine taking nor in its maximum dose were found between the groups.

An evaluation of the use of drugs for pain relief has shown that morphine was significantly more often used under median access – 98.57% against 90.00% in the ministernotomy group (p = 0.029), and in the ministernotomy group, non-steroidal, anti-inflammatory drugs were also significantly more often used – 98.57% versus 78.57% (p<0.001) (Table 5).

TABLE 5. The characteristics of medications used in groups.

Indicator	Median sternotomy (N = 70)	Ministernotomy (N = 70)	p
Preparations for maintaining the hemodynamic			
The number of patients treated with adrenaline, n (%)	21 (30%)	4 (5.71%)	<0.001*
The duration of treatment with adrenaline (hours)	27.23 ± 3.03	4.75 ± 2.75	0.314
The maximum dose of adrenaline, μg/kg/min	0.05 ± 0.001	0.05 ± 0.001	0.910
The number of patients treated with noradrenaline, n (%)	43 (61.43%)	42 (60.0%)	0.863
The duration of treatment with noradrenaline (hours)	37.21 ± 4.93	23.06 ± 4.06	0.236
The maximum dose of noradrenaline, μg/kg/min	0.05 ± 0.01	0.05 ± 0.01	0.913

Indicator	Median sternotomy (N = 70)	Ministernotomy (N = 70)	p
The number of patients treated with dopamine, n (%)	5 (7.14%)	6 (8.57%)	0.753
The duration of treatment with dopamine (hours)	31.70 ± 3.40	18.33 ± 1.95	0.625
The maximum dose of dopamine, μg/kg/min	10.08 ± 2.41	6 ± 2.02	0.750
The number of patients treated with dobutamine, n (%)	6 (8.57%)	6 (8.57%)	1.00
The duration of treatment with dobutamine (hours)	34 ± 9.18	8.33 ± 1.17	0.415
The maximum dose of dobutamine, μg/kg/min	4 ± 1	4.68 ± 0.54	0.894
The number of patients treated with nitroglycerine, n (%)	4 (5.71%)	4 (5.71%)	1.00
The duration of treatment with nitroglycerine (min)	24 ± 4	9 ± 3.11	0.103
The maximum dose of nitroglycerine, μg/ min	0.44 ± 0.007	0.45 ± 0.001	0.916
Preparations for anesthesia			
Morphine, n (%)	69 (98.57%)	63 (90%)	0.029*
Tramadol, n (%)	0 (0%)	3 (4.62%)	0.080
Nonsteroidal anti-inflammatory drugs (other than paracetamol), n (%)	69 (98.57%)	55 (78.57%)	<0.001*
Paracetamol, n (%)	48 (68.57%)	54 (77.14%)	0.254

*p<0.05 – differences between the groups are significant.

A survey of the patients showed an increase in patient satisfaction with the treatment performed after a ministernotomy due to the faster return to daily activities and a better cosmetic effect.

In 7 days after the operations took place, we asked patients whether they were able to perform simple actions (Figure 4).

Just as in large samples of patients, we noted that among the patients from the ministernotomy group, the ability to perform simple actions during the first week after surgery was identified in a significantly greater number of patients (p<0.0001). There were no significant differences between the groups in regard to meal intake.

The results of the patients' surveys regarding the cosmetic effects of both methods are shown in Figure 5.

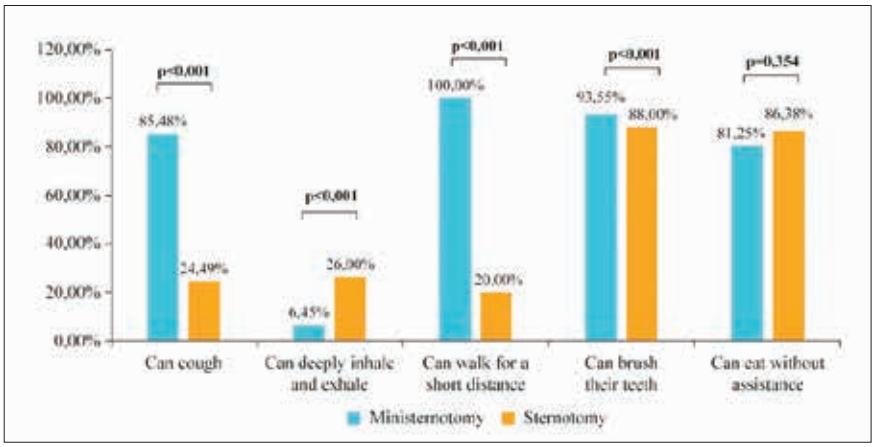

FIG. 4. The ability to perform simple actions in both groups of patients.

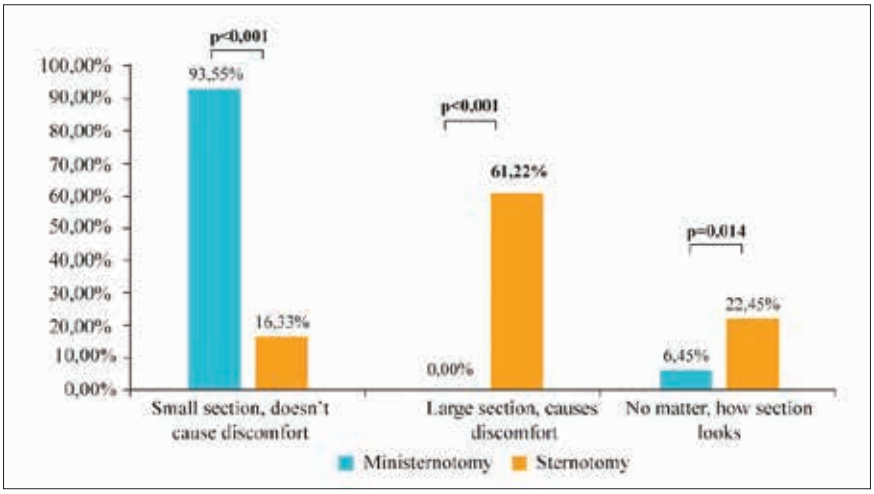

FIG 5. The opinion of patients on the postoperative section.

In the ministernotomy group, there were no patients insufficient with the surgical wound size. At the same time, in the sternotomy group, most patients responded that the section is large and causes discomfort.

To clarify the intensity of the pain syndrome, a patient survey was conducted using the Visual Analog Pain Scale. The results are shown in Figure 6.

We noted similar dynamics of pain syndrome in patients of both groups with a gradual increase in pain by the 4–5th day. At the same time, in patients from the ministernotomy group, the pain sensations from the 1st to the 3rd day were

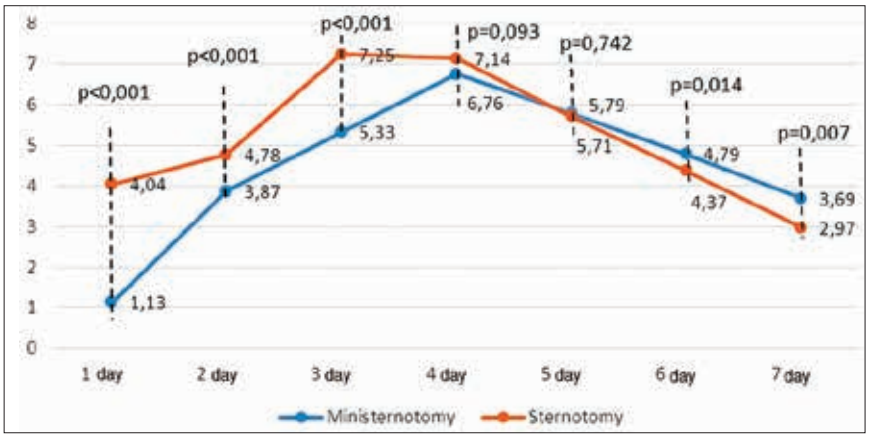

FIGURE 6. The dynamics of the patients' pain assessment according to VAS.

significantly smaller. On the 4–5th day, the pain syndrome intensity in patients from both groups became approximately equal and later decreased, while it was less pronounced in the sternotomy group. As a result of this, on the 6–7th day, its level in this group became reliably lower than in the main group.

To monitor the distant results of surgery, echocardiography indicators, the NYHA stage and clinical symptoms were examined for several years.

On the 30th day, patients were compared by the NYHA severity degree. Thoracalgia was met among the clinical symptoms in both groups with a comparable frequency (Table 6).

TABLE 6. The 30-day patient results

Indicator	Median sternotomy (N = 70)	Ministernotomy (N = 69)	P
NYHA, n (%)			
I	0 (0%)	0 (0%)	---
II	48 (68.6%)	46 (66.7%)	0.810
III	22 (31.4%)	23 (33.3%)	0.810
Clinical complaints, n (%)	(n = 70)	(n = 65)	
Absent	62 (88.6%)	59 (90.7%)	0.676
Thoracalgia	8 (11.4%)	6 (9.2%)	0.676
Pain behind the breastbone	0 (0%)	0 (0%)	---
Dyspnea	0 (0%)	0 (0%)	---
Sleep disturbances	0 (0%)	0 (0%)	---
LVEF %, n (%)			
<30%	0 (0%)	0 (0%)	---
30-50%	4 (5.7%)	8 (11.6%)	0.217
>50%	66 (94.3%)	61 (88.4%)	0.217

*$p<0.05$ – differences between the groups are significant.

Results of the 90th day showed that in the sternotomy group, the number of patients with NYHA I was significantly higher (p = 0.033).

Among the clinical symptoms in both groups, the most common was thoracology. It was met among the clinical symptoms in both groups with a comparable frequency.

Two patients from the minimally invasive access group underwent coronary angiography, while no patients from the median access group underwent this procedure (p = 0.154) (Table 7).

TABLE 7. The 90-day patient results

Indicator	Median sternotomy (N = 70)	Ministernotomy (N = 69)	P
NYHA, n (%)			
I	28 (40%)	16 (23.2%)	0.033*
II	36 (51.4%)	45 (65.2%)	0.099
III	6 (8.6%)	8 (11.6%)	0.554
Clinical complaints, n (%)	(n = 67)	(n = 64)	
Absent	52 (77.6%)	53 (82.8%)	0.635
Thoracalgia	8 (11.9%)	6 (9.4%)	0.635
Pain behind the breastbone	1 (1.5%)	1 (1.7%)	0.948
Dyspnea	0 (0%)	3 (4.7%)	0.135
Edema	1 (1.5%)	0 (0%)	0.337
Palpitation	5 (7.5%)	1 (1.7%)	0.201
Cardiac interventions, n (%)	(n = 69)	(n = 69)	
Pacemaker	4 (5.83%)	3 (4.3%)	0.227
Coronarography	0 (0%)	2 (2.90%)	0.154
Angioplasty	0(0%)	0 (0%)	---
LVEF %, n (%)	(n = 69)	(n = 69)	
<30%	0 (0%)	0 (0%)	---
30–50%	2 (2.9%)	5 (7.3%)	0.245
>50%	67 (97.1%)	64 (92.7%)	0.245

*p<0, 05 – differences between groups are significant.

A year later, no significant differences in the frequency of the occurrence of varying NYHA severity degrees were found in the groups. Under ministernotomy, the number of patients with no complaints was significantly larger. In this group, only one patient complained of experiencing chest pains, while in the median access group, 5.2% of all patients suffered from dyspnea. There were no new cardiac surgeries during the period (Table 8).

TABLE 8. The 360-day patient results.

Indicator	Median sternotomy (N = 58)	Ministernotomy (N = 69)	P
NYHA, n(%)			
I	32 (55.2%)	34 (49.3%)	0.508
II	24 (41.4%)	35 (50.7%)	0.293
III	2 (3.5%)	0 (0.0%)	0.120
Clinical complaints, n (%)	(n = 57)	(n = 61)	
Absent	46 (80.7%)	60 (98.4%)	0.002*
Thoracalgia	8 (14%)	1 (1.6%)	0.078
Angina pectoris	0 (0%)	0 (0%)	---
Dyspnea	3 (5.2%)	0 (0%)	0.070
Palpitation	0 (0%)	0 (0%)	---
Edema	0 (0%)	0 (0%)	---
LVEF %, n (%)	(n = 55)	(n = 54)	
<30%	0 (0%)	0 (0%)	---
30-50%	5 (9.1%)	2 (3.7%)	0.251
>50%	50 (90.9%)	52 (96.3%)	0.251

*p<0, 05 – differences between groups are significant.

In the future, there were no significant differences between the groups (Tables 9–10).

TABLE 9. The 2nd year patient results.

Indicator	Median sternotomy (N = 46)	Ministernotomy (N = 69)	P
NYHA, n (%)	(n = 39)	(n = 69)	
I	30 (76.9%)	51 (73.9%)	0.760
II	9 (23.1%)	18 (26.1%)	0.760
III	0 (0%)	0 (0%)	---
Clinical complaints, n (%)	(n = 41)	(n = 66)	
Absent	40 (97.6%)	66 (100%)	0.202
Thoracalgia	0 (0%)	0 (0%)	---
Pain behind the breastbone	0 (0%)	0 (0%)	---
Dyspnea	0 (0%)	0 (0%)	---
Edema	0 (0%)	0 (0%)	---
Palpitation	1 (2.4%)	0 (0%)	0.202
LVEF %, n (%)	(n = 46)	(n = 34)	
<30%	0 (0.0%)	0 (0.0%)	
30–50%	22 (47.8%)	2 (5.9%)	0.330
>50%	24 (52.1%)	32 (94.1%)	0.330

*p<0, 05 – differences between groups are significant.

TABLE 10. The 3rd year patient results.

Indicator	Median sternotomy (N = 51)	Ministernotomy (N = 69)	P
NYHA, n (%)	(n = 23)	(n = 69)	
I	20 (86.9%)	60 (87.0%)	1.00
II	3 (13%)	9 (13%)	1.00
III	0(0%)	0(0%)	---
Clinical complaints, n (%)	(n = 39)	(n = 20)	
Absent	39 (100%)	20 (100%)	1.00
Thoracalgia	0 (0%)	0 (0%)	---
Pain behind the breastbone	0 (0%)	0 (0%)	---
Edema	0 (0%)	0 (0%)	---
Palpitation	0 (0%)	0 (0%)	---
LVEF %, n (%)	(n = 39)	(n = 25)	
<30%	0 (0%)	0 (0%)	---
30-50%	3 (7.7%)	0 (0%)	0.155
>50%	36 (92.3%)	25 (100%)	0.155

*p<0, 05 – differences between groups are significant.

In the 4th and 5th years after the surgeries, we did not reveal any statistically significant differences between the groups – either in the clinical picture or in echocardiography results. However, it should be noted that we were able to trace the data of only 7 patients in each group, so it's possible that differences may occur with an increase in the sampling number.

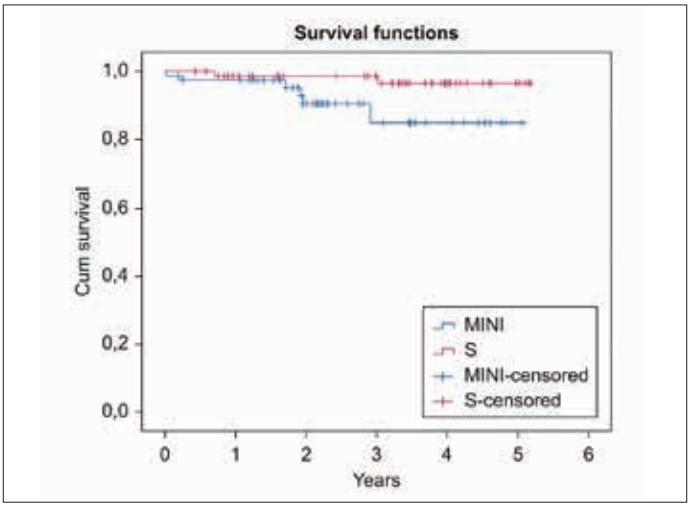

FIG 7. The Kaplan-Meier curves.

Thus, we have observed that the major differences between the two operational techniques are observed in the early postoperative period. With the observation period increasing, the differences between groups are leveled.

These data were confirmed by an analysis using the Kaplan-Meier methodology. It is noted that the five-year survival of the ministernotomy group's patients was 91.4%, and in the median sternotomy group, it was 97.1%. When using the Long Rank test, no significant differences were found: p = 0.054 (Figure 7).

Thus, when comparing identical groups, we observed more favorable postoperative indicators under ministernotomy. In particular, there was a reduction in the duration of artificial lung ventilation, a reduction in the incidence of coagulopathy and its pharmacological treatment, a decreased length of hospital stay and the best subjective feelings of th patients. During the long- term follow-up, we have not found any significant differences between the groups in most cases; thus, ministernotomy can be recommended as priority access when performing surgery to replace the aortic valve.

3.2. Peculiarities of the influence of pre-operative indicators on the course of surgery and on the course of the nearest post-operative period

Since we had identified the differences in surgical and in post-operative phases in two groups of patients, we decided to conduct a correlation analysis to identify whether the preoperative indicators' effect on the surgery course and on the early postoperative period in patients from the ministernotomy and median access groups is the same.

The following table shows the impact of preoperative indicators on the course of surgery in the ministernotomy and sternotomy groups (Tables 11–14).

TABLE 11. The influence of preoperative indicators on the course of surgery in the median sternotomy group.

Indicator	The loweast patient's temperature during surgery, °C	Aorta clamping duration (min)	Extracorporeal circulation duration (min)	Number of cardioplegia cycles	Cardioplegia duration (min)	Surgery duration (min)
Age	0.058	-0.240*	-0.152	-0.071	-0.262*	-0.067
p	0.641	0.049*	0.216	0.565	0.031*	0.587
BMI	0.023	-0.079	-0.118	-0.044	0.090	0.013
p	0.851	0.521	0.337	0.720	0.466	0.920

Indicator	The loweast patient's temperature during surgery, °C	Aorta clamping duration (min)	Extracorporeal circulation duration (min)	Number of cardioplegia cycles	Cardioplegia duration (min)	Surgery duration (min)
Hemoglobin level	-0.295	-0.065	-0.087	-0.052	-0.036	0.199
p	0.153	0.758	0.681	0.805	0.866	0.339
Creatinine level	0.111	0.308	0.376	0.311	0.100	0.251
p	0.597	0.135	0.064	0.130	0.635	0.226
Bilirubin level	0.088	-0.007	-0.038	0.023	-0.122	-0.292
p	0.676	0.974	0.857	0.912	0.561	0.157
Blood clotting	0.091	0.312	0.555*	0.188	0.000	0.379
p	0.666	0.129	0.004*	0.367	0.999	0.062
Euroscore II	-0.015	0.445*	0.477*	0.578*	0.269	0.536*
p	0.945	0.026*	0.016*	0.002*	0.193	0.006*
ISTD	0.142	0.173	0.195	0.121	0.043	0.040
p	0.499	0.407	0.349	0.564	0.840	0.850
LVDD	0.236	-0.137	-0.155	-0.129	-0.065	-0.163
p	0.255	0.512	0.460	0.539	0.756	0.436
LVSD	0.191	-0.063	-0.034	-0.138	-0.093	0.100
p	0.361	0.764	0.870	0.510	0.659	0.634
PG max	0.398*	-0.002	-0.225	0.071	-0.222	-0.249
p	0.049*	0.993	0.280	0.735	0.287	0.230
PG mean	0.264	-0.024	-0.218	0.083	-0.209	-0.284
p	0.202	0.910	0.296	0.692	0.317	0.169

*$p<0.05$ – differences between the groups are significant.

As can be seen from the data presented in the sternotomy group, age affects the duration of aortic clamping (r = -0.240, p = 0.049) and the duration of cardioplegia (r =-0.262, p = 0.031). The duration of cardiopulmonary bypass, to our knowledge, is directly proportional to blood clotting (r = 0.555, p = 0.004).

The largest number of indicators proved to be dependent on the Euroscore. These indicators include the duration of surgery, aortic clamping time, the duration of cardiopulmonary bypass and the number of cardioplegia cycles. In all cases, a correlation of medium strength was observed.

Also, a weak correlation between the level of the maximum gradient on the aortic valve and the lowest patient's body temperature during surgery was found (r = 0.398, p = 0.049).

TABLE 12. The influence of preoperative indicators on the course of surgery in the ministernotomy group.

Indicator	The loweast patient's temperature during surgery, °C	Aorta clamping duration (min)	Extracorporeal circulation duration (min)	Number of cardioplegia cycles	Cardioplegia duration (min)	Surgery duration (min)
Age	-0.007	0.044	0.180	-0.150	-0.127	0.190
p	0.958	0.721	0.139	0.218	0.297	0.131
BMI	0.043	-0.160	-0.251	-0.112	-0.160	0.063
p	0.725	0.191	0.037*	0.360	0.189	0.608
Hemoglobin level	-0.034	0.179	0.255	-0.024	0.214	-0.034
p	0.813	0.205	0.068	0.867	0.127	0.813
Creatinine level	0.287	0.102	0.179	0.069	-0.075	0.287
p	0.039*	0.470	0.204	0.625	0.596	0.039*
Bilirubin level	-0.047	0.026	0.121	0.111	0.079	-0.047
p	0.742	0.857	0.394	0.433	0.578	0.742
Blood clotting	-0.232	0.069	0.038	0.114	-0.128	0.089
p	0.098	0.629	0.788	0.420	0.364	0.522
Euroscore II	-0.130	0.087	0.208	-0.153	-0.088	0.109
p	0.358	0.538	0.139	0.279	0.533	0.430
ISTD	-0.025	0.184	0.151	0.244	0.032	0.124
p	0.862	0.192	0.286	0.081	0.824	0.366
LVDD	0.067	-0.045	-0.006	0.107	0.234	0.123
p	0.639	0.753	0.969	0.452	0.095	0.375
LVSD	0.059	-0.251	-0.156	0.026	0.127	0.102
p	0.679	0.073	0.269	0.855	0.371	0.476

PG max	-0.065	-0.101	-0.212	0.067	0.101	0.108
p	0.651	0.481	0.135	0.641	0.482	0.434
PG mean	-0.203	-0.078	-0.113	0.111	0.227	0.119
p	0.430	0.522	0.522	0.872	0.914	0.372

*p<0.05 – differences between the groups are significant.

It is important to note that there are much less factors influencing operative performance in the ministernotomy group. They include the body mass index, which influences the duration of cardiopulmonary bypass (r = -0.251, p = 0.037), and the creatinine level, which correlates with the lowest body temperature of the patient at the time of the operation and the duration of surgery (r = 0.287, p = 0.039) (see Table 12).

When assessing the impact of preoperative indicators on the course of the post-operative period, we noted the influence of the patient's age at the lowest rates of Hb in the median access group (r = -0.315, p = 0.013). According to our data, the hemoglobin level before surgery influenced the duration of the patient's stay in the intensive care unit (r = -0.408, p = 0.048). The creatinine level also correlated with the duration of patient stay in the intensive care unit (r = 0.458, p = 0.024), the duration of artificial lung ventilation (r = 0.513, p = 0.010) and the duration of hospitalization (r = 0.409, p = 0.047). The Euroscore rating correlated with the duration of artificial lung ventilation (r = 0.494, p = 0.014) and the amount of blood that flowed through drainage during the first 24 hours (r = 0.462, p = 0.023) (see Table 13).

TABLE 13. The influence of preoperative indicators on the course of the postoperative period in the median sternotomy group.

Indciator	Stay duration in an intensive care unit	Ventilation time (h)	Amount of blood flown through drainage	Hospitalization duration (in total)	Hospitalization duration (since the day of surgery)	The lowest hemoglobin level after surgery	The lowest platelets level
Age	-0.090	-0.144	-0.006	0.124	0.198	-0.315	-0.187
p	0.485	0.266	0.961	0.337	0.123	0.013*	0.145
BMI	-0.232	-0.090	0.034	-0.074	-0.217	-0.055	-0.235
p	0.070	0.489	0.795	0.565	0.091	0.670	0.066
Hemoglobin level	-0.408	-0.021	0.261	0.019	0.049	0.236	0.164
p	0.048*	0.921	0.219	0.930	0.821	0.266	0.444

Indciator	Stay duration in an intensive care unit	Ventilation time (h)	Amount of blood flown through drainage	Hospitalization duration (in total)	Hospitalization duration (since the day of surgery)	The lowest hemoglobin level after surgery	The lowest platelets level
Creatinine level	0.537	0.443	0.165	0.208	0.104	-0.127	0.087
p	0.007*	0.030*	0.441	0.329	0.630	0.555	0.687
Bilirubin level	0.090	0.165	-0.044	-0.174	-0.076	-0.256	0.190
p	0.677	0.442	0.837	0.416	0.725	0.227	0.373
Blood clotting	0.458	0.513	0.064	**0.409**	0.270	0.027	0.102
p	0.024*	0.010*	0.765	0.047*	0.202	0.902	0.636
Euroscore II	0.350	0.494	0.462	0.180	-0.014	-0.176	-0.345
p	0.093	0.014*	0.023*	0.400	0.947	0.411	0.099
ISTD	0.193	0.179	0.242	-0.081	-0.167	-0.064	0.293
p	0.366	0.402	0.255	0.707	0.436	0.768	0.165
LVDD	-0.199	-0.058	-0.038	-0.328	-0.281	-0.084	-0.107
p	0.352	0.787	0.859	0.118	0.183	0.698	0.618
LVSD	-0.030	0.101	-0.042	-0.326	-0.322	-0.242	-0.312
p	0.891	0.638	0.847	0.121	0.126	0.255	0.138
PG max	-0.005	-0.198	-0.108	0.168	0.293	0.178	0.043
p	0.980	0.353	0.617	0.432	0.165	0.406	0.843
PG mean	0.049	-0.200	0.015	0.015	0.129	0.116	0.057
p	0.821	0.348	0.946	0.946	0.549	0.589	0.791

*$p<0.05$ – differences between the groups are significant.

In the ministernotomy group, the course of the postoperative period was influenced by the patient's age, which affected the duration of stay in the intensive care unit ($r = 0.268$, $p = 0.030$), and by the level of creatinine, which correlated with the duration of hospitalization ($r = 0.478$, $p = 0.003$). According to our data, the end-systolic dimension of the left ventricle may also affect the duration of hospitalization ($r = 0.365$, $p = 0.026$) and the duration of stay in the intensive care unit ($r = 0.332$, $p = 0.045$) (Table 14).

TABLE 14. The influence of preoperative indicators on the course of the postoperative period in the ministernotomy group.

Indicator	Duration of stay in intensive care unit	Ventilation time (h)	Amount of blood flown through drainage	Hospitalization duration (in total)	Hospitalization duration (since the day of surgery)	The lowest hemoglobin level after surgery	The lowest platelets level
Age	0.268	0.184	0.032	0.167	0.155	0.201	-0.033
p	0.030*	0.139	0.798	0.180	0.215	0.106	0.794
BMI	-0.060	0.063	-0.077	-0.188	-0.084	-0.036	0.012
p	0.632	0.616	0.538	0.130	0.504	0.772	0.926
Hemoglobin level	0.135	0.101	-0.150	0.123	0.163	0.168	0.101
p	0.425	0.552	0.376	0.470	0.335	0.321	0.551
Creatinine level	0.302	-0.042	-0.093	0.478	0.438	0.090	-0.112
p	0.069	0.804	0.585	0.003*	0.007*	0.597	0.511
Bilirubin level	0.079	0.085	-0.233	-0.044	-0.019	-0.340	-0.190
p	0.640	0.618	0.165	0.797	0.910	0.040	0.259
Blood clotting	0.243	0.126	-0.239	0.226	0.213	0.235	0.176
p	0.148	0.458	0.154	0.178	0.206	0.162	0.297
Euroscore II	0.110	-0.038	0.153	0.168	0.233	0.171	0.087
p	0.518	0.823	0.367	0.320	0.165	0.311	0.609
ISTD	-0.057	-0.272	-0.084	-0.013	-0.132	-0.018	0.115
p	0.739	0.103	0.620	0.941	0.435	0.918	0.500
LVDD	0.272	0.144	-0.074	0.368	0.364	-0.056	0.056
p	0.104	0.396	0.666	0.025	0.027	0.742	0.741
LVSD	0.332	0.173	0.032	0.365	0.354	-0.160	-0.193
p	0.045*	0.306	0.850	0.026*	0.031*	0.345	0.253
PG max	-0.013	-0.126	-0.047	0.013	-0.094	-0.032	-0.103
p	0.940	0.457	0.782	0.939	0.579	0.852	0.544
PG mean	-0.156	-0.229	-0.140	-0.203	-0.174	-0.199	-0.114
p	0.355	0.172	0.409	0.229	0.303	0.237	0.502

* $p<0.05$ – differences between the groups are significant.

Thus, we have observed that in the median sternotomy group, a much larger number of parameters influence the course of the operation and the features of the postoperative period; hence, the choice of this access should be more carefully considered by the surgeon, as preoperative indicators may significantly affect the outcome of the operation.

Under ministernotomy, the outcome of the operation depends upon a significantly smaller number of factors, which indirectly indicates that this access is favorable for most categories of patients.

The findings obtained could form the basis for the formation of new criteria for the selection of patients to perform median sternotomy or ministernotomy.

3.3. Clinical results of the implementation of median sternotomy and ministernotomy in patients with the pathology of aortic valve suffering excessive body mass.

3.3.1. Evaluation of operational indicators

In assessing surgery characteristics, it was marked that biological valves were significantly more frequently used in the case of ministernotomy – 48 (85.7%) versus 22 (39.3%) in the cases of a median sternotomy ($p<0.001$). The frequency of the application of valves having a certain size did not differ significantly in the two groups.

An analysis of surgery duration showed that the duration of ministernostomy was significantly longer when compared to median sternotomy; on the average, the difference was nearly 18 minutes, $p = 0.046$.

When performing a ministernotomy, a longer clamping of the aorta during the surgery was required. On average, in the main group of the patients, the aorta was clamped 15 minutes longer than in the control group patients ($p<0.001$).

During the operation, in all patients from the ministernotomy group, an antegrade cardioplegia was performed through the coronary ostium, while a combination of two types of cardioplegia – through the coronary ostium and retrograde cardioplegia – was used in the majority of patients from the median sternotomy group: 29 patients (51.8%) ($p<0.0001$).

In the case of a ministernotomy, the use of extracorporeal circulation lasted 148.3 ± 4.4 minutes, while in the case of sternotomy, it lasted 133.6 ± 2.7 minutes. Thus, on average, in the case of a ministernotomy, the duration of extracorporeal circulation is 15 minutes longer than in the case of sternotomy, $p = 0.023$.

When performing sternotomy, an intra-aortic balloon pump (IABP) was used during the course of surgery of 1 (1.8%) patient, and in the ministernotomy group, extracorporeal membrane oxygenation was conducted for 1 patient (1.8%) in two

days after surgery during the reoperation. There were no cases of transition to a median sternotomy from minimal access.

Operation characteristics of the two groups of patients are presented in Table 15.

TABLE 15. The characteristics of surgery indicators in groups.

Indicator	Median sternotomy (N = 56)	Ministernotomy (N = 56)	P
Surgery duration (min), (mean±SD)	246.02 ± 72.31	264.91 ± 62.03	0.046*
Aortic cross-clamping time (min), (mean±SD)	76.82 ± 23.30	91.88 ± 20.713	<0.001*
Cardiopulmonary bypass time (min), (mean±SD)	133.6 ± 2.7	148.3 ± 4.4	0.023*
Bleeding during surgery, n (%) *Source – aortotomy* *Other source*	1 (1.8%) 1 (1.8%) 0 (0%)	0 (0%) 0 (0%) 0 (0%)	0.316 0.316 ---
Number of cardioplegia cycles	4.31 ± 1.27	4.05 ± 1.03	0.772
Cardioplegia duration (min)	18.14 ± 8.90	17.09 ± 6.98	0.256
Repeated cardioplegia, n(%)	1 (1.8%)	0 (0%)	0.316
Cardioplegia during surgery, n (%) *Retrograde* *Coronary mouth* *Combined*	 3 (5.4%) 24 (42.9%) 29 (51.8%)	 0 (0%) 56 (100%) 0 (0%)	 0.043* <0.001* <0.001*
Cardioplegic solution, n (%) *Pharmaco-cold cardioplegia* *Cold blood cardioplegia*	 30 (53.6%) 26 (46.4%)	 50 (89.3%) 6 (10.7%)	 <0.001* <0.001*
Aortotomy type, n (%) *Transverse aortotomy* *Hockey stick aortotomy*	 5 (8.9%) 51 (91.07%)	 26 (46.4%) 30 (53.6%)	 <0.001* <0.001*
The lowest patient's body temperature during surgery,°C	28.45 ± 2.40	28.05 ± 0.87	0.668
Valve type, n (%) *Biological* *Mechanical*	 22 (39.3%) 34 (60.7%)	 48 (85.7%) 8 (14.3%)	 <0.001* <0.001*
Aortic valve size: n (%) *19 mm* *21 mm* *23 mm* *25 mm* *27 mm* *29 mm*	 1 (1.8%) 3 (5.4%) 27 (48.2%) 23 (41.1%) 2 (3.6%) 0 (0%)	 0 (0%) 6 (10.7%) 23 (41.1%) 22 (39.3%) 5 (8.9%) 0 (0%)	 0.315 0.297 0.447 0.847 0.242 ---
ECMO, n (%)	0 (0%)	1 (1.8%)	0.315
IABP, n (%)	1 (1.8%)	0 (0%)	0.315

*$p<0.05$ – differences between the groups are significant.

3.3.2. Evaluation of postoperative indicators

The duration of artificial lung ventilation in both groups was comparable. For ministernotomy, it was 9.38 ± 1.74 hours, and for median sternotomy – 10.3 ± 1.37 hours (p = 0.744).

The amount of blood flowing through the drain during the first 24 hours also did not differ significantly between the two groups and was 354.46 ± 31.16 ml in the median sternotomy group and 315.18 ± 28.69 in the mini invasive access group (p = 0.356).

Despite the fact that in the ministernotomy group platelets were needed for 7.14% of the patients, and in sternotomy group – for 1.8% of the patients, the differences between the groups were not statistically significant (p = 0.172).

The number of resternotomies after surgery in the two groups did not differ statistically – 3.6% after a median sternotomy and 1.8% after a ministernotomy. The reason for the resternotomies after surgery in the two groups was bleeding from an unknown source.

In the case of a ministernotomy, postoperative renal failure was significantly less common – in 2 patients (3.6%) – as compared with 12 patients in the case of a median sternotomy (21.4%) (p = 0.004). All patients from the ministernotomy group were treated with diuretics. Nine patients from median sternotomy group were treated with diuretics; hemofiltration was used for 2 patients, and hemodialysis was used for 1 patient.

The mortality rate in both groups was comparable – in the case of a sternotomy, 2 patients died (mortality – 3.6%). Mortality in the case of a ministernotomy was 1.8% – 1 patient died. All fatal outcomes have occurred after the surgery. In the case of a median sternotomy, patients died in 3 and 12 days after surgery. In the case of a ministernotomy, the patient died on the 7th day.

No significant differences in hospitalization duration were found (p = 0.161). In patients from the ministernotomy group, it was 18.55 ± 1.40 days, and in patients from the control group – 22.52 ± 1.31 days. The duration of hospitalization after surgery did not differ significantly. Patients spent a comparable number of hours in an intensive care department. A left ventricular ejection fraction ≥50% at discharge was significantly greater in the sternotomy group.

The nearest postoperative outcomes are presented in Table 16.

An evaluation of drug therapy for the hemodynamic support showed that adrenaline was used significantly more often in the sternotomy group – 30.4% versus 5.4% in the ministernotomy group (p<0.001).

Among medicines for anesthesia in the postoperative period, morphine and non-steroidal anti- inflammatory drugs were most often used. There were no statistically

TABLE 16. The characteristics of the postoperative outcomes in the groups.

Indicator	Median sternotomy (N = 56)	Ministernotomy (N = 56)	P
Ventilation time (h), (mean±SD)	10.03 ± 1.37	9.38 ± 1.74	0.744
Blood loss≥1000 mL/24 h, n (%)	2 (3.8%)	1 (1.8%)	0.559
24h chest tube drainage (mL), (mean±SD)	354.46 ± 31.16	315.18 ± 28.69	0.356
ICU stay (h), (mean±SD)	72.46 ± 5.81	70.79 ± 7.39	0.859
Hospitalstay,post-surgery (days), (mean±SD)	15.23 ± 1.57	13.18 ± 1.08	0.280
Hospital stay (days), (mean±SD)	22.52 ± 1.31	18.55 ± 1.40	0.161
Number of patients, who underwent red blood cells transfusion, n (%)	8 (14.3%)	12 (21.4%)	0.641
Red blood cells transfused, in total (ml)	839.38 ± 82.1	510.4 ± 79.4	0.215
Number of patients, who underwent platelets transfusion, n (%)	1 (1.8%)	4 (7.14%)	0.172
Platelets transfused, in total (ml)	120.0 ± 19.8	435.0 ± 177.4	0.881
Number of patients, who underwent fresh frozen plasma transfusion, n (%)	4 (7.41%)	1 (1.8%)	0.416
Fresh frozen plasma transfused, in total (ml)	1193.75 ± 146.17	1040 ± 97.75	0.898
Resternotomy, n (%)	2 (3.6%)	1 (1.8%)	0.539
Cardiac tamponade, n (%)	1 (1.8%)	1 (1.8%)	1.000
Stroke, n (%)	0 (0%)	0 (0%)	---
Cardiac rhythm at discharge, n (%)			
Sinus rhythm	51 (91.07%)	50 (89.3%)	0.076
Auricular fibrillation	2 (3.8%)	3 (5.4%)	0.698
Implantation of pacemaker within the period from surgery till discharge from hospital	3 (5.4%)	3 (5.4%)	1.000
An implantation of pacemaker within the whole postoperative period, n (%)	3 (5.4%)	3 (5.4%)	1.000
Acute renal failure, n (%)	12 (21.43%)	2 (3.6%)	0.004*
Was treated with a diuretics	9 (16.07%)	2 (3.6%)	0.427
Was treated with a hemofiltration	2 (3.6%)	0 (0)%	0.205
Was treated with a hemodialysis	1 (1.8%)	0 (0)%	0.53
Surgical wound infection, n (%)	3 (5.4%)	1 (1.8%)	0.647
Superficial wound infection - treated with antibiotic only	2 (3.6%)	1 (1.8%)	0.089
Mediastinitis - treated with sternum drainage with washing	1 (1.8%)	0 (0%)	0.316

Indicator	Median sternotomy (N = 56)	Ministernotomy (N = 56)	P
30-day mortality, n (%)	2 (3.6%)	1 (1.8%)	0.558
Intraoperative mortality, n (%)	0 (0.0%)	0 (0%)	---
LVEF at discharge, n (%) *<30%* *30-50%* *≥50%*	 0 (0%) 12 (22.2%) 42 (77.8%)	 0 (0%) 22 (40%) 33 (60%)	 --- 0.045* 0.045*
PG max (mmHg)	32.12 ± 15.46	33.45 ± 20.42	0.461
PG mean (mmHg)	25.17 ± 7.03	25.40 ± 12.03	0.372

*p<0.05 – differences between the groups are significant.

significant differences in the use of morphine – 96.4% in the minimal access group and 100% in the median access group (p = 1.00).

Nonsteroidal anti-inflammatory drugs were reliably more frequently used in patients after a median sternotomy, 98.2% vs. 76.8% (p<0.001). Paracetamol was reliably more frequently used in patients from the sternotomy group, 83.9% versus 71.4% (p<0.005) (Table 17).

TABLE 17. The characteristics of drugs used in the groups.

Indicator	Sternotomy (N = 70)	Ministernotomy (N = 70)	p
Preparations for maintaining hemodynamic			
The number of patients treated with adrenaline, n (%)	21 (30.0%)	4 (5.71%)	<0.001*
Duration of treatment with adrenaline (hours)	27.23 ± 3.03	4.75 ± 2.75	0.314
The maximum dose of adrenaline, µg/kg/min	0.05 ± 0.001	0.05 ± 0.001	0.910
The number of patients treated with noradrenaline, n (%)	43 (61.43%)	42 (60%)	0.863
Duration of treatment with noradrenaline (hours)	37.21 ± 4.93	23.06 ± 4.06	0.236
The maximum dose of noradrenaline, µg/kg/min	0.05 ± 0.01	0.05 ± 0.01	0.913
The number of patients treated with dopamine, n (%)	5 (7.14%)	6 (8.57%)	0.753
Duration of treatment with dopamine (hours)	31.70 ± 3.40	18.33 ± 1.95	0.625
The maximum dose of dopamine, µg/kg/min	10.08 ± 2.41	6 ± 2.02	0.750

Indicator	Sternotomy (N = 70)	Ministernotomy (N = 70)	p
The number of patients treated with dobutamine, n (%)	6 (8.57%)	6 (8.57%)	1.00
Duration of treatment with dobutamine (hours)	34 ± 9.18	8.33 ± 1.17	0.415
The maximum dose of dobutamine, µg/kg/min	4 ± 1	4.68 ± 0.54	0.894
The number of patients treated with nitroglycerine, n (%)	4 (5.71%)	4 (5.71%)	1.00
Duration of treatment with nitroglycerine (min)	24 ± 4	9 ± 3.11	0.103
The maximum dose of nitroglycerine, µg/ min	0.44 ± 0.007	0.45 ± 0.001	0.916
Preparations for anesthesia			
Morphine, n (%)	69 (98.57%)	63 (90%)	0.029*
Tramadol, n (%)	0 (0%)	3 (4.62%)	0.080
Nonsteroidal anti-inflammatory drugs (other than paracetamol), n(%)	69 (98.57%)	55 (78.57%)	<0.001*
Paracetamol, n(%)	48 (68.57%)	54 (77.14%)	0.254

*p<0.05 – differences between the groups are significant.

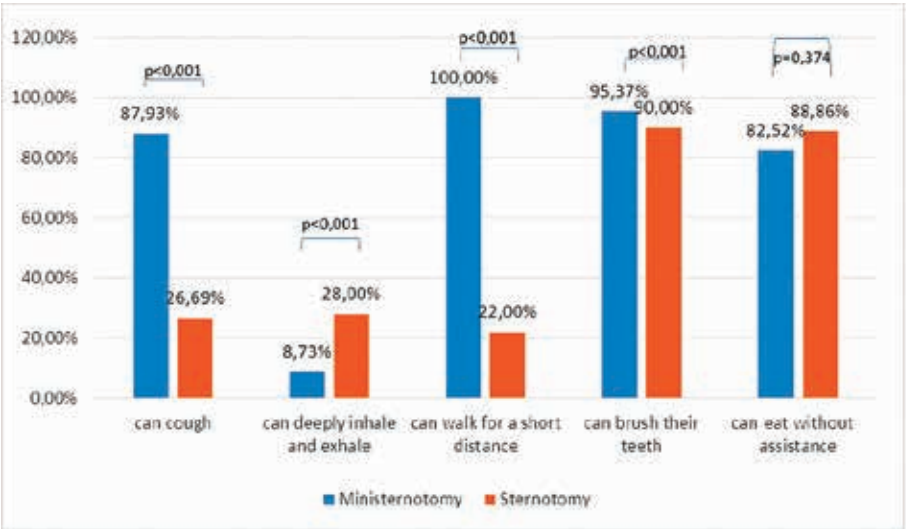

FIGURE 8. The ability to perform simple actions in both groups of patients.

Thus, we see that patients from the ministernotomy group require much less serious therapy with analgesics – that is not only good for the patients' health but also beneficial from the pharmaco-economic perspective.

In addition to standard calculations, we also included a questionnaire of patients in the clinical analysis. As a result of the survey, we have revealed an increase in patient satisfaction with treatment due to a faster return to daily activities and better cosmetic effects.

In 7 days after the surgeries, we asked the patients how many of them could perform simple actions (Fig. 8).

According to the data obtained, among the patients of the main group, the ability to perform simple actions during the first week after the operation was encountered in a significantly greater number of individuals ($p<0.001$). Significant differences were not revealed only with respect to food intake – in the main group, this ability was fixed in 82.52%, and in the control group – in 88.86% of the subjects ($p = 0.374$).

Results of the patient survey regarding the cosmetic effects of both methods are shown in Figure 9.

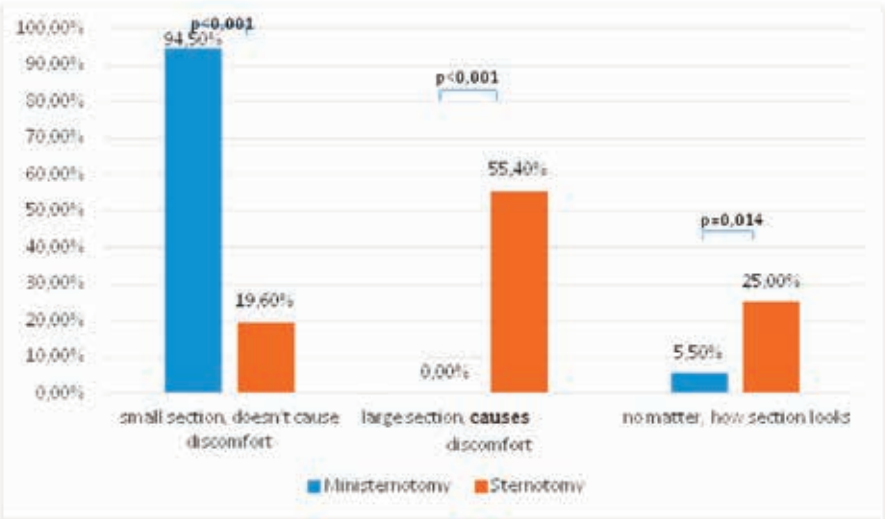

FIGURE 9. The opinion of patients on the postoperative section.

We detected significant differences in the number of patients satisfied with the size of postoperative wound – in ministernotomy group there were 94.5% of such patients ($p<0.001$). Number of patients who do not care how the incision looks like was significantly larger in median sternotomy group ($p = 0.014$).

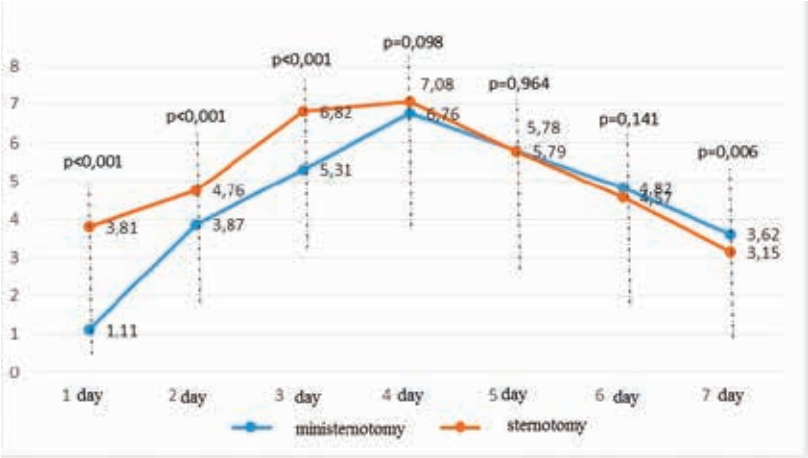

FIGURE 10. Dynamic assessment of pain on a visual analog scale

To clarify the pain intensity, patients' survey was conducted using visual analog pain scale. The results are shown in Figure 10.

As seen from the graph, at the first day after surgery symptoms of pain in patients from the ministernotomy group are minimal. Then a tendency to the increase in pain appears. On the 4th day the statistical differences in the level of pain in patients of the two groups are leveled and in the future their sensations are comparable. However, on the 7th day in median sternotomy group we observed marked reduction in pain, and as a result its level in this group became significantly lower than that in the main group – 3.15 ± 0.71 versus 3.62 ± 1.11, respectively (p = 0.006).

In addition, we have analyzed the impact of excess weight of the body on peri- and postoperative outcomes in the two groups. For main indicators we have calculated the correlation coefficient (Table 18).

As a result of the analysis, any significant correlation between the body mass index and any index in both groups have been not identified.

TABLE 18. The effect of the body mass index in the peri- and postoperative indicators.

Indicator	Median sternotomy		Ministernotomy	
	r^2	p	r^2	p
Surgery duration, min.	0.123	0.382	0.255	0.116
Aortic cross-clamping time, min.	0.042	0.766	0.169	0.303
The duration of cardiopulmonary bypass, min.	0.508	0.721	0.247	0.129
Ventilation time, hours	0.051	0.721	0.121	0.461

Indicator	Median sternotomy		Ministernotomy	
	r^2	p	r^2	p
The amount of blood flowing through the drain, ml	-0.173	0.903	-0.190	0.247
ICU stay, hours	0.156	0.276	0.122	0.941
Hospitalstay,post-surgery (days),	0.089	0.530	0.205	0.211
Hospital stay (days)	.103	0.464	0.203	0.215
LVEF %, n (%) at discharge	0.209	0.136	0.007	0.965

*$p<0.05$ – differences between the groups are significant.

To monitor the distant results of surgery in overweight patients, the echocardiography indicators, NYHA stages and clinical symptoms have been examined within a duration of several years.

Significant differences between patients were not observed at the 30th day. Severity degrees, according to the NYHA, echocardiography indicators and clinical symptoms, were comparable (Table 19).

The 90th day results showed a significant predominance of patients with no clinical complaints in the group of patients who underwent minimally invasive access. No other differences have been identified neither in NYHA severity nor in echocardiography indicators (Table 20).

TABLE 19. The 30-day patient results.

Indicator	Median sternotomy (N = 47)	Ministernotomy (N = 42)	P
NYHA, n (%)			
I	0 (0%)	0 (0%)	---
II	34 (72.3%)	25 (59.5%)	0.202
III	13 (27.7%)	17 (40.5%)	0.202
Clinical complaints, n (%)			
Absent	34 (72.3%)	36 (85.7%)	0.679
Thoracalgia	13 (27.7%)	6 (14.3%)	0.188
Pain behind the breastbone	0 (0%)	0 (0%)	---
Dyspnea	0 (0%)	0 (0%)	---
Sleep disturbances	0 (0%)	0 (0%)	---
LVEF %, n (%)			
<30%	0 (0%)	0 (0%)	---
30-49%	15 (31.9%)	7 (16.7%)	0.217
>50%	32 (68.1%)	35 (83.3%)	0.541
PG max (mmHg)	29.90 ± 11.12	32.82 ± 13.58	0.267
PG mean (mmHg)	18.5 ± 5.50	19.47 ± 6.52	0.562

*$p<0.05$ – differences between the groups are significant.

TABLE 20. The 90-day patient results.

Indicator	Median sternotomy (N = 46)	Ministernotomy (N = 42)	P
NYHA, n (%)			
I	0 (0%)	1 (2.38%)	0.293
II	32 (27.7%)	34 (80.95%)	0.771
III	14 (72.3%)	7 (16.66%)	0.085
Clinical complaints, n (%)			
Absent	28 (60.87%)	34 (80.95%)	0.039*
Thoracalgia	4 (8.69%)	3 (7.14%)	0.788
Pain behind the breastbone	1 (2.2%)	1 (2.4%)	0.948
Dyspnea	8 (17.4%)	3 (7.14%)	0.147
Edema	1 (2.2%)	0 (0%)	0.337
Palpitation	4 (8.7%)	1 (2.4%)	0.201
LVEF %, n (%)			
<30 %	0 (0%)	0 (0%)	---
30-50 %	17 (36.9%)	5 (11.9%)	0.167
>50 %	29 (63.1%)	37 (88.1%)	0.275
PG max (mmHg)	26.39 ± 9.72	29.69 ± 10.76	0.133
PG mean (mmHg)	18.51 ± 6.43	17.76 ± 5.77	0.738

*$p<0.05$ – differences between the groups are significant.

TABLE 21. The 360-day patient results.

Indicator	Median sternotomy (N = 44)	Ministernotomy (N = 46)	P
NYHA, n (%)			
I	20 (45.5%)	19 (41.30%)	0.286
II	17 (38.6%)	27 (58.70%)	0.202
III	7 (15.90%)	0 (0%)	0.157
Clinical complaints, n (%)	(n = 43)	(n = 36)	
Absent	38 (88.37%)	35 (97.2%)	0.002*
Thoracalgia	2 (4.65%)	1 (2.8%)	0.188
Pain behind the breastbone	0 (0%)	0 (0%)	---
Dyspnea	3 (6.98%)	0 (0%)	0.070
Sleep disturbances	0 (0%)	0 (0%)	---
LVEF %, n (%)	(n = 42)	(n = 35)	
<30%	0 (0%)	0 (0%)	---
30-50%	7 (16.7%)	2 (5.7%)	0.247
>50%	35 (83.3%)	33 (94.3%)	0.251
PG max (mmHg)	30.8 ± 11.12	32.82 ±13.58	0.267
PG mean (mmHg)	18.5 ± 5.50	19.35 ± 6.52	0.562

*$p<0,05$ – differences between groups are significant;

In a year after surgery, the number of patients with no complaints was significantly higher in the ministernotomy group. Also, in the control group, such complaints as dyspnoea were more common, but despite the fact that these complaints were not met in the main group, the differences between the groups did not reach any statistical significance. The parameters of the left ventricular ejection fraction in the two groups did not differ significantly (Table 21).

In the second year after the operation, in 100% of cases, clinical complaints were absent in the ministernotomy group. Echocardiography and NYHA groups were comparable in terms of the indices (Table 22).

TABLE 22. The 720-day patient results.

Indicator	Median sternotomy (N =44)	Ministernotomy (N = 55)	P
NYHA, n (%)	(n = 38)	(n = 55)	
I	30 (78.95%)	41 (74.54%)	0.760
II	8 (21.05%)	14 (25.46%)	0.760
III	0 (0%)	0 (0%)	---
Clinical complaints, n (%)	(n = 43)	(n = 52)	
Absent	41 (95.4%)	52 (100%)	0.202
Thoracalgia	0 (0%)	0 (0%)	---
Pain behind the breastbone	0 (0%)	0 (0%)	---
Dyspnea	0 (0%)	0 (0%)	---
Sleep disturbances	2 (4.6%)	0 (0.0%)	0.202
LVEF %, n (%)	(n = 42)	(n = 34)	
<30%	0 (0%)	0 (0%)	---
30-50%	17 (40.5%)	2 (5.9%)	0.347
>50%	25 (59.5%)	32 (94.1%)	0.298
PG max (mmHg)	30.8 ± 11.12	32.82 ± 13.58	0.221
PG mean (mmHg)	20.80 ± 5.71	21.75 ± 3.53	0.573

*p<0, 05 – differences between groups are significant

In the third year after the operation, we found no statistically significant differences between the groups – neither in clinical picture nor in echocardiography results (Table 23).

When assessing survival using the Kaplan-Meier method, it was noted that the five-year survival of patients from the ministernotomy group was 92.86%, and for the patients in the median sternotomy group, it amounted to 82.14%. A reliability evaluation by means of a log-rank test showed no significant difference: p = 0.269 (Figure 11).

Thus, ministernotomy in patients with obesity is a sufficiently safe operation that allows for performing complex surgical interventions on the aortic valve. Since

TABLE 23. The 3rd year patient results.

Indicator	Median sternotomy (N = 44)	Ministernotomy (N = 55)	P
NYHA, n (%)	(n = 30)	(n = 55)	
I	26 (86.7%)	48 (87.27%)	1.000
II	4 (13.3%)	7 (12.73%)	1.000
III	0 (0%)	0 (0%)	---
Clinical complaints, n (%)	(n = 32)	(n = 20)	
Absent	32 (100%)	20 (100%)	1.000
Thoracalgia	0 (0%)	0 (0%)	---
Pain behind the breastbone	0 (0%)	0 (0%)	---
Dyspnea	0 (0%)	0 (0%)	---
Sleep disturbances	0 (0%)	0 (0%)	---
LVEF %, n (%)	(n = 30)	(n = 30)	
<30%	0 (0%)	0 (0%)	---
30-50%	3 (10%)	0 (0%)	0.155
>50%	27 (90%)	30 (100%)	0.155

*p<0, 05 – differences between groups are significant;

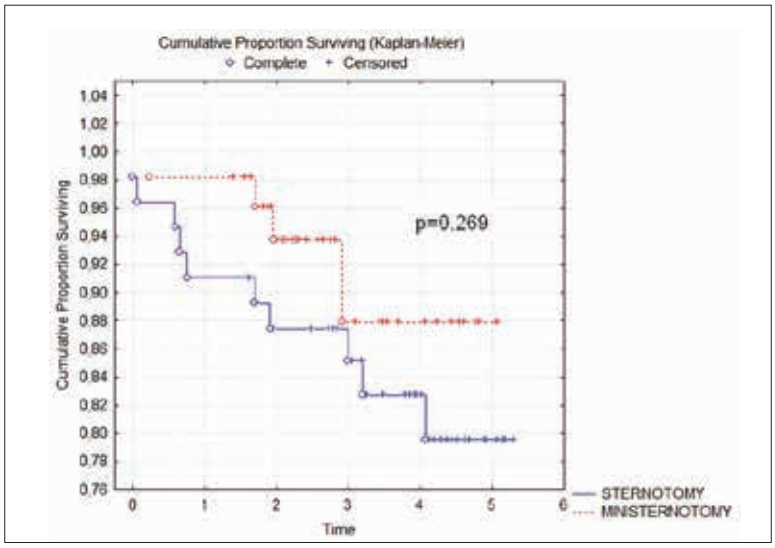

FIGURE 11. Kaplan-Meier curves.

its main outcomes are comparable to those for median sternotomy, and the proportion of patients who are satisfied with the treatment results is higher and the postoperative complications, such as renal insufficiency after ministernotomy, were significantly less frequent, we believe that this method of access can be recommended as the preferred one for aortic valve replacement in patients who are overweight.

Conclusion

The experience of using mini-access in prosthetic surgery has been around for more than 20 years, but discussions about the appropriateness and safety of this technique continue until now despite the large number of scientific papers devoted to this topic.

Despite the fact that many authors tend to favor mini-sternotomy when choosing access, within the literature, one can quite often find very inconsistent data on the advantages of one or the other kind of access. That is why our study was designed to detail the available data. As a result, we did notice some technical points due to which ministernotomy gives way to median access. In particular, these are prolonged aortic cross-clamping and the longer time of artificial circulation, but we concluded on the basis of the data that these features do not affect the early postoperative period and the frequency of complications. Therefore, this method is not inferior in terms of security to median access. In addition, ministernotomy is associated with a smaller level of traumatism, as evidenced by the lower blood volume flowing through the drainage, and, accordingly, a reduced need for a medicamentous correction of coagulopathy and analgesics, a faster recovery of patients and the reduced duration of hospitalization.

Another aspect to which we paid attention at the beginning of the the study was the lack of a sufficient number of studies that reveal the detailed nuances of the course of the early postoperative period and, especially, the long-term periods of the observation of patients or their study based on individual indicators. Therefore, our tasks included a complex study of these aspects. As a result, in a number of studies, including the study of 5-year survival, it was noted that in the long-term follow-up of the vast majority of indicators, ministernotomy is comparable to traditional intervention, and that this is more in favor of the first technique.

The study of the peculiarities of using ministernotomy in a particular groups of patients – with overweight patients, in our instance – has shown that the use of this kind of access is also associated with a decrease in the frequency of complications and the best subjective feelings of patients both immediately after the operation and in the long observation period. That is why mini- invasive access should be recommended as the main kind of access in such patients.

Another point in favor of ministernotomy was that the study of preoperative factors affecting the outcome of the operation demonstrated a lesser level of dependence of ministernotomic results on the initial data, which also makes it the operation of choice. It turns out that ministernotomy use is more justified under many equal conditions.

Thus, ministernotomy can be recommended as a priority operation for aortic valve replacement.

Findings

1. An aortic valve replacement using the ministernotomic approach is as safe and effective as a surgical intervention using the median sternotomy approach.
2. An aortic valve replacement using the ministernotomic approach requires a longer aorta clamp duration and an increased duration of artificial circulation as compared to the median sternotomic approach.
3. Patients of the ministernotomy group required statistically significantly shorter duration of artificial lung ventilation, lower dosage of drugs for coagulopathy correction, as well as adrenalin and morphine; they lost less blood oozing through drains, stayed shorter in an intensive care unit and required transfusion of lower volumes of the red blood mass and fresh-frozen plasma.
4. For patients, who underwent surgery using the ministernotomy approach, the early post- operative period is characterized by the statistically better cosmetic result, less pain in the first 7 days after surgery, early rehabilitation, fewer postoperative complications, shorter stay in hospital and less frequent necessity to prescribe nonsteroidal anti-imflammatory drugs.
5. For patients, who underwent surgery using the ministernotomy approach, the long-term post-operative period is characterized by the statistically higher number of patients with no complaints within a year after surgery. Upon 30 and 90 days, as well as 2 and 3 years after surgery, there were no significant differences between groups. During the said periods, there were no new cardiac interventions performed. Rate of survival of patients in the ministernotomy group was 91.4%, while in the sternotomy group - 97.1%, however, no significant difference was found out between groups.
6. As a result of a correlation analysis, it was found out that a lot more pre-operative indices in patients from the sternotomy group were linked to the process of surgery and the peculiarities of the postoperative period. Those indices include age, blood coagulability, Euroscore, maximum and average gradient, haemoglobin and creatinine levels. In the ministernotomy group, a lower number of factors have had impact on the results of surgery; this indirectly proves that the approach in question is more suitable for a larger category of patients.
7. As a result of an analysis of homogenous patients who were overweight, it was found out that postoperative indices in patients after ministernotomies were better, namely:

a. A decreased risk of acute renal failure;
b. Better subjective sensations for patients after surgery;
c. A statistically higher number of patients in the ministernotomy group have no complaints upon 90 and 360 days after surgery;
d. A better cosmetic result;
e. A smaller demand in drug therapy for haemodynamic support and less consumption of painkillers.

8. Based on the results of an analysis of links between overweight with the process of surgery and postoperative results, it was found that there is no significant correlation between the body-weight index and any of indices.

Practical recommendations

1. In the presence of equal clinical situations in patients and in the absence of contraindications to the implementation of ministernotomy or median sternotomy, ministernotomy should be considered a priority when choosing the technique of conducting an operation.
2. Due to a number of advantages over median access surgery, ministernotomy should be recognized as an operation of choice in patients with an aortic valve pathology in the presence of obesity.
3. An analysis of correlation relationships demonstrated that the course of surgery in the ministernotomy group is less dependent on age; therefore, we recommend that this intervention be carried out not only, for example, for the elderly (as they are patients at risk) but for younger age groups as well.

Publications and presentations at conferences publications

1. Aliahmed HMA, Podkopajev A, Samėnienė P. Comparison of results of aortic valve replacement through median sternotomy and mini sternotomy in overweight patients. *Lietuvos chirurgija* 2017; 16 (3–4):183–187.

2. Aliahmed HMA, Karalius R, Valaika A, Grebelis A, Samėnienė P, Čypienė R. Efficacy of Aortic Valve Replacement through Full Sternotomy and Minimal Invasion (Ministernotomy). *Medicina* 2018; 54(2): 26. DOI: 10.3390/medicina54020026.

Presentations

1. Aliahmed H. The efficacy and safety of ministernotomy for correction of aortic valve disease in patients with overweight. *The 12th International Congress of Update in Cardiology and Cardiovascular Surgery (UCCVS)*, Antalya, Turkey, March 10–13, 2016.

2. Aliahmed H. Comparison of the results of operations for aortic valve replacement techniques median sternotomy and minimal invasion (ministernotomy). *The 7th Joint Scandinavian Conference in Cardiothoracic Surgery*, Bergen, Norway, September 3–5, 2015.

SANTRAUKA

Įvadas

Tiriamoji problema, darbo aktualumas ir reikšmė

Pastaraisiais dešimtmečiais labai padidėjo aortos vožtuvų patologijos dažnis, o tai paskatino ieškoti tausojančių chirurginio gydymo metodų. Tad 1996 metais chirurgų arsenale atsirado nauji minimaliai invaziniai būdai (viršutinė ir apatinė ministernotomija, V formos, Z formos, T formos, J formos ir kt. ministernotomija). Nauji metodai sudarė sąlygas pagerinti gydymo rezultatus, sumažinti komplikacijų dažnį, palyginti su tradicine vidurine sternotomija, tačiau vis dar aktualus išlieka klausimas, kiek iš tikrųjų šie metodai yra geresni už tradicinę sternotomiją.

Pagal intraoperacinius veiksnius operacijos technika nesiskiria nuo standartinei sternotomijai taikomų metodų. Tiek sternotomijos, tiek ministernotomijos pjūviams gali būti naudojami tie patys instrumentai, tačiau atliekant ministernotomiją ypač svarbu kruopščiai praskirti krūtinkaulio kraštus, antraip bus neišvengta krūtinkaulio lūžio prie apatinio pjūvio krašto. Siekiant tinkamai praskirti krūtinkaulio kraštus, naudojamas nedidelis šonkaulių skėtiklis Tuffier, o atliekant įprastinę vidurinę sternotomiją – standartinis torakalinis žaizdų skėtiklis.

Pripažinta, kad aortos vožtuvo keitimas dirbtinės kraujotakos sąlygomis, atliekant vidurinę sternotomiją, užtikrina geriausią prieigą prie širdies, tačiau kartu yra susijęs su didesne sužalojimų tikimybe, kraujavimo rizika ir tolesnio mediastinito vystymusi. Be to, dėl stipraus skausmo sindromo ankstyvuoju pooperaciniu laikotarpiu prireikia nevienkartinio narkotinių analgetikų vartojimo net ir ligonį išrašius į namus, dėl kvėpavimo mechanikos pažeidimo gali išsivystyti kvėpavimo nepakankamumas. Kita vertus, įrodyta, kad minimalios invazijos būdai leidžia sumažinti netenkamo kraujo kiekį, infekcijos išsivystymo tikimybę ir hospitalizacijos trukmę, pagerina kosmetinį rezultatą ir pagreitina paciento gijimą.

Tačiau kai kurie autoriai atkreipia dėmesį ne tik į mini sternotomijos privalumus, bet ir jos trūkumus: ilgesnį pačios operacijos atlikimo laiką, ilgesnį aortos skersinio gnybto uždėjimo laiką ir didesnę dirbtinės kraujotakos taikymo trukmę. Tai yra ypač aktualu rizikos grupei priskiriamiems pacientams, kuriems šie veiksniai gali turėti daug įtakos operacijos rezultatams ir tolesnei savijautai.

Kaupiantis chirurginei patirčiai, visos pirmiau nurodytos problemos gali būti išsprendžiamos. Ministernotomijos rezultatai bus vis geresni, vis sumaniau pasitelkiant minimalios invazijos metodo pranašumus. Todėl kiekvienos klinikos patirtis yra įdomi ir reikšminga.

Nors yra atlikta nemažai tyrimų siekiant palyginti vidurinės (standartinės) sternotomijos rezultatus su minimaliai invazyviu metodu, visi jie iš esmės skirti pačios operacijos niuansams aptarti. Nepakankamai dėmesio skiriama dviejų grupių pacientų medikamentinių preparatų poreikio tyrimui, pooperacinio laikotarpio eigos ypatybėms ir fiziologinių funkcijų atkūrimui. Be to, atliekant šiuolaikinius tyrimus labiausiai akcentuojami trumpalaikiai rezultatai, nors iš tikrųjų būtina atsižvelgti į operacijos metodo įtaką vėlyviesiems rezultatams ir ligonių išgyvenamumui, o tai jau yra ateities tyrimų tema. Taigi turimus rezultatus būtina de-

taliau išanalizuoti pasitelkiant išsamų minimalios ir vidurinės prieigos operacijų palyginimą, atskleidžiantį ne tik pačios operacijos eigos ypatumus, bet ir visus pooperacinio laikotarpio bei tolesnio stebėjimo subtilumus. Be to, dauguma pastaruoju metu atliktų tyrimų pasižymi tuo, kad juose nagrinėjami atskiri gydymo aspektai – arba operacijos eiga, arba ankstyvieji pooperaciniai, arba vėlyvieji rezultatai. Tačiau iš tikrųjų būtina atlikti kompleksinius tyrimus, atsižvelgiant į visus šiuos veiksnius, nes tik visapusiškai išnagrinėjus klausimą bus galima nustatyti vienos ar kitos metodikos privalumus.

Būtina atkreipti dėmesį, kad optimalios prieigos pasirinkimas yra susijęs ne tik su medicininiais, bet ir socialiniais bei ekonominiais aspektais, nes po operacijos atsirandančios specifinės komplikacijos labai pablogina gydymo rezultatus ir dažnai lemia ilgalaikį neįgalumą, o tokiems ligoniams gydyti reikia papildomų finansinių išteklių.

Dar vienas svarbus aspektas – tai būtinybė tikslinti, kokiu atveju taikytina viena ar kita prieiga. Tobulėjant operacijų technikai, keičiasi ir ligoniams tinkamos metodikos parinkimo principai. Tai ypač akivaizdu analizuojant rizikos grupei priskiriamus pacientus. Pavyzdžiui, dar visai neseniai antsvoris buvo kontraindikacija atlikti minimaliai invazyvias operacijas. Tačiau pastaruoju metu šis požiūris buvo peržiūrėtas, nes atlikti tyrimai parodė, kad nutukusių pacientų operacijos minimali prieiga yra siejama su mažesne operacine trauma, mažesniu pooperacinių komplikacijų skaičiumi ir greitesniu pacientų gijimu.

Todėl būtina sukurti naujus pacientų, kuriems galima atlikti ministernotomiją, atrankos principus. Kadangi trūksta duomenų apie vėlyvuosius operacinių intervencijų rezultatus, reikia atlikti papildomus tyrimus.

Kuriant tokio pobūdžio pacientų atrankos strategiją, reikėtų atkreipti dėmesį į prieš operaciją nustatytų antropometrinių, klinikinių ir laboratorinių rodiklių įtaką skirtingais metodais atliekamos chirurginės intervencijos eigai ir ankstyvojo pooperacinio laikotarpio ypatumams, juolab kad tokie tyrimai yra tik pavieniai. Siekiant pasirinkti tinkamiausią intervenciją, labai svarbu išsamiai ir detaliai palyginti minimaliai invazyvia ir tradicine metodika atliktų operacijų ankstyvuosius ir vėlyvuosius rezultatus.

Tyrimo tikslas

Įvertinti ir palyginti aortos vožtuvo keitimo operacijų rezultatus, taikant vidurinės sternotomijos ir minimalios invazijos (ministernotomijos) metodus.

Darbo uždaviniai:

1. Įvertinti ministernotomijos efektyvumą ir saugumą keičiant aortos vožtuvą pacientams, turintiems aortos vožtuvo patologiją, ir palyginti su vidurine (standartine) sternotomija.
2. Atlikti grupių pacientų aortos vožtuvo keitimo operacijos ypatumų lyginamąją analizę, kai taikoma ministernotomija ir vidurinė sternotomija.
3. Atlikti ministernotomijos ir sternotomijos grupių pacientų pooperacinių rodiklių lyginamąją analizę, kai rodikliai yra vertinami pacientų gydymo reanimacijos skyriuje laikotarpiu.

4. Ištirti ankstyviausius pooperacinius klinikinius rezultatus, įskaitant susidariusių komplikacijų dažnį, skausmo pojūčius, pajėgumą atlikti paprastus veiksmus ir hospitalizacijos trukmę.
5. Ištirti lyginamų grupių pacientų vėlesnius klinikinius rezultatus.
6. Įvertinti prieš operaciją ištirtų pacientų antropometrinių, klinikinių ir laboratorinių rodiklių įtaką chirurginės intervencijos eigai ir ankstyvojo pooperacinio laikotarpio ypatumams, kai taikoma mininimali ir vidurinės sternotomijos prieiga.
7. Atlikti antsvorio turinčių pacientų aortos vožtuvo keitimo operacijų, taikant minimalią ir vidurinę sternotomijos prieigą, charakteristikų lyginamąją analizę, įvertinti abiejų grupių pacientų ankstyvuosius ir vėlyvuosius rezultatus.
8. Įvertinti antsvorio poveikį dviejų aptariamų grupių pacientų operacijos ir pooperaciniams rezultatams.

Tyrimo mokslinis naujumas

Novatoriškumu pasižymi visų galimų prieigos būdų poveikio paciento sveikatai kompleksinis tyrimas.

Darbo rezultatai išsamiai atskleidžia pacientų, kuriems taikyta minimali ir vidurinė prieiga, operacijų eigą ir ankstyvojo pooperacinio laikotarpio ypatumus. Ištirtos ne tik visuotinai priimtos charakteristikos, bet ir preparatų poreikis skirtingoms grupėms, gijimo laikotarpio eiga, subjektyvūs paciento pojūčiai.

Darbe pateiktas ypač išsamus klinikinių charakteristikų ir echokardiografijos rodiklių kitimo per trejų metų laikotarpį įvertinimas, charakterizuojantis pacientų širdies ir kraujagyslių sistemos funkcinę būklę ir bendrą savijautą, o tai reikšmingai papildo kitus darbus, kuriuose nebuvo siekiama taip išsamiai apibūdinti šių pacientų pooperacinio laikotarpio eigos.

Koreliacinės analizės būdu įvertintas pacientų, kuriems buvo taikyta minimali ir vidurinė prieiga, priešoperacinių veiksnių poveikis, atsižvelgiant į chirurginės intervencijos eigą ir ankstyviausio pooperacinio laikotarpio ypatumus, o tai gali padėti sukurti naujus vieno ar kito tipo operacijų pasirinkimo kriterijus, priklausomai nuo pradinių paciento charakteristikų.

Praktinė darbo reikšmė

Praktinė šio darbo reikšmė yra ta, kad gautus rezultatus galima pritaikyti kardiologijos ir kardiochirurgijos skyriuose aortos vožtuvo patologiją turinčių pacientų gydymui optimizuoti, siekiant sumažinti chirurginių intervencijų sukeliamą mirtingumą ir sutrumpinti hospitalizavimo trukmę.

Atliktas darbas atskleidė pagrindines ankstyvojo ir vėlyvojo laikotarpio problemas, iškylančias pacientams, kuriems atlikta vidurinė sternotomija ir ministernotomija. Paaiškėjo, kad ministernotomijos poveikis yra labiau tausojantis, todėl ši prieiga rekomenduojama kaip prioritetinis invazijos būdas, kai nustatoma aortos vožtuvo patologija.

Vieno ar kito operacijos metodo pasirinkimo kriterijų tikslinimas atsižvelgiant į pradines paciento charakteristikas leidžia išplėsti ministernotomijos taikymo indikacijas.

Įrodyta, kad ministernotomijos prieigos operacijos ne tik leidžia greičiau grįžti į kasdienį gyvenimą, bet ir lemia palankesnius klinikinius rezultatus bei, tikėtina, pasižymi didesniu ekonominiu efektyvumu dėl trumpesnės hospitalizacijos ir mažesnio kiekio suvartojamų vaistų.

Tiriamieji ir tyrimo metodai

Įtraukimo į tyrimą kriterijai – tik izoliuota aortos vožtuvo patologija.

Pašalinimo iš imties kriterijai:

- Daugelio vožtuvų ydos;
- Įgytos širdies ydos, reikalaujančios korekcijos kartu su AŠ;
- Pacientų amžius < 18 metų;

Tyrimas atliktas nuo 2011 m. sausio 1 d iki 2016 m. sausio 1 d Vilniaus universiteto ligoninėje Santaros klinikose. Tyrimui atlikti gautas Vilniaus regioninio biomedicininių tyrimų etikos komiteto leidimas Nr. 158200-14-715-235.

Tai retrospektyvusis tyrimas pacientų, kuriems buvo atliktas izoliuotas aortos vožtuvo keitimas, pasitelkus sternotomijos arba ministernotomijos metodus. Iš viso buvo ištirti 426 pacientai, kuriems diagnozuota aortos vožtuvo patologija. Jų amžius vyravo nuo 18 iki 88 metų.

70 pacientų buvo atlikta ministernotomija, o 356 pacientams – vidurinė sternotomija. Visos operacijos buvo atliktos nuo 2011 m. sausio 1 d iki 2016 m. sausio 1 d Vilniaus universiteto ligoninėje Santaros klinikose. Duomenys surinkti iš ligos istorijų, chirurginių ir anesteziologinių protokolų, ambulatorinių kortelių.

Pirmame tyrimo etape buvo išanalizuoti 140 intervencijų, atliktų dėl aortos vožtuvo keitimo turintiems sergantiesiems, rezultatai. Antrajame tyrimo etape buvo išanalizuoti 102 intervencijų, atliktų keičiant aortos vožtuvą antsvorio turintiems sergantiesiems, rezultatai

Remiantis panašiausių atvejų analizės metodu (angl. propensity score matching (PSM)), pacientai, panašiai pasiskirstę pagal lytį, amžių, kūno masės indeksą, pagrindinę diagnozę, ligos etiologiją, NYHA vertinimą ir pagal echokardiografijos rezultatus prieš operaciją.

Pagrindinės grupės pacientams buvo atliekamos šio tipo intervencijos: J-mini-sternotomija 44,3%, W-mini-sternotomija 21,4% ir dalinė sternotomija 34,3%.

Statistinė analizė

Statistinis gautų duomenų apdorojimas buvo atliekamas pasitelkus dispersinę analizę, naudojantis IBM SPSS 20.0 (IBM Corp, NY, USA), „Statistica 12“ (Stat soft, Tulsa, OK, USA) programomis bei R „party“ ir „rpart“ programiniais paketais.

Duomenų normaliojo pasiskirstymo vertinimas buvo atliekamas naudojant Liliforso (Lilliefors) kriterijų, kuris parodė, kad duomenys nėra pasiskirstę normaliai, todėl gautiems rezultatams apdoroti buvo taikomi neparametriniai statistikos metodai. Kruskalo ir Voliso (Kruskal–Wallis) kriterijus buvo naudojamas siekiant patikrinti medianų skirtumus, o dviejų grupių skirstiniams palyginti buvo pasitelktas Mano ir Vitnio (Mann–Whitney) U testas.

Siekiant atlikti požymių priklausomumo analizę dviejose nepriklausomose tyrimo objektų grupėse ir patikrinti nulinę statistinę hipotezę, buvo taikomi Mantelio ir Hencelio

(Mantel– Haenszel) chi kvadrato kriterijus ir tikslusis Fišerio (Fisher) kriterijus (mažiems skaičiams su binominiu pasiskirstymu, jeigu stebėjimų skaičius viename ar keliuose lentelės 2×2 langeliuose buvo ≤5).

Chi kvadratas buvo skaičiuojamas pagal formulę

$$\chi^2 = Z^2 = \frac{(n-1)(ad-bc)^2}{n_1 n_0 m_1 m_0}$$

čia: a – skaičius subjektų, kuriems nustatytas ekspozicijos faktorius ir požymis; b – skaičius subjektų, kuriems nustatytas ekspozicijos faktorius ir nenustatytas požymis; c – skaičius subjektų, kuriems nenustatytas ekspozicijos faktorius ir nustatytas požymis; d – subjektų skaičius, kuriems nenustatytas ekspozicijos faktorius ir nenustatytas požymis.

Kriterijaus statistika atitinka Pirsono (Pearson) pasiskirstymą su laisvės laipsniais df=(1-r)(1-c). Gautos reikšmės lyginamos su kritinėmis sritimis ir daroma išvada apie faktorių tarpusavio ryšį arba jo nebuvimą.

Apdorojant tyrimo duomenis, išgyvenamumas įvertintas taikant Kaplano ir Mejerio (Kaplan–Meier) metodą, grupėms palyginti naudotas logaritminio rango (log-rank) kriterijus.

Lentelėse duomenys nurodyti kaip reikšmių vidurkiai ir standartinė paklaida (M±m).

Gauti rezultatai buvo vertinami pagal p reikšmę (p-value) – tai tikimybė, kad gaunamas tyrimo rezultatas yra atsitiktinis. Reikšmė p parodo skirtumų tarp grupių statistinę reikšmę. Įprastai reikšmingumo lygmuo yra p≤0,05.

Rezultatai

Lyginant operacijos rodiklius tiriamųjų grupėse, pastebėta, kad mini-sternotomijos trukmė, lyginant su vidurinės sternotomija, yra vidutiniškai 7 minutėmis ilgesnė, tačiau statistiškai reikšmingai nesiskiria (p=0,856). Buvo nustatyta, kad mini-sternotomijos atlikimo metu reikėjo beveik 8 minutėmis ilgiau taikyti aortos užspaudimą operacijos eigoje (p=0,007). Mini- sternotomijai atlikti taip pat reikėjo ilgiau taikyti ekstrakorporinę kraujotaką – ji tęsėsi beveik 12 minučių ilgiau nei atliekant vidurinės sternotomiją (p=0,049). Statistiškai reikšmingi skirtumai yra susiję su pakartotinės kardioplegijos skaičiumi, vidurinės sternotomijos grupėje ji buvo atlikta 3 (4,3%) pacientams, kai mini-sternotomijos grupėje ji nebuvo atlikta nei vienam pacientui (p=0,042).

Vertinant pooperacinius rodiklius, paaiškėjo, kad mini-sternotomijos grupės pacientams prireikė ženkliai trumpesnės trukmės dirbtinės plaučių ventiliacijos nei sternotomijos grupės pacientams - 9,7±1,7 val. ir 11,7±1,4 val, (p<0,001) atitinkamai. Taip pat buvo nustatyta, kad pirmąsias 24 valandas po operacijos mini-sternotomijos grupėje per drenažą prateka mažesnis kraujo kiekis, lyginant su pilnos prieigos grupės pacientais. Po mini-sternotomijos reikšmingai mažesniam pacientų skaičiui reikėjo taikyti medikamentinę koagulopatijos korekciją.

Bendras hospitalizacijos laikas pilnos prieigos grupėje buvo beveik 4 dienomis ilgesnis (p=0,012). Tikėtina, kad trumpesnė hospitalizacijos trukmė yra vienas iš pagrindinių daugumos minimalios invazijos metodikų privalumų.

Lyginant pooperacinių komplikacijų, tokių kaip pooperacinės žaizdos infekcija, prieširdžių virpėjimas, širdies tamponavimas, emobolija, ūmus inkstų nepakankamumas, pasireiškimo dažnį – reikšmingų skirtumų tarp grupių nebuvo aptikta. Mirtingumas abiejose grupėse statistiškai reikšmingai nesiskyrė. Mini-sternotomijos grupėje mirė 1 pacientas (1,4%), o vidurinės sternotomijos grupėje mirties atvejų nebuvo užfiksuota.

Skausmo intensyvumo analizė, naudojantis vizualine analogine skale (angl. Visual Analog Scale), po atliktos mini-sternotomijos ir tradicinės operacijos atskleidė mini-sternotomijos privalumus tik pirmosiomis keliomis dienomis po operacijos. Remiantis apklausos duomenimis, taip pat nustatytas pacientų pasitenkinimo gautu gydymu didėjimas dėl greitesnio grįžimo prie kasdieninės veiklos ir geresnio kosmetinio rezultato.

Be to, buvo atskleistas įdomus faktas, kad praėjus metams po operacijos, mini- sternotomijos grupės pacientų, nepateikusių skundų, susijusių su pagrindiniu susirgimu ir chirurgine intervencija, skaičius buvo ženkliai didesnis, tačiau atsižvelgiant į tolimesnį kelių metų laikotarpį, šis skirtumas nebebuvo statistiškai reikšmingas.

Lyginant šią minimaliai invazinę metodiką su vidurinės sternotomija, kai atliekamas aortos vožtuvo keitimas pacientams, pasižymintiems antsvoriu, buvo pastebėta, kad mini-sternotomijai atlikti reikia daugiau laiko ir tai sąlygoja ilgesnį aortos užspaudimo laiką operacijos eigoje ir didesnę dirbtinės kraujotakos trukmę.

Remiantis šio tyrimo duomenimis, žaizdos infekcijos bei daugelio kitų komplikacijų dažnis, ženkliai nesiskyrė dviejose grupėse, tačiau taikant minimalios invazijos prieigą, stebima komplikacijų skaičiaus mažėjimo tendencija. Be to, atlikus mini-sternotomiją, užfiksuota beveik 6 kartus mažiau tokių komplikacijų, kaip ūmus inkstų nepakankamumas, pasitaikymo dažnis, o tai turi didelę įtaką paciento gerbūviui ateityje.

Medikamentinės terapijos hemodinamikai palaikyti įvertinimas parodė, kad adrenalinas ženkliai dažniau vartojamas vidurinės sternotomijos grupėje ($p<0,001$). Nesteroidiniai priešuždegiminiai preparatai statistiškai reikšmingai dažniau buvo skiriami pacientams po vidurinės sternotomijos ($p<0,001$). Paracetamolio vartojimas taip pat buvo dažniau skiriamas sternotomijos grupės pacientams ($p<0,005$).

Tai, kad po mini-sternotomijos atlikimo gaunamas geresnis kosmetinis rezultatas, nekelia jokių abejonių. Apibendrinant galima teigti, kad pacientų pasitenkinimas po mini-sternotomijos operacijos buvo žymiai didesnis ne tik dėl geresnio kosmetinio rezultato, bet ir dėl greitesnio grįžimo prie kasdienės veiklos. Vertinant operacijų rezultatus praėjus 90 ir 360-imt dienų nuo operacijos atlikimo paaiškėjo, kad mažiau nusiskundimų turi mini-sternotomijos grupės pacientai, tačiau kadangi imtis nebuvo didelė, gautos išvados turi būti tikslinamos, atliekant daugiau tyrimų. Vertinant tolimesnius rezultatus, susijusius su penkerių metų trukmės pacientų išgyvenamumu po abiejų tipų operacijų atlikimo, esminių skirtumų nebuvo pastebėta.

Išvados

1. Aortos vožtuvo protezavimas pasitelkiant ministernotomiją yra tokia pat saugi ir veiksminga chirurginė operacija, kaip ir vidurinė sternotomija.
2. Aortos vožtuvo protezavimui taikant ministernotomiją reikia ilgesnio aortos užspaudimo laiko ir didesnės trukmės dirbtinės kraujotakos, palyginti su vidurinės sternotomijos grupe.
3. Ministernotomijos grupės pacientų dirbtinės plaučių ventiliacijos trukmė buvo statistiškai reikšmingai trumpesnė, išsiskyrusio per drenus kraujo kiekis mažesnis, jiems reikėjo mažiau vaistų koagulopatijai koreguoti bei mažiau adrenalino ir morfino, reanimacijos skyriuje gulėjo trukmė trumpiau ir jiems mažiau reikėjo perpilti eritrocitų masės bei šviežiai šaldytos plazmos.
4. Ankstyvasis pooperacinis laikotarpis pacientams, operuotiems, naudojant mini sternotomijos metodiką, pasižymi statistiškai geresniu kosmetiniu poveikiu, mažesniu skausmu per pirmas 7 dienas po operacijos, ankstyva reabilitacija, mažesniu pooperacinių komplikacijų skaičiumi, trumpesne hospitalizacijos trukme, ir rečiau buvo skiriami Nesteroidiniai vaistai nuo uždegimo.
5. Vėlesnis pooperacinis laikotarpis pacientams, operuotiems, naudojant ministernotomijos metodiką, pasižymi statistiškai didesniu skaičiumi pacientų, neturinčių jokių nusiskundimų metų laikotarpiu po ministernotomijos. Praėjus 30 ir 90 dienų bei 2 ir 3 metams po operacijos, reikšmingų skirtumų tarp grupių nebuvo. Jokių naujų širdies intervencijų šiais laikotarpiais neatlikta. Ministernotomijos grupės pacientų išgyvenamumas sudarė 91,4 %, sternotomijos grupės – 97,1 %, tačiau reikšmingų skirtumų tarp grupių neaptikta.
6. Atlikus koreliacinę analizę nustatyta, kad sternotomijos grupėje gerokai daugiau priešoperacinių rodiklių turi ryšį su operacijos eiga ir pooperacinio laikotarpio ypatumais. Šiems rodikliams priklauso amžius, kraujo krešumas, EuroSCORE, maksimalus ir vidutinis gradientas, hemoglobino ir kreatinino lygis. Ministernotomijos grupėje mažesnis skaičius veiksnkų turėjo įtakos operacijos rezultatams, o tai netiesiogiai rodo, jog ši prieiga yra tinkamesnė didesnei kategorijai pacientų.
7. Remiantis antsvorio tuinčių homogeniškų pacientų grupių analize nustatyta, kad geresni pooperaciniai šių pacientų rodikliai yra atlikus ministernotomiją. Tai yra:
   a. sumažėja ūminio inkstų nepakankamumo išsivystymo dažnis;
   b. geresni subjektyvūs pacientų pojūčiai po operacijos;
   c. statistiškai daugiau ministernotomijos grupės pacientų neturi nusiskundimų praėjus 90 ir 360 dienų po operacijos;
   d. geresnis kosmetinis rezultatas;
   e. mažesnis medikamentinės terapijos poreikis hemodinamikai palaikyti ir mažesnis analgetikų vartojimas.
8. Remiantis antsvorio sąsajų su operacijos eigos ir pooperaciniais rezultatais analizės išvadomis nustatyta, kad abiejose grupėse nėra reikšmingos kūno masės indekso koreliacijos nė su vienu iš rodiklių.

Praktinės rekomendacijos

1. Jeigu pacientai pasižymi vienodomis klinikinėmis charakteristikomis ir jiems nėra jokių vienos ar kitos prieigos kontraindikacijų, renkantis chirurginės intervencijos metodą prioritetinis turėtų būti ministernotomija.
2. Dėl daugelio ministernotomijos privalumų, palyginti su vidurinės prieigos operacija, šis metodas turėtų būti pripažintas tinkamu pasirinkimu operuojant antsvorio turinčius ar nutukusius pacientus dėl aortos vožtuvo patologijos.
3. Koreliacinių ryšių analizė parodė, kad ministernotomijos grupės pacientų operacijos eiga mažiau priklauso nuo amžiaus. Todėl ji gali būti rekomenduojama ne tik rizikos grupės, pavyzdžiui, vyresnio amžiaus, asmenims, bet ir jaunesnio amžiaus pacientams.

Sandra Kutkienė

THE ASSESSMENT AND MODIFICATION OF DYSLIPIDEMIAS: EFFECTS ON ETIOPATHOGENESIS, THE PREVENTION OF CARDIOVASCULAR DISEASES AND THE RELATIONSHIP WITH OTHER ATHEROSCLEROTIC RISK FACTORS

DISLIPIDEMIJŲ ĮVERTINIMO IR MODIFIKAVIMO ĮTAKA ŠIRDIES IR KRAUJAGYSLIŲ LIGŲ ETIOPATOGENEZEI BEI PREVENCIJAI IR SĄSAJOS SU KITAIS ATEROSKLEROZĘ SKATINANČIAIS RIZIKOS VEIKSNIAIS

Biomedicinos mokslai, medicina (06 B)
Biomedical sciences, medicine (06 B)

Abbreviations

ABP – arterial blood pressure
AD – atherogenic dyslipidemia
AH – arterial hypertension
AIxHR index – heart rate adjusted augmentation
Apo – apolipoprotein
BIA – bioelectrical impedance analysis
BMI – body mass index
CAC – Coronary artery Calcium
CAVI – cardio-ankle vascular index
CCA – common carotid artery
CHD – coronary heart disease
CI – confidence interval
CT – computed tomography
CVD – cardiovascular disease
DBP – diastolic blood pressure
DM – diabetes mellitus
EAS – European Atherosclerosis Society
ECG – electrocardiogram
ESC – European Society of Cardiology
FCH – Familial combined hyperlipidemia
FEM – femoral artery
FH – familial hypercholesterolemia
HDL – high-density lipoproteins
HDL-C – high-density lipoprotein cholesterol
HR – heart rate
IMT – intima-media thickness
LBM – lean body mass
LCCA – left common carotid artery
LDL – low-density lipoproteins
LDL-C – low-density lipoprotein cholesterol
LitHiR Risk – Lithuanian High Cardiovascular
Lp(a) – lipoprotein(a)
OR – odds ratio
PBF – percentage body fat
PCSK9 – Proprotein convertase subtilisin/kexin type 9
PH – Polygenic hypercholesterolemia
PWV – pulse wave velocity
RA – radial artery
RCCA – right common carotid artery
SBP – systolic blood pressure
SCORE – Systematic COronary Risk Evaluation
SD – standard deviation
SD – severe dyslipidemia
SH – severe hypercholesterolemia
TBW – total body water
TC – total cholesterol
TG – triglycerides
VFA – visceral fat area
vs. – against *(versus)*
VUH SK – Vilnius University Hospital Santaros Klinikos
WC – waist circumference
WHR – waist-hip ratio

1. Introduction

Cardiovascular disease (CVD) is the leading cause of death globally, with one-third of deaths attributed to CVD worldwide. Lithuania has the highest level of deaths from coronary heart disease (CHD) in Europe and is classified as a high-risk country in the 2016 European guidelines on CVD prevention. The main risk factors for CVD are elevated levels of blood lipids, high blood pressure, tobacco use, diabetes mellitus, unhealthy eating habits, a low level of physical activity, overweight and obesity. To tackle these risk factors is proven to be the key approach for lowering cardiovascular mortality. Various genetic, pathological, observational and interventional studies have established the important role of dyslipidemia in the development of CVD. Dyslipidemia is a multifactorial disorder, which emerges due to an interplay between genetic, lifestyle and environmental factors and is defined as having an increased concentration of total cholesterol (TC) or an increased low-density lipoprotein cholesterol (LDL-C), or a high concentration of plasma triglycerides (TG), or low high-density lipoprotein cholesterol (HDL-C). A proper treatment of dyslipidemia has been shown to reduce the risk of CVD by 30% in a 5-year period. Also, dyslipidemia is often found together with multiple other cardiovascular risk factors, especially hypertension and obesity. It is well-known that coexisting multiple risk factors tend to increase the risk of CVD synergistically because of the additional adverse effects on the vascular endothelium.

The estimated prevalence of dyslipidemia among middle-aged Lithuanians is very high. An elevation of LDL-C, known as severe hypercholesterolemia (SH), is the most common type of dyslipidemia. It constitutes a major risk factor for the development of atherosclerosis and receives most attention as an established treatment goal. According to an analysis of data from the Lithuanian High Cardiovascular Risk (LitHiR) program (hereinafter referred to as "the program"), elevated TC and increased LDL-C are the lipid abnormalities most commonly found among middle-aged Lithuanians without established CVD: 80.5% have an increased TC, and 75.7% – an increased LDL-C. Patients with severely elevated LDL-C might either have genetic disorders, such as Familial hypercholesterolemia (FH), Polygenic hypercholesterolemia (PH) and Familial combined hyperlipidemia (mixed hyperlipidemia) (FCH), or non-genetic explanations, including certain secondary causes of SH (e.g., the nephrotic syndrome, cholestasis or untreated hypothyroidism) as well as lifestyle factors. It is evident that only a small proportion of severely hypercholesterolemic subjects will have identified FH mutations, so the recognition of SH is important on the population-basis, as extremely elevated LDL-C levels drive the clinical risk for these patients. An analysis of the serum lipid profile could provide an initial approximate differentiation between various types of dyslipidemia

and help distinguish subjects for further evaluation for the familial forms of dyslipidemia, as opportunistic screening in community laboratories for potential FH has been proven to be effective.

Epidemiological studies found low HDL-C to be a common lipid abnormality across European populations. Low HDL-C is not prevalent in Lithuania, as most people have increased TC or LDL-C concentrations, even though dyslipidemia is a very common risk factor in Lithuania. Although low levels of HDL-C have been proven to be a major risk factor for coronary heart disease (CHD) many decades ago, recent genetic studies suggest that the causal relationship is weak. Also, therapeutic interventions aimed at increasing plasma HDL-C levels showed no real benefits on CVD morbidity and mortality. These revelations highlight the fact that measuring plasma HDL-C concentration might not be enough and emphasize the importance of exploring lipoprotein quality and function.

1.1 Aim of the Research

The aim of this study is to evaluate the prevalence and trends in the morbidity of different types of dyslipidemia, as well as any associations with other cardiovascular risk factors, among middle- aged Lithuanians without any established cardiovascular diseases and to develop recommendations for evaluating and long-term monitoring of patients with severe dyslipidemia in Lithuania.

1.2 Objectives of the Research

1. To evaluate the prevalence, trends in morbidity, diagnosis and treatment of different types of dyslipidemia as well as its associations with other cardiovascular risk factors in the program's population.
2. To assess and compare the cardiovascular risk profiles of patients with dyslipidemia and those of the control group.
3. To assess and compare the cardiovascular risk profiles of different types of dyslipidemia.
4. To evaluate the prevalence and trends in morbidity of severe dyslipidemia as well as to assess and compare the cardiovascular risk profiles of patients with different types of severe dyslipidemia.
5. To evaluate the cardiovascular risk profile of patients with severe hypercholesterolemia and establish recommendations for evaluating and long-term monitoring of patients with severe dyslipidemia.
6. To assess the quality and function of high-density lipoproteins in patients with severe dyslipidemia and the control group.

1.3 Statements to be Defended

1. Lithuanians have a distinct cardiovascular risk profile with dyslipidemia being one of the most important cardiovascular risk factors.
2. Dyslipidemia is often found together with multiple other cardiovascular risk factors.
3. A delayed diagnosis and poor control of dyslipidemia is one of the main problems in the treatment of middle-aged Lithuanians and should thus receive more attention.
4. The diagnosis, treatment and monitoring of severe, possibly familial, dyslipidemia is delayed and inadequate.
5. The HDL function is insufficient among middle-aged Lithuanians.

1.4 Scientific Novelty of the Research

More than ten years ago, in 2006, the LitHiR primary prevention program was started due to an unfavorable situation of cardiovascular morbidity and mortality in our country. The design of the program is compatible with the ESC/EAS Guidelines suggesting that risk-factor screening, including the lipid profile, may be considered in men ≥40 and women ≥50 years of age. The program includes men aged 40–54 and women aged 50–64 without overt CVD from all regions of Lithuania. Patients are evaluated in primary health care institutions, and high-risk subjects are reffered to specialized cardiovascular prevention units. The goals of this program are to decrease the prevalence of cardiovascular risk factors, reduce CVD-related morbidity and mortality as well as increase the early diagnosis of dyslipidemia, hypertension and other cardiovascular risk factors. Analysis of data from 23 204 patients evaluated in primary health care centers revealed that dyslipidemia was diagnosed for 89.7% of middle-aged adults without overt CVD, and severe dyslipidemia was determined for 12.1% of the program's population (Rinkūnienė, 2014).

Also, dyslipidemia remained a very prevalent and poorly controlled risk factor in Lithuania during the period of 2009–2012 (Rinkūnienė, 2014). While continuing and extending the work of E. Rinkūnienė, it was decided to focus on the early diagnosis and management of dyslipidemia while also assessing the prevalence of severe dyslipidemia and its associations with other cardiovascular risk factors: arterial hypertension (AH), smoking, diabetes mellitus (DM), metabolic syndrome (MetS) and obesity. The aim was to evaluate and compare the cardiovascular risk profiles of different types of dyslipidemia. While focusing on severe dyslipidemia, it was intended to create recommendations for the evaluation and long-term monitoring of patients with severe dyslipidemia in Lithuania. The analysis of the program's population showed that only 16.7% of middle-aged subjects have decreased plasma

HDL-C (Rinkūnienė, 2014). This revelation served as an encouragement for performing a quality evaluation of HDL particles in order to assess the function of HDL and possibly re-evaluate the causes of high cardiovascular morbidity in Lithuania.

Main Novelties of the Research:

- Retrospective data analysis for the period of 2009–2016;
- The sample of the restrospective data analysis consists of 92 373 forms;
- A detailed evaluation and comparison of cardiovascular risk profiles as well as associations with other cardiovascular risk factors of various types of dyslipidemia;
- Recommendations given for the evaluation and long-term monitoring of patients with severe dyslipidemia in Lithuania;
- The detection and evaluation of cases of familial hypercholesterolemia;
- An assessement of the quality of high-density lipoproteins in order to evaluate the function of HDL and help determine the reasons of high cardiovascular morbidity in Lithuania.

2. Study subjects and methods

The study was conducted at Vilnius University Hospital (VUH) Santaros Klinikos during the period of 2014–2018. Permission No. 158200-15-816-329 was issued by the Vilnius Regional Biomedical Research Ethics Committee.

2.1 Retrospective Analysis of the Primary Prevention Program Electronic Database

2.1.1 Sample Size and the Selection of Participants

A retrospective study describes the analysis of the lipid profile in a randomly selected group of 92 373 subjects included in the electronic database of the primary prevention program during the period of 2009–2016. The LitHiR program is funded by the Ministry of Health and has obtained the Local Research Ethics Committee's approval. It includes men aged 40–54 and women aged 50–64 years without overt CVD from all regions of Lithuania. This program is conducted in 398 out of 420 (94.8%) primary health care centers, uniformly covering the whole country. In 2016, 256 625 adults were examined in the primary health care centers, covering about 37.5% of the whole target population. The program consists of subjects selected in three different ways: enlisting patients of proper age in primary health care centers, inviting patients who fit the program's enrollment criteria after looking at their existing medical histories and enrolling patients informed about the program via mass

media. The exclusion criteria are: a) a proven (clinically evident) coronary heart disease (CHD); b) a proven (clinically evident) cerebrovascular disease; c) a proven (clinically evident) peripheral artery disease; d) an end-stage oncological disease; e) any other end-stage somatic disease.

2.1.2 Study Design and Methods

Primary care physicians filled specially designed protocols providing information about subjects included in the program. Each participant underwent a physical examination, which consisted of an anthropometry (height, weight, waist circumference, waist-to-hip ratio and body mass index (BMI), defined as weight in kilograms divided by height (in meters squared)) as well as blood pressure and pulse readings. Histories of cardiovascular risk factors, other diseases and medications were obtained during interviews. Serum TC, HDL- C, TG and plasma glucose levels were evaluated by commercially available kits using venous blood samples at the standardized laboratories in the participating centers. LDL-C levels were calculated using the Friedewald formula for individuals with TG <4.5 mmol/l. Tests were performed in the morning, and it was suggested that the participants not to eat for at least 12 hours before the scheduled tests. Secondary causes of dyslipidemia were not ruled out. Less than 5% of subjects in the database were reported to use lipid-lowering medications. Metabolic syndrome (MetS) was assessed according to the National Cholesterol Education Program III modified criteria. Arterial hypertension (AH) was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg, or the diagnosis of hypertension was documented in a medical record. Obesity was identified whenever the BMI ≥30, and abdominal obesity was determined when waist circumference was >102 cm for men and >88 cm for women. The overall cardiovascular risk was calculated according to the risk estimation Systematic Coronary Risk Evaluation (SCORE) system.

2.1.3 Grouping of the Participants

Participants were divided into groups according to their lipidogram parameters, and different lipid profiles were distinguished. Dyslipidemia was considered if serum TC >5 mmol/l, or LDL-C >3 mmol/l, or HDL-C <1.0 mmol/l in men and <1.2 mmol/l in women, or TG >1.7 mmol/l. Severe dyslipidemia was described as TC ≥7.5 mmol/l, or LDL-C ≥6 mmol/l, or TG ≥4.5 mmol/l. Severe hypercholesterolemia was described as LDL-C ≥6 mmol/l, and severe hypertriglyceridemia was defined as TG ≥4.5 mmol/l. Atherogenic dyslipidemia (AD) was defined as TG >1.7 mmol/l and HDL-C <1.2 mmol/l in women and <1.0 mmol/l in men. Low HDL-C was described as HDL-C <1.2 mmol/l in women and <1.0 mmol/l in men, normal

HDL-C – from 1.2 mmol/l in men and 1.0 mmol/l for women to 1.55 mmol/l, high HDL-C – ≥1,55 mmol/l in both men and women. The control group was defined as TC<5mmol/l and LDL-C<3 mmol/l, HDL-C >1.0 mmol/l for men, >1.2 mmol/l for women, and TG <1.7 mmol/l for both men and women.

We performed a thorough analysis and comparison of selected dyslipidemia profiles as well as the trends of the prevalence of different dyslipidemias, for the period of 2009–2016. Study data has been further analyzed by dividing all subjects into appropriate groups based on age. Men: 40–44 years, 45–49 years, 50–54 years; women: 50–54 years, 55–59 years, 60–64 years.

2.2 Prospective Analysis of the Selected Group of Patients with Severe Dyslipidemia and the Control Group

2.2.1 The Selection and Grouping of Participants

A total of 213 participants were enrolled in the prospective study. It included men and women from 18 up to 60 years of age. The average age of subjects was 49.15 ± 8.01 years. We collected data from 110 (51.6%) patients (N=54 women and 56 men) with severe dyslipidemia and 103 (48.4%) controls (N=51 women and 52 men) without dyslipidemia or established CVD. Written informed consent was obtained from all participants before their inclusion in the study.

Severe dyslipidemia was considered as serum TC ≥7.5 mmol/L or LDL-C ≥6 mmol/L. Subjects with possible secondary causes of severe dyslipidemia (uncontrolled hypothyroidism, diabetes mellitus, nephrotic syndrome, renal insufficiency, cholestasis, viral hepatitis, liver cirrhosis, alcoholism, anorexia), pregnancy, terminal stage cancer and any terminal stage disease were excluded from this study. Controlled thyroid dysfunction and diabetes mellitus diagnosed later than dyslipidemia were not considered as exclusion criteria.

Included in the control group of this study were only subjects without dyslipidemia, without any evident cardiovascular diseases (myocardial infarction, an unstable angina, a stable angina with a positive cardiac stress test, a coronary artery pathology identified during cardiac catheterization or a coronary computed tomography angiography, coronary artery bypass surgery, percutaneous coronary intervention), with no cerebrovascular disorders (a previous acute ischemic or hemorrhagic stroke, a diagnosed stenosis of carotid arteries), no peripheral artery diseases (acute ischemic syndromes, chronic limb ischemia, aortic aneurysm), no disorders that may impact the concentrations of blood lipids (an uncontrolled hypothyroidism, diabetes mellitus, a nephrotic syndrome, a renal insufficiency, cholestasis, viral hepatitis, liver cirrhosis, alcoholism, anorexia), pregnancy, terminal stage cancer and any terminal stage disease.

2.2.2 Study Design and Methods

Thorough cardiovascular disease risk estimations (history of smoking, arterial hypertension, physical activity, dietary habits, body composition analysis) were completed. Various diagnostic tests, including coronary artery calcium score evaluations, echocardiographies, abdominal ultrasounds and ultrasounds of the tendons, were performed. Anthropometric data (height, weight, waist circumference) were gathered, and the heart rate, arterial blood pressure were measured. Blood cholesterol, lipoprotein and apolipoprotein analyses was performed in the Center of Laboratory Medicine in VUH Santaros Klinikos. All the tests and procedures were carried out in the morning, and the participants were advised not to eat for at least 12 hours beforehand. The recommended values of measured lipids and lipoproteins are shown in Table 1.

TABLE 1. Recommended values of measured lipids and lipoproteins.

Measurement		Recommended value
Total cholesterol (mmol/l)	<5	
Triglycerides (mmol/l)	≤1.7	
HDL cholesterol (mmol/l)	>1 male, >1.2 female	
LDL cholesterol (mmol/l)	<3	
ApoA1 (g/l)	1.1–2.05 male, 1.25–2.15 female	
ApoB (g/l)	0.55–1.40 male, 0.55–1.25 female	
ApoA2 (g/l)	0.26–0.51	
ApoE (mg/l)	23-63	
ApoB/ApoA1	0.35–1.0 male, 0.3–0.9 female	
Lipoprotein(a) (g/l)	<0.3	

Abbrevations: HDL – high-density lipoprotein; LDL – low-density lipoprotein; Apo – apolipoprotein.

Arterial hypertension was considered as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg, or the diagnosis of hypertension was documented in a medical record.

Different anthropometric parameters were measured by applying the required anthropometric tools and following the rules of accurate measurement. Abdominal obesity was determined as waist circumference >102 cm for men and >88 cm for women. The waist- hip ratio reference range was 0.75-0.9 for men and 0.70-0.85 for women. The BMI was calculated by dividing body weight (in kilograms) by height (in meters squared). While assessing the BMI, the following groups were distinguished: ideal – 22, normal – 20–25 for men and 18.5–24 for women, overweight –

25–29.9, obese – 30–40 and severely obese – >40. Bioelectrical impedance analyses were performed using a bioimpedance analyzer IOI353, using which the individual, dressed in light clothing, would stand barefoot on metal plates while the following parameters would be measured: lean body mass (LBM), skeletal muscle mass (SMM), mineral mass, body fat mass (BFM), percentage body fat (PBF), visceral fat area (VFA), total body water (TBW) and protein mass. Also, analyses of the abdominal region were performed. The measurements were evaluated and grouped in accordance with the recommendations posed by manufacturers of the body composition analyzer.

Abdominal ultrasounds were performed using an abdominal transducer of 3.5 MHz. Patients were assessed lying in the supine position or on the left side. During the ultrasound examination, the presence of conditions associated with severe dyslipidemia were evaluated: atherosclerotic lesions of the abdominal aorta, fatty liver, pancreatic steatosis and gallstones.

Morphological assessments of the aortic valve were based on transthoracic echocardiography parasternal long and short axis view. We evaluated the number of cusps, cups mobility, thickness and calcification.

For the ultrasound examinations of the Achilles and wrist tendons, a linear transducer of 9 MHz was used. The Achilles tendons were accessed from a myotendinous junction to the site of the calcaneal insertion in sagittal and transverse planes. Patients were assessed lying in prone position with both feet hanging over the edge of the table. Measurements of the tendon thickness (anteroposterior diameter) were made at the level of the medial malleolus. The Achilles tendons were considered normal if their thickness and echogenicity was uniform in both planes and the AP diameter was less than 6.4 mm for females and 6.8 mm for males. Tendinosis was diagnosed if a fusiform thickening of the Achilles tendon without the disruption of tendon fibers was found with or without intratendinous hypoechoic foci.

Images for the CAC scoring were acquired following a standard protocol with 2.5 mm collimation, sequential acquisition and electrocardiographic gating. Imaging was performed with the 64 slice multidetector CT (GE LightSpeed VCT, Milwaukee, Wisconsin, US). Advantage Workstation (version 4.6, GE Healthcare, US) software was used for post processing of the images. The CAC scores were calculated according to the Agatston's method and later with respect to age, sex and ethnicity converted to percentiles.

Arterial stiffness was evaluated using the applanation tonometry system SphygmoCor v.8.0, and the cardio-ankle vascular index (CAVI) technique using the VaSera VS 1000 device. Brachial blood pressure was recorded, and the distance between the surface markings of the sternal notch and the femoral artery was measured. The data and the simultaneously recorded ECG allowed the system to compute the main

parameters of arterial stiffness: pulse wave velocity (PWV) and aortic augmentation index (AIxHR). PWV was measured in femoral (FEM) and radial (RA) arteries. Normal PWV was defined as <10 m/s. The cardio-ankle vascular index (CAVI) was calculated automatically using two main parameters: the β- parameter of arterial stiffness and the cardio-ankle index. Cuffs were applied bilaterally to the upper arms and ankles, and electrodes were fixed to each wrist, and a heart sound sensor was placed on the second intercostal sternum. The arteriosclerosis detector was equipped with both measurement and calculation systems to calculate the CAVI automatically: optimal <8.0, moderately increased – 8.0–9.0, severely increased – >9.0.

Duplex scannings of the carotid arteries for the assessment of the presence of atherosclerotic plaques and the measurement of carotid intima-media thickness (IMT) was performed using high-resolution echo-tracking technology (Logiq 700, General Electric). The following parameters were measured: right and left side common carotid artery (CCA) wall distention, CCA IMT, CCA stiffness and plaques in carotid arteries. A plaque was described as an intima- media thickness of more than 1.5 mm.

Additionally, for patients with a very high probability of familial hypercholesterolemia, genetic testing was performed. In the Center of Medical Genetics of VUH Santaros Klinikos, next-generation sequencing (NGS) analyses of genomic DNA, isolated from the patients' peripheral blood, were performed using the TruSight Cardio Sequecing panel (Illumina Inc., San Diego, CA) to identify the known genes associated with inherited cardiac conditions.

A detailed evaluation of HDL-C quality and quantity was performed for 93 (48 women and 45 men) randomly selected participants. Cholesterol efflux capacity was measured using the Cholesterol efflux fluorometric assay kit (BioVision, Inc., CA, US) according to the manufacturer's protocol. HDL cholesterol efflux was calculated and categorized into tertiles – below average, average or above average.

2.2.3 Statistical Analysis

Continuous variables were expressed by means and standard deviations (SD). Frequencies (%) are reported for categorical data. A Mantel-Haenszel chi-square test for trends was used to analyze the trends of the prevalence for categorical variables ("*p* for trend"). To evaluate linear associations between continuous variables, ANOVA for linear trends was used ("*p* for trend"). Continuous variables were compared using the Kruskal–Wallis univariate analysis of variance (ANOVA). Categorical variables were compared with the help of a Chi-square test or Fisher exact test.

Correlation analysis was performed to assess the linear association between characteristics, and a Spearman's correlation coefficient (r) was applied. Correlation was considered to be either weak (r <0.3), moderate (0.3≤ r ≤0.7) or strong (r>0.7).

The impact of factors on the change of the likelihood of morbidity was defined by assessing the ratios of odds (OR) together with confidence intervals (CI). A p- value of <0.05 was considered significant. Statistical analyses were performed using SPSS 23.0 (SPSS Inc., Chicago, Illinois, US).

3. Results

3.1 Results of the Retrospective Analysis

3.1.1 An Evaluation of the Prevalence, Trends in Morbidity, Diagnosis and Treatment of Dyslipidemia

The prevalence of any type of dyslipidemia remained stable and high, affecting 89.7% (n=82 893) (from 89.1% in 2009 to 89.5% in 2016) of middle-aged adults participating in the Primary Prevention Program. The tendencies of prevalence of any type of dyslipidemia in the program's population and in different gender and age groups during the period of 2009–2016 are shown in Figures 1–3.

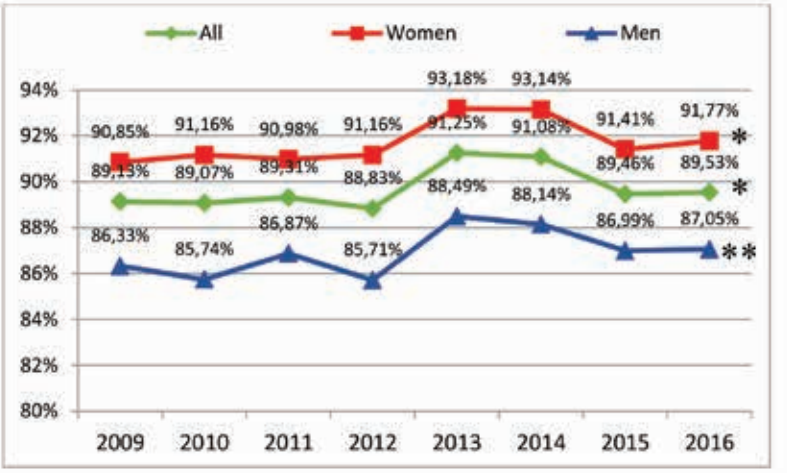

FIGURE 1. Trends in the prevalence of any type of dyslipidemia among middle-aged Lithuanian adults from 2009 to 2016.
* p<0.001; ** p=0.005.

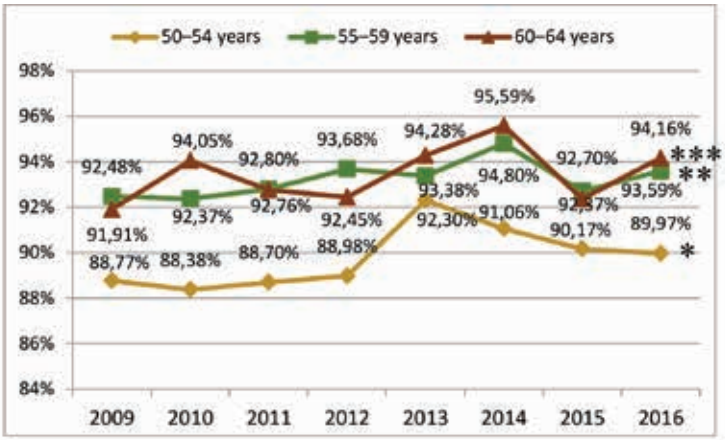

FIGURE 2. Trends in the prevalence of any type of dyslipidemia among middle-aged Lithuanian women from 2009 to 2016.
* p<0.001; ** p=0.021; *** p=0.070.

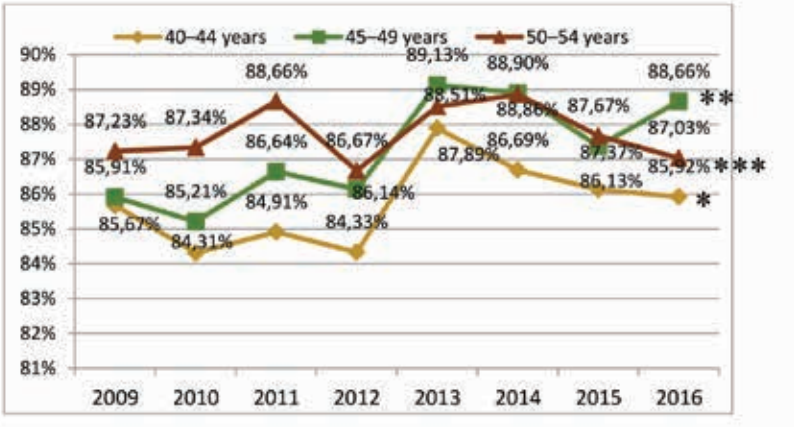

FIGURE 3. Trends in the prevalence of any type of dyslipidemia among middle-aged Lithuanian men from 2009 to 2016.
* p=0.09; ** p<0.001; *** p=0.711.

Based on the analysis of a digital database, primary care physicians diagnosed dyslipidemia for 66.5% (n=61 441) of the examined patients during the period of 2009–2016. The prevalence of dyslipidemia and the percentage of newly diagnosed dyslipidemia during the period of 2009–2016 are shown in Figure 4.

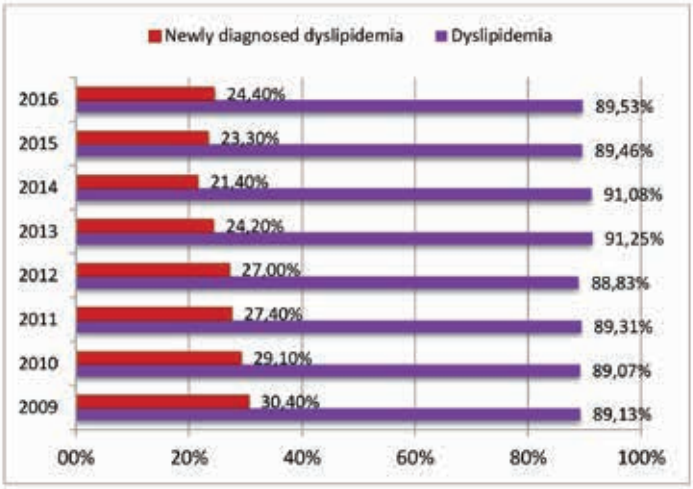

FIGURE 4. The prevalence of dyslipidemia and the percentage of newly diagnosed dyslipidemia for 2009–2016.

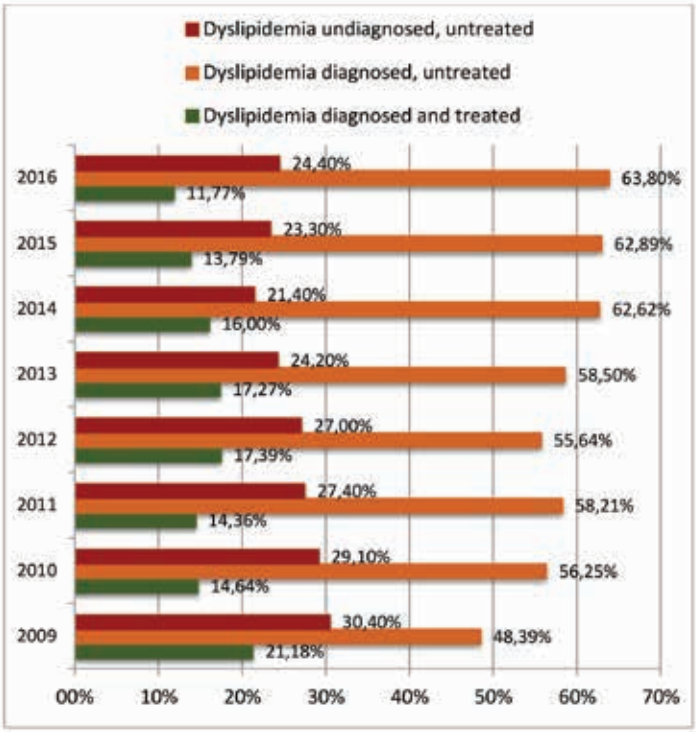

FIGURE 5. Treatment of dyslipidemia in the program's population during the period of 2009–2016.

Lipid-lowering medications were prescribed to 15.9% of subjects with dyslipidemia. Of the patiens with the diagnosis of dyslipidemia, 58.3% did not receive any treatment. In this study, dyslipidemia was newly diagnosed for 25.9% of the participants according to the laboratory analysis. These patients did not receive lipid-lowering drugs, as they were not considered to have dyslipidemia by primary care physicians. An evaluation of dyslipidemia treatment in the program's population is presented in Figure 5. In the cohort receiving lipid-lowering treatment, only 6.7% of the patients reached treatment goals (LDL-C <3 mmol/l). The achievement of treatment goals in middle-aged Lithuanians during the period of 2009–2016 is presented in Figure 6.

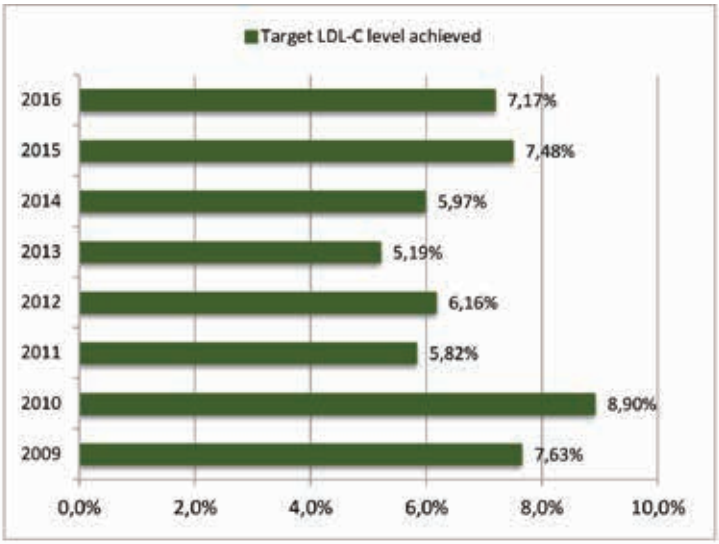

FIGURE 6. The achievement of treatment goals (LDL-C < 3 mmol/l) in patients with dyslipidemia receiving lipid-lowering medications for 2009–2016. Abbrevations: LDL-C – low-density lipoprotein cholesterol.

3.1.2 Basic Characteristics and Lipidogram Parameters of the Study Participants

This study included 92 373 adults without overt cardiovascular disease – 53 961 (58.4%) women and 38 412 (41.6%) men. The average age of the sample group was 52.15 (± 6.21) years. Women and men varied in age because of the different study enrollment criteria: the average age of men was 46.96 (± 4.39) years, and women – 55.85 (± 4.40) years. A larger part – 81.7% – of subjects had TC >5 mmol/L; 79.3% had LDL-C >3 mmol/L; 30.4% had TG >1.7 mmol/L, and 13.7% had low HDL-C (<1.0 mmol/L in men and <1.2 mmol/L in women). The distribution of the lipido-

gram parameters among the studied population is shown in Figures 7–10. Any type of dyslipidemia was diagnosed in 89.7% of middle-aged adults without overt cardiovascular disease. When analyzing the prevalence of other major cardiovascular risk factors, the most frequent were arterial hypertension (AH) – 54.5%, abdominal obesity – 43.7%, metabolic syndrome (MetS) – 31.5% and obesity – 34.6%. Un-

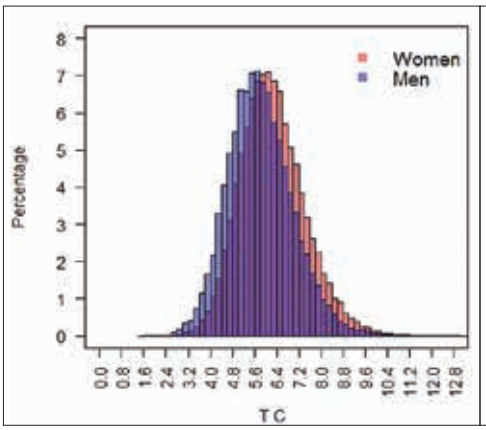

FIGURE 7. The distribution of TC concentrations among the program's population during the period of 2009–2016 (n=92 373).

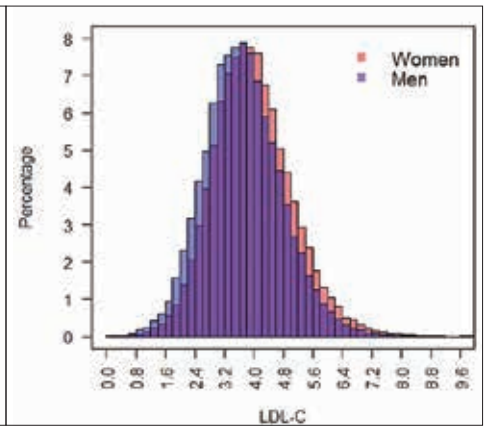

FIGURE 8. The distribution of LDL-C concentrations among the program's population during the period of 2009–2016 (n=92 373).

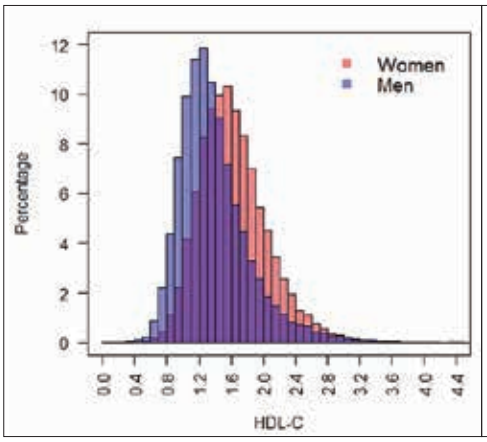

FIGURE 9. The distribution of HDL-C concentrations among the program's population during the period of 2009–2016 (n=92 373).

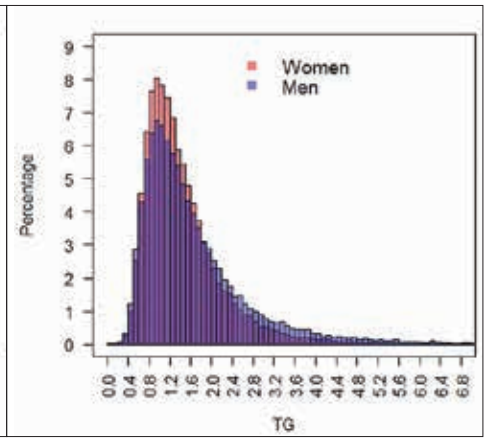

FIGURE 10. The distribution of TG concentrations among the program's population during the period of 2009–2016 (n=92 373).

healthy dietary patterns were determined in 61.5% of the studied participants, while insufficient physical activity – in 51.2% of subjects. Baseline characteristics and the prevalence of cardiovascular risk factors of the whole study population are shown in Table 2.

3.1.3 Cardiovascular Risk Assessment in Middle-Aged Patients with and without Dyslipidemia

The group with dyslipidemia consisted of 82 893 (89.7%) subjects, and the group without dyslipidemia included 9 480 (10.3%) adults. All the major risk factors, including AH, abdominal obesity, MetS, diabetes mellitus (DM) and obesity, except for smoking, were more prevalent in patients with dyslipidemia compared to patients without it (p <0.001) (Table 2). The average SCORE index of the whole study population was 1.87, while patients with dyslipidemia had a higher SCORE compared to the control group (Table 2).

TABLE 2. The baseline characteristics and trends of cardiovascular risk factors in the study's population.

Characteristics	All patients		With dyslipidemia		Without dyslipidemia		p
	n=92 373		n=82 893		n=9 480		
	Mean	SD	Mean	SD	Mean	SD	
Age (years)	52.15	6.21	52.34	6.20	50.54	6.05	<0.001
WC (cm)	93.72	13.5	94.07	13.5	90.64	13.08	<0.001
BMI (kg/m^2)	28.60	5.41	28.78	5.39	27.04	5.38	<0.001
SBP (mmHg)	133.52	16.3	133.87	16.4	130.4	15.44	<0.001
DBP (mmHg)	82.76	9.48	82.95	9.50	81.15	9.14	<0.001
HR (bpm)	71.95	8.78	72.00	8.78	71.50	8.72	<0.001
Glucose(mmol/l)	5.52	1.22	5.54	1.23	5.35	1.08	<0.001
TC (mmol/l)	6.08	1.21	6.28	1.12	4.40	0.45	<0.001
LDL-C (mmol/l)	3.87	1.08	4.04	1.00	2.42	0.43	<0.001
HDL-C (mmol/l)	1.54	0.46	1.54	0.47	1.58	0.37	<0.001
TG (mmol/l)	1.59	1.16	1.66	1.19	0.93	0.31	<0.001
Non-HDL-C	4.54	1.21	4.74	1.11	2.83	0.48	<0.001
TG/HDL-C	1.22	1.55	1.29	1.62	0.63	0.28	<0.001
SCORE index	1.87	1.68	1.95	1.71	1.20	1.18	<0.001

Characteristics	All patients		With dyslipidemia		Without dyslipidemia		p
	n=92 373		n=82 893		n=9 480		
	Mean	SD	Mean	SD	Mean	SD	
Frequencies	n	%	n	%	n	%	p
DM (%)	9897	10.7	9207	11.1	690	7.3	<0.001
AH (%)	50317	54.5	46216	55.8	4101	43.3	<0.001
Abdominal obesity (%)	40408	43.7	37547	45.3	2861	30.2	<0.001
Smoking (%)	21218	23.0	18703	22.6	2515	26.5	<0.001
Metabolic syndrome (%)	29094	31.5	28219	34.0	875	9.2	<0.001
RF ≥3 (%)	53971	58.4	49819	60.1	4152	43.8	<0.001
CHD history (%)	24025	26.0	21837	26.3	2188	23.1	<0.001
Diet (unbalanced) (%)	56800	61.5	51783	62.5	5017	52.9	<0.001
Physical activity (insufficient) (%)	47268	51.2	43074	52.0	4194	44.2	<0.001
BMI <25 (kg/m^2) (%)	24891	26.9	21037	25.4	3854	40.7	<0.001
BMI 25-30 (kg/m^2) (%)	35589	38.5	32209	38.9	3380	35.7	<0.001
BMI 30-40 (kg/m^2) (%)	28778	31.2	26776	32.3	2002	21.1	<0.001
BMI >40 (kg/m^2) (%)	3115	3.4	2871	3.5	244	2.6	.001

Abbreviations: SD – standard deviation; WC – waist circumference; BMI – body mass index; SBP – systolic blood pressure; DBP – diastolic blood pressure; HR – heart rate; TC – total cholesterol; LDL-C – low-density lipoprotein cholesterol; HDL-C – high-density lipoprotein cholesterol; TG – triglycerides; DM – diabetes mellitus; AH – arterial hypertension; RF – risk factors; CHD – coronary heart disease.

3.1.4 Cardiovascular Risk Assessment in Middle-Aged Men with and without Dyslipidemia

The study included 38 412 men aged 40–54 years: 33 403 (87.0%) with dyslipidemia and 5 009 (13.0%) without dyslipidemia. The mean value of TC in men was 6.07 ± 1.10mmol/l, LDL-C – 3.92 ± 0.98 mmol/l, HDL-C – 1.39 ± 0.47 mmol/l and TG – 1.86 ± 1.45 mmol/l. The prevalence of different cardiovascular risk factors in dyslip-

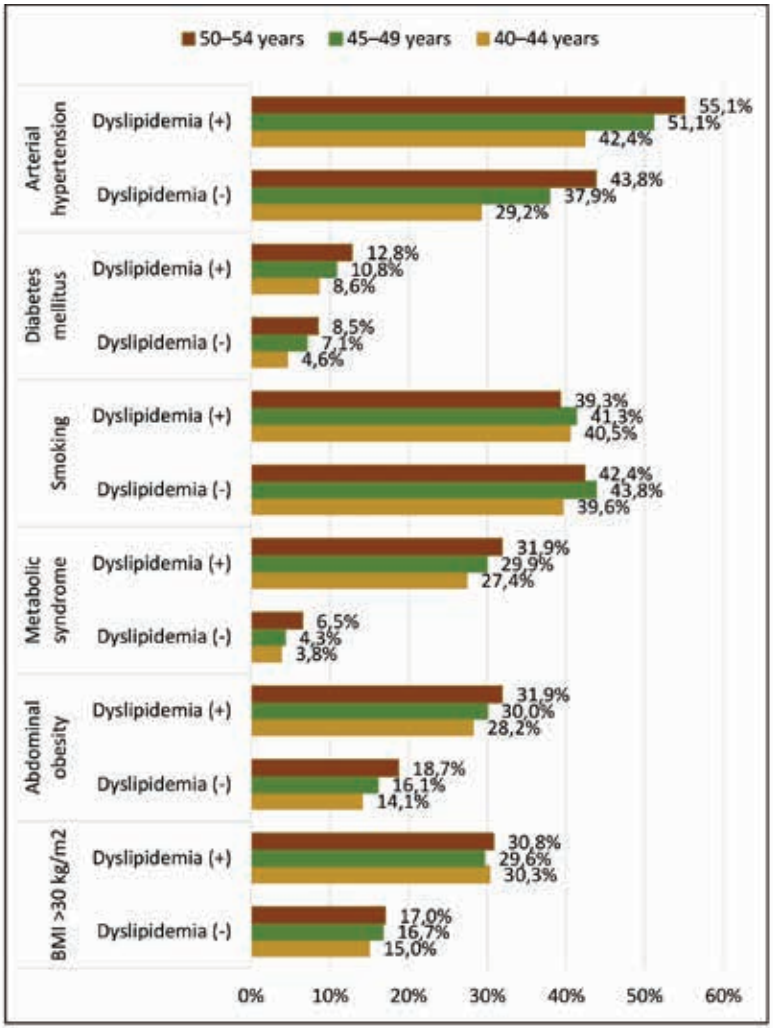

FIGURE 11. The prevalence of different cardiovascular risk factors in men of different ages with and without dyslipidemia. Abbrevations: BMI – body mass index.

idemic and non-dyslipidemic men is presented in Figure 11. Men with dyslipidemia possessed all the main risk factors – except for smoking – significantly more often than the males without dyslipidemia: AH (49.6% vs. 36.6%, p<0.001), DM (10.8% vs. 6.6%, p<0.001), abdominal obesity (30.1% vs. 16.2%, p<0.001), MetS (29.8% vs. 4.8%, p<0.001) and obesity (30.3% vs. 16.1%, p<0.001). The prevalence of DM, AH, abdominal obesity, MetS and obesity increased with age in both dyslipidemic and

non-dyslipidemic men's groups. Males with dyslipidemia had a higher prevalence of DM, AH, abdominal obesity, MetS and obesity in all age groups in comparison with the control group (Figure 11). Smoking was more prevalent in men aged 40–54 without dyslipidemia compared to the group with dyslipidemia (41.8% vs. 40.3%, p <0.001). The highest frequency of smoking in males without dyslipidemia was observed in those aged 45–49 years (43.8%), followed by the groups of men aged 50–55 (42.4%) and 40–44 (39.6%). The prevalence of smoking was lower in men older than 50 years compared to younger males in both dyslipidemic and non-dyslipidemic groups (Figure 11). Also, men with dyslipidemia tended to have histories of CHD in their families, an unbalanced diet and insufficient physical activity more often than those without dyslipidemia (23.9% vs. 20.6%, p<0.001; 65.5% vs. 54.1%, p<0.001; 47.3% vs. 39.5%, p<0.001, respectively).

3.1.5 Cardiovascular Risk Assessment in Middle-Aged Women with and without Dyslipidemia

The study included 53 961 women aged 50–64: 49 490 (91.7 %) with dyslipidemia and 4 471 (8.3%) without dyslipidemia. The mean laboratory values of women were: TC – 6.41 ± 1.11 mmol/l, LDL-C– 4.12 ± 1.01 mmol/l HDL-C – 1.63 ± 0.45 mmol/l and TG – 1.53 ± 0.96 mmol/l. The prevalence of different cardiovascular risk factors in dyslipidemic and non-dyslipidemic women of different ages is shown in Figure 12. Women aged 50–64 with dyslipidemia had all the main cardiovascular risk factors significantly more frequently than females without dyslipidemia (DM (11.3% vs. 8.0%, p<0.001), AH (59.9% vs. 50.7%, p<0.001), abdominal obesity (55.6% vs. 45.8%, p<0.001), smoking (10.6% vs. 9.4%, p<0.001), MetS (36.9% vs. 14.2%, p<0.05) and obesity (39.5% vs. 32.1%, p<0.001)). The prevalence of DM, AH, abdominal obesity, MetS and obesity increased with age in both dyslipidemic and non-dyslipidemic groups (figure 12). Subjects with dyslipidemia had a higher prevalence of DM, AH, abdominal obesity, MetS and obesity in all age groups in comparison with the control group (Figure 12). Women with dyslipidemia reported smoking more often in all age groups compared to women without dyslipidemia (p <0.001). The frequency of smoking was lower in older women in both dyslipidemic and non- dyslipidemic groups compared to younger females (Figure 12).

3.1.6 The Prevalence of Severe Dyslipidemia in the Study Population

Severe dyslipidemia was diagnosed for 13.4% (n=12 334) of study subjects (n=92 373). Severe dyslipidemia made up 14.9% (n=12 334) of the dyslipidemia cases (n=82 893). Severe dyslipidemia was more prevalent among women compared

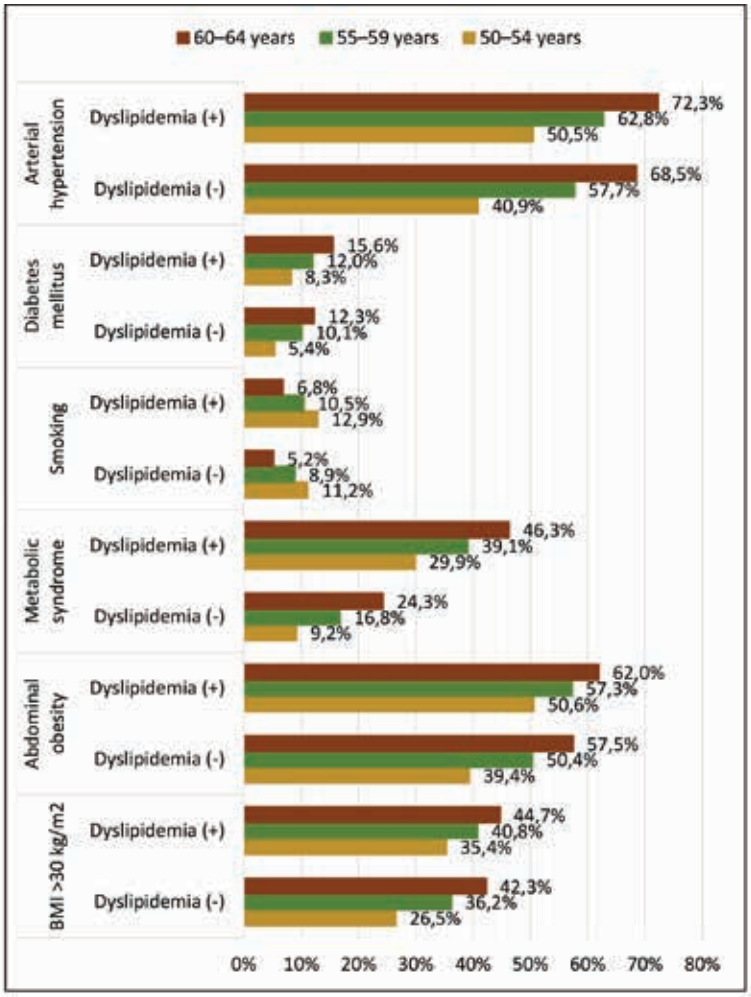

FIGURE 12. The prevalence of different cardiovascular risk factors in women of different ages with and without dyslipidemia.
Abbrevations: BMI – body mass index.

to men (14.63% vs. 10.5%, p <0,001, respectively). While assessing the trends of the prevalence of severe dyslipidemia from 2009 to 2016, the prevalance decreased from 12.2% in 2009 to 11.6% in 2016 (p <0.013). Trends in the prevalence of severe dyslipidemia among men and women in different age groups over the period of 2009–2016 are shown in Figures 13–15.

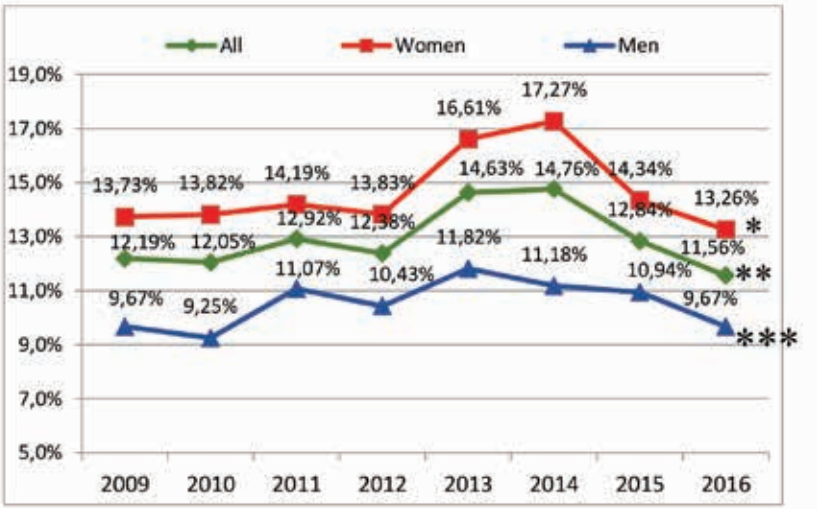

FIGURE 13. Trends in the prevalence of any type of severe dyslipidemia among middle-aged Lithuanian adults from 2009 to 2016.
* p=0.004; ** p=0.013; *** p=0.078.

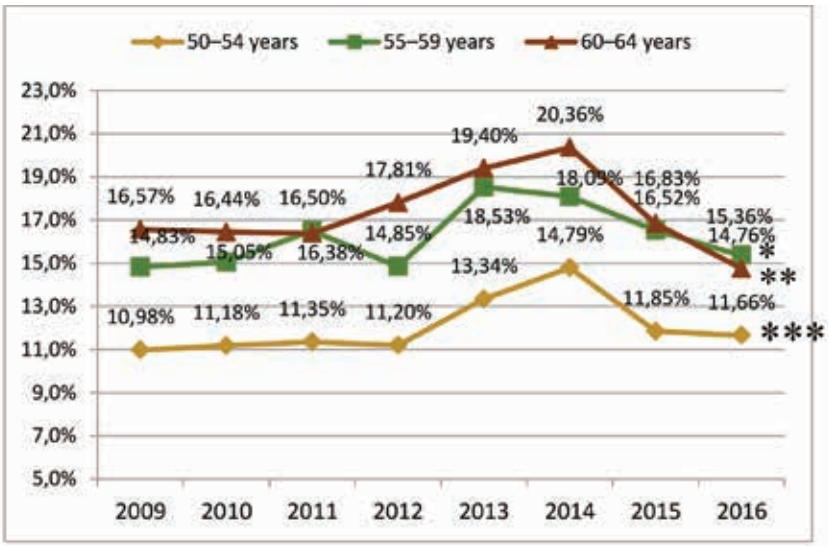

FIGURE 14. Trends in the prevalence of any type of severe dyslipidemia among middle-aged Lithuanian women (50–64 years) from 2009 to 2016.
* p=0.016; ** p=0.191; *** p=0.006.

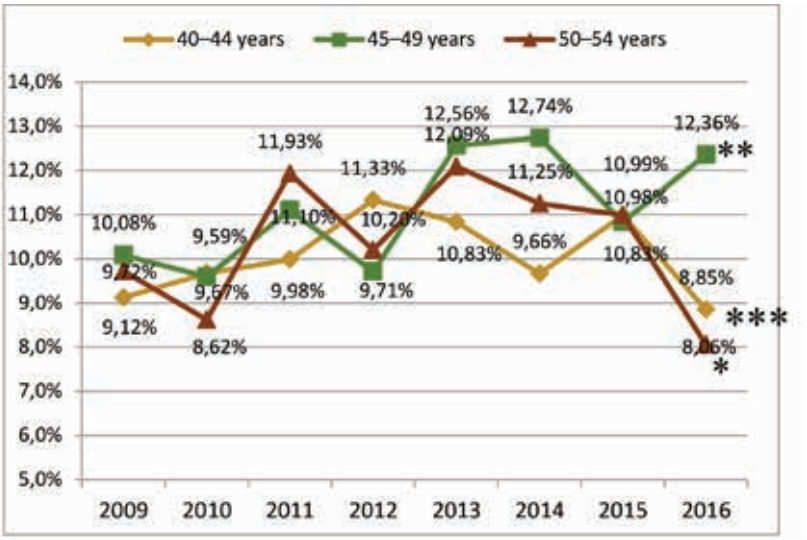

FIGURE 15. Trends in the prevalence of any type of severe dyslipidemia among middle-aged Lithuanian men (40–54 years) from 2009 to 2016.
* p=0.005; ** p=0.894; *** p=0.679.

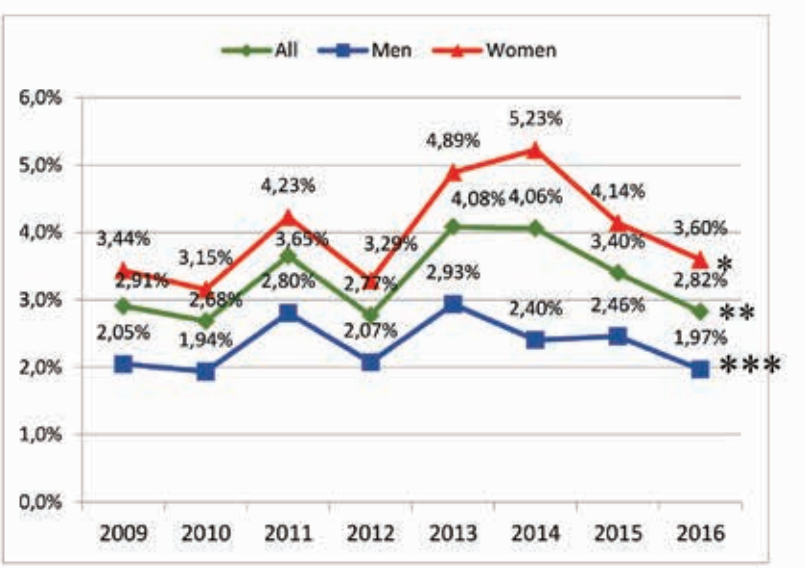

FIGURE 16. Trends of the prevalence of severe hypercholesterolemia (LDL-C ≥6.0 mmol/l) among middle-aged Lithuanians from 2009 to 2016.
* p<0.001; ** p=0.003; *** p=0.574.

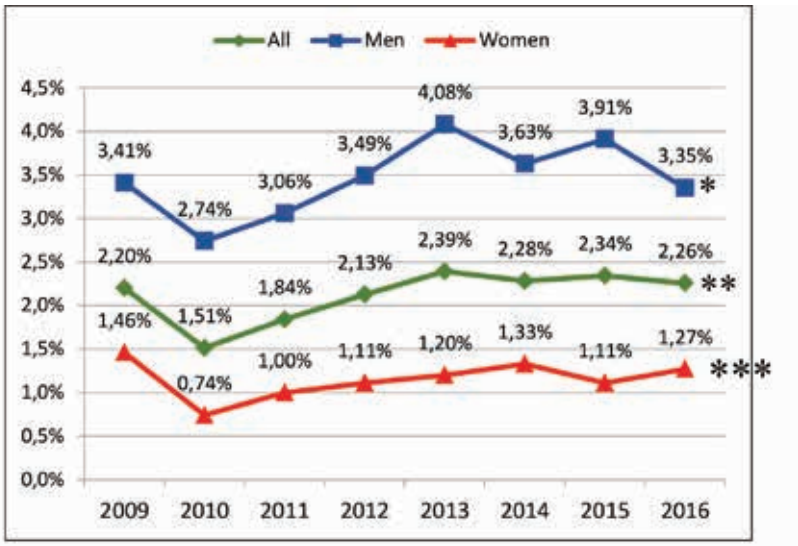

FIGURE 17. Trends of the prevalence of severe triglyceridemia (TG ≥4.5 mmol/l) among middle-aged Lithuanians from 2009 to 2016.
* p=0.017; ** p<0.001; *** p=0.358.

3.1.7 The Prevalence of Severe Hypercholesterolemia and Severe Hypertriglyceridemia in the Study

Population Severe hypercholesterolemia (LDL-C ≥ 6 mmol/l) was detected in 3.2% (2 956) of the subjects, while severe hypertriglyceridemia (TG ≥4.5 mmol/l) was observed in 2.0% (1 827) of the subjects. The prevalence of severe hypercholesterolemia in the overall population decreased from 2.91% to 2.82% (p=0.003), and the prevalence of severe hypertriglyceridemia increased from 2.20% to 2.26% (p= 0.001) in the overall population over the period of 2009–2016 (Figures 16, 17). Patients with severe hypercholesterolemia were significantly older in comparison with the severe hypertriglyceridemia group (54.14±6.22 years vs. 49.37±6.10 years, p<0.001). There were no statistically significant changes in the prevalence of LDL-C ≥6 mmol/l or TG ≥4.5 mmol/l in different age groups.

3.1.8 An Analysis and Comparison of the Cardiovascular Risk Profiles of Patients with Severe Hypercholesterolemia and Severe Hypertriglyceridemia

This study included 2 956 patients with severe hypercholesterolemia and 1 827 subjects with severe hypertriglyceridemia (a total of 4 783). The two groups were compared by demographic characteristics, laboratory parameters and the frequency

TABLE 3. The comparison of baseline characteristics and the cardiovascular risk profile of patients with severe hypercholesterolemia and severe hypertriglyeridemia.

Characteristics	Total		LDL-C ≥6 mmol/l		TG ≥4.5 mmol/l		*p-value*
	n=4 783		n=2 956		n=1 827		
	Mean	SD	Mean	SD	Mean	SD	
Age (years)	52.32	6.60	54.14	6.22	49.37	6.10	<0.001
WC (cm)	96.91	13.24	93.04	11.98	103.18	12.77	<0.001
BMI (kg/m²)	29.65	5.07	28.61	4.69	31.34	5.22	<0.001
SBP (mmHg)	136.68	17.17	135.14	16.78	139.17	17.50	<0.001
DBP (mmHg)	84.51	9.95	83.51	9.58	86.12	10.31	<0.001
HR (bpm)	72.87	8.94	72.33	8.80	73.75	9.11	<0.001
Glucose (mmol/l)	5.92	1.84	5.54	1.06	6.54	2.54	<0.001
TC (mmol/l)	8.22	1.44	8.89	0.91	7.14	1.47	<0.001
LDL-C (mmol/l)	5.43	1.76	6.61	0.65	3.52	1.25	<0.001
HDL-C (mmol/l)	1.39	0.46	1.57	0.40	1.12	0.40	<0.001
TG (mmol/l)	3.78	3.18	1.92	0.78	6.79	3.29	<0.001
Non-HDL-C	6.83	1.30	7.33	0.87	6.02	1.46	<0.001
SCORE index	2.82	2.34	2.99	2.35	2.55	2.30	<0.001
Frequencies	n	%	n	%	n	%	
DM (%)	851	17.8	335	11.3	516	28.2	<0.001
AH (%)	3065	64.1	1801	60.9	1264	69.2	<0.001
Abdominal obesity (%)	2537	53.0	1425	48.2	1112	60.9	<0.001
Smoking (%)	1351	28.2	675	22.8	676	37.0	<0.001
Metabolic syndrome (%)	2665	55.7	1270	43.0	1395	76.4	<0.001
RF ≥3 (%)	3490	73.0	1957	66.2	1533	83.9	<0.001
CHD history (%)	1436	30.0	902	30.5	534	29.2	0.346
Diet (unbalanced) (%)	3272	68.4	1903	64.4	1369	74.9	<0.001
Physical activity (insufficient) (%)	2769	57.9	1614	54.6	1155	63.2	<0.001
BMI <25 (kg/m²) (%)	795	16.6	643	21.8	152	8.3	<0.001
BMI 25-30 (kg/m²) (%)	1972	41.2	1321	44.7	651	35.6	<0.001
BMI 30-40 (kg/m²) (%)	1859	38.9	929	31.4	930	50.9	<0.001
BMI >40 (kg/m²) (%)	157	3.3	63	2.1	94	5.1	<0.001

Abbreviations: SD – standard deviation; WC – waist circumference; BMI – body mass index; SBP – systolic blood pressure; DBP – diastolic blood pressure; HR – heart rate; TC – total cholesterol; LDL-C – low-density lipoprotein cholesterol; HDL-C – high-density lipoprotein cholesterol; TG – triglycerides; DM – diabetes mellitus; AH – arterial hypertension; RF – risk factors; CHD – coronary heart disease.

of cardiovascular risk factors (Table 3). Patients with severe hypercholesterolemia were older compared to severe triglyceridemia group (54.14 years vs. 49.37 years, p <0,001). While assessing the prevalence of major CVD risk factors, all cardiovascular risk factors, except the family history of CHD, were more prevalent among patients with severe hypertriglyceridemia compared with subjects with severe hypercholesterolemia (Table 3).

3.1.9 The Characteristics of Subjects with Different Low-Density Lipoprotein Cholesterol Levels

According to our database, 19.8% of subjects without overt cardiovascular disease (n=18 290, 49.6% women and 50.4% men, p<0.01) had LDL-C less than 3 mmol/l. From 2009 to 2016 a significant decrease in number of subjects with normal LDL-C (<3 mmol/l) levels was observed in the whole study population (from 21.9% to 19.3%, p=0.001) and both gender groups (men's group from 25.5% to 22.9%, p=0.001, women's group from 19.7% to 16.1%, p=0.001). The distribution of LDL-C levels in the middle- aged Lithuanian adults over the period of 2009-2016 is shown in figure 18. In the study population, LDL-C >3 mmol/l was more frequently determined in women compared to men (Figure 19). We additionally evaluated TG levels for patients with elevated LDL-C. 1.4% (n=1293) of adults with disregarded TG levels had LDL-C 6.5–8.49 mmol/l, while both LDL-C 6.5–8.49 mmol/l and TG ≤1.7 mmol/l were found in 0.6% (n=554) of the subjects (Figure 20).

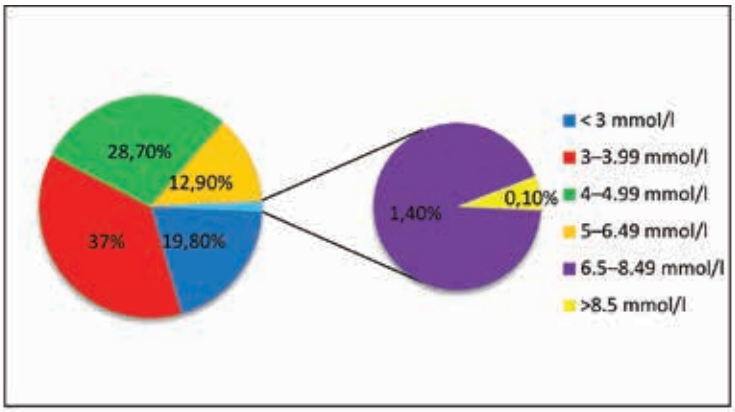

FIGURE 18. The distribution of LDL-C levels in middle-aged Lithuanian adults during the period 2009–2016.

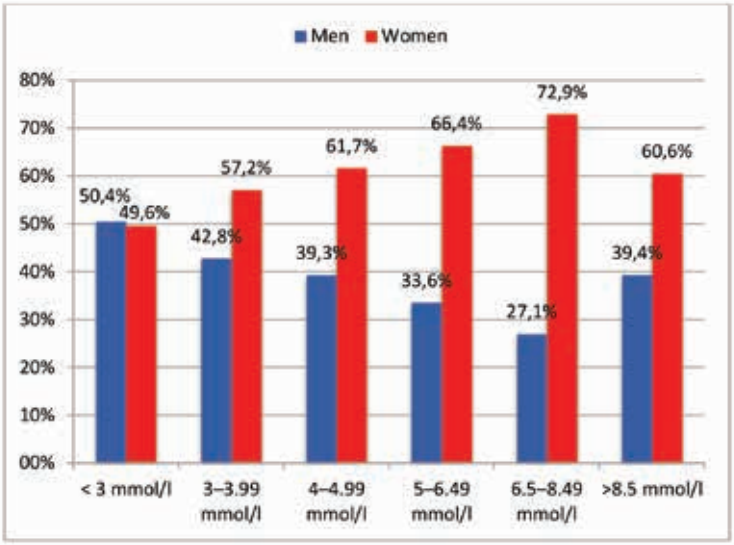

FIGURE 19. The differences in LDL-C levels in middle-aged Lithuanian men and women during the period 2009–2016.

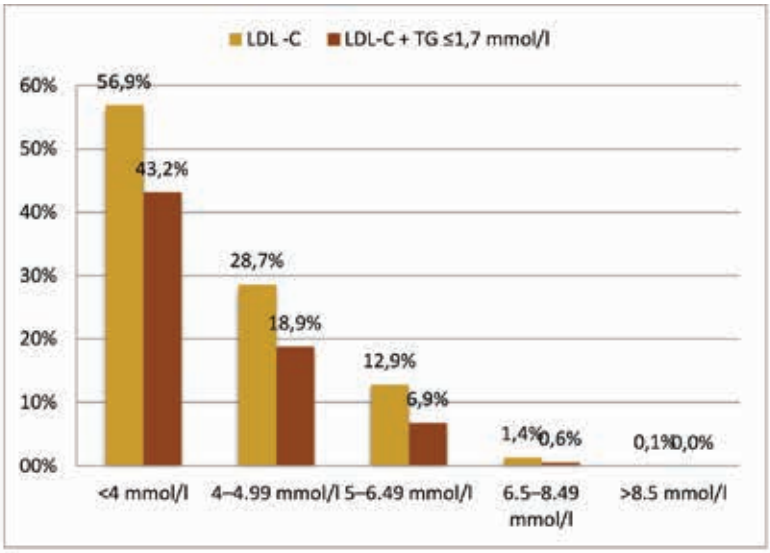

FIGURE 20. The distribution of LDL-C levels and LDL-C levels with normal TG levels in middle-aged Lithuanian adults during the period of 2009–2016.
Abbrevations: LDL-C – low-density lipoprotein cholesterol; TG – triglycerides.

3.1.10 The Characteristics of Subjects with Different Triglycerides Levels

Of all subjects, 68.9% (n=63 644, 61.4% women and 38.6% men, p<0.01) had the concentration of triglycerides that amounted to less than 1.7 mmol/l. During the period of 2009–2016, the prevalence of normal TG levels (<1.7 mmol/l) decreased from 69.69% to 69.09% (p <0.001) in all study participants. This pattern was also seen in men (from 64.31% to 63.63%, p=0.002), but the mean TG values remained consistent in women (from 73.0% to 74.0%, p=0.778). The distribution of different

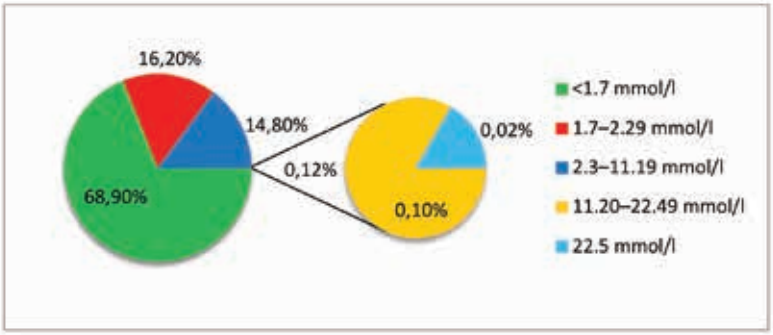

FIGURE 21. The distribution of TG levels in middle-aged Lithuanian adults during the period of 2009–2016.

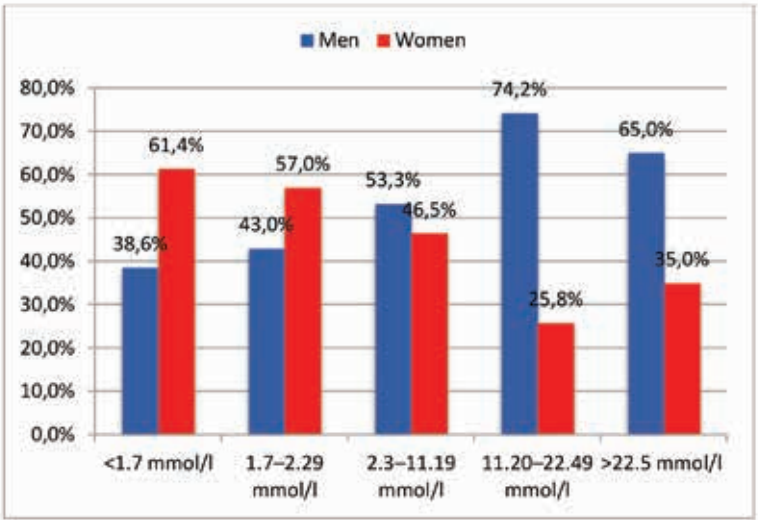

FIGURE 22. The differences in TG levels in middle-aged Lithuanian men and women during the period of 2009–2016.

TG levels in the middle-aged Lithuanian population over the period of 2009–2016 is shown in Figure 21. The TG levels of <2.3 mmol/l were more commonly found in women, while the TG levels of >11.2 mmol/l were more common among men (Figure 22).

3.1.11 The Cardiovascular Risk Profile of Patients with Atherogenic Dyslipidemia, Hypertriglyceridemia and Low HDL-C

In the program's population, 8.1% (n=7 489) of subjects had atherogenic dyslipidemia (54.5% of them were women and 45.5% men). Isolated high TG (>1.7 mmol/l) was observed in 22.3% of adults, and the prevalence of isolated low HDL-C (<1.2 mmol/l in women and <1.0 mmol/l in men) was 5.6%. The mean age in the atherogenic dyslipidemia group was 52.03 ± 6.60 years. The average values of triglycerides were lower in the older men and women with atherogenic dyslipidemia compared to younger subjects. Demographic, anthropometric and laboratory characteristics of participants with AD, hypertriglyceridemia and low-HDL-C levels groups are shown in Table 4. Participants in the low-HDL-C group were statistically significantly older (52.41 ± 6.33 years) in comparison with other groups. Participants with AD tended to have higher prevalences of AH (69.0%), DM (22.6%), abdominal obesity (67.6%), MetS (88.9%), an unbalanced diet (71.0%), low physical activity (64.2%) and the percentage of people having more than 3 risk factors (86.1%). In addition, the prevalence of smoking (26.1%) and CHD in the first degree relatives (29.1%) was higher in the AD group than in the low-HDL-C group. Participants in low-HDL group had a more favorable risk profile: a lower prevalence of DM (12.5%), AH (56.9%), MetS (52.7%), smoking (22.3%), an unbalanced diet (62.2%) and low physical activity (56.5%). Participants in the hypertriglyceridemia group had higher values of total cholesterol (6.74 ± 1.24mmol/l), LDL-C (4.28 ± 1.16mmol/l), HDL-C (1.45 ± 0.34mmol/l), non-HDL cholesterol (5.29 ± 1.22mmol/l) and the SCORE index (2.27 ± 1.94) in comparison with other groups.

3.1.12 The Associations of Cardiovascular Risk Factors with Different Types of Dyslipidemia (Atherogenic Dyslipidemia, Hypertriglyceridemia and Low HDL-C)

According to the program's database, all analyzed risk factors, including the main risk factors, such as DM (OR: 2.74, 95% CI: 2.58-2.90), AH (OR: 1.96, 95% CI 1.87-2.01), obesity (OR: 2.92, 95% CI: 2.78-3.10) and smoking (OR: 2.74, 95% CI: 2.58-2.90), were significantly associated with AD and hypertriglyceridemia (Table 5). There was no association between isolated low HDL-C levels and smoking, CHD history in first-degree relatives and an unbalanced diet.

TABLE 4. A comparison of demographic characteristics, laboratory parameters and the cardiovascular risk factors of participants with atherogenic dyslipidemia, hypertriglyceridemia and low HDL-C.

Characteristics	Atherogenic dyslipidemia		Hyper-triglyceridemia		Low HDL-C		p-value	p-value (AD vs. HTG)	p-value (AD vs. HDL-C)	p-value (HTG vs. HDL-C)
	n=7 489		n=20 593		n=5 153					
	Mean	SD	Mean	SD	Mean	SD				
Age (years)	52.03	6.6	51.71	6.44	52.41	6.33	<0.001	0.001	0.004	<0.001
BMI (kg/m²)	31.91	5.63	30.21	5.30	29.99	5.95	<0.001	<0.001	<0.001	0.027
SBP (mmHg)	137.61	16.95	136.85	16.79	133.87	16.36	<0.001	0.002	<0.001	<0.001
DBP (mmHg)	84.93	9.71	84.66	9.73	82.87	9.31	<0.001	0.093	<0.001	<0.001
HR (bpm)	73.02	8.70	72.67	8.77	72.52	8.88	0.002	0.009	0.005	0.519
Glucose (mmol/l)	6.06	1.90	5.76	1.47	5.55	1.27	<0.001	<0.001	<0.001	<0.001
TC (mmol/l)	6.16	1.25	6.74	1.24	5.10	1.05	<0.001	<0.001	<0.001	<0.001
LDL-C (mmol/l)	3.89	1.12	4.28	1.16	3.53	0.97	<0.001	<0.001	<0.001	<0.001
HDL-C (mmol/l)	0.95	0.15	1.45	0.34	0.98	0.15	<0.001	<0.001	<0.001	<0.001
TG (mmol/l)	3.16	2.09	2.55	1.22	1.24	0.29	<0.001	<0.001	<0.001	<0.001
Non-HDL-C (mmol/l)	5.20	1.23	5.29	1.22	4.11	1.02	<0.001	<0.001	<0.001	<0.001
TG/HDL-C ratio	3.58	4.11	1.86	1.04	1.32	0.55	<0.001	<0.001	<0.001	<0.001
SCORE index	2.19	1.95	2.27	1.94	1.63	1.45	<0.001	0.008	<0.001	<0.001
Atherogenic index (log (TG/HDL-C))	0.48	0.22	0.23	0.17	0.10	0.14	<0.001	<0.001	<0.001	<0.001

Characteristics	Atherogenic dyslipidemia		Hyper-triglyceridemia		Low HDL-C		p-value	p-value (AD vs. HTG)	p-value (AD vs. HDL-C)	p-value (HTG vs. HDL-C)
	n=7 489		n=20 593		n=5 153					
	Mean	SD	Mean	SD	Mean	SD				
Frequencies	n	%	n	%	n	%	p	p	p	p
DM (%)	1696	22.6	3258	15.8	644	12.5	<0.001	<0.001	<0.001	<0.001
AH (%)	5170	69.0	13373	64.9	2934	56.9	<0.001	<0.001	<0.001	<0.001
Abdominal obesity (%)	5062	67.6	11485	55.8	2764	53.6	<0.001	<0.001	<0.001	0.018
Smoking(%)	1953	26.1	5319	25.8	1148	22.3	<0.001	<0.001	<0.001	<0.001
MetS (%)	6657	88.9	11809	57.3	2714	52.7	<0.001	<0.001	<0.001	<0.001
RF ≥3 (%)	6448	86.1	15051	73.1	3473	67.4	<0.001	<0.001	<0.001	<0.001
CHD history (%)	2176	29.1	5716	27.8	1311	25.4	<0.001	<0.001	<0.001	0.003
Diet (unbalanced) (%)	5319	71.0	14086	68.4	3204	62.2	<0.001	<0.001	<0.001	<0.001
Physical activity	4811	64.2	11737	57.0	2910	56.5	<0.001	<0.001	<0.001	<0.001
(insufficient) (%)										
BMI <25 (kg/m^2) (%)	591	7.9	2996	14.5	1001	19.4	<0.001	<0.001	<0.001	<0.001
BMI 25-30 (kg/m^2) (%)	2451	32.7	7950	38.6	1877	36.4	<0.001	<0.001	<0.001	<0.001
BMI 25-30 (kg/m^2) (%)	3844	51.3	8706	42.3	1952	37.9	<0.001	<0.001	<0.001	<0.001
BMI 25-30 (kg/m^2) (%)	603	8.1	941	4.6	323	6.3	<0.001	<0.001	<0.001	<0.001

Abbreviations: HDL-C – high-density lipoprotein cholesterol; SD – standard deviation; AD – atherogenic dyslipidemia; HTG – hypertrygliceridemia; WC – waist circumference; BMI – body mass index; SBP – systolic blood pressure; DBP – diastolic blood pressure; HR – heart rate; TC – total cholesterol; LDL-C – low-density lipoprotein cholesterol; TG – triglycerides; DM – diabetes mellitus; AH – arterial hypertension; MS – metabolic syndrome; RF – risk factors; CHD – coronary heart disease.

TABLE 5. The associations of cardiovascular risk factors with different types of dyslipidemia (atherogenic dyslipidemia, hypertriglyceridemia and low HDL-C).

Risk factor	Group	Atherogenic dyslipidemia		Hypertriglyceridemia		Low HDL-C	
		OR (95 % CI)	p-value	OR (95 % CI)	p-value	OR (95 % CI)	p-value
DM	Total	2.74 (2.58-2.90)	<0.001	1.84 (1.76-1.93)	<0.001	1.20 (1.11-1.31)	0.001
	Men	2.13 (1.94-2.34)	<0.001	1.69 (1.57-1.81)	<0.001	1.08 (0.94-1.25)	0.293
	Women	3.29 (3.05-3.55)	<0.001	2.02 (1.90-2.15)	<0.001	1.28 (1.15-1.42)	0.001
AH	Total	1.96 (1.87-2.01)	<0.001	1.75 (1.69-1.80)	<0.001	1.11 (1.05-1.18)	0.001
	Men	1.70 (1.59-1.83)	<0.001	1.78 (1.70-1.87)	<0.001	0.89 (0.82-0.98)	0.014
	Women	2.40 (2.23-2.59)	<0.001	1.92 (1.84-2.02)	<0.001	1.27 (1.18-1.37)	0.001
Smoking	Total	1.20 (1.14-1.27)	<0.001	1.22 (1.18-1.27)	<0.001	0.96 (0.90-1.03)	0.225
	Men	1.07 (0.99-1.14)	0.081	0.95 (0.91-0.99)	0.043	1.04 (0.95-1.14)	0.378
	Women	1.29 (1.17-1.42)	<0.001	1.27 (1.19-1.36)	<0.001	0.94 (0.83-1.06)	0.296
Obesity	Total	2.92 (2.78-3.10)	<0.001	1.87 (1.81-1.93)	<0.001	1.52 (1.44-1.61)	0.001
	Men	3.09 (2.88-3.32)	<0.001	2.19 (2.09-2.30)	<0.001	1.34 (1.22-1.47)	0.001
	Women	3.75 (3.46-4.06)	<0.001	2.29 (2.19-2.40)	<0.001	1.67 (1.54-1.80)	0.001
MetS	Total	22.27 (20.69-23.97)	<0.001	4.24 (4.10-4.38)	<0.001	2.57 (2.43-2.72)	0.001
	Men	17.51 (15.97-	<0.001	3.97 (3.78-4.17)	<0.001	1.72 (1.57-1.89)	0.001
	Women	38.52 (33.68-44.04)	<0.001	5.23 (5.00-5.48)	<0.001	3.31 (3.07-3.57)	0.001

Risk factor	Group	Atherogenic dyslipidemia		Hypertriglyceridemia		Low HDL-C	
		OR (95 % CI)	p-value	OR (95 % CI)	p-value	OR (95 % CI)	p-value
CHD history	Total	1.18 (1.12-1.25)	<0.001	1.12 (1.08-1.16)	<0.001	0.97 (0.90-1.03)	0.340
	Men	1.30 (1.20-1.40)	<0.001	1.20 (1.14-1.26)	<0.001	0.96 (0.86-1.07)	0.427
	Women	1.13 (1.05-1.21)	<0.001	1.11 (1.06-1.16)	<0.001	0.97 (0.89-1.05)	0.414
Diet (unbalanced) (%)	Total	1.59 (1.51-1.68)	<0.001	1.47 (1.43-1.52)	<0.001	1.03 (0.97-1.09)	0.297
	Men	1.47 (1.36-1.58)	<0.001	1.49 (1.42-1.56)	<0.001	0.96 (0.88-1.06)	0.407
	Women	1.68 (1.56-1.80)	<0.001	1.42 (1.36-1.49)	<0.001	1.09 (1.01-1.17)	0.029
Physical activity (insufficient)	Total	1.80 (1.71-1.89)	<0.001	1.35 (1.31-1.40)	<0.001	1.25 (1.19-1.33)	<0.001
	Men	1.79 (1.67-1.92)	<0.001	1.42 (1.35-1.48)	<0.001	1.13 (1.04-1.24)	0.007
	Women	1.87 (1.74-1.99)	<0.001	1.40 (1.34-1.46)	<0.001	1.33 (1.23-1.43)	0.001
RF ≥ 3	Total	1.47 (1.38-1.57)	<0.001	1.58 (1.51-1.64)	<0.001	0.90 (0.84-0.96)	0.002
	Men	1.99 (1.76-2.25)	<0.001	1.56 (1.46-1.67)	<0.001	1.19 (1.04-1.36)	0.011
	Women	1.26 (1.16-1.36)	<0.001	1.45 (1.37-1.52)	<0.001	0.82 (0.76-0.89)	0.001

Abbreviations: OR – odds ratio; HDL-C – high-density lipoprotein cholesterol; DM – diabetes mellitus; AH – arterial hypertension; MetS – metabolic syndrome; RF – risk factors; CHD – coronary heart disease.

Further statistical analysis according to gender revealed that arterial hypertension, diabetes mellitus, obesity, metabolic syndrome, an unbalanced diet and CHD history in first-degree relatives were significantly associated with AD and isolated hypertriglyceridemia in both men and women. An unbalanced diet was significantly associated with low-HDL in men; however, smoking did not show any significant association with AD and low-HDL in men. There was a significant association between an unbalanced diet and low-HDL in women (OR: 1.09, 95% CI 1.01-1.17).

3.1.13 The Distribution of High-Density Lipoprotein Cholesterol Levels among Study Population and Associations with Other Cardiovascular Risk Factors

Of all subjects, 42.8% (n=39 496) had normal levels of HDL-C, 43.6% (n=40 235) – high concentrations of HDL-C, and 13.7% (n=12 642) of the population had low HDL-C. The mean age in the low HDL-C group was 52.19 ± 6.49 years, in the normal HDL-C group – 51.00 ± 6.34, and 53.27 ± 5.76 years (p <0.001) in the high HDL-C group (Table 6). Men in the high HDL-C group were older compared to men with low or normal HDL-C (p<0.001). In contrast, women with low HDL-C were older than women with normal or high HDL-C (p<0.001) (Figure 23).

The baseline characteristics and comparison of cardiovascular risk profiles of patients with low, normal and high HDL-C levels are shown in Table 6. The mean

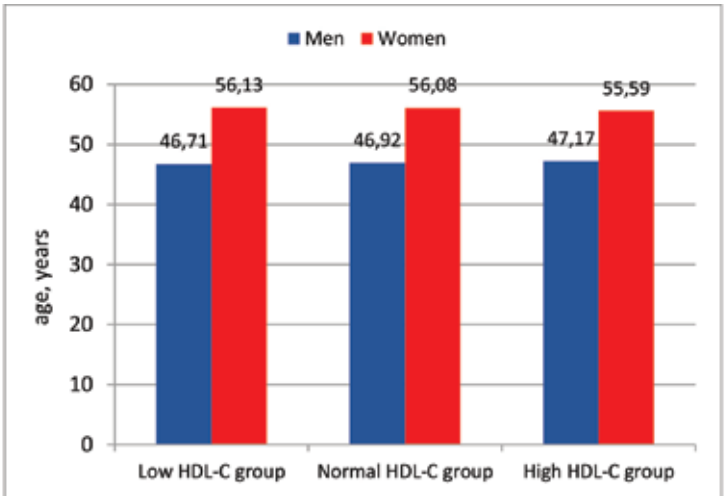

FIGURE 23. Age differences (in years) between the analyzed groups of HDL-C levels (p=0.001).
Abbreviations: HDL-C – high-density lipoprotein cholesterol.

TABLE 6. A comparison of baseline characteristics and the cardiovascular risk profiles of patients with low, normal and high HDL-C levels.

Characteristics	Low HDL-C		Normal HDL-C		High HDL-C		p	p (1 gr. vs. 2 gr.)	p (1 gr. vs. 3 gr.)	p (2 gr. vs. 3 gr.)
	n=12642		n=39496		n=40235					
	Mean	SD	Mean	SD	Mean	SD				
Age (years)	52.19	6.49	51.00	6.34	53.27	5.76	<0.001	<0.001	<0.001	<0.001
WC (cm)	99.99	13.96	96.23	13.01	89.28	12.42	<0.001	<0.001	<0.001	<0.001
BMI (kg/m^2)	31.12	5.84	29.20	5.29	27.22	4.98	<0.001	<0.001	<0.001	<0.001
SBP (mmHg)	136.08	16.81	133.97	16.11	132.27	16.27	<0.001	<0.001	<0.001	<0.001
DBP (mmHg)	84.09	9.60	83.26	9.51	81.86	9.33	<0.001	<0.001	<0.001	<0.001
HR (bpm)	72.81	8.78	71.84	9.10	71.78	8.44	<0.001	<0.001	<0.001	0.579
Glucose (mmol/l)	5.85	1.69	5.57	1.24	5.36	0.97	<0.001	<0.001	<0.001	<0.001
TC (mmol/l)	5.72	1.28	5.97	1.19	6.31	1.18	<0.001	<0.001	<0.001	<0.001
LDL-C (mmol/l)	3.75	1.08	3.95	1.04	3.84	1.10	<0.001	<0.001	<0.001	<0.001
HDL-C (mmol/l)	0.97									
TG (mmol/l)	2.38	1.87	1.70	1.06	1.23	0.72	<0.001	<0.001	<0.001	<0.001
Non-HDL-C	4.76	1.27	4.66	1.18	4.36	1.20	<0.001	<0.001	<0.001	<0.001
TG/HDL-C	2.66	3.37	1.33	0.91	0.66	0.42	<0.001	<0.001	<0.001	<0.001
SCORE index	1.96	1.78	1.88	1.69	1.84	1.63	<0.001	<0.001	<0.001	0.001

Characteristics	Low HDL-C		Normal HDL-C		High HDL-C		p	p (1 gr. vs. 2 gr.)	p (1 gr. vs. 3 gr.)	p (2 gr. vs. 3 gr.)
	n=12642		n=39496		n=40235					
	Mean	SD	Mean	SD	Mean	SD				
Atherogenic index (log (TG/HDL-C))	0.32	0.27	0.06	0.23	-0.24	0.22	<0.001	<0.001	<0.001	<0.001
Frequencies	n	%	n	%	n	%				
DM (%)	2340	18.5	4559	11.5	2998	7.5	<0.001	<0.001	<0.001	<0.001
AH (%)	8104	64.1	22058	55.8	20155	50.1	<0.001	<0.001	<0.001	<0.001
Abdominal obesity (%)	7826	61.9	17998	45.6	14584	36.2	<0.001	<0.001	<0.001	<0.001
Smoking (%)	3101	24.5	9917	25.1	8200	20.4	<0.001	0.582	<0.001	<0.001
MetS (%)	9371	74.1	12356	31.3	7367	18.3	<0.001	<0.001	<0.001	<0.001
RF ≥3 (%)	9921	78.5	23879	60.5	20171	50.1	<0.001	<0.001	<0.001	<0.001
CHD history (%)	3487	27.6	10243	25.9	10295	25.6	<0.001	0.001	<0.001	0.798

Abbreviations: HDL-C – high-density lipoprotein cholesterol; SD – standard deviation; WC – waist circumference; BMI – body mass index; SBP – systolic blood pressure; DBP – diastolic blood pressure; HR – heart rate; TC – total cholesterol; LDL-C – low-density lipoprotein cholesterol; TG – triglycerides; DM –diabetes mellitus; AH – arterial hypertension; MetS – metabolic syndrome; RF – risk factors; CHD – coronary heart disease.

concentration of TC was the highest (6.31 ± 1.18) in patients with high HDL-C compared to subjects with low or normal HDL-C, p<0.001. Mean levels of LDL-C were the highest in patients with normal HDL-C (3.95 ± 1.04, p<0.001), while the highest mean concentration of TG was found in subjects with low HDL-C (2.38 ± 1.87, p<0.001). All cardiovascular risk factors, except for smoking, were most prevalent among the patients with low HDL-C (p<0.001): AH (64.1%), DM (18.5%), abdominal obesity (61.9%), MetS (74.1%), family history of CHD (27.6%), an unhealthy diet (67.4%), insufficient physical activity (61.1%) and a BMI of 30–40 (kg/m^2) (45.8%). Smoking was most prevalent among subjects with normal HDL-C (25.1%), followed by low HDL-C (24.5%) and high HDL-C (20.4%) (p<0.001) (Table 6).

3.1.14 The Associations of Cardiovascular Risk Factors with Lipidogram Parameters

All analyzed cardiovascular risk factors, except for DM, were associated with increased and severely increased levels of TC and LDL-C (Tables 7, 8). DM was significantly related only to severely increased concentration of TC (OR: 1.317, 95% CI: 1.227-1.414). Smoking was determined less frequently together with moderate hypercholesterolemia (OR: 0.895, 95% CI: 0.86-0.93) while more frequently with severe hypercholesterolemia (OR: 1.213, 95% CI: 1.1-1.337) (Table 8). All evaluated CVD risk factors were associated with increased and severely increased levels of triglycerides (Table 9). All analyzed CVD risk factors, including DM (OR: 1.68, 95% CI: 1.59-1.775), AH (OR: 1.311, 95% CI 1.257-1.368), obesity (OR: 1.744, 95% CI: 1.67-1.821) and smoking (OR: 1.202, 95% CI: 1.144-1.264), were significantly related to low levels of HDL-C (Table 10).

TABLE 7. The associations of cardiovascular risk factors with different total cholesterol concentrations.

Risk factor	Group	5.2 mmol/l> TC <7.5 mmol/l			TC ≥7.5 mmol/l		
		OR (CI)	p-value	*p-value	OR (CI)	p-value	*p-value
Insufficient physical activity	Women	1.04 (0.995;1.088)	0.079	<0.001	1.119 (1.054;1.187)	<0.001	<0.001
	Men	1.174 (1.123;1.228)	<0.001		1.39 (1.287;1.502)	<0.001	
	Total	1.105 (1.071;1.14)	<0.001		1.214 (1.159;1.273)	<0.001	
Unbalanced diet	Women	1.243 (1.189;1.299)	<0.001	0.001	1.388 (1.307;1.474)	<0.001	<0.001
	Men	1.381 (1.32;1.445)	<0.001		1.772 (1.63;1.927)	<0.001	

Risk factor	Group	5.2 mmol/l> TC <7.5 mmol/l			TC ≥7.5 mmol/l		
		OR (CI)	p-value	*p-value	OR (CI)	p-value	*p-value
	Total	1.309 (1.268;1.351)	<0.001		1.515 (1.443;1.59)	<0.001	
DM	Women	0.915 (0.853;0.981)	0.013	<0.001	1.14 (1.042;1.248)	0.004	<0.001
	Men	1.134 (1.051;1.222)	0.001		1.714 (1.529;1.923)	<0.001	
	Total	1.012 (0.962;1.066)	0.643		1.317 (1.227;1.414)	<0.001	
AH	Women	1.086 (1.038;1.135)	<0.001	<0.001	1.365 (1.285;1.451)	<0.001	<0.001
	Men	1.384 (1.324;1.447)	<0.001		1.867 (1.727;2.018)	<0.001	
	Total	1.227 (1.189;1.266)	<0.001		1.537 (1.466;1.612)	<0.001	
Smoking	Women	1.12 (1.039;1.207)	0.003	0.086	1.385 (1.26;1.523)	<0.001	0.011
	Men	1.037 (0.992;1.085)	0.111		1.185 (1.096;1.281)	<0.001	
	Total	1.059 (1.019;1.101)	0.004		1.257 (1.184;1.335)	<0.001	
Obesity	Women	1.013 (0.97;1.059)	0.554	<0.001	1.067 (1.005;1.132)	0.033	<0.001
	Men	1.267 (1.205;1.332)	<0.001		1.599 (1.472;1.737)	<0.001	
	Total	1.118 (1.082;1.156)	<0.001		1.217 (1.16;1.277)	<0.001	
CHD history	Women	1.07 (1.018;1.125)	0.008	0.106	1.274 (1.193;1.36)	<0.001	0.959
	Men	1.136 (1.078;1.198)	<0.001		1.279 (1.171;1.398)	<0.001	
	Total	1.101 (1.061;1.141)	<0.001		1.277 (1.212;1.345)	<0.001	
MetS	Women	1.178 (1.123;1.236)	<0.001	<0.001	1.83 (1.721;1.946)	<0.001	<0.001
	Men	1.637 (1.551;1.727)	<0.001		3.001 (2.763;3.26)	<0.001	
	Total	1.365 (1.317;1.415)	<0.001		2.164 (2.06;2.273)	<0.001	

Risk factor	Group	5.2 mmol/l> TC <7.5 mmol/l			TC ≥7.5 mmol/l		
		OR (CI)	p-value	*p-value	OR (CI)	p-value	*p-value
BMI >30	Women	0.948 (0.907;0.992)	0.021	<0.001	0.891 (0.839;0.947)	<0.001	<0.001
	Men	1.298 (1.234;1.365)	<0.001		1.583 (1.456;1.72)	<0.001	
	Total	1.093 (1.057;1.13)	<0.001		1.079 (1.028;1.133)	0.002	
RF >=3	Women	1.901 (1.807;2)	<0.001	<0.001	2.555 (2.369;2.755)	<0.001	<0.001
	Men	2.321 (2.206;2.442)	<0.001		3.627 (3.26;4.035)	<0.001	
	Total	2.103 (2.029;2.179)	<0.001		2.906 (2.734;3.089)	<0.001	

* *p* – homogeneity of variance

Abbreviations: TC – total cholesterol; OR – odds ratio; DM – diabetes mellitus; AH – arterial hypertension; CHD – coronary heart disease; MetS – metabolic syndrome; BMI – body mass index; RF – risk factors.

TABLE 8. The associations of cardiovascular risk factors with different low-density lipoprotein cholesterol concentrations.

Risk factors	Group	3mmol/l > LDL-C< 6 mmol/l			LDL-C ≥6 mmol/l		
		OR (CI)	p-value	*p-value	OR (CI)	p-value	*p-value
Insufficient physical activity	Women	1.118 (1.069;1.169)	<0.001	0.578	1.219 (1.11;1.339)	<0.001	0.953
	Men	1.138 (1.086;1.193)	<0.001		1.225 (1.069;1.404)	0.004	
	Total	1.128 (1.092;1.164)	<0.001		1.221 (1.13;1.319)	<0.001	
Unbalanced diet	Women	1.305 (1.248;1.364)	<0.001	0.834	1.414 (1.285;1.556)	<0.001	0.234
	Men	1.296 (1.235;1.359)	<0.001		1.573 (1.358;1.824)	<0.001	
	Total	1.3 (1.259;1.344)	<0.001		1.461 (1.348;1.583)	<0.001	
DM	Women	0.987	0.718	0.573	1.027	0.722	0.039

Risk factors	Group	3mmol/l > LDL-C< 6 mmol/l			LDL-C ≥6 mmol/l		
		OR (CI)	p-value	*p-value	OR (CI)	p-value	*p-value
		(0.92;1.059)			(0.887;1.189)		
	Men	1.017 (0.942;1.099)	0.665		1.339 (1.091;1.643)	0.005	
	Total	1.001 (0.95;1.054)	0.978		1.118 (0.992;1.26)	0.067	
AH	Women	1.2 (1.147;1.255)	<0.001	0.029	1.457 (1.323;1.604)	<0.001	0.561
	Men	1.289 (1.23;1.351)	<0.001		1.531 (1.335;1.755)	<0.001	
	Total	1.242 (1.203;1.283)	<0.001		1.48 (1.368;1.602)	<0.001	
Smoking	Women	0.975 (0.907;1.048)	0.494	0.005	1.375 (1.198;1.578)	<0.001	0.015
	Men	0.863 (0.823;0.904)	<0.001		1.08 (0.942;1.239)	0.271	
	Total	0.895 (0.86;0.93)	<0.001		1.213 (1.1;1.337)	<0.001	
Obesity	Women	1.257 (1.203;1.315)	<0.001	0.012	1.315 (1.198;1.445)	<0.001	0.652
	Men	1.376 (1.304;1.451)	<0.001		1.37 (1.179;1.592)	<0.001	
	Total	1.305 (1.262;1.351)	<0.001		1.33 (1.228;1.44)	<0.001	
CHD history	Women	1.082 (1.029;1.137)	0.002	0.087	1.351 (1.221;1.493)	<0.001	0.655
	Men	1.155 (1.092;1.222)	<0.001		1.295 (1.108;1.513)	0.001	
	Total	1.114 (1.073;1.156)	<0.001		1.334 (1.226;1.452)	<0.001	
MetS	Women	1.447 (1.378;1.519)	<0.001	0.539	2.266 (2.06;2.493)	<0.001	0.974
	Men	1.481 (1.4;1.565)	<0.001		2.26 (1.958;2.608)	<0.001	
	Total	1.461 (1.409;1.516)	<0.001		2.264 (2.091;2.451)	<0.001	

Risk factors	Group	3mmol/l > LDL-C< 6 mmol/l			LDL-C ≥6 mmol/l		
		OR (CI)	p-value	*p-value	OR (CI)	p-value	*p-value
BMI>30	Women	1.182 (1.129;1.238)	<0.001	<0.001	1.023 (0.928;1.127)	<0.001	0.005
	Men	1.391 (1.318;1.468)	<0.001		1.323 (1.137;1.539)	<0.001	
	Total	1.268 (1.224;1.313)	<0.001		1.099 (1.013;1.193)	0.023	
RF >=3	Women	2.212 (2.104;2.326)	<0.001	0.061	2.955 (2.603;3.355)	<0.001	0.613
	Men	2.371 (2.25;2.498)	<0.001		3.129 (2.607;3.756)	<0.001	
	Total	2.288 (2.206;2.372)	<0.001		3.011 (2.713;3.342)	<0.001	

* *p* – homogeneity of variance

Abbreviations: LDL-C – low-density lipoprotein cholesterol; OR – odds ratio; DM – diabetes mellitus; AH – arterial hypertension; CHD – coronary heart disease; MetS – metabolic syndrome; BMI – body mass index; RF – risk factors.

TABLE 9. The associations of cardiovascular risk factors with different triglycerides levels.

Risk factor	Group	1,7 mmol/l < TG < 4,5 mmol/l			TG ≥ 4,5mmol/l		
		OR (CI)	p	*p	OR (CI)	p	*p
Insufficient physical activity	Women	1.601 (1.539;1.666)	<0.001	0.851	2.041 (1.72;2.421)	<0.001	0.732
	Men	1.61 (1.541;1.682)	<0.001		2.115 (1.89;2.367)	<0.001	
	Total	1.605 (1.559;1.653)	<0.001		2.091 (1.903;2.297)	<0.001	
Unbalanced diet	Women	1.564 (1.502;1.629)	<0.001	0.827	1.958 (1.641;2.336)	<0.001	0.558
	Men	1.553 (1.483;1.627)	<0.001		2.09 (1.838;2.376)	<0.001	
	Total	1.559 (1.512;1.608)	<0.001		2.044 (1.842;2.268)	<0.001	
DM	Women	2.868 (2.712;3.033)	<0.001	<0.001	5.694 (4.791;6.766)	<0.001	0.010
	Men	1.976 (1.844;2.118)	<0.001		4.282 (3.756;4.881)	<0.001	
	Total	2.463 (2.358;2.572)	<0.001		4.719 (4.252;5.239)	<0.001	

Risk factor	Group	1,7 mmol/l < TG < 4,5 mmol/l			TG ≥ 4,5mmol/l		
		OR (CI)	p	*p	OR (CI)	p	*p
AH	Women	2.241 (2.149;2.338)	<0.001	<0.001	2.741 (2.275;3.304)	<0.001	0.745
	Men	1.919 (1.836;2.005)	<0.001		2.643 (2.354;2.968)	<0.001	
	Total	2.085 (2.022;2.149)	<0.001		2.673 (2.422;2.949)	<0.001	
Smoking	Women	1.303 (1.226;1.384)	<0.001	<0.001	1.921 (1.555;2.374)	<0.001	<0.001
	Vyras	0.959 (0.918;1.003)	0.066		1.211 (1.084;1.352)	0.001	
	Total	1.064 (1.026;1.102)	0.001		1.316 (1.192;1.453)	<0.001	
Obesity	Women	2.949 (2.826;3.076)	<0.001	0.885	3.999 (3.295;4.852)	<0.001	0.268
	Men	2.962 (2.825;3.106)	<0.001		4.527 (4.047;5.063)	<0.001	
	Total	2.954 (2.862;3.049)	<0.001		4.345 (3.936;4.797)	<0.001	
CHD history	Women	1.131 \ (1.083;1.18)	<0.001	0.001	1.201 (1.011;1.427)	<0.001	0.069
	Men	1.264 (1.202;1.33)	<0.001		1.461 (1.293;1.651)	<0.001	
	Total	1.185 (1.147;1.224)	<0.001		1.366 (1.236;1.508)	<0.001	
MetS	Women	10.946 (10.463;11.45)	<0.001	0.703	18.426 (14.939;22.727)	<0.001	0.035
	Men	10.796 (10.223;11.401)	<0.001		23.895 (21.034;27.146)	<0.001	
	Total	10.885 (10.514;11.27)	<0.001		21.792 (19.49;24.366)	<0.001	
BMI >30	Moteris	2.559 (2.46;2.662)	<0.001	<0.001	3.07 (2.61;3.61)	<0.001	<0.001
	Vyras	3.121 (2.976;3.273)	<0.001		4.691 (4.193;5.247)	<0.001	
	Total	2.772 (2.689;2.857)	<0.001		4.029 (3.673;4.421)	<0.001	
RF >=3	Moteris	3.925 (3.679;4.186)	<0.001	<0.001	6.454 (4.514;9.229)	<0.001	0.983
	Vyras	3.081 (2.901;3.273)	<0.001		6.483 (5.214;8.06)	<0.001	
	Total	3.478 (3.328;3.634)	<0.001		6.475 (5.375;7.8)	<0,001	

* *p* – homogeneity of variance

Abbreviations: TG – triglycerides; OR – odds ratio; DM – diabetes mellitus; AH – arterial hypertension; CHD – coronary heart disease; MetS – metabolic syndrome; BMI – body mass index; RF – risk factors.

TABLE 10. The associations of cardiovascular risk factors with different high-density lipoprotein cholesterol levels.

Risk factor	Group	HDL-C < 1mmol/l; HDL-C <1,2 mmol/l			HDL-C ≥1,55 mmol/l		
		OR (CI)	p-value	*p-value	OR (CI)	p-value	*p-value
Insufficient physical activity	Women	1.324 (1.251;1.402)	<0.001	0.144	0.699 (0.673;0.726)	<0.001	0.017
	Men	1.408 (1.326;1.495)	<0.001		0.751 (0.717;0.787)	<0.001	
	Total	1.363 (1.308;1.421)	<0.001		0.719 (0.699;0.741)	<0.001	
Unbalanced diet	Women	1.21 (1.142;1.281)	<0.001	0.842	0.785 (0.755;0.815)	<0.001	<0.001
	Men	1.22 (1.145;1.3)	<0.001		0.91 (0.868;0.954)	<0.001	
	Total	1.214 (1.164;1.267)	<0.001		0.832 (0.807;0.857)	<0.001	
DM	Women	1.729 (1.61;1.858)	<0.001	0.21	0.525 (0.493;0.558)	<0.001	<0.001
	Men	1.61 (1.478;1.754)	<0.001		0.729 (0.672;0.792)	<0.001	
	Total	1.68 (1.59;1.775)	<0.001		0.595 (0.566;0.625)	<0.001	
AH	Women	1.376 (1.297;1.461)	<0.001	0.022	0.622 (0.598;0.646)	<0.001	<0.001
	Men	1.248 (1.176;1.324)	<0.001		0.777 (0.742;0.813)	<0.001	
	Total	1.311 (1.257;1.368)	<0.001		0.681 (0.661;0.701)	<0.001	
Smoking	Women	1.227 (1.123;1.339)	<0.001	0.59	1.124 (1.056;1.196)	<0.001	<0.001
	Men	1.191 (1.121;1.265)	<0.001		1.401 (1.338;1.467)	<0.001	
	Total	1.202 (1.144;1.264)	<0.001		1.294 (1.247;1.343)	<0.001	
Obesity	Women	1.641 (1.544;1.745)	<0.001	0.004	0.452 (0.435;0.47)	<0.001	<0.001
	Men	1.861 (1.751;1.977)	<0.001		0.391 (0.368;0.415)	<0.001	
	Total	1.744 (1.67;1.821)	<0.001		0.432 (0.418;0.446)	<0.001	

Risk factor	Group	HDL-C < 1mmol/l; HDL-C <1,2 mmol/l			HDL-C ≥1,55 mmol/l		
		OR (CI)	p-value	*p-value	OR (CI)	p-value	*p-value
CHD anamnesis	Women	1.043 (0.982;1.109)	0.17	0.337	0.978 (0.938;1.02)	0.304	<0.001
	Men	1.091 (1.019;1.168)	0.012		0.806 (0.762;0.852)	<0.001	
	Total	1.064 (1.017;1.113)	0.007		0.911 (0.881;0.942)	<0.001	
MetS	Women	6.297 (5.897;6.723)	<0.001	0.124	0.409 (0.392;0.426)	<0.001	0.056
	Men	5.861 (5.498;6.248)	<0.001		0.379 (0.354;0.405)	<0.001	
	Total	6.087 (5.814;6.373)	<0.001		0.399 (0.385;0.413)	<0.001	
BMI>30	Women	1.572 (1.487;1.661)	<0.001	<0.001	0.456 (0.438;0.474)	<0.001	<0.001
	Men	1.881 (1.771;1.999)	<0.001		0.353 (0.332;0.375)	<0.001	
	Total	1.703 (1.634;1.774)	<0.001		0.42 (0.406;0.433)	<0.001	
RF>=3	Women	2.452 (2.231;2.696)	<0.001	0.748	0.561 (0.535;0.588)	<0.001	<0.001
	Men	2.4 (2.195;2.624)	<0.001		0.74 (0.702;0.78)	<0.001	
	Total	2.424 (2.272;2.587)	<0.001		0.633 (0.611;0.656)	<0.001	

* *p-value* – homogeneity of variance

Abbreviations: HDL-C – high-density lipoprotein cholesterol; OR – odds ratio; DM – diabetes mellitus; AH – arterial hypertension; CHD – coronary heart disease; MetS – metabolic syndrome; BMI – body mass index; RF – risk factors.

3.2 Results of the Prospective Analysis

3.2.1 A Comparison of the Baseline Characteristics of Patients with and without Severe Dyslipidemia

The group with severe dyslipidemia consisted of 110 (51.6%) patients, while the control group comprised 103 (48.4%) participants. The study included a total of 105 (49.3%) women and 108 (50.7%) men. Patients with and without SD did not differ significantly in age (48.75 ± 9.07 vs. 49.57 ± 6.71, p=0.453, respectively). Af-

ter assesing the gender aspect, women with SD were the same age as women in the control group (53.28 ± 6.79 vs. 52.12 ± 6.24, p=0.365) as well as men in the control group (44.39 ± 8.89 vs. 47.08 ± 6.24, p=0.074). Among subjects with SD, women were older compared to men (53.28 ± 6.79 vs. 44.39 ± 8.89, p<0.001), and, likewise, women in the control group were older than men without SD (52.12 ± 6.24 vs. 47.08 ± 6.24, p<0.001). In the study population, levels of TC, LDL-C and TG were higher in subjects with SD compared to the controls, while patients without SD had higher levels of HDL-C (Table 11).

In men and women without SD, their levels of TG (0.93 ± 0.37 vs. 0.99 ± 0.35, p=0.40) and LDL-C (2.71 ± 0.44 vs. 2.87 ± 0.50, p=0.094) did not differ significantly between genders, while in the control group, women had higher TC (4.88 ± 0.64 vs. 4.42 ± 0.50, p<0.001) and HDL-C (1.56 ± 0.31 vs. 1.28 ± 0.30, p<0.001) than to men. Among subjects with SD, men and women had similar levels of TC (7.41 ± 2.14 vs. 8.10 ± 2.04, p=0.083), LDL-C (5.04 ± 2.15 vs. 5.72 ± 2.02, p=0.089) and TG (2.56 ± 1.98 vs. 2.06 ± 1.60, p=0.146), while women with SD had higher mean concentrations of HDL-C compared to men with SD (1.46 ± 0.36 vs. 1.09 ± 0.24, p<0.001) (Table 11).

TABLE 11. The baseline characteristics of subjects with and without severe dyslipidemia (n=213).

Characteristics	Group	Severe dyslipidemia group		Control group		p- value
		n=110		n=103		
		Mean	SD	Mean	SD	
Age (years)	Total	48.75	9.07	49.57	6.71	0.453
	Men	44.39	8.89	47.08	6.24	0.074
	Women	53.28	6.79	52.12	6.24	0.365
TC (mmol/l)	Total	7.75	2.11	4.65	0.62	<0.001
	Men	7.41	2.14	4.42	0.50	<0.001
	Women	8.10	2.04	4.88	0.64	<0.001
LDL-C (mmol/l)	Total	5.37	2.10	2.79	0.48	<0.001
	Men	5.04	2.15	2.71	0.44	<0.001
	Women	5.72	2.02	2.87	0.50	<0.001
HDL-C (mmol/l)	Total	1.27	0.36	1.42	0.33	0.002
	Men	1.09	0.24	1.28	0.30	<0.001
	Women	1.46	0.36	1.56	0.31	0.125

Characteristics	Group	Severe dyslipidemia group		Control group		p- value
		n=110		n=103		
		Mean	SD	Mean	SD	
TG (mmol/l)	Total	2.32	1.81	0.96	0.36	<0.001
	Men	2.56	1.98	0.93	0.37	<0.001
	Women	2.06	1.60	0.99	0.35	<0.001
ApoA1 (mmol/l)	Total	1.64	0.30	1.71	0.32	0.116
	Men	1.52	0.27	1.60	0.25	0.109
	Women	1.76	0.29	1.82	0.35	0.390
ApoA2	Total	0.36	0.18	0.32	0.06	0.047
(mmol/l)	Men	0.35	0.08	0.31	0.05	0.017
	Women	0.38	0.24	0.34	0.07	0.236
ApoB (mmol/l)	Total	1.50	0.48	0.76	0.18	<0.001
	Men	1.44	0.51	0.73	0.13	<0.001
	Women	1.55	0.44	0.78	0.23	<0.001
ApoB/Apo A1	Total	0.93	0.32	0.45	0.14	<0.001
	Men	0.96	0.34	0.47	0.12	<0.001
	Women	0.89	0.28	0.43	0.15	<0.001
ApoE (mg/l)	Total	67.58	27.95	43.33	13.55	<0.001
	Men	67.39	34.00	39.17	11.03	<0.001
	Women	67.77	20.33	47.66	14.65	<0.001
Lp(a) (g/l)	Total	0.25	0.37	0.13	0.19	0.006
	Men	0.23	0.35	0.14	0.18	0.095
	Women	0.26	0.39	0.13	0.20	0.034

Abbreviations: SD – standard deviation; TC – total cholesterol; LDL-C – low-density lipoprotein cholesterol; HDL-C – high-density lipoprotein cholesterol; TG – triglycerides; Apo – apolipoprotein; Lp(a) – lipoprotein(a).

Of all subjects with severe dyslipidemia, 8.2% (n=9) were diagnosed with SD for the first time. Of all patients with SD, 29.1% (n=32) received lipid-lowering treatment before the study (25.9% (n=14) women and 32.1% (n=18) men), although their SD was not controlled. The use of lipid-lowering medications in the study population with SD is presented in Figure 24.

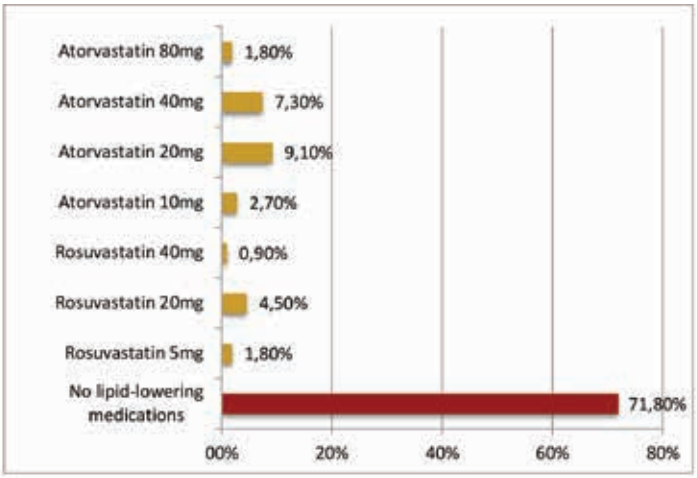

FIGURE 24. Use of lipid-lowering drugs in the study group with SD.

3.2.2 Types of Hyperlipoproteinemia in Patients with Severe Dyslipidemia

According to the Frederickson Classification of Lipid Disorders, most of the study participants with SD had hyperlipoproteinemia type II a (58.2%), followed by type II b (20.9%) and type IV (5%). None of the examined patients had hyperlipoproteinemia type I. The tendency of women having hyperlipoproteinemia type II a more frequently compared to men was observed (68.5% vs. 48.2%), while type II b was more prevalent among men (26.8% vs. 14.8%). The distribution of the types of hyperlipoproteinemia in patients with SD is shown in Figure 25.

3.2.3 A Comparison of the Cardiovascular Risk Profiles of Patients with and without Severe Dyslipidemia

While assessing the frequency of cardiovascular risk factors in the study population, the prevalence of the following risk factors was such: AH – 38.5% (n=82), smoking – 24.9% (n=53), alcohol consumption – 71.4% (n=152), insufficient physical activity – 49.3% (n=105), family history of CHD – 43.2% (n=92). The comparison of the prevalence of CVD risk factors in the study groups is presented in Table 12. Patients with SD more frequently had AH, a family history of CHD and insufficient physical activity compared to subjects in the control group. Alcohol consumption was more prevalent among patients without SD, while smoking showed no significant difference between the groups (Table 12).

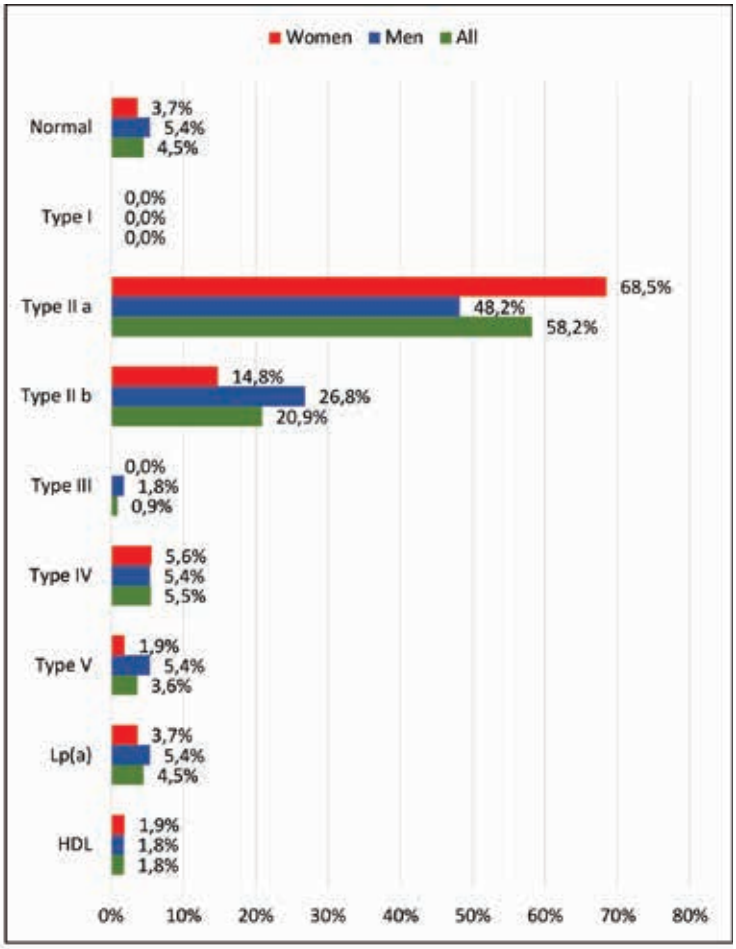

FIGURE 25. The distribution of the types of hyperlipoproteinemia in patients with severe dyslipidemia, p=0.533 (n=110).
Abbreviations: HDL – high-density lipoproteins; Lp(a) – lipoprotein(a).

TABLE 12. A comparison of the prevalence of cardiovascular risk factors in patients with and without severe dyslipidemia (n=213).

Characteristics	Group	Severe dyslipidemia group		Control group		p-value
		n=110		n=103		
		n	%	n	%	
AH (%)	Total	52	47.3	30	29.1	0.007
	Men	19	33.9	11	21.2	0.139
	Women	33	61.1	19	37.3	0.015
Smoking (%)	Total	30	27.3	23	22.3	0.404
	Men	17	30.4	15	28.8	0.864
	Women	13	24.1	8	15.7	0.283
Alcohol consumption (%)	Total	68	61.8	84	81.6	0.001
	Men	40	71.4	42	80.8	0.257
	Women	28	51.9	42	82.4	0.001
Family history of CHD (%)	Total	71	64.5	21	20.4	<0.001
	Men	38	67.9	8	15.4	<0.001
	Women	33	61.1	13	25.5	<0.001
Insufficient physical activity (%)	Total	65	59.1	40	38.8	0.003
	Men	31	55.4	18	34.6	0.031
	Women	34	63.0	22	43.1	0.042
Family history of DM (%)	Total	18	16.4	16	15.5	0.869
	Men	9	16.1	7	13.5	0.703
	Women	9	16.7	9	17.6	0.894

Abbreviations: AH – arterial hypertension; CHD – coronary heart disease; DM – diabetes mellitus.

3.2.3.1 The Associations between Arterial Hypertension and Severe Dyslipidemia

AH was more prevalent among patients with SD compared to subjects in the control group (47.3% vs. 29.1%, p = 0,007, respectively) (Table 12). Subjects with SD were diagnosed with AH for approximately 8.9 years (8.9 ± 8.05), while patients without SD – for 7.5 years (7.5 ± 6.83), p=0.425. Patients with SD used antihypertensive drugs less frequently compared to the control group (73.1% (n=38) vs. 96.7% (n=29), p=0.008, respectively). The control of arterial hypertension was achieved in

55.8% (n=29) of subjects with severe dyslipidemia, and in70.0 % (n=21) of patients without SD, p=0.203. The chances of having severe dyslipidemia were two times higher among patients with AH (OR=2.18; 1.24-3.84; p=0.007). Subjects with AH more frequently had TC >5mmol/l, (75.6% vs. 55.0%, p=0.002), LDL-C >3 mmol/l (70.7% vs. 55.0%, p=0.022) and TG >1.7mmol/l (46.3% vs. 26.0%, p=0.002) compared to participants without AH.

3.2.3.2 The Associations between Smoking and Severe Dyslipidemia

The prevalence of smoking was similar in both SD and control groups (27.3% (n=30) vs. 22.3% (n=23), p=0.404, respectively). The average time of smoking was 19.08 (± 9.09) years in the SD group and 18.62 years (± 8.75) in the control group, p=0.836. The mean number of cigarettes smoked per day was 10.53 ± 6.10 in patients with SD and 10.28 ± 6.06 in the control group, p=0.867.

3.2.3.3 The Associations between Alcohol Consumption and Severe Dyslipidemia

The prevalence of alcohol consumption was lower in patients with severe dyslipidemia compared to the control group (61.8% (n=68) vs. 81.6% (n=84), p=0,001, respectively). A significant difference was not found between men with and without severe dyslipidemia (71.4% (n=40) vs. 80.8% (n=42), p=0.257), while women with severe dyslipidemia had a lower prevalence of alcohol consumption in comparison with healthy women (51.9% (n=28) vs. 82.4% (n=42), p=0.001). Patients who consume alcohol had smaller chances of having severe dyslipidemia (OR=0.37; 0.20-0.69; p=0.002).

3.2.3.4 The Associations between Menopause and Severe Dyslipidemia

The prevalence of menopause was higher in women with severe dyslipidemia compared to women in the control group (81.1% (n=43) vs. 58.8% (n=30), p=0.013, respectively). The average time of menopause was similar in both groups (SD+: 5.58 ± 3.20 years, SD-: 6.97 ±4.12 years, p=0.129). Menopause was associated with a greater chance of having severe dyslipidemia (OR=3.010; 1.24-7.30; p=0.015). Patients with menopause more frequently had TC>5mmol/l (75.3% vs. 51.6%, p=0.017), LDL-C >3 mmol/l (70.7% vs. 55.0%, p=0.022), TG >1,7mmol/l (39.7% vs. 12.9%, p=0.007), ApoE >63 mg/l (36.6% vs. 16.1%, p=0.039).

3.2.3.5 Associations between Family History of CHD and Severe Dyslipidemia

Family history of CHD was more prevalent among subjects with severe dyslipidemia compared to the control group (64.5 % (n=71) vs. 20.4 % (n=21), p<0.001). Family history of CHD was related to an approximately seven times higher probability of having severe dyslipidemia (OR=7.109; 3.83-13.19; p<0.001). Patients with histories of CHD in their families more frequently had TC >5mmol/l (82.6 % vs. 47.9 %, p <0.001), LDL-C >3 mmol/l (78.3 % vs. 47.9 %, p<0.001), TG >1,7mmol/l. (51.1 % vs. 20.7 %, p<0.001), ApoE>63 mg/l (42.7 % vs. 20.8 %, p=0.001).

3.2.3.6 The Associations between Physical Activity and Severe Dyslipidemia

Of all patients with severe dyslipidemia, 59.1% (n=65) had insufficient or no physical activity in comparison with the 38.8% (n=40) in the control group (p=0,003). Insufficient physical activity more than twice increases the chances of having SD (OR=2.28; 1.31-3.94; p=0.003).

3.2.3.7 The Associations between Dietary Patterns and Severe Dyslipidemia

In the severe dyslipidemia group, less subjects ate fruit 1–2 times per day compared to the control group (53.6% vs. 70.9%, p=0.035). Men with SD chose to eat pickled vegetables 1–2 times per day (28.6% vs. 42.3%, p=0.026) and grains 1–2 times per day (17.9% vs. 35.3%, p=0.041) less frequently than those in the control group. Patients with SD consumed more eggs per week compared to subjects without SD (Figure 26). The consumption of dairy products, bread, fish products, fresh and pickled vegetables, grains and fat, as well as salt intake, were similar in both groups. Also, the frequency of eating, regular eating patterns, snacking, use of food supplements and vitamins were similar in both groups. Men in the control group tended to read specialized literature about nutrition and health more frequently than men with SD (23.3% vs. 12.7%, p=0.044), while women with SD consumed less food high in saturated fat and cholesterol compared to the control group (1.9% vs. 13.7%, p=0.022). Men with SD were more likely to use iodised salt in order to prevent Iodine deficiency compared to men in the control group (64.3% vs. 42.3%, p=0.022).

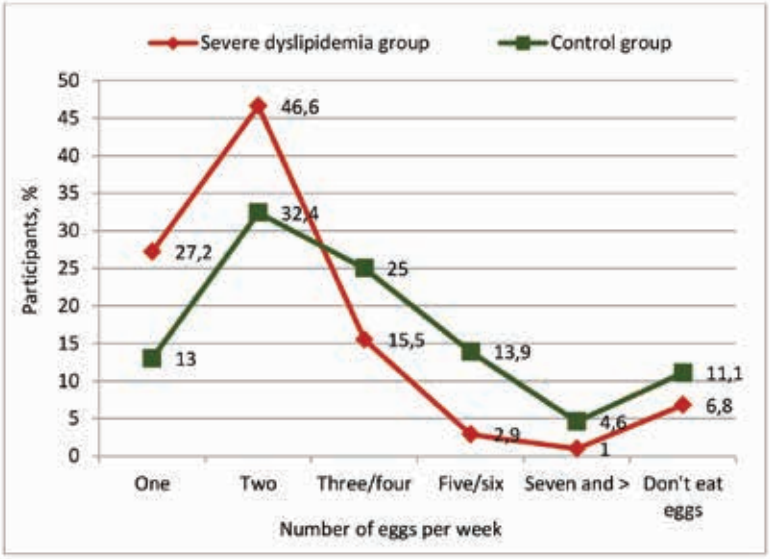

FIGURE 26. Number of eggs consumed per week (p=0.001).

3.2.4 The Associations of Anthropometric Measurements and Severe Dyslipidemia

A detailed analysis of the parameters of body composition analysis in patients with and without severe dyslipidemia is presented in Table 13. After comparing the assessments of LBM (%), SMM (%), protein mass (%) and BFM (%) between men and women with SD, significant differences were not found. More men with severe dyslipidemia had decreased mineral mass in comparison with women with severe dyslipidemia (53.6% vs. 3.7%, <0.001).

TABLE 13. An assessment of the parameters of body composition analysis in patients with and without severe dyslipidemia.

Characteristics	Group	Under	Optimal	Over	p-value
BFM (%)	SD+	1.80	12.70	85.50	<0.001
	SD–	8.70	30.10	61.20	
LBM (%)	SD+	84.40	13.80	1.80	0.001
	SD–	62.10	29.10	8.80	
SMM (%)	SD+	84.50	13.60	1.90	0.001
	SD–	62.10	31.10	6.80	

Characteristics	Group	Under	Optimal	Over	p-value
Protein (%)	SD+	80.90	17.30	1.80	<0.001
	SD–	47.60	44.70	7.70	
Minerals (%)	SD+	29.10	70.90	0.00	0.142
	SD–	20.40	79.60	0.00	
TBW (%)	SD+	18.20	81.80	0.00	0.121
	SD–	10.70	89.30	0.00	

Abbreviations: SD+ – with severe dyslipidemia; SD– – without severe dyslipidemia; BFM – Body Fat Mass; LBM – Lean Body Mass; SMM – Sceletal Muscle Mass; TBW – Total Body Water.

After evaluating the abdominal region with a body composition analyzer, significant differences in the distribution of fat in the abdomen in study groups were not found (p=0.078) (Figure 27).

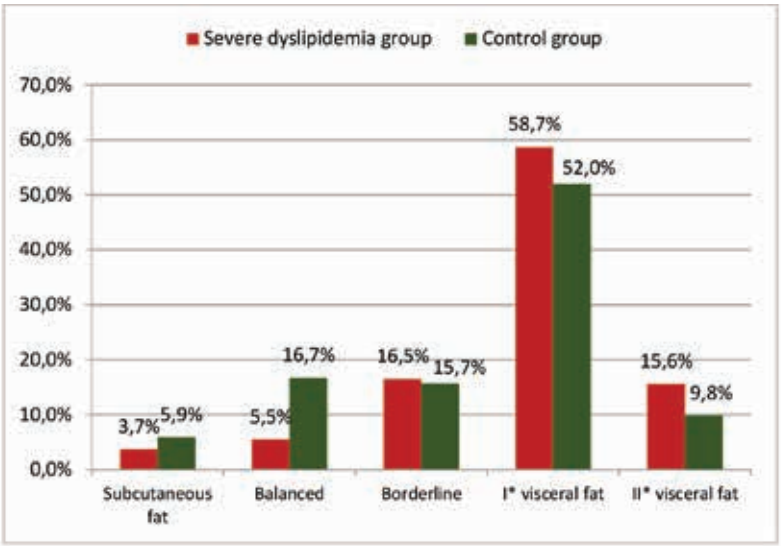

FIGURE 27. A comparison of abdominal region analysis in study groups (p=0.078).

After assessing fat distribution in the abdominal region in men and women, significant differences were found between genders (p=0.017) (Figure 28).

The distribution of visceral fat was similar in both the severe dyslipidemia and control groups (p=0.141) (Figure 29). Also, no differences were found between men and women (p=0.817). The prevalence of increased WHR was similar in study

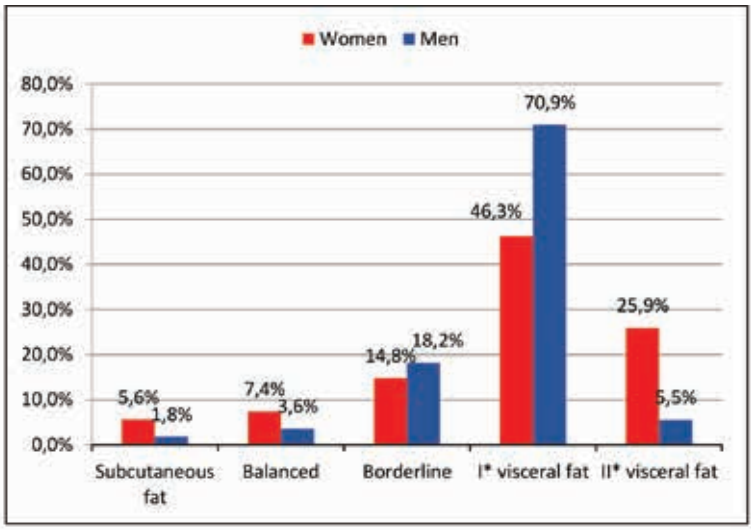

FIGURE 28. A comparison of the abdominal region analysis in different genders (p=0.017).

groups (67.3% vs. 68.9%, *p*=0.795), but increased WC was more frequently found among subjects with severe dyslipidemia compared to the control group (57.3% vs. 40.8%, p=0.016). Among patients with severe dyslipidemia, more women had increased WC compared to men (68.5% vs. 46.4%, p=0.019).

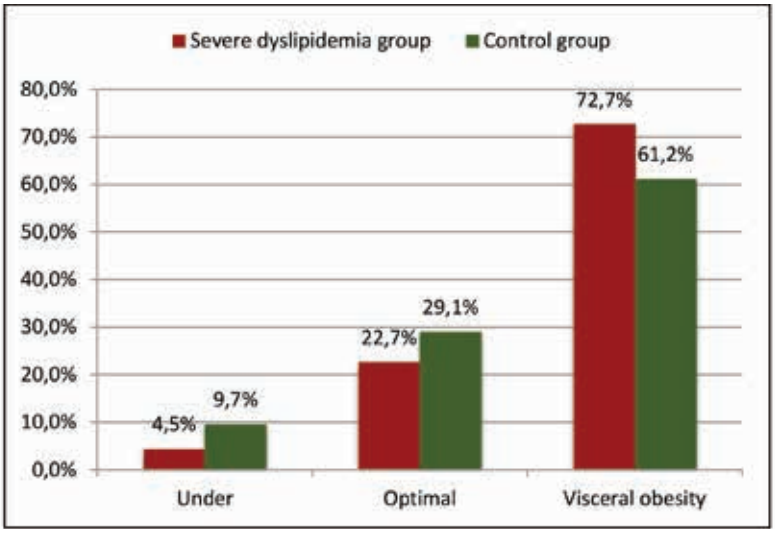

FIGURE 29. Visceral fat evaluation in the study population (p=0.141).

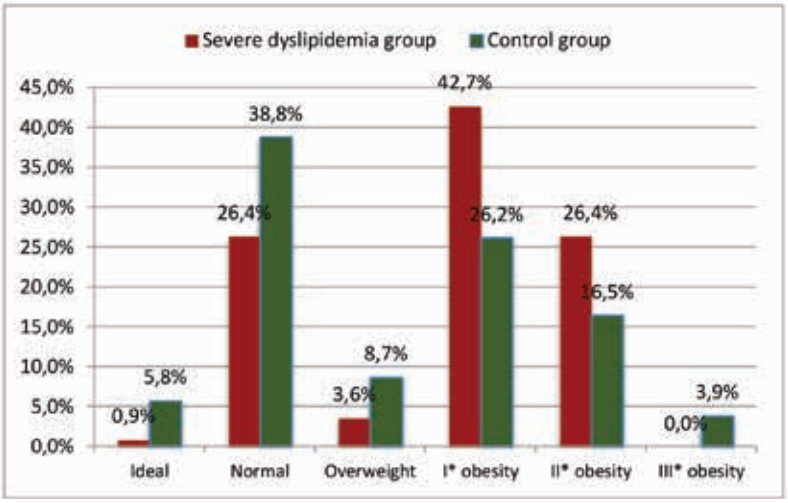

FIGURE 30. The distribution of BMI values in the study population (p=0.002).

The evaluation of BMI in both study groups is presented in Figure 30 (p=0.002). No significant differences between women and men with SD were determined.

An assessment of the body fat percentage (PBF) in patients with and without severe dyslipidemia is shown in Figure 31, p=0.008. Significant differences in the distribution of PBF categories were found between men and women (Figure 32, p=0.011).

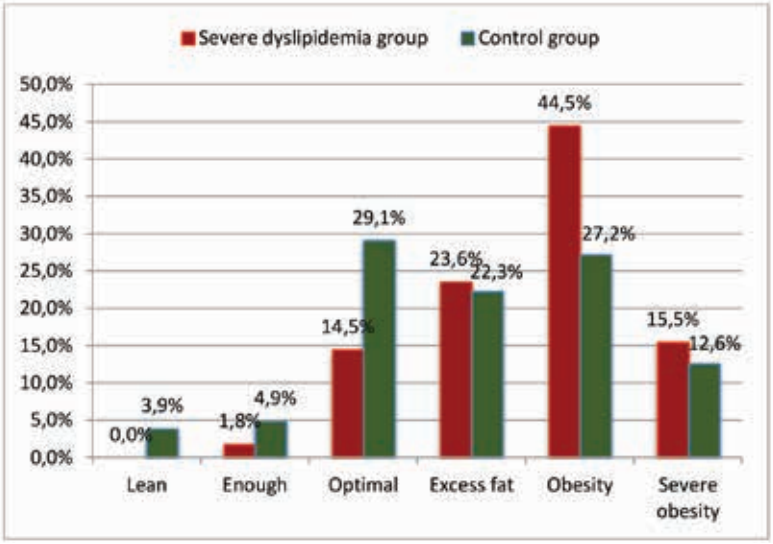

FIGURE 31. The assessment of the percentage of body fat in patients with and without severe dyslipidemia (p=0.008).

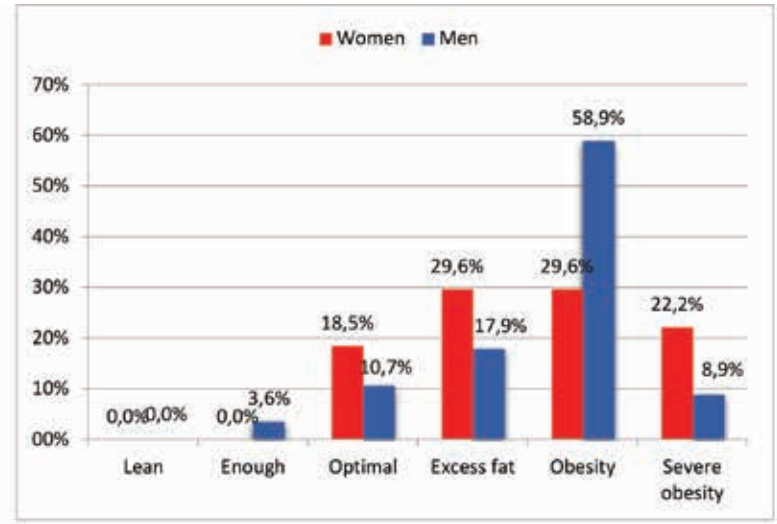

FIGURE 32. The assessment of the percentage of body fat in men and women with severe dyslipidemia (p=0.011).

Results of the correlation analysis between plasma lipid concentration and anthropometric measurements indicated that the TC level was significantly related to the parameters of the body composition analysis – body fat mass (BFM) and PBF (Table 14). Also, a moderate correlation was observed between LDL-C concentration and body fat mass (BFM), and a weak relation was found between LDL-C and PBF. No significant associations were found between TC or LDL-C and traditional anthropometric parameters (BMI, WC, WHR) (Table 14). Negative correlations were determined between most of the analyzed parameters and HDL- C levels, the strongest of them being HDL-C to WHR (r=-0.442, $p<0.001$). TG levels were associated with BMI, WC, WHR, BFM, PBF, visceral fat analysis (VFA) and body fat distribution; the strongest relation was observed between TG and BMI (r=0.402, $p<0.001$) as well as TG and PBF (r=0.402, $p<0.001$). Lp(a) concentration was not related to any of the analyzed anthropometric parameters (Table 14). Lipid measurements showed most correlations with BFM or PBF (7 out of 10 parameters). Out of the traditional anthropometric parameters, most correlations were found between BMI and measured lipids (6 out of 10). The ApoB/apoA1 ratio, as well as ApoA1, were associated with most anthropometric measurements (11 out of 12), followed by HDL-C (10 out of 12). Spearman's correlation coefficients between anthropometric measurements or body composition analysis parameters and plasma lipid concentration are presented in Table 14.

TABLE 14. Spearman's correlation coefficients between anthropometric measurements or body composition analysis parameters and plasma lipid concentration.

Characteristics	TC	LDL-C	HDL-C	TG	Apo A1	Apo B	Apo A2	Apo E	Apo B/ apo A1	Lp(a)
BMI	0.099	0.094	-0.331**	0.402**	-0.224**	0.191**	0.030	0.195**	0.242**	0.094
WC	0.039	0.014	-0.373**	0.383**	-0.269**	0.132	0.071	0.126	0.211**	0.004
WHR	-0.037	0.000	-0.442**	0.301**	-0.317**	0.086	0.018	0.058	0.183**	0.003
PBF	0.239**	0.208**	0.131	0.241**	0.153*	0.240**	0.138*	0.317**	0.132	0.060
BFM	0.382**	0.370**	-0.059	0.402**	-0.017	0.424**	0.152*	0.365**	0.369**	0.124
LBM	-0.022	0.014	-0.275**	0.024	-0.259**	0.015	-0.071	-0.157*	0.140*	0.044
Muscle	-0.027	0.009	-0.291**	0.025	-0.265**	0.010	-0.077	-0.155*	0.137*	0.035
Protein	-0.026	0.010	-0.275**	0.018	-0.263**	0.010	-0.068	-0.163*	0.136*	0.045
Minerals	0.026	0.051	-0.184**	0.046	-0.215**	0.044	-0.019	-0.087	0.138*	0.062
TBW	-0.026	0.011	-0.281**	0.016	-0.272**	0.009	-0.073	-0.164*	0.140*	0.041
Body fat distribution	0.100	0.122	-0.270**	0.310**	-0.179**	0.192**	0.060	0.194**	0.220**	0.017
VFA	0.035	0.077	-0.374**	0.311**	-0.264**	0.149*	0.034	0.129	0.217**	-0.001

* statistically significant as $p<0.05$; ** statistically significant as $p<0.01$;Abbreviations: TC – total cholesterol; LDL-C – low-density lipoprotein cholesterol; HDL-C – high-density lipoprotein cholesterol; TG – triglycerides; Apo – apolipoprotein; Lp(a) – lipoprotein(a); BMI – body mass index; WC – waist circumference; WHR – waist-hip ratio; PBF – percent body fat; BFM – body fat mass; LBM – lean body mass; TBW – total body water; VFA – visceral fat analysis.

3.2.5. The Usefulness of the Ultrasonographic Evaluation in Patients with Severe Dyslipidemia

An ultrasonographic evaluation revealed that an Achilles tendon pathology was present in 42.7% of subjects with severe dyslipidemia and in 29.1% of healthy controls (p=0.039, Table 15). A more pronounced association was present in women, where the frequency of Achilles tendinopathy (AT) reached 24.1% among SD patients and only 2.0% in controls (p=0.001, Table 15). Severe dyslipidemia increased the odds of AT by 1.815 (95% CI, 1.028-3.206). Wrist tendon pathologies in those with severe hypercholesterolemia were comparable to the controls (p=0.366, Table 15). Neither the aortic valve nor mitral valve pathology were associated with SD (p=0.856, p=0.300 respectively, Table 15). The frequency of liver steatosis (p=0.457), pancreatic steatosis (p=0.852) and gall bladder stones (p=0.056) differed slightly among groups but did not reach any statistical significance (Table 15). Table 16 represents the ultrasonographic differences according to gender. The prevalence of the Achilles tendon pathology was higher in males despite the presence (SD^+) or absence (SD^-) of severe hypercholesterolemia (SD^+ 60.7% vs. 24.1%, SD^- 55.8% vs. 2.0%, p<0.001). Furthermore, men showed a higher proportion of subjects with wrist tendon pathologies (SD^+ 17.9% vs. 0%, p<0.001, SD^- 11.5 % vs. 0 %, p=0.012) and aortic valve atherosclerotic lesions (SD^+ 25.0 % vs. 5.6 %, p=0,005, SD^- 26.9 % vs. 2.0 %, p<0.001).

TABLE 15. The ultrasonographic characteristics of the study population.

Characteristics	SD+ (n=110)	SD– (n=103)	p-value
Achilles tendon lesions			
Total	47 (42.7%)	30 (29.1%)	0.039
Women	13 (24.1%)	1 (2.0%)	0.001
Men	34 (60.7%)	29 (55.8%)	0.602
Wrist tendon lesions			
Total	10 (9.1%)	6 (5.8%)	0.366
Women	0 (0%)	0 (0%)	
Men	10 (17.9%)	6 (11.5%)	0.356
Atherosclerotic lesions of abdominal aorta			
Total	37 (33.6%)	40 (39.2%)	0.399
Women	17 (31.5%)	11 (22.0%)	0.276
Men	20 (35.7%)	29 (55.8%)	0.036
Fatty liver			
Total	32 (29.1%)	25 (24.3%)	0.457

Characteristics	SD+ (n=110)	SD– (n=103)	p-value
Women	14 (25.9%)	7 (13.7%)	0.407
Men	18 (32.1%)	18 (34.6%)	0.534
Pancreatic steatosis			
Total	29 (26.4%)	26 (25.2%)	0.852
Women	11 (20.4%)	9 (17.6%)	0.722
Men	18 (32.1%)	17 (32.7%)	0.951
Gall bladder stones			
Total	7 (6.4%)	9 (8.7%)	0.056
Women	3 (5.6%)	4 (7.8%)	0.277
Men	4 (7.1%)	5 (9.6%)	0.292
Aortic valve lesions			
Total	17 (15.5%)	15 (14.6%)	0.856
Women	3 (5.6%)	1 (2.0%)	0.336
Men	14 (25.0%)	14 (26.9%)	0.820

Abbreviations: SD+ – severe dyslipidemia positive; SD– – severe dyslipidemia negative.

TABLE 16. The differences of ultrasonographic characteristics according to gender.

Characteristics	Women (n=105)	Men (n=108)	p-value
Achilles tendon lesions			
SD+	13 (24.1%)	34 (60.7%)	<0.001
SD–	1 (2.0%)	29 (55.8%)	<0.001
Total	14 (13.3%)	63 (58.3%)	<0.001
Wrist tendon lesions			
SD+	0%	10 (17.9%)	<0.001
SD–	0%	6 (11.5%)	0.012
Total	0%	16 (14.8%)	<0.001
Atherosclerotic lesions of abdominal aorta			
SD+	17 (31.5%)	20 (35.7%)	0.639
SD–	11 (22.0%)	29 (55.8%)	<0.001
Total	28 (26.9%)	49 (45.4%)	0.005
Fatty liver			
SD+	14 (25.9%)	18 (32.1%)	0.788
SD–	7 (13.7%)	18 (34.6%)	0.075
Total	21 (20.0%)	36 (33.3%)	0.153
Pancreatic steatosis			
SD+	11 (20.4%)	18 (32.1%)	0.161

Characteristics	Women (n=105)	Men (n=108)	p-value
SD–	9 (17.6%)	17 (32.7%)	0.079
Total	20 (19.0%)	35 (32.4%)	0.026
Gall bladder stones			
SD^+	3 (5.6%)	4 (7.1%)	0.794
SD–	4 (7.8%)	5 (9.6%)	0.977
Total	7 (6.7%)	9 (8.3%)	0.888
Aortic valve lesions			
SD+	3 (5.6%)	14 (25.0%)	0.005
SD–	1 (2.0%)	14 (26.9%)	<0.001
Total	4 (3.8%)	28 (25.9%)	<0.001

Abbreviations: SD+ – severe dyslipidemia positive; SD– – severe dyslipidemia negative.

3.2.4. The Usefulness of Vascular Imaging and the Indices of Arterial Stiffness in the Evaluation of Patients with Severe Dyslipidemia

Changes in the vascular ultrasound, such as atherosclerotic plaques or increased IMT, were found in the carotid arteries of 59.6% (n=127) of all patients. Carotid plaques were determined for 50.7% (n=108), while increased IMT was discovered in 34.3% (n=73) of subjects. Changes in carotid arteries were more prevalent among patients with severe dyslipidemia – 74.1% (atherosclerotic plaques: 66.4% vs. 33.0%, p<0,0001; increased IMT: 44.5% vs. 23.3%, p=0.001) compared to the 43.1% in the control group, p<0.001. The parameters of carotid arteries are presented in Table 17. SD was significantly related to the presence of atherosclerotic plaques (OR=4.00; 2.264-7.081, p<0.001) and increased IMT (OR=2.64;1.463-4.778, p=0.001) in the carotid arteries. Severe dyslipidemia was associated with a decreased distention (OR=0.99; 0.995-0.999; p=0.004) and increased stiffness (OR=1.56; 1.242-1.967, p<0.001) of the right common carotid artery (CCA) as well as increased IMT (OR=1.00; 1.002-1.006; p=0.001), decreased distention (OR=0.99; 0.996-1.000, p=0.05) and increased stiffness (OR=1.30; 1.070-1.584, p=0.008) of the left CCA.

The possibility of having SD was three times higher (OR=2.98; 1.710-5.219; p<0.001), when the right CCA distention was <402 mm, three times higher (OR=3.03; 1.730-5.296; p<0.001), when the right CCA stiffness was >3.25 mm, 2.5 times higher (OR=2.54; 1.460-4.421; p=0.001), when the left CCA IMT was >601.5 μm, three times higher (OR=2.845; 1.576-5.137; p=0.001), when the left CCA stiffness was >3.75 mm and almost three times higher (OR=2.69; 1.449-4.997, p=0.002), when the left CCA distention was <478.5 mm.

TABLE 17. A comparison of the parameters of the carotid arteries of both study groups.

Characteristics	Group	SD+ n=110		SD- n=103		p-value
		Mean	SD	Mean	SD	
RCCA IMT (μm)						
	Total	626.3	131.7	623.0	123.8	0.849
	Men	600.6	24.3	636.2	114.6	0.126
	Women	652.5	134.9	609.4	132.3	0.102
RCCA stiffness (mm)						
	Total	3.8	1.6	3.0	1.1	<0.001
	Men	3.4	1.6	2.7	0.9	0.008
	Women	4.2	1.5	3.3	1.3	0.001
RCCA distention (mm)						
	Total	393.5	160.5	459.2	154.1	0.003
	Men	441.0	188.9	517.7	163.6	0.027
	Women	344.2	105.2	399.6	118.1	0.012
LCCA IMT (μm)						
	Total	676.5	147.4	607	130.2	<0.001
	Men	650.2	149.5	603.0	128.1	0.082
	Women	703.8	142.1	611.2	133.5	0.001
LCCA stiffness (mm)						
	Total	3.8	1.5	3.2	1.4	0.007
	Men	3.6	1.4	3.0	1.4	0.036
	Women	4.0	1.6	3.5	1.3	0.078
LCCA distention (mm)						
	Total	390.1	147.6	430.7	148.3	0.046
	Men	421.5	172.0	497.2	146.2	0.016
	Women	357.4	109.5	362.8	117.4	0.807

Abbreviations: SD – standard deviation; SD+ – severe dyslipidemia positive; SD– – severe dyslipidemia negative; LCCA – left common carotid artery; RCCA – right common carotid artery; IMT – intima–media thickness.

After evaluating other parameters of vascular stiffness, instances of a higher PWV in the femoral artery was more frequently found in the severe dyslipidemia group compared to the control group (8.09 ± 1.36 m/s vs. 7.36 ± 1.29 m/s, p<0.001) (Table 18). Patients with SD had higher AIxHR than healthy controls (26.34 ± 10.01 vs. 12.53 ± 10.97, p<0.001) (table 18). Chances of having SD were more than 13 times higher (OR=13.508; 6.936-26.307; p<0.001) when AIxHR was <22.5%, and chances of having SD were 3.5 times higher (OR=3.578; 1.885-6.791; p<0.001) when PWV in the femoral artery was >8.35 m/s.

TABLE 18. A comparison of the parameters of the arterial stiffness of both study groups.

Characteristics		SD+		SD-		p-value
		n=110		n=103		
		Mean	SD	Mean	SD	
AIx/HR (%)						
	Total	12.53	10.97	26.34	10.01	<0.001
	Men	12.63	10.24	27.83	10.06	<0.001
	Women	12.07	11.77	24.80	9.81	<0.001
FEM (m/s)						
	Total	7.36	1.29	8.09	1.36	<0.001
	Men	7.33	1.02	8.23	1.44	<0.001
	Women	7.39	1.53	7.96	1.27	0.044
RA (m/s)						
	Total	8.58	1.04	8.48	0.99	0.507
	Men	8.69	1.06	8.59	0.80	0.608
	Women	8.47	1.01	8.37	1.16	0.658
R-CAVI						
	Total	7.67	1.59	7.70	1.39	0.871
	Men	7.29	1.69	7.60	1.40	0.303
	Women	8.05	1.40	7.80	1.38	0.348
L-CAVI						
	Total	7.54	1.49	7.49	1.53	0.822
	Men	7.23	1.65	7.52	1.70	0.363
	Women	7.86	1.23	7.46	1.34	0.114

Abbreviations: SD – standard deviation; SD+ – severe dyslipidemia positive; SD– – severe dyslipidemia negative; Aix/HR – heart rate adjusted augmentation index; FEM – pulse wave velocity in femoral artery; RA – pulse wave velocity in radial artery; R-CAVI – right cardio-ankle vascular index; L- CAVI – left cardio-ankle vascular index.

3.2.6 The Usefulness of Evaluating the Coronary Artery Calcium Score in Patients with Severe Dyslipidemia

Figure 33 demonstrates the distribution of participants according to their CAC score percentiles. Table 19 represents the baseline lipid profile and the apolipoproteins of subjects with a CAC score ≥25th percentile. There were no significant correlations between the biochemical parameters and CAC percentiles except for lipoprotein(a). An increase in lipoprotein(a) was associated with CAC score percentiles (p=0.038) (Table 19).

TC and LDL-C demonstrated a tendency to increase as the percentiles of CAC score increased; however, this was not statistically significant (p=0.704 and p=0.667, respectively) (Figure 2). Concentrations of HDL- C and TG did not correlate with the percentiles either (p=0.443 and p=0.773, respectively) (Figure 34).

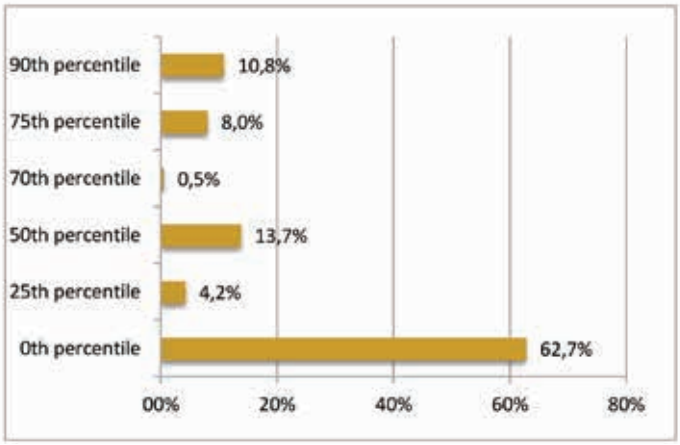

FIGURE 33. The distribution of all participants according to their CAC score percentiles.

TABLE 19. The associations between CAC percentiles and lipid profile components.

Characteristics	25th percentile (%)	50th percentile (%)	75th percentile (%)	90th percentile (%)	p- value	Chi-square
ApoA1 F <1.25 g/l; M <1.1 g/l	0	6.9	11.8	13.0	0.775	1.787
ApoA2 <0.26 g/l	33.3	6.9	0	13.0	0.095	7.901
ApoB/ApoA1 F >0.9; M >1.0	25.0	34.5	47.1	52.2	0.479	3.495

Characteristics	25th percentile (%)	50th percentile (%)	75th percentile (%)	90th percentile (%)	p- value	Chi-square
ApoB F >1.25 g/l; M >1.4 g/l	33.3	41.4	64.7	52.2	0.371	4.268
ApoE >63 mg/l	25.0	46.4	41.2	59.1	0.413	3.950
TC >5 mmol/L	66.7	86.2	82.4	78.3	0.219	5.743
Lp(a) >0.3 g/l	11.1	31.0	0	39.1	0.038	10.163
LDL-C >3 mmol/l	55.6	75.9	70.6	69.6	0.772	1.804
Low HDL-C F <1.2 mmol/l; M <1.0 mmol/l	33.3	37.9	17.6	21.7	0.258	5.294
TG >1.7 mmol/l	44.4	51.7	41.2	52.2	0.808	1.607

Abbreviations: Apo – apolipoprotein; TC – total cholesterol; Lp(a) – lipoprotein(a); LDL-C – low-density lipoprotein cholesterol; HDL-C – high- density lipoprotein cholesterol; TG – triglycerides; F – female; M – male.

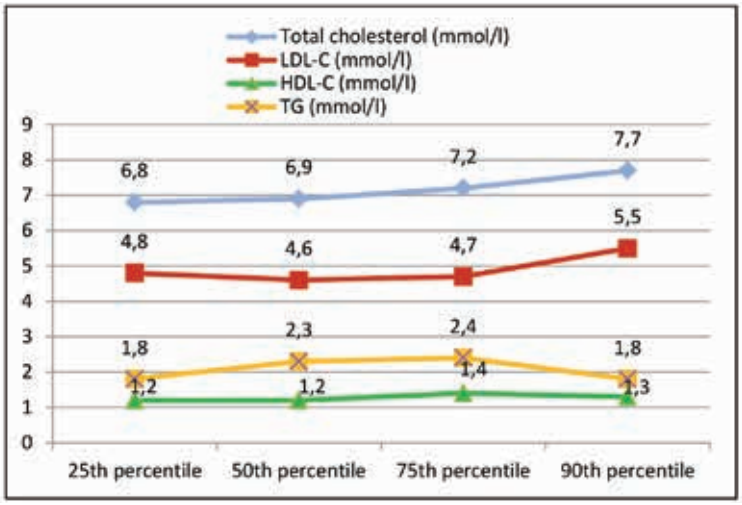

FIGURE 34. Blood lipid components and their association with the CAC score ≥25th percentile, TC (p=0.704), LDL-C (p=0.667), HDL-C (p=0.443), TG (p=0.773). Abbreviations: LDL-C – low-density lipoprotein cholesterol; HDL-C – high- density lipoprotein cholesterol; TG – triglycerides.

In 0th CAC percentiles group, 62 (47%) subjects out of 133 had severe hypercholesterolemia. In total, there were 79 (37.2%) subjects with elevated (≥25th) CAC percentiles. Out of them, 47 (59.5%) had severe dyslipidemia and 32 (40.5%) did not. However, the CAC score did not differ between the groups (severe dyslipidemia (+) 140.30 ± 185.72 vs. severe dyslipidemia (–) 87.84 ± 140.65, p=0.146). There was a comparable difference in how the participants of these groups were distributed among different percentile groups (p=0.044) according to their age, gender, race/ethnicity (Figure 35). Eigtheen women and 61 men had elevated CAC percentiles; however, the percentiles did not differ significantly between the genders either (p=0.075). Neither women nor men demonstrated percentile differences between severe dyslipidemia and control groups (women p=0.272, men p=0.706). There were no gender differences in the severe dyslipidemia group separately as well (p=0.238).

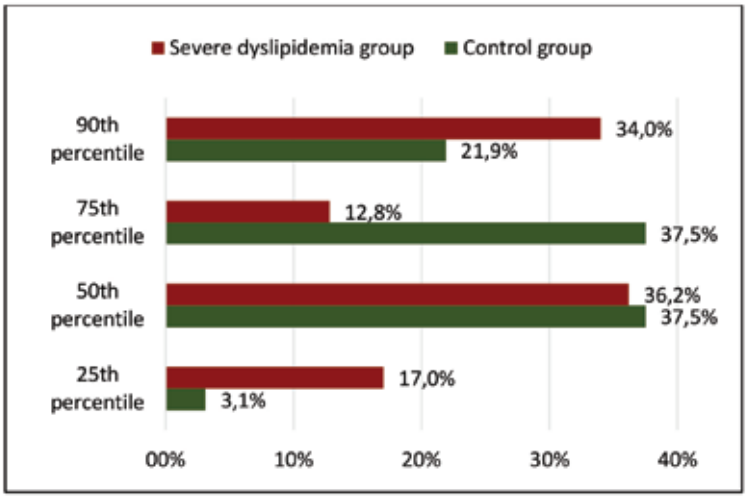

FIGURE 35. The distribution of the CAC score's ≥25th percentile in severe hypercholesterolemia and control groups, p=0.044.

The CAC percentiles did not differ between hypertensive and normotensive patients (p=0.875), smokers and non-smokers (p=0.083), subjects with and without any family history of CHD (p=0.576). CAC percentiles were not associated with physical activity (insufficient physical activity group p=0.512) or any family history of DM (p=0.219) as well. The CAC percentiles did not differ between subjects with and without ultrasonographically evident Achilles tendinopathies (p=0.480). Furthermore, the body composition analysis did not reveal any significant association between the CAC percentiles and visceral obesity (p=0.17), body mass index (p=0.20) or an increased waist-hip ratio (p=0.25) as well.

3.2.6. The Evaluation of the Quality and Quantity of HDL-C and Use in Clinical Practice

3.2.6.1 The Evaluation of HDL-C Concentration in the Study Population

A detailed examination of the quality and quantity of HDL-C was performed on 93 randomly selected participants from both study groups. We found 70.2 % (n=33) of decreased HDL-C concentrations in the SD group. Patients with SD more frequently had decreased levels of HDL-C (30%, n=33) compared to healthy controls (13.6%, n=14), p=0.004. If assessed in terms of gender, more men with SD had decreased HDL-C (33.9%, n=19) compared to the control group (13.5%, n=7), p=0.013. No significant difference in HDL-C levels between women with SD (25.9%, n=14) and healthy women (13.7%, n=7) was found (p=0.118). A weak but significant association was found between age and HDL-C concentration (r=0.180, p=0.008). Chances of having severe dyslipidemia are approximately two times higher (OR 2.433, CI:1.366-4.334), when HDL-C <1.19 mmol/l (sensivity – 46.4%, specifity – 73.8%, area under the curve – 2.6%). In order to define the increased, normal and decreased HDL-C concentration, in this study, we calculated the 33th percentiles, which divide all measurements in three equal parts (Table 20).

TABLE 20. The descriptive statistics of HDL-C (n=93).

Characteristics		HDL-C (mmol/l)
Mean		1.28
Median		1.27
Standard deviation		0.32
Smallest observation		0.75
Largest observation		2.16
Percentiles	33.33	1.08
	66.66	1.40

Abbreviations: HDL-C – high-density lipoprotein cholesterol.

A normal concentration of HDL-C was determined for most of the evaluated men and women, irrespective of the severe dyslipidemia status (Figure 36). While analyzing the group with increased HDL-C, more women had increased HDL-C compared to men (13 vs. 4; p=0.015) (Figure 37). After evaluating the different age groups of study population, significant differences in HDL-C levels were not found (Figure 38).

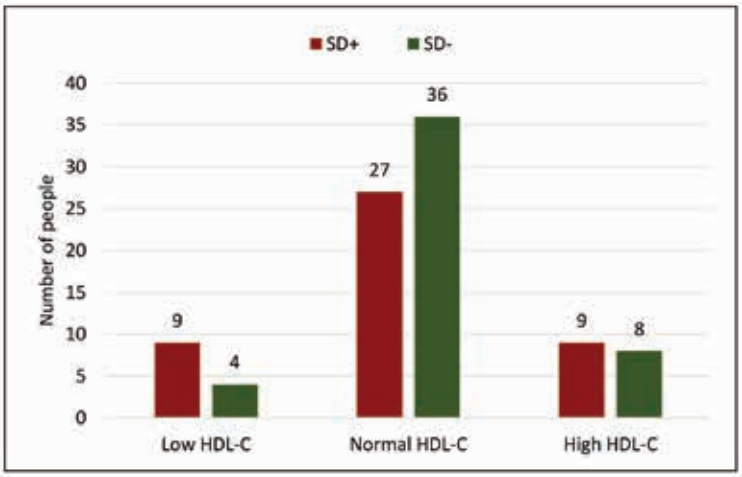

FIGURE 36. The associations between HDL-C concentration and severe dyslipidemia in the study population, p=0.205 (n=93).

Abbreviations: SD+ – patients with severe dyslipidemia; SD– – patients without severe dyslipidemia

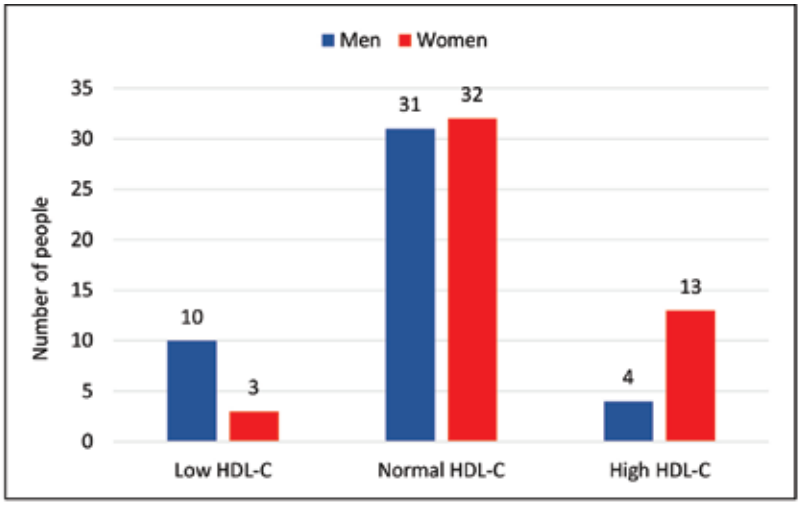

FIGURE 37. The associations between HDL-C concentration and gender in the study population, p=0.015 (n=93).

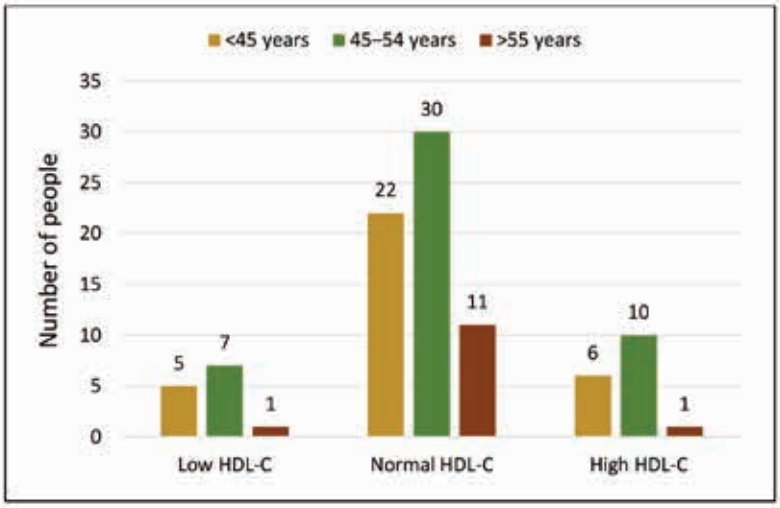

FIGURE 38. The associations between HDL-C concentration and age in the study population p=0.715 (n=93).

3.2.6.2 Relations of HDL-C Concentration with Other Cardiovascular Risk Factors

Normal levels of HDL-C were most frequently found among patients with I* obesity (Figure 39, p=0,02). Associations between HDL-C levels and other cardiovascular risk factors (abdominal obesity, AH, smoking, family history of CHD) were not found. Normal HDL-C concentrations were more prevalent among patients consuming alcohol in comparison with those who do not drink alcoholic beverages, p=0.004 (Figure 40). Although physically active subjects tended to have normal levels of HDL-C more frequently than people with insufficient physical activity, significant differences were not found (Figure 41).

A strong positive connection was found between ApoA1 and HDL-C concentrations in both severe dyslipidemia (r=0.866) and control (r=0.63) groups (Table 21). In patients without SD, an increasing HDL-C is associated with a decreasing ApoB/ApoA1 ratio (r=-0.56). Increasing TG and BMI were associated with decreasing HDL-C concentrations. An increase in waist circumference was related to a decrease in HDL-C levels in women with SD (r=-0.309). In subjects with SD, a significant decrease of the HDL function was observed while the concentration of HDL-C had increased (Table 21).

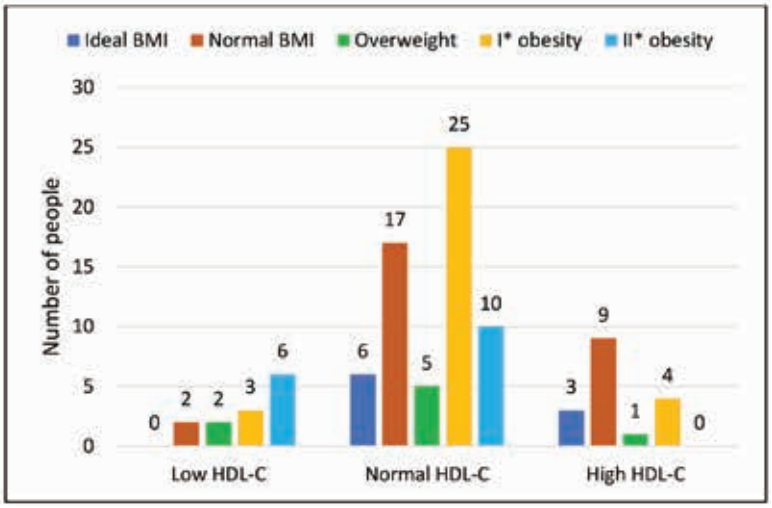

FIGURE 39. The associations between HDL-C concentration and BMI in the study population, p=0.02 (n=93).

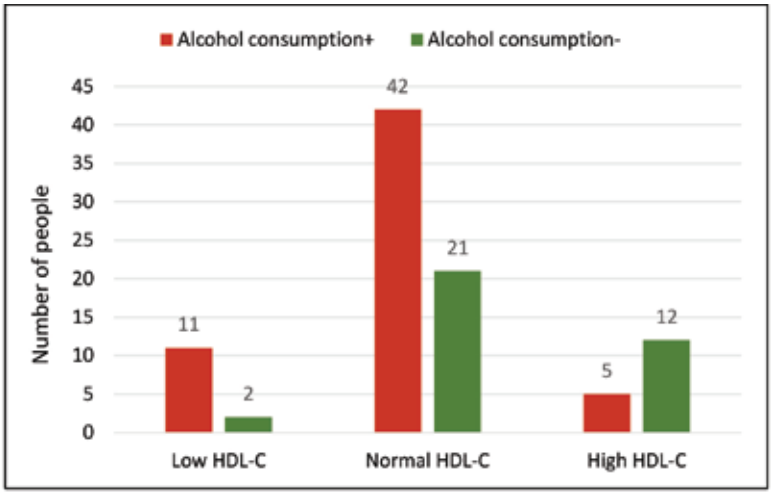

FIGURE 40. The associations between HDL-C concentration and alcohol consumption in the study population, p=0.004 (n=93).

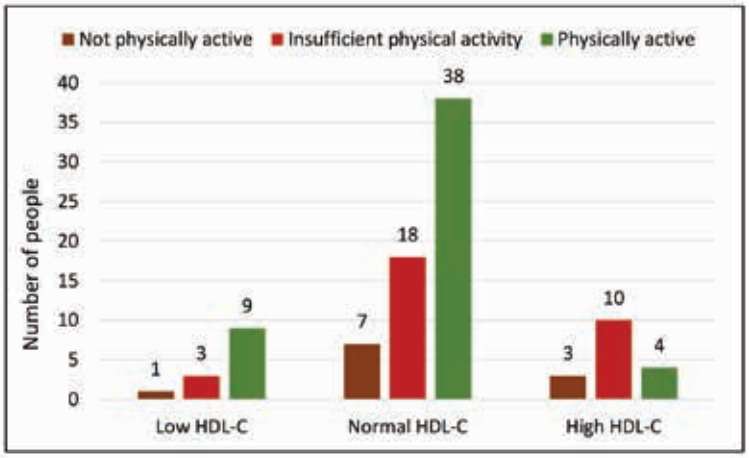

FIGURE 41. The associations between HDL-C concentration and physical activity in the study population, p=0.068 (n=93).

TABLE 21. Spearman's correlation coefficients between HDL-C concentration and other characteristics in groups with and without severe dyslipidemia.

Characteristics	HDL-C (mmol/l)			
	SD–	SD+	SD+	
			Men	Women
HDL-C function (%)	-0.146	-0.335*	-0.123	-0.198
Age (years)	0.130	0.061	0.059	-0.030
TC (mmol/l)	0.342*	0.277	-0.008	0.327*
TG (mmol/l)	-0.380*	-0.608*	-0.582*	-0.217
LDL-C (mmol/l)	-0.057	0.412*	0.083	0.232
Apo A1 (mmol/l)	0.866*	0.630*	0.713*	0.755*
Apo B (mmol/l)	-0.097	0.275	-0.065	0.126
Apo A2 (mmol/l)	0.410*	0.418*	0.317*	0.455*
Apo E (mmol/l)	0.140	-0.314*	-0.160	0.050
Apo B/Apo A1	-0.567*	-0.015	-0.325*	-0.104
Lp(a) (g/l)	-0.263	0.232	-0.141	-0.015
BMI (kg/m2)	-0.327*	-0.531*	-0.461*	-0.441*
WC (cm)	0.183	-0.583*	-0.189	-0.309*

* statistically significant as p<0.05;

Abbreviations: HDL-C – high-density lipoprotein cholesterol; TC – total cholesterol; TG – triglycerides; LDL-C – low-density lipoprotein cholesterol; Apo – apolipoprotein; Lp(a) – lipoprotein(a); BMI – body mass index; WC – waist circumference.

Irrespective of age, a strong negative relation between HDL-C and TG was observed (r=-0.588; r=-0.326; r=-0.775) as well as a positive connection between HDL-C and Apo A1 concentrations (r=0.864; r=0.669; r=0.803) (Table 22). A negative association between the Apo B/Apo A1 ratio and HDL-C concentration decreased with age (Table 22). Age did not have any effect on the HDL function in this study.

Table 22. Spearman's correlation coefficients between HDL-C concentration and other characteristics across different age groups.

Characteristics	HDL-C (mmol/l)		
	<45y	45-54y	≥55y
HDL function (%)	-0.217	-0.267	0.006
Age (years)	-0.330	0.282	-0.365
TC (mmol/l)	0.110	0.226	-0.177
TG (mmol/l)	-0.588*	-0.326*	-0.775*
LDL-C (mmol/l)	0.032	0.168	-0.083
Apo A1 (mmol/l)	0.864*	0.669*	0.803*
Apo B (mmol/l)	0.127	0.065	-0.349
Apo A2 (mmol/l)	-0.014	0.600*	0.511
Apo E (mg/l)	-0.108	0.040	-0.481
Apo B/Apo A1	-0.374*	-0.143	-0.535
Lp(a) (g/l)	-0.295	0.138	0.194
BMI (kg/m2)	-0.561*	-0.400*	-0.398
WC (cm)	-0.272	-0.273	-0.209

* statistically significant as p<0,05;

Abbreviations: HDL-C – high-density lipoprotein cholesterol; TC – total cholesterol; TG – triglycerides; LDL-C – low-density lipoprotein cholesterol; Apo – apolipoprotein; Lp(a) – lipoprotein(a); BMI – body mass index; WC – waist circumference.

3.2.6.3 An Evaluation of the HDL Function in the Study Population (n=93)

A below average HDL function was found in 67.7% (n=63) of subjects (n=93) (Figure 42). An average function of HDL in the study population was 47.5%. The descriptive statistics of the HDL function are presented in Table 23. Significant differences in the HDL function between men and women were not detected (Figure 43). After an evaluation of men and women with SD, a below average HDL function was found to be more common among women, although the difference was not statistically significant (Figure 44).

TABLE 23. The descriptive statistics of the HDL function in the study population (n=93).

HDL function (%)		Result
Mean		44.75
Standard deviation		11.61
Smallest observation		13.06
Largest observation		69.15
Deciles	66.6 (2/3)	49.21
	75 (3/4)	51.83

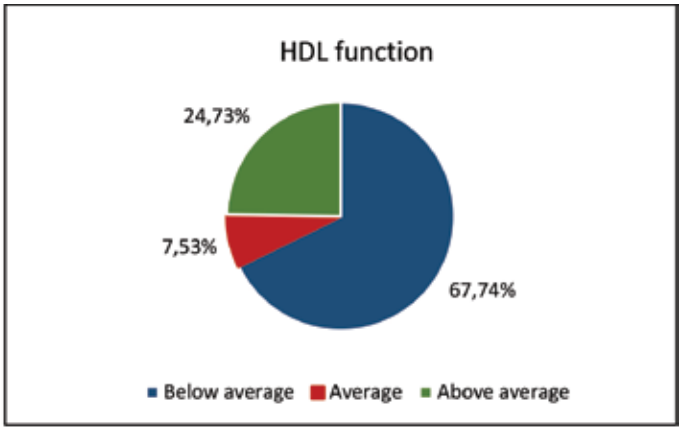

FIGURE 42. An evaluation of the HDL function in the study population (n=93).

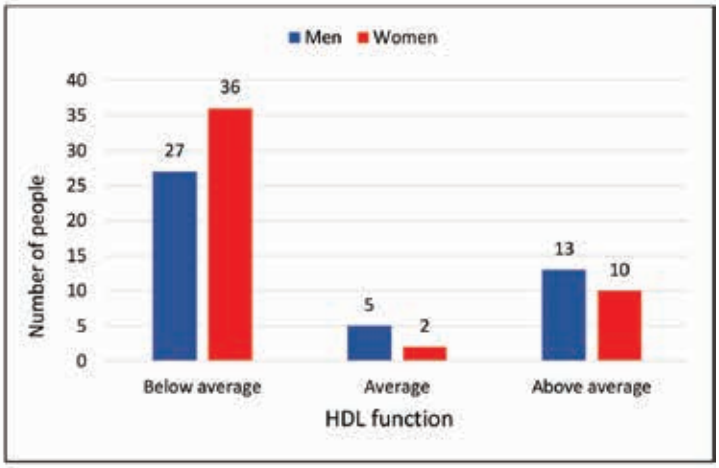

FIGURE 43. The distribution of the HDL function among different genders, p=0.238 (n=93).

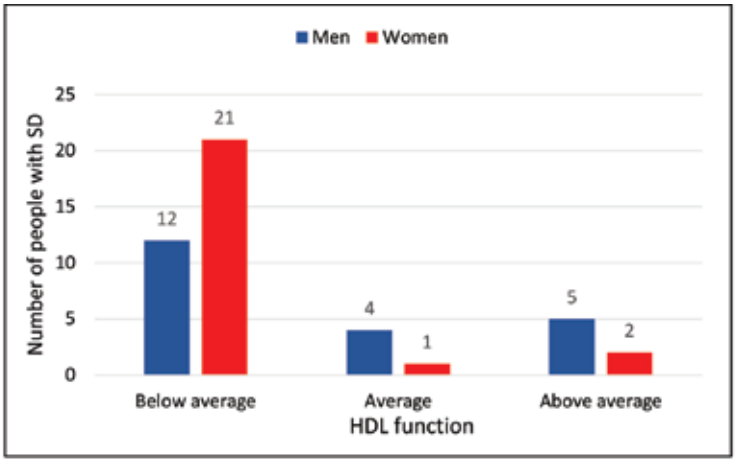

FIGURE 44. The distribution of the HDL function among men and women with severe dyslipidemia, p=0.068 (n=45).

In this study, we have found a significant negative connection between HDL-C concentration and the HDL function (r=-0.228) (Table 24). After evaluating men and women separately, no significant relation was established (Table 25).

TABLE 24. The associations of the HDL function with HDL-C concentration in the study population.

	Sample	HDL-C
HDL function (%)	Total	-0.228 (0.028*)
	Women	-0.198 (0.177)
	Men	-0.123 (0.421)

* statistically significant as p<0.05;

Abbreviations: HDL-C – high-density lipoprotein cholesterol.

TABLE 25. The associations of the HDL function with severe dyslipidemia.

	Gender	SD+	SD –
HDL function (%)	Men	-0.112 (0.601)	-0.146 (0.497)
	Women	-0.02 (0.933)	-0.146 (0.497)

* statistically significant as p<0.05;

Abbreviations: HDL-C – high-density lipoprotein cholesterol; SD+ – patients with severe dyslipidemia; SD – – patients without severe dyslipidemia.

3.2.6.4 The Associations of the HDL Function with Other Cardiovascular Risk Factors

Patients with normal BMI and I* obesity had a below average HDL function more frequently compared to people with overweight and II* obesity (Figure 45). Significant associations of the HDL function and other cardiovascular risk factors

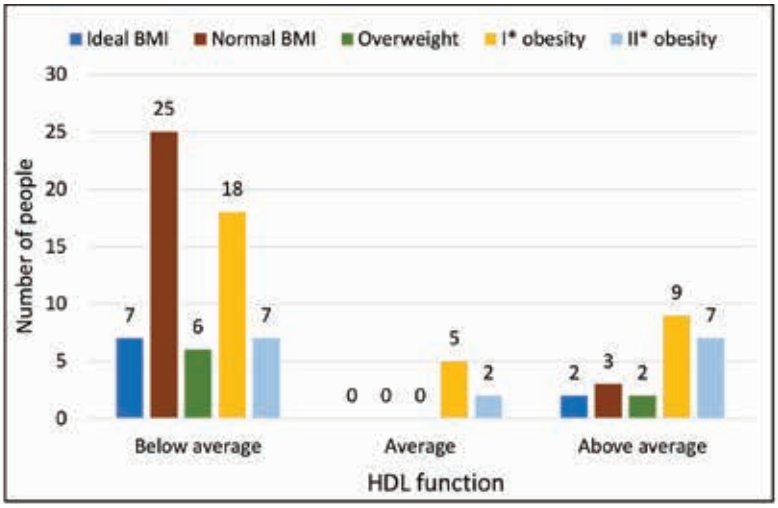

FIGURE 45. The associations of BMI with the HDL function in the study population, p=0.05 (n=93).

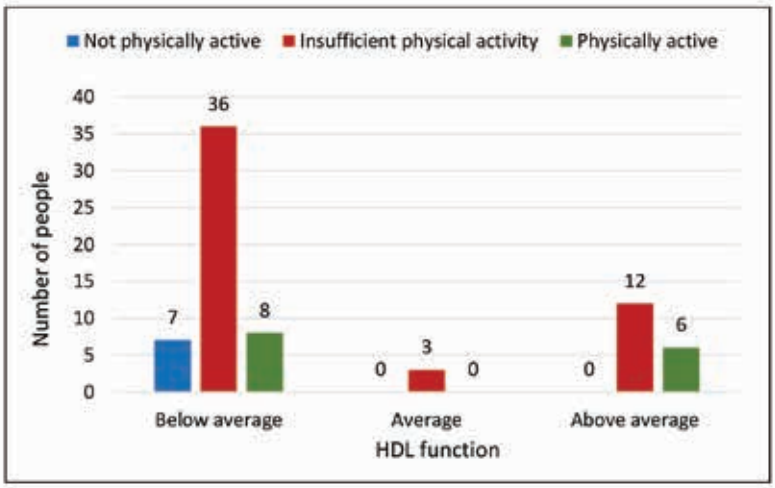

FIGURE 46. The associations of physical activity with the HDL function in the study population, p=0.197 (n=93).

(abdominal obesity, AH, smoking, a family history of CHD, alcohol consumption) were not established. Although, among physically active subjects, a below average HDL function was more common compared to patients with insufficient physical activity, this difference was not statistically significant (Figure 46).

After assessing the HDL function among all men and women and in relation to other cardiovascular risk factors, significant differences were not found. But while analyzing the group of men with SD, we found that that men with SD and a normal BMI more frequently had a below average HDL function (Figure 47).

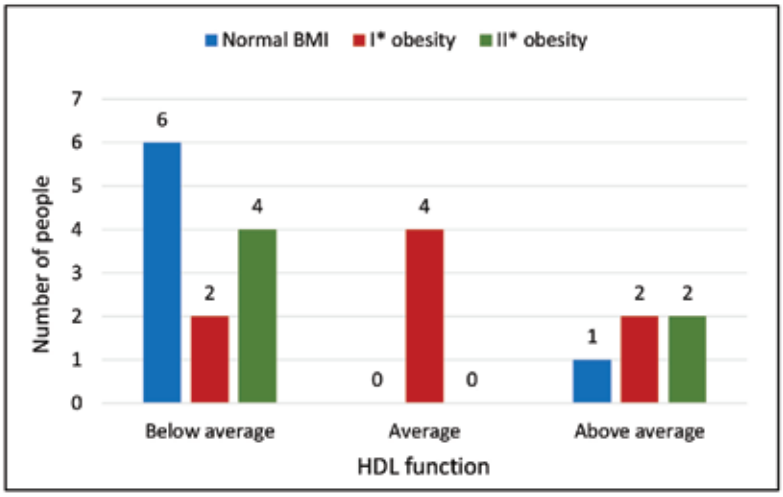

FIGURE 47. The HDL function in different groups of men with severe dyslipidemia according to BMI, p=0.049 (n=21).

3.2.7. Genetic Testing in Patients with Severe Dyslipidemia

The group with severe dyslipidemia consisted of 110 (51.6%) patients, but genetic testing was decided to be performed in 13 subjects – this was done after clinical evaluations of the probability of familial hypercholesterolemia (FH) in accordance with the Dutch Lipid Clinic Network criteria. LDL receptor (LDLR) mutations were found in four patients, confirming the diagnosis of FH. Five patients tested negative for any evaluated mutations associated with FH. The remaining four people did not agree to analysis due to personal reasons. Mutations in Proprotein convertase subtilisin/kexin type 9 (PCSK9) gene and ApoB gene were not detected.

Conclusions

1. Dyslipidemia is very common (89.7%) and one of the most important cardiovascular risk factors with an increasing prevalence being observed during the period of 2009–2016 in Lithuania (from 89.1% to 89.5%). The diagnosis and treatment of dyslipidemia is delayed and inadequate.
2. Dyslipidemia is associated with a greater probability of possessing all major CVD risk factors (diabetes mellitus, arterial hypertension, abdominal obesity, metabolic syndrome and obesity), except smoking, compared to adults without dyslipidemia. An unbalanced diet, an insufficient level of physical activity and a family history of CHD were also more common among subjects with dyslipidemia.
3. Atherogenic dyslipidemia is associated with an unfavorable cardiovascular risk profile. Subjects with atherogenic dyslipidemia more frequently possess other cardiovascular risk factors compared to people with isolated hypertriglyceridemia or low HDL-C.
4. The prevalence of severe hypertriglyceridemia increased from 2.2% to 2.3%, while a decreased prevalence was found for severe dyslipidemia (from 12.1% to 11.6%) as well as severe hypercholesterolemia (from 2.9% to 2.8%) for the period of 2009–2016 in Lithuania. Severe hypertriglyceridemia was associated with having other major CVD risk factors more often (except for family history of CHD) compared to severe hypercholesterolemia.
5. In the prospective part of the study, patients with severe dyslipidemia more frequently happened to be obese and have arterial hypertension, histories of CHD in their families, menopause and insufficient levels of physical activity compared to a control group. For the evaluation and long-term monitoring of patients with severe dyslipidemia, useful investigations include a body composition analysis, an Achilles tendon ultrasonography, a carotid artery ultrasound as well as genetic testing (if available).
6. An insufficient function of HDL was observed in 67.7% of study subjects. An inverse relationship was determined between the HDL function and the plasma concentration of HDL-C (r=-0.228).

Practical recommendations

- The diagnosis and treatment of dyslipidemia, as well as the patient-physician relationship, should be improved, as these are one of the most important health problems of middle-aged Lithuanians.
- Dyslipidemia is often detected alongside other cardiovascular risk factors and requires special attention while assessing the cardiovascular risk of a particular patient.

- It is necessary to pay more attention to diagnosis, treatment and long-term monitoring as well as the evaluation of cardiovascular risks and the management of patients with severe dyslipidemia.
- Body composition analysis is necessary and useful for evaluating patients with severe dyslipidemia as well as for monitoring their body composition changes while making lifestyle interventions or other preventative measures required to manage cardiovascular risks.
- The ultrasound imaging of the Achilles tendon and carotid arteries is useful for evaluating and monitoring patients with severe dyslipidemia.
- The CAC score is not an appropriate diagnostic tool in the algorithm of severe dyslipidemia examination.
- While suspecting familial hypercholesterolemia, genetic testing is helpful for establishing a definite diagnosis, finding the best treatment options, maintaining a better patient-physician relationship and screening family members for the index case.
- Further extensive studies are needed to analyze the function of HDL in Lithuania and support the findings of this study.

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2. **Kutkiene S.** Prevalence of cardiovascular risk factors in middle-aged subjects of high risk Lithuanian population. The 84th EAS Congress, May 29–June 1, 2016, Innsbruck, Austria (oral presentation).

3. **Kutkiene S.** Gender differences in cardiovascular risk profile in middle-aged subjects of high risk Lithuanian population during the period 2009-2014. The 3rd International Conference "Evolutionary Medicine: Pre-Existing Mechanisms and Patterns of Current Health Issues," June 14–17, 2016, Vilnius, Lithuania.

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16. **Kutkiene S.**, Petrulioniene Z., Kasiulevicius V., Sausdravas R., Sarapnickiene I., Gargalskaite U., Saulyte A., Staigyte J., Kovaite M., Rinkuniene E., Dzenkeviciute V. Case-control study of dietary habits among Lithuanian adults with severe dyslipidemia. 5. The 26th Nordic- Baltic Congress of Cardiology, June 1–3, 2017, Vilnius, Lithuania (poster presentation).

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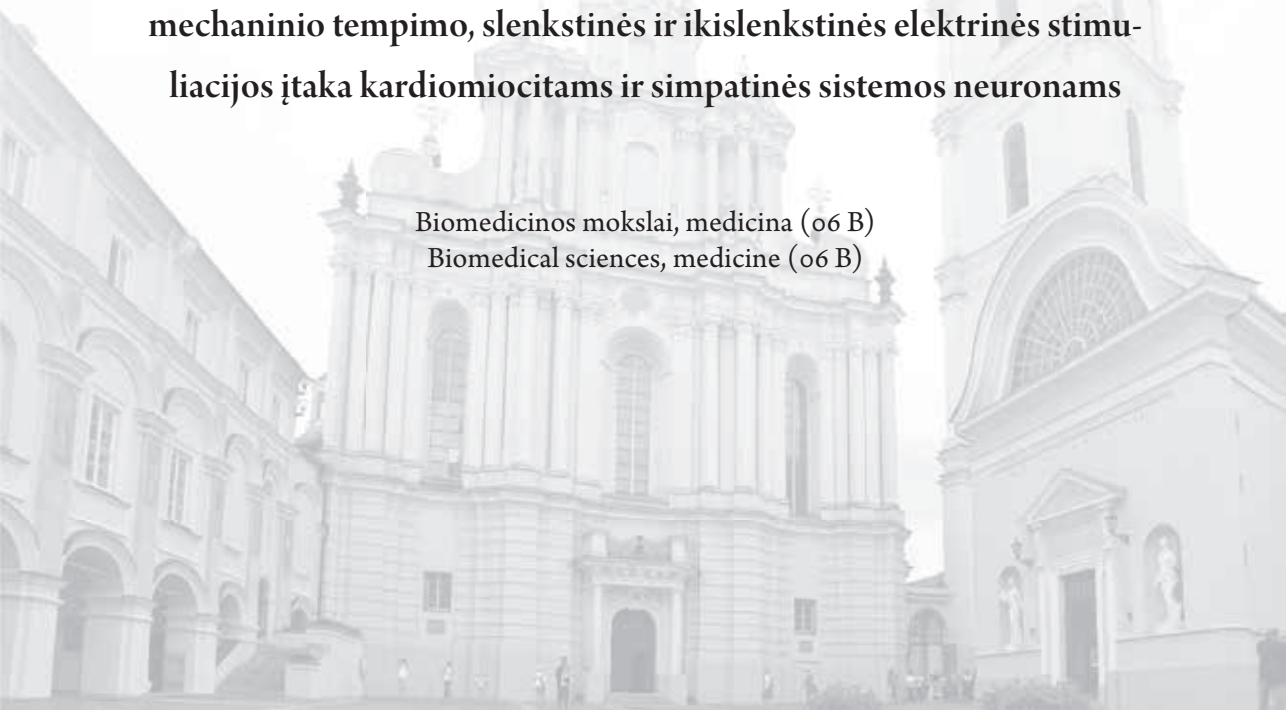
Gediminas Račkauskas

SUB THRESHOLD HIGH FREQUENCY ELECTRICAL FIELD STIMULATION EFFECT FOR ANGIOGENESIS AND VEGF EXPRESSION IN CARDIOMYOCYTES AND HEART TISSUE. Remodelling in the heart: impact of mechanical stretch, threshold and sub-threshold electrical stimulation on cultivated cardiomyocytes and sympathetic neurons

IKISLENKSTINIO DIDELIO DAŽNIO ELEKTRINIO LAUKO STIMULIACIJOS EFEKTAS ANGIOGENEZEI IR KRAUJAGYSLIŲ ENDOTELIO AUGIMO VEIKSNIO SINTEZEI KARDIOMIOCITUOSE. Širdies remodeliacija: mechaninio tempimo, slenkstinės ir ikislenkstinės elektrinės stimuliacijos įtaka kardiomiocitams ir simpatinės sistemos neuronams

Biomedicinos mokslai, medicina (06 B)
Biomedical sciences, medicine (06 B)



1. Abbreviations

AF – atrial fibrillation
CAD – coronary artery disease
DCM – dilated cardiomyopathy
HCAECs – human coronary artery endothelial cell
HFES – high-frequency bipolar electrical field stimulation
IA – immunoadsorption
IHD – ischemic heart disease
NGF – nerve growth factor
NRVMs – neonatal rat ventricular myocytes
PCI – percutaneous coronary intervention
SES – subthreshold electrical stimulation
SCG – superior cervical ganglia
SN – sympathetic neurons
VEGF – vascular endothelial growth factor
WB – Western blot

2 Introduction

2.1 Scientific relevance

Arterial occlusive diseases cause serious ischemic diseases in various organs, such as the heart, brain, and leg, and therapeutic angiogenesis is thought to be beneficial for such conditions. Local administration of recombinant angiogenic growth factors, such as basic fibroblast growth factor and vascular endothelial growth factor (VEGF), salvaged ischemic areas of myocardium and hindlimb in animal models. However, the clinical application requires large amounts of these recombinant proteins and is not feasible at this time. Instead of recombinant proteins, use of gene therapy, in vivo transfection of angiogenic growth factor gene, has been attempted to treat these diseases.

VEGF, a dimeric endothelial cell – specific growth factor, is thought to be a principal angiogenic factor that stimulates migration, proliferation, and expression of various genes in endothelial cells. VEGF is synthesized by cells around vasculature and affects endothelial cells as a paracrine factor. The expression of VEGF is upregulated by hypoxia and various cytokines. One part of our present study revealed that low-voltage electrical stimulation of heart cells induced de novo synthesis of VEGF protein and increase proliferation.

The cardiovascular autonomous neural system represents an integrative fiber network that regulates cardiovascular hemodynamic and electrophysiological processes. During normal physiology, the autonomic nervous system represents a short latency responder that quickly compensates for temporary changes of cardiac function (eg, pumping force, blood pressure, and heart rate). However, when compensation becomes permanent during cardiovascular disease (atrial fibrillation, dilatative or ischemic cardiomyopathy and others) specifically sympathetic activation catalyzes a further deterioration of the disease process. Besides functional effects on

heart rate, inotropy, vasomotion, and cardiac electrophysiology, the sympathetic/parasympathetic nervous systems could be markedly affected be the electrical stimulation and produce vide spectrum of biologically active molecules.

2.2. Study hypothesis

Subthreshold electrical stimulation has been shown to induce an improvement of angiogenesis mediated by increased VEGF and NGF protein expression.

2.3 The aim of the study

To evaluate growth factors response to various stimulation techniques in the field of neurocardiology.

2.4. Study tasks

To investigate the effect of sub threshold electrical stimulation on vascular endothelial growth factor regulation in cultured neonatal rat ventricular myocytes, in the aim to reveal new techniques for therapeutic angiogenesis in ischemic heart disease.

To assess the role of autoantibodies in dilated cardiomyopathy inducing the expression of vascular endothelial growth factor in cardiomyocytes. Effect on contractility, vascular endothelial growth factor secretion as a cell response to tension.

To investigate high frequencies and irregularity effects of stimulation on ventricular myocytes and nerve growth factor.

To evaluate the response of sympathetic neurons to mechanical stretching in the evaluation of biologically active molecules: vascular endothelial growth and neuronal growth factors.

2.5. What is new

Up to now some studies has been shown, that high frequency threshold and sub threshold electrical stimulation can induce biologically active molecules production in non-cardiac tissue. Data from this work show, that ventricular myocytes can produce biologically active proteins which has positive effect on cells proliferations and viability.

3. Literature review

Ischemic heart disease (IHD) due to coronary artery disease (CAD) remains the leading cause of death in the Western world (1). Revascularization of existing

arteries and the formation of new collateral vessels may be a promising strategic aim in order to improve the function of organs suffering from hypoxia. Vascular endothelial growth factor (VEGF) plays a pivotal role in angiogenesis and neovascularization. There is accumulating evidence that VEGF is expressed in the heart and that its expression is markedly increased in response to hypoxia or cyclic mechanical stretch (2, 3). These (patho) physiological stimuli recent studies have shown that subthreshold electrical stimulation (SES) augments VEGF expression in cultured skeletal muscle cells. Moreover, in vivo SES has been shown to induce vascular angiogenesis in a hindlimb ischemia model in rats mediated by VEGF (4, 5). Since SES might be delivered to hypoxic cardiac tissue in vivo (e.g., with catheters or wires) this proof-of-principle study investigated the effect of SES on VEGF expression in cultured neonatal rat ventricular myocytes (NRVMs).

Circulating autoantibodies that target diverse myocardial structures have been identified as major predisposing factors for dilated cardiomyopathy (DCM) (6). Patients with DCM show elevated serum levels of vascular endothelial growth factor whose source is unknown (7). Even though a large number of studies suggest that VEGF is beneficial in ischemic cardiomyopathies due to its well-investigated effects on angiogenesis and neovascularisation (8-10), to the best knowledge of the authors there is no data available reporting a beneficial role of VEGF in DCM. In contrast, recent data indicate that an un-physiological expression and secretion of VEGF might be harmful via its pleiotropic effects on all cell types involved in cardiac remodelling. Accumulating data suspect VEGF as a cardiac transcriptional regulator, which is crucially involved in physiological and pathological hypertrophy signalling in cardiomyocytes (11). In this context, Zhou et al. could convincingly demonstrate that VEGF signalling plays a pivotal role in phenylephrine induced cardiomyocyte hypertrophy (12). Additionally, Tsoporis and colleagues have recently shown that VEGF, which is released by cardiomyocytes promotes proliferation of cardiac fibroblasts (13). It is still unclear whether these different observations can be predictably translated into cardiac remodelling. Nevertheless, since VEGF levels are increased in DCM patients, the question raises whether VEGF is just compensatory elevated or it is directly involved in cardiac remodelling pathways due to unknown mechanisms (14-16).

The current options to inhibit or to reverse the process of structural remodelling in the failing heart are limited. Drugs targeting the beta1-adrenergic or the angiotensin signalling pathways can improve survival but have a narrow therapeutic range due to side-effects including bradycardia and/or hypotension (17,18). There is accumulating evidence that the therapy of immunoadsorption (IA) leads to a long-term improvement of cardiac function in patients suffering from DCM (19-22). Whether autoantibodies in DCM influence cardiac VEGF expression, and whether

an un-physiological VEGF secretion might promote remodelling mechanisms due to hypertrophy signalling and cardiac fibroblast proliferation is rather unexplored.

Atrial fibrillation (AF) is associated with a 1.5- to 1.9-fold mortality risk after adjustment for pre-existing cardiovascular conditions (23). Many factors may contribute to this worse outcome like arterial embolism or hemodynamic deterioration. Recently, an irregular ventricular response during AF has been shown to increase sympathetic nerve activity in vivo (24, 25). This raises the hypothesis whether the irregular activation of cardiomyocytes may affect neural growth and activity. Nerve growth factor (NGF) plays a key role in the structural and functional integrity of sympathetic neurons and regulates survival, differentiation and neurite outgrowth (26). For example, in NGF knockout mice the volume of the sympathetic ganglia is remarkably reduced (27). In contrast, overexpression of NGF in the heart results in cardiac hyperinnervation and hyperplasia of stellate ganglia neurons (28). Up to now molecular mechanisms, which regulate cardiac NGF expression are not fully understood.

Mechanical stretch has been shown to increase vascular endothelial growth factor expression in cultured myocytes (29). Beside the biomechanical forces that occur in the developing or diseased heart, cells also from tissues such as lung, skeletal muscles and even cells within the scope of neoplastic genesis are subjected to biomechanical environments sufficient to produce stretches beyond the normal ranges (30-32). In the heart, mechanical overload contributes to VEGF augmentation via a TGF-beta signalling pathway (33). The heart is comprehensively innervated by the autonomic nervous system including sympathetic and parasympathetic nerve fibbers controlling numerous cardiac regulating mechanisms. Therefore, the finding those sympathetic neurons (SN) possess the ability to produce and secrete angiogenic factors including VEGF is of obvious importance (34). However, whether stretch also increases neuronal VEGF expression still remains to be clarified.

Beside its manifold effects on endothelial cells with the consequence of forming new collaterals VEGF also has direct multiple effects on SN (35). For instance, in superior cervical ganglia (SCG) of new-born rats, nerve growth factor (NGF) induces capillary sprouting via the release of VEGF (36). The NGF induced VEGF upregulation in SN is mediated by TrkA receptor activation (37). Taken together, these findings indicate that neuronal cells may regulate their own VEGF requirements depending on the pre-existing biomechanical forces in an autocrine/paracrine fashion by a NGF and TrkA signalling pathway. Likewise, NGF has been shown to promote angiogenesis in ischemic hind limbs (38) and to recover cardiac function after myocardial infarction (MI) (39). During MI intracardiac cells are exposed to increasing stretch. Stretch is one important contributor to neuronal NGF expression (40), whereas cardiomyocytes respond to stretch with a down-regulation of NGF (41).

Up to now, it is quite uncertain if the beneficial effects observed by NGF signalling in terms of increasing wall stress (e.g. MI) are due to a direct NGF function or whether stretch-induced NGF secretion promotes neuronal VEGF expression in an autocrine/paracrine fashion subsequently contributing to neovascularisation and recovering heart function.

4. Methods

4.1. Cell Cultures of Neonatal Rat Ventricular Myocytes (NRVMs)

All animal experiments were approved by the local and state Ethics in Animal Research Committee (University Hospital RWTH Aachen, Germany, TV-No. 10596 A). NRVMs were isolated and cultured as described previously by Simpson et al. with little modifications according to the protocol by Zobel et al. (42-44). After cervical dislocation, hearts were obtained from 1- to 3-day old Sprague – Dawley rats with no consideration to the gender (Charles River, Sulzfeld, Germany) and digested with collagenase II (1.0 mg/ml, 280 U/mg, C2-22; Biochrom, Berlin, Germany), trypsin (0.5 mg/ml, trypsin 1:250, L-11-002; PPA, Colbe, Germany), and 1% penicillin–streptomycin (P-4458; Sigma-Aldrich, Steinheim, Germany). Myocytes were purified from fibroblasts by passage through a Percoll gradient (P-1644; Sigma-Aldrich). Myocytes were plated onto six-well dishes (140685; Nunc, Schwerte, Germany) at a density of 3×10^5 cells per well. Cells were grown in DMEM/Ham's F-12 + l-glutamine (FG-4815; Biochrom) supplemented with 10% horse serum (B15-021; PPA) and 5% fetal bovine serum (A-15-101; PPA). After 24 h, the serum medium was removed, and the cells were washed and maintained in serum-free DMEM/Ham's F-12 + l-glutamine.

Human coronary artery endothelial cells (HCAECs) were grown in endothelial cell growth medium (C-22020; PromoCell GmbH, Heidelberg, Germany) until starting the experiments with NRVM cell-conditioned medium. The tissue used by PromoCell for the isolation of human cell cultures is derived from donors who have signed an informed consent form (this being done by the donor himself, an authorized agent, or a legal agent), which outlines in detail the purpose of the donation and the procedure for processing the tissue.

4.2. Application of Subthreshold Electrical Simulation (SES)

Three days after cell isolation, serum-containing medium was changed to serum-free medium, and thereafter NRVMs were stimulated for 48 h with low-voltage,

high- frequency bipolar electrical field stimulation (HFES) using the C-PaceEP external pacing system (IonOptix, Wageningen, The Netherlands). The stimulus strength (0.5 V/cm, impulse duration 1 ms) was chosen to yield an electrical field that was well below the threshold that elicited contraction of the cultured myocytes. The lowest capture threshold in the different preparations was 1.0 V/ cm. Lack of contraction of the myocytes was checked every 12 h during the experiment by microscopic inspection of the cultures. The frequencies chosen were 5 Hz, 25 Hz, 50 Hz, and 99 Hz. Cell cultures without HFES served as controls.

4.3. ELISA

For VEGF ELISA, each well was normalized to 300,000 cells, and SES steps from 5 Hz up to 25, 50, and 99 Hz were analyzed. The conditioned media was collected at 0, 24, and 48 h after exposure to SES, and protein in the cell culture supernatant was concentrated by using the vivaspin columns from Sartorius (Göttingen, Germany). Samples were assayed by a VEGF ELISA kit from R&D Systems (Minneapolis, MN, USA) with normalized protein amounts according to the manufacturer's instructions. Protein amount was quantified by using the NanoDrop 1000 from Thermo Scientific (Wilmington, DE, USA).

4.4. Western Blot

Western blots (WB) to assess VEGF and KDR proteins were performed with normalized protein amounts. For WB, cells were homogenized in lysis buffer containing 20 mM Tris-HCl (pH 8.0; #9090.2; Carl-Roth, Karlsruhe, Germany), 1% nonident P-40 (#74.385; Merck, Darmstadt, Germany), and 10% glycerol (#50405-1; Biomol, Hamburg, Germany). Cell lysates were cleared by centrifugation at 17,000×*g* for 20 min. Extracts (100 µg) were subjected to electrophoretic separation through a 10% SDS-polyacrylamide gel (SDS #51430-2, Biomol/ polyacrylamide gel #3029.1; Carl-Roth) and subsequently transferred to a polyvinylidene difluoride membrane (#RPN303F; GE-Healthcare Life Sciences, Freiburg, Germany). The membrane was blocked overnight with 5% bovine serum albumin (#01400-1; Biomol) in phosphate buffered saline (#L-1825; Biochrom) containing 0.1% Tween 20 (PBST; #TW0020; Rockland, ME, USA). Primary rabbit anti-GAPDH (1:1,000; #2118; Cell Signaling, Danvers, MA, USA), rabbit anti-VEGF (1:150; 19003-1-AP; Acris Antibodies, Herford, Germany), and rabbit anti-KDR (200 µg/ml, 1:100; sc-315; Santa Cruz, Heidelberg, Germany) were incubated overnight. Blots were washed three times with PBST and incubated with goat anti-rabbit horseradish peroxidase-conjugated antibody (GE Healthcare) for 1 h. Finally, the ECL Advance Western Blotting Detection Kit (GE Healthcare Life Sciences) was used to visualize the bands with the advanced

luminescent image analyzer LAS-3000 from Fujifilm (Tokyo, Japan). Relative densitometry analyses of the bands were performed with the Multi-Gauche V3.0 software from Fujifilm.

4.5. Antibodies

Cells were fixed in 4% paraformaldehyde (8.187.15; KMF, Lohmar, Germany), permeabilized with Triton X-100 (50800-1; Biomol), and blocked with 1% BSA (01400-1; Biomol). Cells were incubated with the primary antibody, (1:150) rabbit anti-VEGF (200 μg/ml, 1:150; sc-507; Santa Cruz) mouse anti-Trop T-C (200 μg/ml; sc-20025; Santa Cruz) overnight at 4°C and then with a goat anti-rabbit (1:100) and goat anti-mouse (1:100) secondary antibody for 2 h coupled to Alexa Fluor 488 (VEGF) (1 mg/ml; A11008; Invitrogen) or Alexa Fluor 647 (Trop T-C) (1 mg/ml; A20990; Invitrogen). Nuclei were stained with 4′,6-diamidino-2-phenylindole dihydrochlo- ride (10236276001; Roche Mannheim, Germany). To visualize fluorescence signals, the Axiovert 200M micro- scope and the AxioVision Rel. 4.5 software from Zeiss (Jena, Germany) were used.

Primary antibodies for Western blot: rabbit anti-GAPDH (1:1,000; #2118; Cell Signaling), rabbit anti-VEGF (1:150; 19003-1-AP; Acris Antibodies), rabbit anti-KDR (200 μg/ml, 1:100; sc-315; Santa Cruz).

4.6. RNA Preparation, First-Strand cDNA Synthesis, and Quantitative Real-Time Reverse Transcription-PCR

Total RNA was extracted from NRVMs using the Qiagen RNeasy Mini Kit (Venio, The Netherlands) and following the manufacturer's instructions. A total of 1 μg of RNA were reverse-transcribed using random hexamers from the Fermentas First strand cDNA Synthesis Kit (#K1622; Fermentas GmbH, St. Leon- Rot, Germany).

4.7. Quantitative Real-Time Reverse-Transcription-PCR. Primers and Probes for Quantitative Real-Time Reverse-Transcription-PCR

Real-time PCR was performed in 96-well plates on the Step-One plus Sequence Detection System (ABI) as described previously (9). Data were collected with instrument spectral compensation by Applied Biosystems SDS 1.2.3 software (Applied Biosystems, Foster City, CA, USA).

PCR primers and fluorogenic probes for the target gene and the endogenous control were purchased as Assays-On- Demand (Applied Biosystems). The assay numbers for the endogenous control and target genes were as follows: Rn00560865_

m1 (b-2 microglobulin), Rn01533872_m1 (Ngfb), Rn00561661_m1 (Nppa). The assay numbers were as follows: Rn01511602_m1 (VEGF), Rn00564986_m1 (KDR), Rn00560865_m1 (b-2 microglobulin).

4.8. Investigation of Human Coronary Artery Endothelial Cell Proliferation

To reveal the proliferation of HCAECs due to an effect of biologically active VEGF secreted by NRVMs exposed to SES, we used a colorimetric Assay kit from PromoCell (WST-8). WST-8 is bio reduced by cellular dehydrogenases to an orange formazan product that is soluble in cell culture medium. The amount of formazan is directly proportional to the number of living cells. Therefore, this method allows sensitive determination of the number of viable cells in cell culture.

HCAECs were incubated for 48 h with NRVM- conditioned medium. The frequencies of 25 Hz and 5 Hz were analysed, due to the fact that the dominant effect of VEGF induction was observed at 25 Hz (compared to 5 Hz and control).

4.9 DCM patient selection, mechanical stretch and Tunel assay investigation

DCM patients (n 1⁄4 5, mean age 50.3 ± 3.5 years) with severe left ventricular dysfunction (LVEF < 30%, New York Heart Association class III) were treated with IA on five consecutive days. After the final IA session, all patients received 0.5 g/kg polyclonal IgG (Venimmun N) to restore IgG plasma levels (45). The same polyclonal IgG pool was used for cell culture control purposes in a matched concentration, and is indicated as healthy control IgG (HC-IgG) in the appropriate graphs. All DCM patients had a history of heart failure of at least 1 year and had a stable dosage of oral medications including angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, diuretics, aldosterone antagonists and b-blockers for at least 6 months. Ischemic disease was excluded by angiography. The IA therapy was performed with protein A Immunoadsorba columns (Fresenius Medical Care AG, Bad Hombug, Germany). Column eluent (CE) from IA columns containing the eliminated IgG was collected and prepared for cell culture experiments as described elsewhere (22). The Nanodrop ND-1000 (PeqLab, Erlangen, Germany) was used to determine the protein concentration of the CE. Aliquots of CE were stored at 80 C until use. Serum IgG levels of our DCM patients were monitored and an average reduction in IgG levels of 91.7% could be observed, as measured at the end of the last session on the fifth day of IA. Ischemic cardiomyopathy (ICM) patients (n 1⁄4 7) showed similar parameters regarding ejection fraction, LV-dimensions and the NYHA classifications as compared to DCM patients (data not shown).

For mechanical stretch NRCM were incubated on collagen-coated silicone membranes and static stretch was introduced to attached myocytes by applying a gradual increase of tension as described previously (46). Stretch was increased stepwise every 24 h from 3% to 13% (steps: 3, 7, 13%).

The TUNEL assay was performed using the APO-BrdU TUNEL Assay Kit (Invitrogen), according to the manufacturer's instructions. Cell apoptosis was determined by counting the number of TUNEL- positive nuclei and expressed as percentage of total cells counted (47).

4.10. Statistical Analysis

All values are expressed as mean±SEM. Data were processed using Statistical Package for Social Sciences release 14.0 for Windows (IBM, Somers, NY, USA). The statistical significance of differences was evaluated by the Student's *t*-test for two groups and one-way ANOVA followed by LSD post hoc test for multiple groups. A value of $p < 0.05$ was accepted as statistically significant.

5. Results

5.1. SES Augments VEGF Expression and Secretion in Neonatal Rat Ventricular Myocytes

To analyze the effect of SES on cardiomyocyte VEGF expression, NRVMs in cell culture were stimulated for 48 h at 5 Hz, 25 Hz, 50 Hz, and 99 Hz. VEGF protein amount was measured from cell culture medium at 24 and 48 h of stimulation. HFES elicited an increase in VEGF expression both in the cytoplasm and supernatant. A dominant effect of SES was observed at 25 Hz with a 1.8-fold increase of VEGF protein amount in the cytoplasm (Fig. 1). In parallel, VEGF in the cell culture supernatant rose significantly with an almost bell-shaped dose–response curve and a maximum at 25 Hz. The effect of HFES on VEGF expression was sustained for at least 48 h at all HFES frequencies (Fig. 2). Figure 3 shows VEGF immunocytofluorescence images of the VEGF expression in cardiomyocytes, which were exposed to subthreshold high-frequency electrical fields in vitro.

In order to evaluate which was the relative contribution of augmented VEGF gene expression on elevated VEGF protein expression, we analysed the VEGF mRNA content of cell lysates.

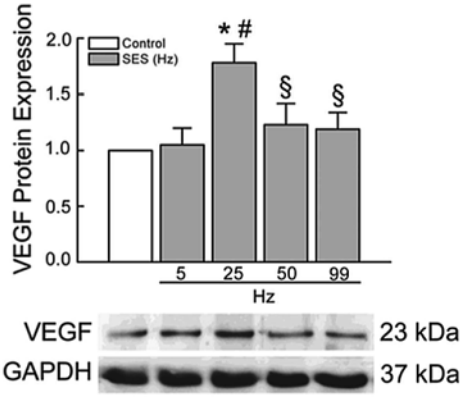


FIGURE 1. Frequency dependence of relative VEGF protein expression in cell lysates during 48 h of subthreshold electrical stimulation (SES). Results are representative of at least five independent experiments. *p<0.05 versus control. #p<0.05 versus 5 Hz. §p < 0.05 versus 25 Hz.

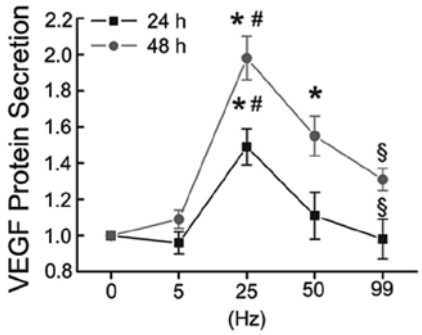


FIGURE 2. Extracellular secretion of VEGF protein normalized to control. Medium was collected at 24 and 48 h during continuous electrical stimulation, and VEGF protein was determined by ELISA. Results are representative of at least five independent experiments. *p<0.05 versus control. #p<0.05 versus 5 Hz. §p < 0.05 versus 25 Hz.

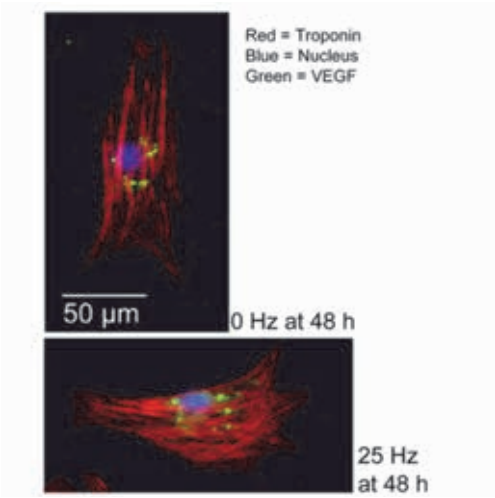


FIGURE 3. Representative images of NRVMs in cell culture exposed to SES. These images show abundance of VEGF protein.

5.2. Changes of VEGF mRNA and VEGF Receptor During HFES in NRVMs

HFES led to significant decreases in VEGF mRNA, which was significant for 25, 50, and 99 Hz with a maximum at 25 Hz (Fig. 4). Since this might be due to a negative feedback loop in response to the increased VEGF protein levels both in the cytoplasm and in the supernatant, we measured the expression of the membranous VEGF receptor (KDR) both on protein (Fig. 5) and mRNA levels (Fig. 6). SES significantly decreased the expression of the KDR receptor protein at 25 Hz and though not significant at 50 and 99 Hz. Likewise, the KDR receptor mRNA expression was significantly augmented during SES at 25, 50, and 99 Hz (Fig. 6). Of note, the relative suppression of VEGF mRNA expression and KDR protein and gene expression followed the frequency dependency as observed for the induction of VEGF protein with a maximum at 25 Hz.

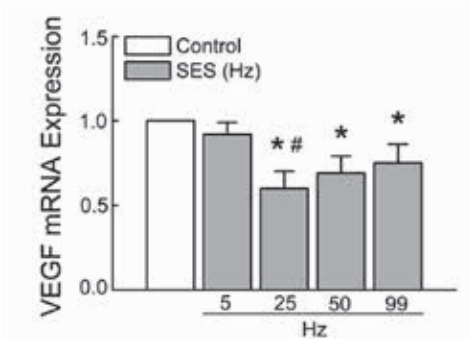


FIGURE 4. Relative expression of VEGF mRNA in cultured NRVMs. Cells were electrically stimulated for 48 h at indicated frequencies. Thereafter, total cDNA was extracted, and RT-PCR for VEGF mRNA was performed. Results are representative of at least four independent experiments. *p < 0.05 versus control. #p < 0.05 versus 5 Hz.

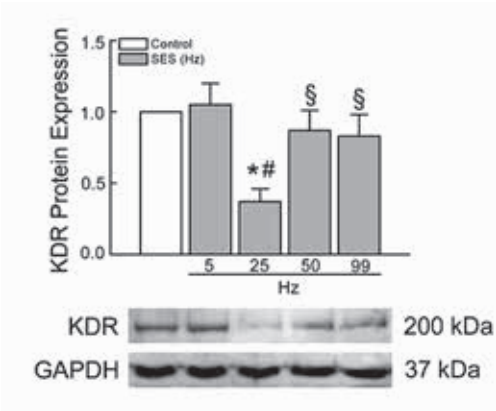


FIGURE 5. Relative KDR protein expression. Results are representative of at least five independent experiments. *p<0.05 versus control. #p < 0.05 versus 5 Hz. §p < 0.05 versus 25 Hz.

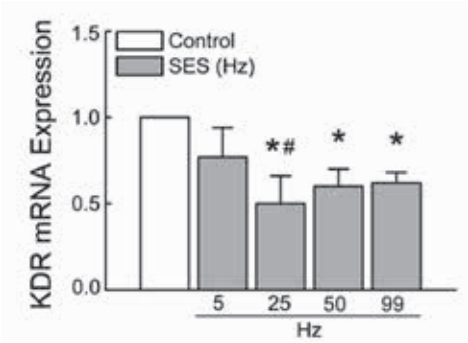


FIGURE 6. Relative expression of KDR mRNA in cultured NRVMs. Cells were electrically stimulated for 48 h at indicated frequencies. Results are representative of at least four independent experiments. *p<0.05 versus control. #p<0.05 versus 5 Hz.

5.3 SES-Induced VEGF Expression Is Biologically Active and Promotes Human Coronary Artery Endothelial Cell Proliferation

To investigate whether the observed VEGF induction by SES is effective in promoting endothelial cell proliferation, HCAECs were incubated with NRVM-conditioned medium (48 h of SES at 5 Hz, 2 5Hz, 50 Hz, and 99 Hz, respectively). We found that medium conditioned by 25 Hz SES augmented the growth of HCAECs, whereas the medium conditioned by 5 Hz SES did not elicit a significant growth response of HCAECs in vitro (Fig. 7).

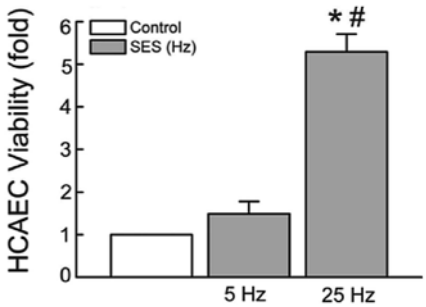


FIGURE 7. Growth response of HCAECs incubated with cell conditioned medium of NRVMs exposed to SES. Conditioned culture supernatant, 25 Hz, was sufficient to promote endothelial cell proliferation. Results are representative of at least four independent experiments. *p < 0.05 versus control. #p < 0.05 versus 5 Hz.

5.4. Augmented serum levels of VEGF in patients with DCM decrease after IA

Since increased myocardial wall stress can affect VEGF expression, the VEGF serum levels of DCM patients were compared to that of patients with ischemic cardiomyopathy. The left ventricular ejection fraction as well as left ventricular end-systolic and end-diastolic diameters of the two groups showed no significant differences (data not shown). In our small number of patients we observed significantly

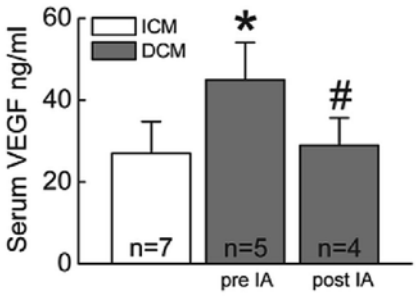


FIGURE 8. A Serum levels of VEGF decrease after the IA therapy. VEGF serum levels in patients with DCM showed significantly higher baseline values as compared to ICM patients. After the IA therapy, serum VEGF levels showed almost the same concentration in both groups. In our small number of patients, this decrease was not paralleled by statistically significant alterations in echocardiographic parameters (data not shown). The number of patients post IA decreased to n ¼ 4, since one of the 5 DCM patients who initially participated the study received a heart transplantation during the study period. *p < 0.05 vs. ICM, #p < 0.05 vs. DCM.

higher baseline VEGF serum levels in the group of DCM as compared to the patients in the group of ICM. Four weeks after the IA therapy we observed a sizeable decrease of serum VEGF levels in the group of DCM patients compared to the baseline levels (Fig. 8). At this point, the serum VEGF levels in both groups (DCM vs. ICM) showed nearly the same VEGF concentrations, without any significant differences in the above-mentioned echocardiographic parameters.

5.5. Pre-treatment of NRCM by DCM-IgG induce the expression of VEGF

In cell culture, we first investigated the dose response of DCM- IgG on NRCM with respect to apoptosis. We observed that the degree of apoptosis increased slightly beyond baseline levels at a DCM-IgG concentration of 500 ng/ml (Fig. 9).

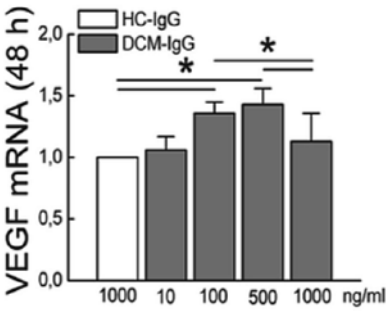


FIGURE 9. Dose dependent effect of DCM-IgG on the rate of apoptosis in NRCM.NRCM were incubated with healthy control (HC) IgG and DCM-IgG. Apoptosis was determined by TUNEL assay. At a dosage of 500 ng/ml DCM-IgG a slight increase in apoptosis could be observed, which became significant at a concentration of 1000 ng/ml. Pretreatment of NRCM with HC-IgG remained without effect on apoptosis. HC-IgG ¼ polyclonal IgG (Venimmun N) to restore IgG plasma levels, DCM-IgG ¼ IgG from patients with DCM. *p < 0.05 vs. HC- IgG and DCM-IgG 10, 100, 500 ng/ml. All n ¼ 5 cell preparations.

Thus, to avoid side effects due to cell death all further experiments were carried out with DCM-IgG concentrations not above a dosage of 100 ng/ml. At this concentration, NRCM did not show an increase in apoptosis, but a significant increase in VEGF expression, both on mRNA and protein levels (Fig. 10 A, B). At a DCM-IgG concentration beyond 500 ng/ml this effect was reversed, with VEGF levels receding to the range of control cells, possibly due to an increase in cell death.

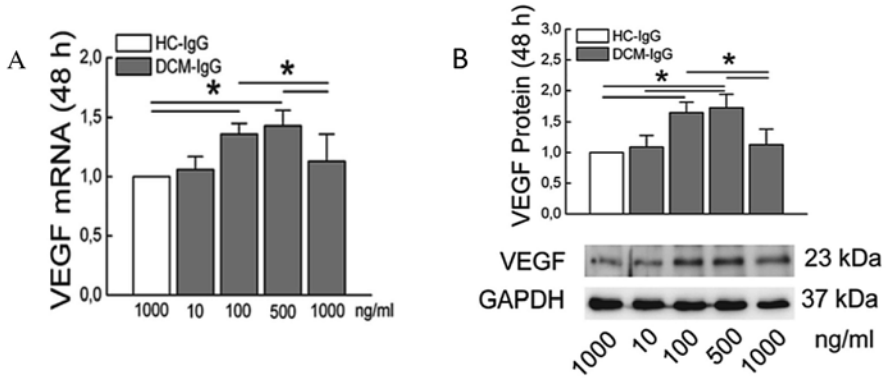


FIGURE 10 (A, B). The expression of VEGF is increased in NRCM due to DCM-IgG stimulation. Treatment of NRCM with DCM-IgG at a concentration of 100 ng/ml lead to an increase in VEGF expression both on mRNA and protein levels as compared to the group of NRCM treated with healthy control IgG at a concentration of 1000 ng/ml. At a DCM-IgG concentration of 1000 ng/ml VEGF levels decreased probably as a result of cell apoptosis.

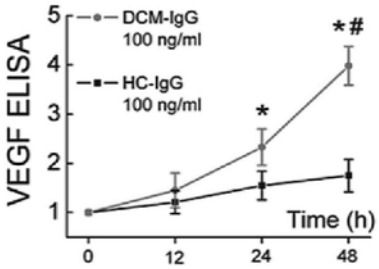


FIGURE 11. The time dependent increase in VEGF secretion due to DCM-IgG stimulation was determined by ELISA experiments. A significant increase in VEGF secretion could be observed after 24 h of 100 ng/ml DCM-IgG stimulation as compared to 0 h. After 48 h of 100 ng/ml DCM-IgG stimulation the increase in VEGF expression became also significant if compared to the control cell group, which was stimulated by HC-IgG at the same concentration of 100 ng/ml. A, B: *p < 0.05, C: *p < 0.05 vs. 0 h, #p < 0.05 vs. control cells. All n ¼ 5 cell preparations.

Using the ELISA technique the secretion of VEGF in the cell culture supernatant could be investigated in a time dependent manner. At a DCM-IgG concentration of 100 ng/ml, the increase in VEGF secretion became statistically significant after 24 h as compared to 0 h, and after 48 h this difference was significant as compared to the control cells treated by HC-IgG (Fig. 11). NRCM treated with 100 ng/ml of IgG from healthy donors did not show any relevant alteration of VEGF secretion during a period of 48 h.

5.6. Mechanical stretch, but not contraction rate increases VEGF expression in NRCM

DCM is accompanied by an increase in heart rate and mechanical overload. Therefore we analyzed the effects of both parameters on the expression of VEGF on a cellular basis. At a DCM-IgG con- centration of 100 ng/ml a significant increase in contraction rate of NRCM could be observed by microscopic examination (Fig. 12). Higher DCM-IgG concentrations resulted in a decrease in contraction rate, probably due to cell damage ultimately leading to apoptosis, whereas control cells that were incubated with equal IgG concentrations from healthy donors remained unaffected.

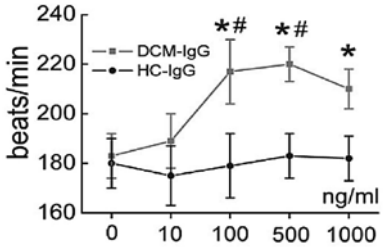


FIGURE 12. Mechanical stretch, but not contraction rate contributes to the increase in VEGF expression in NRCM. The contraction rate of cultured cardiac myocytes increased dose dependently with a maximum at 100 ng/ml of DCM-IgG stimulation.

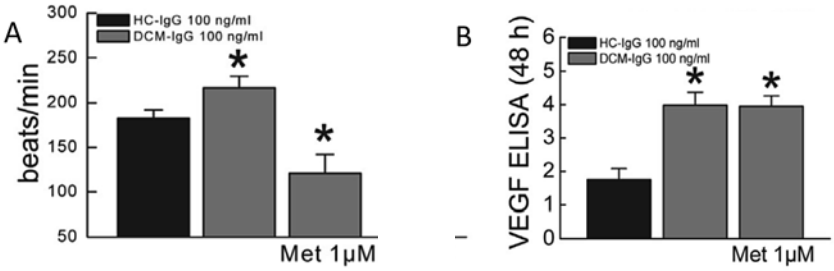


FIGURE 13. Blockade of the beta-1 adrenergic signalling by metoprolol decreased the contraction rate without any effect on VEGF expression (A, B).

Pretreatment of NRCM with metoprolol, a selective beta1-adrenergic receptor blocker, resulted in a decrease in contraction rate without any influence on VEGF expression (Fig. 13 A, B).

By contrast, mechanical stretch lead to an increase in NRCM VEGF expression (Fig. 14). Coincubation of stretched cells with DCM-IgG at a concentration of 100 ng/ml showed an additive effect on VEGF induction (VEGF protein expression; control: 1 ± 0.0 vs. stretch 13%: 1.75 ± 0.15 (P < 0.05) vs. stretch 13% þ 100 ng/ml DCM-IgG: 1.95 ± 0.23).

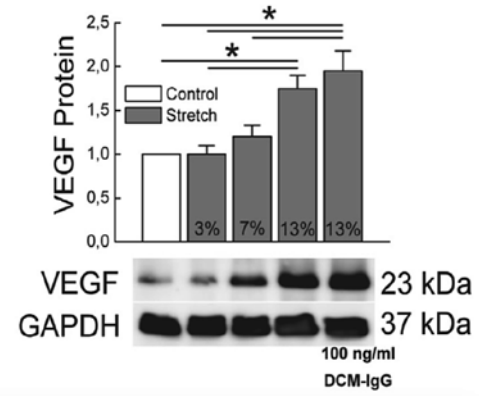


FIGURE 14. Mechanical stretch-induced VEGF induction was enhanced by DCM-IgG stimulation (D, right panel 13% stretch with 100 ng/ml DCM-IgG). Met 1⁄4 Metoprolol. A: *p < 0.05 vs. 0 ng/ml of DCM-IgG, #p < 0.05 vs. HC-IgG. B, C: *p < 0.05 vs. HC-IgG, #p < 0.05 vs. DCM-IgG. D: *p < 0.05. All n 1⁄4 5 cell preparations.

5.7. Arrhythmic stimulation increases NGF expression in ventricular myocytes

We first analyzed NGF mRNA expression in cardiomyocytes exposed to increasing frequency (0, 5 and 50 Hz) and irregular stimulation (5, 25 and 50% SD). At a constant rate of 5 Hz, we observed a slight but insignificant increase of NGF expression at a low degree of irregularity (5% SD). The increase in NGF mRNA expression became significant as irregularity rose to 25% SD (Fig. 15). During irregu-

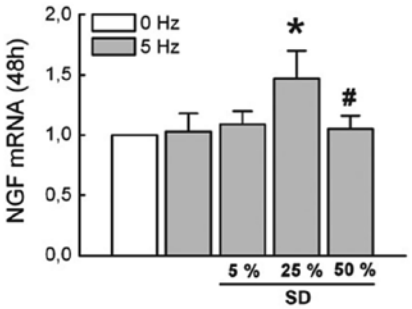


FIGURE 15. Expression of NGF in ventricular myocytes depends on stimulation rate and irregularity. Panel demonstrate that an irregular stimulation with 25% SD significantly increases NGF mRNA expression in ventricular myocytes.

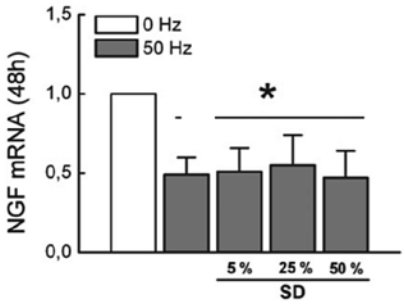


FIGURE 16. As frequency rises, NGF mRNA expression decreases in a significant manner. All PCR data are derived from at least n=3 experiments. *P<0.05 vs. 0 Hz. #P<0.05 vs. 5 Hz+25% SD or 50 Hz.

lar stimulation with 50% SD NGF mRNA expression returned to control levels. By contrast, at a constant rate of 50 Hz NGF mRNA expression decreased significantly independent of the applied irregularity. Variability up to 50% SD remained without effect during stimulation with 50 Hz (Fig. 16).

Next, we performed highly sensitive ELISA experiments with cardiomyocyte cell-conditioned medium to analyze the secretion of NGF in the cell culture supernatant. We found that the increase in NGF mRNA expression by 5 Hz at 25% SD was paralleled by higher NGF levels in the cell culture supernatant (Fig. 17). On the

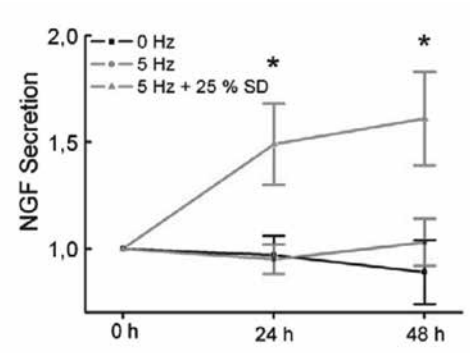


FIGURE 17. NGF mRNA increase is accompanied by a higher NGF secretion into the cell culture supernatant. ELISA experiments revealed higher NGF levels in the cell culture supernatant as irregularity rose to 25% SD at a constant rate of 5 Hz. These experiments also revealed time dependency. NGF increase showed slightly higher values at 48 h as compared to 24 h.

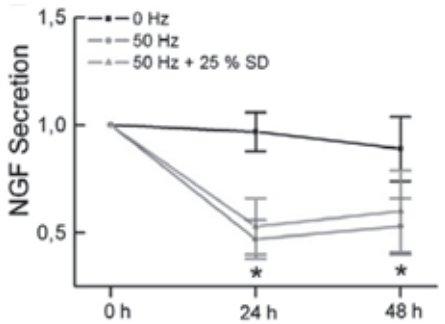


FIGURE 18. HFES with 50 Hz over a stimulation period of 48 h decreased NGF levels in the cell culture supernatant significantly. This response peaked at 24 h with a slight increase after 48 h of HFES stimulation. Irregularity with 25% SD showed no significant effect on NGF secretion. All ELISA data are derived from at least n = 3 experiments. *P < 0.05 vs. 0 Hz. #P<0.05 vs. 5 Hz+25 SD or 50 Hz+25% SD.

other hand, HFES with 50 Hz significantly decreased the amount of NGF in the cell culture supernatant (Fig. 18). This effect was not altered by irregularity. The ELISA data could be confirmed by Western blot experiments. Arrhythmic stimulation significantly increased NGF protein levels in cardiomyocytes.

5.8. The NGF increase during arrhythmic stimulation returns to control levels after cessation of the arrhythmia

Fig. 19 depicts cardiomyocytes in cell culture, double stained for troponin-t (red) and NGF (green). This image demonstrates the abundance of NGF that is expressed by ventricular myocytes. To analyze, whether the effect of arrhythmic pacing on NGF expression in cardiomyocytes is sustained after cessation of the irregular stimulation, we performed further experiments using an altered stimulation program. After an initially arrhythmic stimulation with 5 Hz at 25% SD for the first 24 h, we switched off the irregular stimulation and continued at 5 Hz without the variability of 25% SD for further 24 h. We found that the initial (first 24 h) increase in NGF expression under conditions of arrhythmic pacing returned almost to baseline levels after termination of the irregular stimulation (Fig. 20).

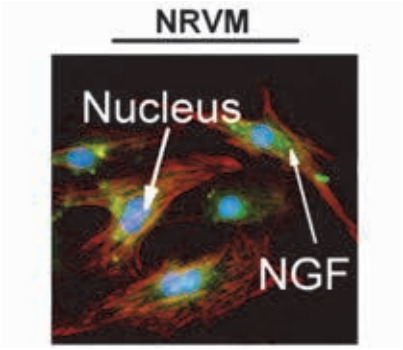


FIGURE 19. IF images showing NRVM with NGF expression and SCG cell cultures with GAP-43 expression. Panel A demonstrates the abundance of NGF expressed by ventricular myocytes.

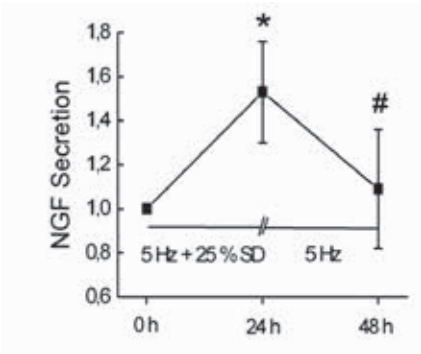


FIGURE 20. The levels of NGF in the cell culture supernatant of 5 Hz + 25% SD stimulated NRVM cells are sufficient to promote neurite outgrowth of cultured sympathetic neurons (C, D). ELISA and WB data are derived from at least n=3 cell preparations. *P<0.05 vs. 5 Hz+25% SD at 0 h (ELISA) or 5 Hz at 48 h (WB). #P<0.05 vs. 5 Hz+25 SD at 24 h (ELISA).

5.9. Increased NGF levels due to arrhythmic stimulation are sufficient to promote neurite outgrowth in sympathetic neurons

To determine whether the increase in cardiomyocyte NGF expression and secretion in terms of irregular stimulation is sufficient to in- duce neurite outgrowth in sympathetic neurons, primary cultures of sympathetic neurons were incubated for 48 h with cardiomyocyte cell-conditioned medium that were collected after 48 h of electrical stimulation. The higher amount in NGF protein levels, resulting from arrhythmic stimulation contributed to an increase in neurite outgrowth in a significant manner as shown by Immunocytofluorescence and Western blot experiments for GAP-43 protein expression, an established marker for neuronal growth (Fig. 21A, B).

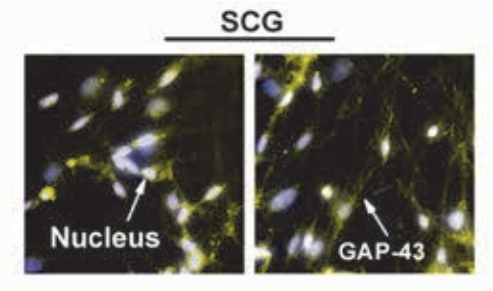


FIGURE 21 A. The levels of NGF in the cell culture supernatant of 5 Hz + 25% SD stimulated NRVM cells are sufficient to promote neurite outgrowth of cultured sympathetic neurons.

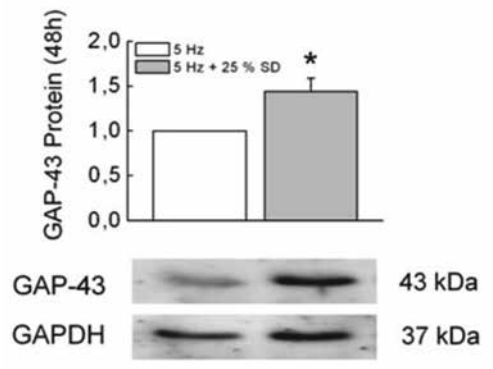


FIGURE 21B. WB data are derived from at least n=3 cell preparations. *P<0.05 vs. 5 Hz+25% SD at 0 h or 5 Hz at 48 h (WB).

5.10. Mechanical stretch increases neuronal VEGF expression

SN were exposed to a gradual increase of stretch as described in the method section. Stretch over a time period of 72 h resulted in a significant increase in VEGF gene expression (Fig. 22).

The higher VEGF mRNA content was paralleled by an increase in VEGF protein expression as confirmed by Western blotting (Fig. 23).

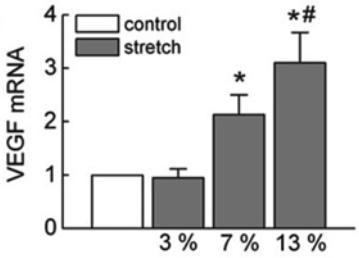


FIGURE 22. Mechanical stretch of SN increases VEGF mRNA and protein expression in a time and dose depended manner. SN were exposed to a gradual increase of mechanical stretch over a time period of 72 h. (A) Real-time PCR showed a time and dose depended increase in VEGF mRNA expression by stretch up to 13%.

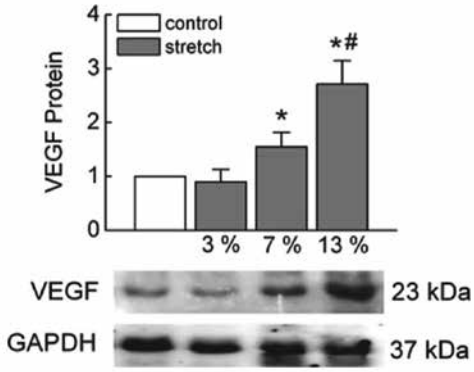


FIGURE 23. The increase in mRNA expression was paralleled by an almost 2,5-fold increase in VEGF protein expression after 72 h of stretch by 13%.

Morphological nerve sprouting due to stretch by 13% could be detected by microscopical images as shown in Fig. 24.

Using highly sensitive VEGF ELISA kits we further analyzed the secretion of VEGF in the cell culture supernatant of SN. These experiments revealed a time and dose dependency of stretch-induced neuronal VEGF expression. During the first 24 h of stretch by 3%, VEGF amounts remained on baseline levels. From 24 to 48 h of

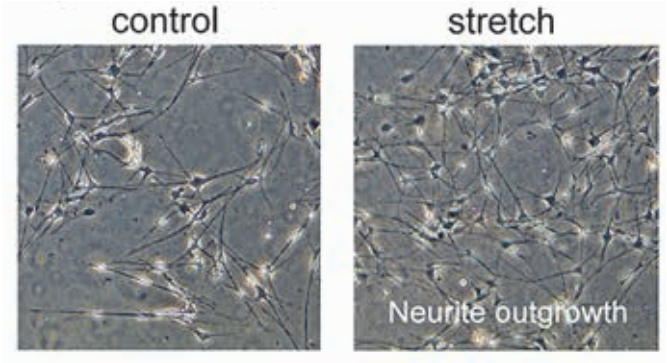


FIGURE 24. Microscopic images showing control and stretched neurons in cell culture.

stretch by 7% we observed a slight but insignificant increase in VEGF expression, which became highly significant after 72 h of stretch by 13% (data not shown).

5.11. Stretch-induced neuronal VEGF secretion is biologically active

To investigate whether neuronal VEGF secreted due to stretch shows a biological activity, we performed cell viability experiments with HCAEC as described in the method section. Conditioned medium from stretched neurons showed significant higher cell viability levels in HCAEC as compared to HCAEC incubated with conditioned medium from neurons not exposed to stretch (Fig. 25).

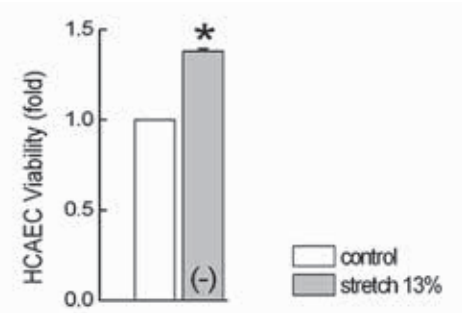


FIGURE 25. To demonstrate the biological activity of neuronal VEGF secreted in terms of mechanical stretch, endothelial cells from human coronary arteries (HCAEC) were incubated with SN cell-conditioned medium for 48 h.

6. Discussion

The principal findings of our study are (a) SES of cultured NRVMs elicits a significant increase in VEGF protein expression, (b) the dominant frequency of VEGF induction by SES was 25 Hz, (c) NRVM VEGF secretion due to SES is biologically active and promotes HCAEC proliferation in vitro.

The most dominant effect of SES on VEGF expression was present at 25 Hz with an almost twofold increase in extracellular VEGF protein levels. A frequency dependency of VEGF expression during in vitro SES was previously described in other cell lines with the optimal electrical frequency varying for different cell types, that is, 50 Hz for skeletal muscle cells and 24 Hz for smooth muscle cells (48). The exact mechanism by which HFES augments cellular protein expression is unclear. In myocardial or cardiac neuronal cells, the functional excitatory effects of electrical stimulation (e.g., muscle cell contraction or neuronal firing) depends on the membrane depolarization, which in turn is determined by its refractory period, with higher frequencies being typically effective in cardiac neuronal cells due to its shorter refractory periods. Trophic effects of SES have also been described in sympathetic

or parasympathetic cardiac neuronal cells (reference our group). High-frequency stimulation led to an increased NGF and NT-3 protein and gene expression with subsequent induction of neuronal cell growth and hypertrophy in vitro and in vivo (49). However, as opposed to the present study, this effect was dependent on neuronal cell membrane depolarization, since suprathreshold current was delivered, and the effect was blunted by lidocaine (50).

In this study, subthreshold electrical stimuli that were not capable of myocardial depolarization. Thus, different electrical effects than only cell membrane depolarization may be operative.

Most notably, SES seems to represent a ubiquitous mechanism by which cellular expression of growth factors can be enhanced. This is further supported by the observation of a frequency-dependent increase in insulin- like growth factor-2 protein and gene expression in osteosarcoma cells (51). In fact, this effect may not solely be restricted to the induction of growth factors, since an increase in constitutive NO synthase during SES of cardiomyocytes has also been described (48).

Theoretically, SES could increase the stability of VEGF protein. However, Kanno et al. showed that electrical stimulation did not affect the stability of VEGF mRNA. This is evidence that the augmentation of VEGF mRNA by electrical stimulation occurs predominantly at the transcriptional level. In addition, transient electrical stimulation (2 h vs. 24 h) led to almost identical VEGF mRNA levels indicating that the initial mRNA increase elicited by SES was sufficient to induce a sustained augmentation of VEGF protein synthesis. This is further sup- ported by the observation of a later onset of the increase in VEGF protein expression 12 h after initiation of SES with a subsequent maximum at 48 h (4).

In the present study, the VEGF mRNA amount was decreased after 48 h of SES despite an increase in VEGF protein. Since KDR receptor mRNA and protein were decreased, a negative feedback mechanism of increased VEGF protein levels on VEGF mRNA expression via downregulated KDR expression might be operative. In fact, Hang and colleagues have already shown that the VEGF mRNA expression gradually decreases during prolonged electrical stimulation and that VEGF-induced angiogenesis may be under a negative feedback control (52). Thus, if a limited exposure of SES is capable of inducing an increase in VEGF mRNA sufficient to induce a sustained VEGF protein expression, the refectory downregulation of VEGF mRNA expression may already occur within the 48-h stimulation period, thus yielding overall lower mRNA levels than in the control group.

Of note, a negative feedback loop secondary to SES - induced VEGF upregulation might counteract the beneficial effect of SES on therapeutic angiogenesis. Since, in a potential clinical application scenario, SES may only be applied on a short-term basis (e.g., via an intracardiac wire or catheter), the induction of angiogenesis may

still be sufficient for a clinically meaningful effect on revascularization. Such a hypothesis is supported by the present study in which a short-term SES exposure of 48 h led to a VEGF protein increase sufficient to induce a cellular growth in vitro.

There are several compelling studies showing the beneficial effects of chronically applied electrical field stimulation to various cell culture and animal models. A study by Shafy and colleagues demonstrated that chronic electrical stimulation via a cardiac resynchronization therapy device contributes to cell differentiation of autologous cultured myoblasts, injected into the infracted areas in a sheep model of myocardial infarction. The authors could show that this technical approach resulted in an improvement in myocardial function (53). Recently Haneef et al. furthermore demonstrated that electrostimulation of stem cells is a safe and effective approach for cell survival and differentiation into cardiac cells (54). Regarding the role of VEGF, Spadaccio and coworkers revealed in an in vivo model of myocardial infarction that continued electrical stimulation contributes to an improvement in myocardial function paralleled by an increase in angiogenesis through endothelial progenitor cell migration and VEGF production (43). These novel studies encourage the investigation of myocardial tissue engineering to develop new therapeutic tools for ischemic heart disease. Our results are completely in line with these findings. Moreover, in the field of new therapeutic approaches for the treatment of heart diseases, it is likely safer to have the opportunity to perform SES to avoid side effects like arrhythmias.

Autoimmune mediated angiogenic signaling has been described e.g. in systemic lupus erythematosus or myasthenia gravis (55, 56). In the latter, the authors could demonstrate a relevant positive correlation of VEGF levels in patients with myasthenia gravis as compared to healthy controls. Furthermore, in cancer research pro- and anti- angiogenic factors are known to be crucially involved in tumor progression and disease development (57, 58). VEGF is the main potent angiogenic factor responsible for the formation of new vessels. Up to now, the induction of VEGF in cancer cells is not fully understood. Recently, Lombardi and colleagues could convincingly demonstrate that breast cancer IgG increased the expression of VEGF in tumor cells. This response was promoted via muscarinic receptor signaling. These findings and our present results clearly demonstrate that autoantibodies in various diseases and pathological circumstances are potent inducers of growth factors, especially VEGF (59).

VEGF and its two receptors VEGFR-1 and VEGFR-2 play a pivotal role in angiogenesis under physiological or pathological circumstances and are involved in neuronal and myocardial development and morphogenesis (60). Studies in animal models revealed that even small perturbations in VEGF levels affect cardiovascular development and embryonic survival (61,62). Abnormal VEGF conditions may favor the initiation and progression of cardiac remodeling due to hypertrophy of

cardiomyocytes (11,12). Furthermore it appears of great importance that VEGF, which is released by cardiomyocytes enhances cardiac fibroblast proliferation in a paracrine manner (13). A pathological proliferation of fibroblasts may favor the progression of myocardial fibrosis that might further trigger VEGF induction due to reduction in oxygen tension as a result of microcirculatory disorders. This vicious cycle might perpetuate the progression of pathological remodeling driven by an undirected and non-physiological VEGF release. This consideration is further enhanced by the finding that mechanical overload, an ubiquitous stimulus in DCM, also leads to an increase in VEGF expression by cardiomyocytes that is further enhanced by such autoantibodies.

The present study extends the knowledge about the possible link between autoimmune signaling in DCM and VEGF induction in cardiomyocytes. This novel finding may contribute to new pharmacological approaches to modulate myocardial remodeling in DCM on a molecular basis.

NGF is not only the major neurotrophic factor for the survival and differentiation of sympathetic neurons, it even functions as a pro-survival factor for cardiomyocytes that regulates apoptosis (63) and plays a pivotal role in cardiac repair following myocardial infarction (64). Thus, NGF seems to be a double-edged sword, since pathological NGF regulation is also involved in nerve sprouting and sudden cardiac death (65). The mechanisms by which cardiac NGF expression is up- or downregulated are poorly understood despite its obvious crucial role in the neurophysiology of the heart. Recently, our group could show that mechanical stretch, an ubiquitous stimulus in cardiac tissue contributes to hypertrophy and NGF downregulation in cardiomyocytes via the Cn-NFAT pathway (66).

Stretch occurs in the cardiovascular system in different forms and degrees (cyclic stretch, static baseline stretch and stretch due to increasing shear forces). During MI or arterial hypertension intracardiac cells are exposed to increasing baseline stretch. Regarding neurotrophic regulation in terms of stretch, cardiomyocytes decrease NGF expression whereas SN respond to stretch with an upregulation of various neurotrophins including NGF (40, 41). In the present study we showed that stretch of SN goes along with VEGF induction in a time and dose dependent fashion. NGF signaling seem to play a considerable role in this scenario, whereas a significant influence of NT-3 or GDNF on neuronal VEGF induction could be ruled out. In addition, neuronal VEGF levels secreted under conditions of mechanical stretch showed a biological activity as confirmed by HCAEC viability experiments. As one cellular source, the single cardiomyocyte responds to stretch with an upregulation of VEGF (29). Since the autonomic nervous system of the heart plays an essential role in the regulation of diverse physiological and pathological phenomena, the knowledge that SN also possess the ability to produce and secrete VEGF is of obvious importance (34).

The sympathetic nervous system mediates its regulatory mechanisms on the cardiovascular system via postganglionic sympathetic neurons, which are innervating blood vessels and the heart. The vascular sympathetic innervation of vessels has a major impact on blood pressure and blood flow; hence alterations in vascular sympathetic innervation are associated with the development and maintenance of cardiovascular diseases (67). VEGF and its receptors have been reported to promote vascular sympathetic innervation (68, 69). Therefore, VEGF regulates not only the formation of collateral vessels as a potent angiogenic factor in terms of hypoxia or other biochemical and biomechanical forces, but also has a strong influence on the physiology of cardiovascular regulating systems.

NGF plays a pivotal role in different scenarios hence on its multifunctional properties. It is known to be the main neurotrophic factor regulating survival and differentiation of SN (70). Current studies identified NGF also as a pro-survival factor for cardiomyocytes (71). In addition, NGF promotes angiogenesis in ischemic hind limbs (10) and improves cardiac function after MI (39). These observations are likely due to a crosstalk regulation between NGF and VEGF. During MI intracardiac cells are exposed to increasing stretch. On the one hand, stretch contributes to NGF depletion in cardiomyocytes (41), whereas SN respond to stretch with an upregulation of various neurotrophins including NGF and CNTF (40). Whether stretch-induced neuronal VEGF expression plays a beneficial role in different in vivo scenarios like MI remains to be clarified.

Taken together, current studies indicate that VEGF has direct effects on the nervous system in terms of neuronal growth, survival and neuroprotection. Conversely, NGF, a neurotrophin that plays a crucial role in promoting neurotrophic effects in sympathetic neurons, has been identified as a novel angiogenic molecule exerting a variety of effects on endothelial cells and in the cardiovascular system in general. This crosstalk of neurotrophic effects of VEGF and angiogenic effects of NGF has been thoroughly reviewed by Lazarovici et al. (72).

More details of related scientific observations are provided and summarized at the provided original articles.

7. Conclusions

Cardiomyocytes stimulated by low-voltage electrical fields produce potent amounts of the angiogenetic factor VEGF, which has positive proliferation effects on HCAECs in vitro. This is a very potent step forward to the treatment of IHD. Angiogenesis due to SES may be one optional alternative treatment of CAD.

The IA therapy decreases elevated VEGF serum levels in patients with DCM. DCM-IgG induce VEGF expression in cultured NRCM. Stretch-induced VEGF production is further enhanced by such autoantibodies, whereas the increase in contraction rate due to DCM-IgG induced beta1-adrenergic stimulation does not seem to play a role in VEGF induction.

Cardiomyocytes control their local NGF under conditions of high rate or irregular stimulation.

SN respond to stretch with an increase in VEGF expression and secretion. Neuronal VEGF augmentation due to stretch shows a time and dose dependency.

8. Study limitations

We did not investigate the mechanisms by which SES - induced VEGF augmentation affected the decrease in VEGF mRNA (negative feedback loop). Future experiments using KDR receptor antibodies during SES and analysis of the intracellular signaling cascades leading to the downregulation of VEGF mRNA may further elucidate these mechanisms.

In the present study, we used an in vitro model of cultured CM from neonatal rats. The expression pattern of VEGF may vary significantly between neonatal and adult rat cardiomyocytes. In addition, we did not analyze the SES response in hypoxic myocytes, as they may be found in the infarction border zone.

Therapeutic angiogenesis after myocardial infarction remains a promising, but challenging, task for cardiovascular medicine. Its importance stems from the high morbidity and mortality due to sequels of occlusive CAD like arrhythmias and heart failure. Several attempts to induce angiogenesis have been investigated (like stem cells, local administration of recombinant angiogenetic growth factors: fibroblast growth factor, VEGF).

The current proof-of-concept study shows that SES is capable of boosting endogenous VEGF expression in ventricular myocytes, which in turn is able to induce proliferation of endothelial cells, thus potentially inducing angiogenesis. SES may be delivered via stimulating catheters positioned either at the endocardial site of the LV or via stimulating wires, which may be introduced into the coronary artery (e.g., after revascularization or even in nonoccluded small vessels not amenable to PCI) or accompanying venous vessels (coronary sinus tributaries). The fact that this approach does not rely on the introduction of cells or proteins in myocardial targets by injection or infusion and stimulation from a single electrode pair may cover a larger area makes this approach potentially attractive for further in vivo studies in animal models of CAD.

SANTRAUKA

Santrumpos

BSADEL – bipoliarinė stimuliacija aukšto dažnio elektros lauku

DKMP – dilatacinė kardiomiopatija

IA – imunoadsorpcija

IES – ikislenkstinė elektrinė stimuliacija

IŠL – išeminė širdies liga

KEAV – kraujagyslių endotelio augimo veiksnys

NAV – nervų augimo veiksnys

PKI – perkutaninė koronarinė intervencija

PV – prieširdžių virpėjimas

SSN – simpatinės sistemos neuronai

VAL – vainikinių arterijų liga

VKG – viršutiniai kaklo ganglijai

WB – *Western Blot*

ŽNSM – žiurkių naujagimių skilvelių miocitai

ŽVAEL – žmogaus vainikinių arterijų endotelio ląstelės

1. Įvadas

1.1. Tyrimo hipotezė

Ikislenkstinė elektrinė stimuliacija skatina angiogenezės vystymąsi dalyvaujant KEAV ir NAV baltymams.

1.2. Tyrimo tikslas

Įvertinti augimo veiksnių atsaką į įvairias stimuliavimo technikas neurokardiologijos srityje.

1.3. Tyrimo uždaviniai

Ištirti ikislenkstinės elektrinės stimuliacijos poveikį kraujagyslių endotelio augimo veiksnio reguliavimui dirbtinai užaugintuose žiurkių naujagimių skilvelių miocituose, siekiant atskleisti naujas terapinės angiogenezės technikas išeminės širdies ligos atvejais.

Įvertinti autoantikūnų vaidmenį dilatacinės kardiomiopatijos atvejais, indukuojant kraujagyslių endotelio augimo veiksnio raišką kardiomiocituose; įvertinti poveikį kontrakcijai ir kraujagyslių endotelio augimo veiksnio sekrecijai kaip ląstelių atsaką į tempimą.

Ištirti didelių dažnių, nereguliarios stimuliacijos poveikį kraujagyslių miocitams ir nervų augimo veiksniui.

Įvertinti simpatinės sistemos neuronų atsaką į mechaninį tempimą vertinant biologiškai aktyvias medžiagas: kraujagyslių endotelio augimo ir neuronų augimo veiksniai.

1.4. Tyrimo naujumas

Iki šių dienų atlikti tyrimai parodė, kad aukšto dažnio slenkstinė ar ikislenkstinė elektrinė stimuliacija gali sukelti biologiškai aktyvių molekulių gamybą ne širdies audiniuose. Šio tyrimo duomenys rodo, kad skilvelio miocitai gali gaminti bioaktyvius baltymus, turinčius teigiamos įtakos ląstelių proliferacijai ir gyvybingumui.

2. Literatūros apžvalga

Vainikinių arterijų ligos (VAL) sukelta išeminė širdies liga (IŠL) išlieka pagrindine mirties priežastimi Vakarų pasaulyje (1). Esamų arterijų revaskuliarizacija ir naujų kolateralinių kraujagyslių susidarymas gali būti perspektyvus strateginis tikslas, siekiant pagerinti organų su hipoksiniais pažeidimais funkciją. Pagrindinį vaidmenį angiogenezės ir naujų kraujagyslių susidarymo procese vaidina kraujagyslių endotelio augimo veiksnys (KEAV). Pateikiama vis daugiau įrodymų, kad KEAV sintezė vyksta širdyje ir jo raiška ženkliai padidėja esant hipoksijai ar cikliškam mechaniniam tempimui (2, 3). Šių (pato)fiziologinių stimulų tyrimai pastaruoju metu parodė, kad ikislenkstinė elektrinė stimuliacija (IES) padidina KEAV sintezę dirbtinai išaugintose skeleto raumenų ląstelėse. Be to, naudojant žiurkių užpakalinių galūnių išemijos modelį su KEAV, nustatyta, kad IES *in vivo* skatina kraujagyslių angiogenezę (4, 5). Kadangi IES gali būti taikoma hipoksiniam širdies audiniui *in vivo* (pvz., kateteriais ar vielomis), šio kontrolinio-klinikinio tyrimo metu buvo tiriamas IES poveikis KEAV sintezei dirbtinai išaugintuose žiurkių naujagimių skilvelių miocituose (ŽNSM).

Nustatyta, kad cirkuliuojantys autoantikūnai, veikiantys įvairias miokardo struktūras, yra pagrindinis dilatacinės kardiomiopatijos (DKMP) rizikos veiksnys (6). Pacientams su DKMP nustatyta padidėjusi kraujagyslių endotelio augimo veiksnio koncentracija kraujo serume ir tokio padidėjimo šaltinis nėra žinomas (7). Nors, remiantis daugelio tyrimų rezultatais, KEAV yra naudingas išeminės kardiomiopatijos atvejais dėl savo plačiai ištirto poveikio angiogenezei ir neovaskuliarizacijai (8-10), autorių žiniomis, duomenų apie teigiamą KEAV vaidmenį DKMP atvejais nėra. Priešingai, pastarųjų metų duomenys rodo, kad nefiziologinė KEAV ekspresija ir sekrecija gali būti žalinga dėl pleiotropinio poveikio visoms širdies remodeliacijoje dalyvaujančių ląstelių rūšims. Pateikiama vis daugiau duomenų, kad KEAV gali būti širdies transkripcijos reguliatorius, darantis esminį vaidmenį perduodant fiziologinės ir patologinės hipertrofijos signalus kardiomiocituose (11). Šiuo aspektu Zhou ir kt. galėtų įtikinamai pademonstruoti, kad KEAV yra lemiamas veiksnys, rodantis fenilefrino sukeltą kardiomiocitų hipertrofiją. (12). Tsoporis su kolegomis neseniai atlikti tyrimai taip pat atskleidė, kad kardiomiocitų sintezuojamas KEAV skatina širdies fibroblastų plitimą (13). Vis tik nėra aišku, ar šiuos skirtingus pastebėjimus galima prognostiškai panaudoti širdies remodeliacijai. Tačiau, kadangi pacientų su DKMP KEAV lygis yra padidėjęs, kyla klausimas, ar KEAV padidėjimas yra tik kompensacinis, ar tai yra nežinomu mechanizmu tiesiogiai susiję su širdies remodeliacija (14-16).

Dabartinės galimybės sustabdyti ar pakeisti širdies struktūros remodeliaciją esant širdies nepakankamumui yra ribotos. Nors beta1-adrenerginiai ar angiotenzino blokatoriai gali pa-

gerinti išgyvenamumą, bet jų terapinis taikomumas yra gana siauras dėl sukeliamo pašalinio poveikio, įskaitant bradikardiją ir (arba) hipotenziją (17,18). Pateikiama vis daugiau įrodymų, kad gydymas imunoadsorpcijos (IA) metodu ilgam pagerina širdies funkciją pacientams, kuriems nustatyta DKMP (19-22). Tačiau yra mažai tyrinėta, ar autoantikūnai DKMP atveju turi įtakos širdies KEAV ekspresijai ir ar nefiziologinė KEAV sekrecija gali skatinti remodeliacijos mechanizmus dėl hipertrofijos signalų ir širdies fibroblastų proliferacijos.

Teigiama, kad prieširdžių virpėjimas (PV) padidina mirtingumo riziką nuo 1,5 iki 1,9 karto (23). Prastesnes išeitis, tokias kaip arterijų embolija arba hemodinamikos sutrikimai, gali sąlygoti daugelis veiksnių. Pastaraisiais metais nustatyta, kad nereguliarus skilvelių atsakas PV metu padidina simpatinės nervų sistemos aktyvumą *in vivo* (24, 25). Todėl keltina hipotezė, kad nereguliari kardiomiocitų aktyvacija gali turėti neigiamos įtakos nervų augimui ir nervų sistemos aktyvumui. Struktūriniam ir funkciniam simpatinės sistemos neuronų vientisumui labai svarbus nervų augimo veiksnys (NAV), reguliuojantis išgyvenamumą, diferenciaciją ir aksonų ataugimą (26). Pavyzdžiui, pelių su „išjungtu“ NAV simpatinės sistemos ganglijų kiekis ženkliai sumažėja (27). Ir priešingai, per didelė KEAV ekspresija širdyje sukelia širdies inervacijos padidėjimą ir žvaigždinių mazgų (ganglijų) neuronų hiperplaziją (28). Molekuliniai mechanizmai, reguliuojantys širdies NAV sintezę, ir šiandien nėra visiškai aiškūs.

Nustatyta, kad mechaninis tempimas padidina kraujagyslių endotelio augimo veiksnio koncentraciją dirbinai išaugintuose miocituose (29). Apart biomechaninių jėgų, atsirandančių besivystančioje ar nesveikoje širdyje, audinių, tokių kaip plaučiai, skeleto raumenys, ląstelės ir netgi su neoplastine geneze susijusios ląstelės patenka į biomechaninę aplinką, kurioje vyksta normos ribas viršijantys tempimai (30-32). Mechaninė širdies perkrova prisideda prie KEAV padidėjimo per transformuojančio augimo veiksnio beta (**TAF-β)** signalų perdavimo kelius (33). Širdį visapusiškai inervuoja autonominė nervų sistema, įskaitant simpatinės ir parasimpatinės nervų sistemos nervines skaidulas, kontroliuojančias įvairius širdies reguliavimo mechanizmus. Taigi, atradimas, kad šie simpatinės sistemos neuronai (SSN) gali gaminti ir išskirti angiogeninius veiksnius, tarp jų ir KEAV, yra neabejotinai reikšmingas (34). Tačiau vis dar nėra išaiškinta, ar tempimas taip pat padidina ir neuroninę KEAV raišką.

Be daugialypio poveikio endotelio ląstelėms, dėl kurių susiformuoja naujos kolateralinės kraujagyslės, KEAV tiesioginis poveikis SSN taip pat yra daugialypis (35). Pavyzdžiui, nustatyta, kad nervų augimo veiksnys, išskirdamas KEAV, sukelia žiurkių naujagimių viršutinių kaklo ganglijų kapiliarų susidarymą (36). NAV sukeltas KEAV aktyvumo padidėjimas SSN yra susijęs su TrkA receptoriaus aktyvavimu (37). Apibendrinant, minėti rezultatai rodo, kad neuronų ląstelės gali reguliuoti savo pačių KEAV poreikį NAV ir TrkA signalų perdavimo keliais, priklausomai nuo ankstesnių autokrininiu ar parakrininiu būdu veikusių biomechaninių jėgų. Be to, nustatyta, kad NAV skatina angiogenezę (38) ir širdies funkcijos atsistatymą po miokardo infarkto (MI) (39). MI metu intrakardinės ląstelės vis labiau tempiamos. Tempimas yra svarbus neuronų NAV raiškos veiksnys (40), o kardiomiocitų atsakas į tempimą yra NAV aktyvumo slopinimas (41). Iki šiol nėra aišku, ar stebimas NAV signalų teigiamas poveikis, pasireškiantis didesniu sienelių įtempimu (pvz., MI atveju), atsiranda dėl tiesioginės NAV

funkcijos, ar tai tempimo sukelta NAV sekrecija skatina neuronų KEAV raišką autokrininiu ar parakrininiu būdu, taip skatindama naujų kraujagyslių susiformavimą ir širdies funkcijos padidėjimą.

3. Metodika

3.1. Žiurkių naujagimių skilvelių miocitų (ŽNSM) kultūros

Visi bandymai su gyvūnais buvo patvirtinti vietos ir valstybinio Gyvūnų tyrimo etikos komiteto (Aachen RWTH Universiteto ligoninė, Vokietija, TV-No. 10596 A). ŽNSM buvo atskirti ir auginami Simpson ir kt. anksčiau aprašytu būdu su nedideliais pakeitimais pagal Zobel ir kt. (42-44) protokolą. Atlikus stuburo slankstelių dislokaciją, paimtos 1-3 dienų amžiaus Sprague–Dawley žiurkių širdys, neatsižvelgiant į žiurkių lytį (Charles River, Sulzfeld, Vokietija) ir suskaidytos paveikiant kolagenaze II (1,0 mg/ml, 280 U/mg, C2-22; Biochrom, Berlynas, Vokietija), tripsinu (0,5 mg/ml, trypsin 1:250, L-11-002; PPA, Colbe, Vokietija) ir 1 proc. penicilino ir streptomicino tirpalu (P-4458; Sigma-Aldrich, Steinheim, Vokietija). Miocitai buvo atskirti nuo fibroblastų, naudojant Percoll tankio gradientą (P-1644; Sigma-Aldrich). Miocitai buvo išsėti į šešių duobučių plokšteles (indus) (140685; Nunc, Schwerte, Vokietija), esant 3×10^5 ląstelių tankiui duobutėje. Ląstelės buvo auginamos DMEM/Ham's F-12 + L-glutamino terpėje (FG-4815; Biochrom) su 10 proc. arklių serumu (B15-021; PPA) ir 5 proc. jaučio embriono kraujo serumu (A-15-101; PPA). Po 24 val. serumo terpė buvo pašalinta, ląstelės praplautos ir toliau laikomos DMEM/Ham's F-12 + L-glutamino terpėje be serumo.

Žmogaus vainikinės arterijos endotelio ląstelės (ŽVAEL) buvo užaugintos endotelio ląstelių augimo terpėje (C-22020; PromoCell GmbH, Heidelberg, Vokietija) prieš pradedant bandymus su NŽSM kondicionuota terpe. „PromoCell“ naudotas žmogaus ląstelių kultūrų izoliavimo audinys paimtas iš donorų, pasirašiusių asmens informavimo ir sutikimo formą (formą pasirašė patys donorai, jų įgalioti atstovai ar juridiniai atstovai), kurioje išsamiai aprašytas audinio aukojimo tikslas ir audinio apdorojimo procedūra.

3.2. Ikislenkstinė elektrinė stimuliacija (IES)

Praėjus trims dienoms po ląstelių izoliacijos, terpė su serumu buvo pakeista terpe be serumo ir NŽSM buvo stimuliuojami 48 val. ikislenkstine ir didelio dažnio bipoliarinės elektrinio lauko stimuliacijos būdu (ADES), naudojant C-PaceEP išorinio stimuliavimo sistemą (IonOptix, Wageningen, Nyderlandai). Impulso stiprumas (0,5 V/cm, impulso trukmė - 1 ms) pasirinktas taip, kad sukurtų elektrinį lauką, gerokai silpnesnį nei slenkstinė reikšmė, sukelianti išaugintų miocitų susitraukimą. Mažiausia stimuliavimo srovė įvairiuose preparatuose buvo 1.0 V/cm. Miocitų kontrakcijos nebuvimas buvo tikrinamas kas 12 val. bandymo metu, tikrinant pasėlius mikroskopu. Buvo pasirinkti dažniai: 5 Hz, 25 Hz, 50 Hz ir 99 Hz. Kontrolei naudotos ląstelės, kurioms netaikyta didelio dažnio elektrinė stimuliacija.

3.3. ELISA analizė

Atliekant KEAV imunofermentinę analizę (ELISA), ląstelių skaičius kiekvienoje duobutėje buvo normalizuotas iki 300 000 ir atlikta IES analizė, naudojant dažnius nuo 5 Hz iki 25 Hz, 50 Hz ir 99 Hz. Kondicionuota terpė buvo surinkta po 0, 24 ir 48 val. po IES ir ląstelių kultūros baltymas buvo koncentruotas „Sartorius" centrifugavimo kolonėlėse (VivaSpin) (Göttingen, Vokietija). Pavyzdžių analizė atlikta su „R&D Systems" KEAV ELISA rinkiniu (Minneapolis, MN, JAV), normalizavus baltymų kiekius pagal gamintojo instrukcijas. Baltymų kiekis nustatytas naudojant „Thermo Scientific" prietaisą NanoDrop 1000 (Wilmington, DE, JAV).

3.4. *Western Blot* metodas

KEAV ir KDR (kenazės domeno įterpimo receptoriaus) normalizuotų baltymų kiekių tyrimai atlikti *Western Blot* (WB) metodu. WB analizei atlikti ląstelės buvo homogenizuotos lizės buferiniame tirpale, turinčiame 20 mM Tris-HCl (pH 8,0; #9090.2; Carl-Roth, Karlsruhe, Vokietija), 1 proc. nejoninio detergento Nonidet P-40 (#74.385; Merck, Darmstadt, Vokietija) ir 10% glicerolio (#50405-1; Biomol, Hamburg, Vokietija). Ląstelių lizatai nuskaidrinti 20 min. centrifuguojant 17 000×g. Ekstraktai (100 µg) separuoti atliekant SDS-poliakrilamido gelio (10 proc.) (SDS #51430-2, Biomol/ polyacrylamide gel #3029.1; Carl-Roth) elektroforezę ir perkelti ant polivinilideno difluorido membranos (#RPN303F; GE-Healthcare Life Sciences, Freiburg, Vokietija). Membrana per naktį blokuota su 5 proc. galvijų serumo albuminu (#01400-1; Biomol) buferiniame fosfato druskų tirpale (#L-1825; Biochrom), turinčiame 0,1 proc. Tween 20 (PBST; #TW0020; Rockland, ME, JAV). Triušių anti-GAPDH pirminiai antikūnai (1:1 000; #2118; Cell Signaling, Danvers, MA, JAV), triušių anti-KEAV (1:150; 19003-1-AP; Acris Antibodies, Herford, Vokietija) ir triušių anti-KDR (200 µg/ml, 1:100; sc-315; Santa Cruz, Heidelberg, Vokietija) buvo inkubuojami per naktį. Blotai buvo perplauti 3 kartus su buferiniu fosfato druskų tirpalu, turinčiu 0,1 proc. Tween 20, ir 1 val. inkubuoti su anti-triušio antikūnais, gautais iš ožkos ir konjuguotais su krienų peroksidaze (GE Healthcare). Galiausiai, juostelės buvo išryškintos naudojant ECL Advance Western Blotting Detection Kit rinkinį (GE Healthcare Life Sciences) ir „Fujifilm" (Tokyo, Japonija) pažangų liuminescencinį vaizdo analizatorių LAS-3000. Atitinkama juostelių densitometrija atlikta „Fujifilm" kompiuterine programa Multi-Gauche V3.0.

3.5. Antikūnai

Ląstelės buvo užfiksuotos 4 proc. formaldehido tirpale (8.187.15; KMF, Lohmar, Vokietija), permeabilizuotos Triton X-100 (50800-1; Biomol) ir blokuojamos 1 proc. galvijų serumo albumino tirpale (01400-1; Biomol). Ląstelės buvo per naktį inkubuojamos su pirminiais antikūnais, (1:150) triušių anti-KEAV (200 µg/ml, 1:150; sc-507; Santa Cruz) ir pelių anti-Trop T-C (200 µg/ ml; sc-20025; Santa Cruz) 4°C temperatūroje, o po to 2 val. inkubuojamos su iš ožkos gautais anti-triušio (1:100) ir iš ožkos gautais anti-pelių (1:100) antriniais antikūnais

kartu su Alexa Fluor 488 (KEAV) (1 mg/ml; A11008; Invitrogen) arba Alexa Fluor 647 (Trop T-C) (1 mg/ml; A20990; Invitrogen). Branduoliai buvo užpilti 4',6-diamidino-2-fenilindolo dihidrochloridu (10236276001; Roche Mannheim, Vokietija). Fluorescenciniai signalai vizualizuoti Axiovert 200M mikroskopu, naudojant „Zeiss" (Jena, Vokietija) kompiuterinę programą AxioVision Rel. 4.5.

Pirminiai antikūnai, naudoti taikant *Western Blot* metodą: triušių anti-GAPDH (1:1,000; #2118; Cell Signaling), triušių anti-KEAV (1:150; 19003-1-AP; Acris Antibodies), triušių anti-KDR (200 μg/ml, 1:100; sc-315; Santa Cruz).

3.6. RNR paruošimas, kopijinės DNR (kDNR) pirmos grandies sintezė ir realaus laiko kiekybinė atvirkštinės transkripcijos PGR

Bendroji RNR išskirta iš ŽNSM Qiagen RNeasy Mini Kit rinkiniu (Venio, Nyderlandai), laikantis gamintojo instrukcijų. 1 μg RNR atvirkštinė transkripcija atlikta „Fermentas" kDNR pirmos grandies sintezės rinkiniu, naudojant atsitiktinius heksamerus (#K1622; Fermentas GmbH, St. Leon- Rot, Vokietija).

3.7. Realaus laiko kiekybinė atvirkštinė transkripcija - PGR. Realaus laiko kiekybinės atvirkštinės transkripcijos - PGR pradmenys ir zondai

Realaus laiko polimerazinė grandininė reakcija (PGR) buvo vykdoma 96 duobučių PGR plokštelėse, taikant pirmiau aprašytą Step-One plus sekos aptikimo sistemą (ABI) (9). Duomenys buvo renkami spektrinės kompensacijos metodu, naudojant „Applied Biosystems" kompiuterinę programą SDS 1.2.3 (Applied Biosystems, Foster City, CA, JAV).

PGR pradmenys ir fluorogeniniai zondai tiksliniams genams ir endogeninei kontrolei buvo perkami kaip užsakomieji tyrimai (Applied Biosystems). Endogeninės kontrolės ir tikslinių genų tyrimų numeracija: Rn00560865_m1 (b-2 microglobulin), Rn01533872_m1 (Ngfb), Rn00561661_m1 (Nppa). Tyrimų numeriai: Rn01511602_m1 (KEAV), Rn00564986_m1 (KDR), Rn00560865_m1 (b-2 microglobulin).

3.8. Žmogaus vainikinės arterijos endotelio ląstelių proliferacijos tyrimas

Siekdami nustatyti ŽVAEL proliferaciją dėl NŽSM, kuriems taikyta IES, išskiriamo bioaktyvaus KEAV, mes naudojome „PromoCell" kolorimetrinės analizės rinkinį (WST-8). WST-8 yra ląstelių dehidrogenazių biologiškai redukuojamas iki oranžinės spalvos formazano junginio, kuris tirpsta ląstelių pasėlių terpėje. Kadangi formazano kiekis yra tiesiogiai proporcingas gyvų ląstelių skaičiui, šis metodas leidžia tiksliai nustatyti gyvybingų ląstelių skaičių ląstelių pasėlyje.

ŽVAEL buvo inkubuojamos 48 val. NŽSM kondicionuotoje terpėje. 25 Hz ir 5 Hz dažniai buvo analizuoti, kadangi dominuojantis KEAV indukcijos poveikis stebėtas taikant 25 Hz dažnį (lyginant su 5 Hz dažniu ir kontrole).

3.9. Pacientų su DKMP atranka, mechaninis tempimas ir Tunel analizės metodas

Pacientams su DKMP (n 1⁄4 5, amžiaus vidurkis 50,3 ± 3,5 m.), kuriems pasireiškia ryški kairiojo skilvelio disfunkcija (KSIF < 30%, Niujorko širdies asociacijos (NYHA) II funkcinė klasė), buvo taikoma IA terapija penkias dienas iš eilės. Po paskutinio IA seanso visiems pacientams buvo skirtas polikloninis IgG (0,5 g/kg) koncentracijai kraujo plazmoje atstatyti (45). Tokie patys atitinkamos koncentracijos polikloniniai IgG buvo naudoti ląstelių pasėlių kontrolės tikslais ir yra nurodyti atitinkamose diagramose kaip sveiki kontroliniai IgG (SK-IgG). Visiems pacientams su DKMP yra buvęs širdies nepakankamumas prieš vienerius metus ar daugiau metų ir jie visi ne trumpiau nei 6 mėn. stabiliai vartojo geriamuosius vaistus, įskaitant angiotenziną konvertuojančių fermentų inhibitorius ar angiotenzino receptorių blokatorius, diuretikus, aldosterono antagonistus ir **β**-blokatorius. Pacientai, kuriems atlikus angiografiją buvo nustatyta išeminė liga, į tyrimą buvo neįtraukti. IA terapijos metu pacientams buvo skiriamos A baltymas Immunoadsorba (Fresenius Medical Care AG, Bad Hombug, Vokietija). IA eliuentas (KE) su išplautu IgG buvo surinktas ir paruoštas kituose šaltiniuose aprašytiems ląstelių pasėlių bandymams (22). KE baltymo koncentracijai nustatyti naudotas prietaisas Nanodrop ND-1000 (PeqLab, Erlangen, Vokietija). KE alikvotinės dalys iki naudojimo buvo laikomos 80°C temperatūroje. Pacientų su DKMP IgG koncentracija serume buvo stebima ir nustatytas IgG koncentracijos redukcijos vidurkis buvo 91,7 proc., matuojant penktą dieną po paskutinio IA terapijos seanso. Pacientų su išemine kardiomiopatija (IKMP) (n 1⁄4 7) išmetimo frakcijos, KS dydžiai ir NYHA funkcinės klasės parametrai buvo panašūs į pacientų su DKM parametrus (duomenys nepateikti).

Mechaniniam tempimui atlikti NŽKM buvo inkubuojami ant kolagenu dengtų silikoninių membranų ir atliktas prisitvirtinusių miocitų statinis tempimas, palaipsniui didinant tempimą, kaip aprašyta pirmiau (46). Tempimas buvo laipsniškai didinamas kas 24 val. nuo 3 proc. iki 13 proc. (3, 7, 13 proc. laipsniais).

TUNEL analizė buvo atlikta „APO-BrdU“ TUNEL analizės rinkiniu pagal gamintojo instrukcijas. Ląstelių apoptozė buvo nustatyta suskaičiavus TUNEL-teigiamų branduolių skaičių, išreikštą procentu nuo visų suskaičiuotų ląstelių (47).

3.10 Statistinė analizė

Visos reikšmės išreikštos vidurkiu ± SN. Duomenys apdoroti naudojant statistinį duomenų analizės paketą SPSS 14.0 for Windows (IBM, Somers, NY, JAV). Statistinis skirtumų reikšmingumas įvertintas pagal porinį Stjudento t kriterijų ir vieno faktoriaus analizę ANOVA, atliekant post-hoc mažiausio reikšmino skirtumo palyginimus tarp įvairių grupių. Rezultatas laikomas statistiškai reikšmingu, kai $p < 0,05$.

4. Rezultatai

4.1. IES padidina žiurkių naujagimių KEAV ekspresiją ir sekreciją

Siekiant nustatyti IES poveikį kardiomiocitų KEAV ekspresijai, ŽNSM ląstelių kultūra buvo stimuliuojama 48 val. 5 Hz, 25 Hz, 50 Hz ir 99 Hz dažniais. KEAV baltymų kiekis ląstelių pasėlių terpėje matuotas po 24 ir 48 stimuliavimo valandų. Didelio dažnio elektrinė stimuliacija (DDES) sukėlė KEAV ekspresijos padidėjimą tiek citoplazmoje, tiek ir ląstelių išorėje. IES dominuojantis efektas stebėtas taikant 25 Hz dažnį, kai KEAV baltymų kiekis citoplazmoje padidėjo 1,8 karto. KEAV sekcija į ląstelės išorę taip pat reikšmingai padidėjo, sudarant beveik varpo formos dozės-efekto kreivę, kurios aukščiausias taškas pasiektas taikant 25 Hz dažnį. DDES poveikis KEAV ekspresijai išliko ne mažiau kaip 48 val., nepriklausomai nuo stimuliacijos dažnio. Skirtingi KEAV baltymo kiekiai matomi imunocitofluorescenciniuose vaizduose.

Mes atlikome ląstelių lizatų KEAV informacinės RNR (iRNR) turinio analizę, kad įvertintume padidėjusios KEAV genų ekspresijos santykinį poveikį padidėjusiai KEAV baltymų raiškai.

4.2. KEAV iRNR ir KEAV receptoriaus pokyčiai DDES ŽNSM metu

DDES sukėlė ženklų KEAV iRNR sumažėjimą, kuris buvo statistiškai reikšmingas taikant 25 Hz, 50 Hz, 99 Hz dažnius ir maksimalus esant 25 Hz dažniui. Kadangi taip galėjo atsitikti dėl neigiamo grįžtamojo poveikio atsako į padidėjusius KEAV baltymo kiekius tiek citoplazmoje, tiek ir ląstelės išorėje, mes išmatavome membranos KEAV receptoriaus (KDR) raišką tiek baltymų, tiek ir iRNR atžvilgiu. IES reikšmingai sumažino KDR receptoriaus baltymų raišką esant 25 Hz dažniui ir nereikšmingai - taikant 50 Hz ir 99 Hz dažnius, tuo tarpu kai KDR receptoriaus iRNR reikšmingai padidėjo IES metu taikant 25 Hz, 50 Hz ir 99 Hz dažnius. Pažymėtina, kad KEAV iRNR ekspresijos ir KDR baltymų bei genų ekspresijos santykinio sumažėjimo priklausomybė nuo dažnių buvo tokia pati, kaip ir KEAV baltymų indukcijos atveju, t.y. reikšmingiausias dydis stebėtas taikant 25 Hz dažnį.

4.3. IES indukuota KEAV ekspresija yra biologiškai aktyvi ir skatina žmogaus vainikinių arterijų endotelio ląstelių proliferaciją

Siekiant nustatyti, ar stebimas IES indukuotas KEAV veiksmingai skatina endotelio ląstelių proliferaciją, ŽVAEL buvo inkubuojamos NŽSM kondicionuotoje terpėje (IES trukmė 48 val., atitinkamai taikant 5 Hz, 25 Hz, 50 Hz ir 99 Hz dažnius). Tyrimo metu stebėta, kad terpėje, kondicionuotoje 25 Hz dažnio IES, ŽVAEL augimas padidėjo, o 5 Hz dažnio IES kondicionuotoje terpėje reikšmingo atsakomojo ŽVAEL augimo *in vitro* nestebėta.

4.4. Pacientų su DKMP padidėjusi KEAV koncentracija serume sumažėja po IA terapijos

Kadangi padidėjęs širdies sienelės tempimas gali turėti įtakos KEAV raiškai, pacientų, sergančių DKMP, KEAV koncentracija serume buvo palyginta su pacientais, kuriems diagnozuota išeminė kardiomiopatija (IKMP). Reikšmingų kairiojo skilvelio išstūmimo frakcijos bei KS galinio sistolinio ir galinio diastolinio diametrų skirtumų tarp dviejų grupių nenustatyta (duomenys nepateikti). Nedidelėje mūsų pacientų su DKMP grupėje pradinė KEAV koncentracija serume buvo žymiai didesnė, lyginant su pacientais IKMP grupėje. Po keturių savaičių IA terapijos buvo stebėtas reikšmingas KEAV koncentracijos serume sumažėjimas pacientų DKMP grupėje, lyginant su pradine koncentracija. Šiame etape KEAV koncentracija serume abejose grupėse buvo beveik vienoda (lyginant DKMP grupę su IKMP grupe), o reikšmingų aukščiau minėtų echokardiografinių skirtumų nenustatyta.

4.5. Pirminis ŽNKM veikimas DKMP-IgG skatina KEAV raišką

Tirdami ląstelių kultūrą, pirmiausia buvo siekiama išsiaiškinti su DKMP-IgG doze susijusią ŽNKM reakciją apoptozės atžvilgiu. Pastebėjome, kad apoptozės lygis šiek tiek padidėjo, lyginant su pradiniu lygiu, kai DKM-IgG koncentracija buvo 500 ng/ml. Taigi, siekiant išvengti šalutinio poveikio dėl ląstelių žūties, visi tolimesni bandymai buvo vykdomi naudojant DKMP-IgG, kurių koncentracija neviršijo 100 ng/ml. Esant tokiai koncentracijai ŽNKM apoptozė nepadidėja, tačiau reikšmingai padidėja KEAV raiška iRNR ir baltymo lygmenyse. DKMP-IgG koncentracijai viršijus 500 ng/ml, stebėtas atvirkštinis poveikis, kai KEAV koncentracija sumažėjo iki kontrolinių ląstelių ribų galimai dėl padidėjusios ląstelių žūties.

Taikant ELISA metodą, galima ištirti KEAV sekreciją ląstelių išorėje priklausomai nuo laiko. Kai DKMP-IgG koncentracija yra 100 ng/ml, KEAV sekrecijos padidėjimas tampa statistiškai reikšmingas po 24 val., lyginant su 0 val., o po 48 val. šis skirtumas yra reikšmingas lyginant su kontrolinėmis ląstelėmis, kurios buvo veikiamos SK-IgG. ŽNKM, kurie buvo veikiami 100 ng/ml koncentracijos sveikų donorų IgG, po 48 val. intervalo nerodė jokių reikšmingų KEAV sekrecijos pokyčių.

4.6. KEAV raiška ŽNKM padidėja ne dėl susitraukimo dažnio, bet dėl mechaninio tempimo

Sergant DKMP, padidėja širdies susitraukimų dažnis ir mechaninė perkrova. Todėl mes tyrėme, kokią įtaką šie du parametrai daro KEAV ekspresijai ląstelių lygmenyje. Kai DKMP-IgG koncentracija 100 ng/ml, mikroskopu stebimas ŽNKM susitraukimo dažnio padidėjimas. Didinant DKMP-IgG koncentraciją, susitraukimų dažnis mažėja, tikėtina, dėl ląstelių pažeidimo ir galutinės apoptozės, o kontrolinės ląstelės, kurios buvo inkubuojamos su tokios pačios koncentracijos sveikų donorų IgG, lieka nepažeistos. ŽNKM, kurie prieš tai buvo paveikti selektyviu beta1-adrenerginių receptorių antagonistu - metoprololiu, susitraukimų dažnis sumažėjo be jokio poveikio KEAV ekspresijai. Ir priešingai, mechaninis tempimas

padidina ŽNKM KEAV raišką. Inkubuojant ištemptas ląsteles su 100 ng/ml koncentracijos DKMP-IgG, pasiektas papildomas KEAV indukcijos efektas (KEAV baltymo raiška; kontrolė: 1 ± 0,0, palyginus su 13% tempimu: 1,75 ± 0,15 ($P < 0,05$), palyginus su 13% tempimu þ 100 ng/ml DKM-IgG: 1,95 ± 0,23).

4.7. Nereguliarus stimuliavimas padidina skilvelių miocitų NAV ekspresiją

Buvo analizuota NAV iRNR raiška kardiomiocituose, didinat stimuliacijos dažnį (0 Hz, 5 Hz ir 50 Hz) ir stimuliuojant nereguliariai (5, 25 ir 50 proc. SN). Taikant pastovų 5 Hz dažnį, stebėtas nedidelis, statistiškai nereikšmingas NAV ekspresijos padidėjimas, esant nedideliam nereguliarumo laipsniui (5 proc. SN). NAV iRNR ekspresijos padidėjimas tapo reikšmingas, kai nereguliarumo laipsnis pasiekė 25 proc. SN. Kai nereguliari stimuliacija pasiekė 50 proc. SN, NAV iRNR raiška vėl sumažėjo iki kontrolės lygio. Tuo tarpu esant pastoviam 50 Hz dažniui, NAV iRNR raiška reikšmingai sumažėjo, nepriklausomai nuo stimuliavimo nereguliarumo. Stimuliuojant 50 Hz dažniu ir keičiant nereguliarumo laipsnį iki 50 proc. SN, jokio poveikio nenustatyta.

Siekiant įvertinti NAV sekreciją ląstelių išorėje, buvo atlikti tyrimai ELISA metodu. Didžiausias poveikis buvo stebėtas taikant 5 Hz dažnį su 25 proc. SN. Kita vertus, taikant 50 Hz ADES, NAV lygis reikšmingai sumažėja. Šiam poveikiui nereguliarumas įtakos neturėjo. ELISA metodu gautus duomenis patvirtina bandymai *Western Blot* metodu. Neritmiška stimuliacija reikšmingai padidina NAV baltymų kiekį kardiomiocituose.

4.8. Atliekant nereguliarią stimuliaciją, NAV padidėjimas grįžta į kontrolinį lygį stimuliaciją nutraukus

Siekiant nustatyti, ar neritmiškos stimuliacijos poveikis NAV ekspresijai kardiomiocituose išlieka nutraukus nereguliarią stimuliaciją, buvo atlikti tyrimai, keičiant stimuliavimo programą. Po pradinės neritmiškos stimuliacijos, kurios metu buvo taikytas 5 Hz dažnis su 25 proc. SN pirmas 24 val., vėliau stimuliacija buvo tęsiama ne be 25 proc. SN. Tyrimas parodė, kad nereguliarios stimuliacijos sukeltas pradinis NAV ekspresijos padidėjimas (per pirmas 24 val.) beveik grįžo iki pradinio kontrolinio lygmens nutraukus nereguliarią stimuliaciją.

4.9. NAV koncentracijos padidėjimas dėl neritmiško stimuliavimo yra pakankamas simpatinės sistemos aksonų ataugimui skatinti

Siekiant nustatyti, ar kardiomiocitų NAV ekspresijos ir sekrecijos padidėjimas, pasiektas atliekant neritmišką stimuliavimą, yra pakankamas, kad skatintų simpatinės sistemos aksonų ataugimą, pirminiai simpatinės sistemos neuronų pasėliai buvo inkubuojami 48 val. kardiomiocitų ląstelėmis kondicionuotoje terpėje, paimtoje po 48 val. elektrinės stimuliacijos. Neritmiško stimuliavimo sukelta didesnė NAV baltymo koncentracija reikšmingai padidino aksonų ataugimą, nustatytą atliekant baltymo GAP-43 (neuronų augimo markerio) raiškos bandymus imunocitofluorescenciniu ir *Western Blot* metodu.

4.10. Mechaninis tempimas padidina neuronų KEAV raišką

SSN tempimas buvo laipsniškai didinamas, kaip aprašyta skyriuje „Metodika". KEAV genų raiška reikšmingai padidėjo po 72 val. tempimo. Bandymai *Western Blot* metodu patvirtino, kad kartu su KEAV iRNR koncentracija padidėja ir KEAV baltymų raiška. Naudodami didelio jautrumo tyrimų rinkinius ELISA, buvo ištirta KEAV sekrecija SSN ląstelių išorėje. Šie bandymai parodė, kad tempimo indukuota neuronų KEAV raiška priklauso nuo laiko ir dozės. Per pirmas 24 val. tempiant 3 proc., KEAV kiekis liko pradiniame lygmenyje. Nuo 24 val. iki 48 val. tempiant 7 proc., stebėtas nereikšmingas KEAV ekspresijos padidėjimas, kuris tapo statistiškai reikšmingu po 72 val. 13 proc. tempimo (duomenys nepateikiami).

4.11. Tempimo sukelta neuronų KEAV sekrecija yra biologiškai aktyvi

Siekiant nustatyti, ar tempimo sukelta neuronų KEAV sekrecija yra biologiškai aktyvi, buvo atlikta ląstelių gyvybingumo bandymus su ŽVAEL „Metodikos" skyriuje aprašytu būdu. Kondicionuotoje ištemptų neuronų terpėje buvo stebėtas ženkliai didesnis ląstelių gyvybingumas ŽVAEL, lyginant su ŽVAEL inkubuotų neuronų, kuriems tempimas netaikytas, terpėje.

5. Rezultatų aptarimas

Pagrindiniai mūsų tyrimo rezultatai yra: a) IES reikšmingai padidina išaugintų ŽNSM KEAV baltymų raišką, b) dominuojantis IES poveikis KEAV raiškai stebėtas taikant 25 Hz dažnį, c) IES indukuota ŽNSM KEAV sekrecija yra biologiškai aktyvi ir skatina ŽVAEL proliferaciją *in vitro*.

Stipriausias IES poveikis KEAV raiškai stebėtas taikant 25 Hz dažnį, kuomet ekstraląstelinė KEAV baltymų koncentracija padidėjo beveik dvigubai. KEAV ekspresijos priklausomybė nuo dažnio, atliekant IES *in vitro*, buvo aprašyta anksčiau, atliekant kitų ląstelių junginių bandymus, t.y. taikant 50 Hz dažnį skeleto raumenų ląstelėms ir 24 Hz dažnį lygiųjų raumenų ląstelėms (48). Tikslus mechanizmas, kaip DDES padidina ląstelių baltymų raišką, nėra aiškus. Miokardo ar širdies neuronų ląstelėse funkcinis elektrostimuliacijos dirginamasis poveikis (pvz., raumenų ląstelių susitraukimas arba neuronų iškrova) priklauso nuo membranos depoliarizacijos, kurią, savo ruožtu, lemia refrakterinis laikotarpis. Todėl aukštesnių dažnių, kurių refrakterinis laikotarpis yra trumpesnis, poveikis širdies neuronų ląstelėms paprastai būna stipresnis. IES trofinis poveikis taip pat buvo aprašytas, atliekant simpatinės ir parasimpatinės sistemos neuronų ląstelių bandymus (žr. mūsų grupę). Aukštų dažnių stimuliacija padidino NAV, baltymo NT-3 ir genų ekspresiją, kuri, savo ruožtu, paskatino neuronų ląstelių augimą ir hipertrofiją *in vitro* ir *in vivo* (49). Tačiau, priešingai nei šiame tyrime, minėtas poveikis priklausė nuo neuronų ląstelių depoliarizacijos, kadangi buvo naudojama ikislenkstinė srovė ir poveikį slopino lidokainas (50).

Šiame tyrime ikislenkstinė elektros stimuliacija negalėjo sukelti miokardo depoliarizacijos, todėl dėl skirtingo elektros srovės poveikio galėjo vykti tik ląstelių membranos depoliarizacija.

Ypač atkreiptinas dėmesys, kad IES yra universalaus pobūdžio mechanizmas, galintis padidinti ląstelių augimo veiksnio raišką. Šį teiginį taip pat patvirtina nuo dažnio priklausantis į insuliną panašaus augimo veiksnio–2 baltymų ir genų ekspresijos padidėjimas osteosarkomos ląstelėse (51). Minėtas poveikis neapsiriboja vien tik augimo veiksnių indukcija, kadangi šaltiniuose taip pat yra aprašyta didesnė nuolatinė azoto oksido sintazė IES metu (48).

Teoriškai, IES galėtų padidinti KEAV baltymo stabilumą. Tačiau, Kanno ir kt. atlikti tyrimai parodė, kad elektrinė stimuliacija neturėjo poveikio KEAV iRNR stabilumui. Tai įrodo, kad KEAV iRNR padidėjimas dėl elektrinės stimuliacijos iš esmės vyksta transkripciniame lygmenyje. Be to, atlikus stimuliaciją nenusistovėjusiu elektriniu lauku (2 val., plg. su 24 val.) gautas beveik identiškas KEAV iRNR lygis, kas rodo, kad pradinio IES sukelto iRNR padidėjimo pakako indukuoti ilgalaikį KEAV baltymų sintezės padidėjimą. Šią išvadą taip pat patvirtina pastebėjimai, kad KEAV baltymų ekspresija pradeda didėti vėliau, po 12 val. nuo IES pradžios, o maksimalus padidėjimas stebėtas po 48 val. (4).

Šiame tyrime KEAV iRNR lygis sumažėjo po 48 val. IES, nors KEAV baltymo koncentracija padidėjo. Kadangi KDR receptoriaus iRNR ir baltymai sumažėjo, galėjo suveikti padidėjusios KEAV baltymų koncentracijos neigiamo grįžtamojo ryšio KEAV iRNR ekspresijai mechanizmas (per KDR kiekio sumažėjimą). Iš tiesų, Hang su kolegomis jau nustatė, kad KEAV iRNR raiška palaipsniui mažėja tęsiant elektrinę stimuliaciją ilgą laiką ir kad KEAV indukuota angiogenezė gali priklausyti nuo neigiamo grįžtamojo ryšio (52). Taigi, jei riboto IES taikymo sukelto KEAV iRNR padidėjimo pakanka ilgalaikei KEAV baltymų raiškai indukuoti, reflektorinis KEAV iRNR raiškos mažėjimas gali prasidėti jau per 48 val. stimuliacijos laikotarpį ir todėl bendras iRNR lygis bus žemesnis nei kontrolinėje grupėje.

Pažymėtina, kad IES indukuoto KEAV padidėjimo antrinis neigiamas grįžtamasis ryšys gali neutralizuoti teigiamą IES poveikį terapinei angiogenezei. Kadangi pagal galimą klinikinio pritaikymo scenarijų IES gali būti vykdoma tik trumpą laiką (pvz., intrakardialiniais kateteriais), angiogenezės indukcijos gali pakakti klinikiniu požiūriu reikšmingam revaskuliarizacijos poveikiui pasiekti. Šią hipotezę patvirtina ir mūsų atliktas tyrimas, kurio metu trumpalaikė 48 val. IES sukėlė KEAV baltymų padidėjimą, kurio pakako ląstelių augimui indukuoti *in vitro*.

Kai kurių tyrimų rezultatai įtikinamai rodo teigiamą sistemingai taikomos elektrinio lauko stimuliacijos poveikį įvairių ląstelių pasėliams ir gyvūnų modeliams. Shafy ir kt. atliktas tyrimas parodė, kad sisteminga elektrinė stimuliacija širdies resinchronizacijos terapijos prietaisu padidina autologinių mioblastų, įšvirkštų į avies modelio miokardo infarkto pažeistas sritis, diferenciaciją. Autoriai įrodė, kad šios technikos taikymas pagerino miokardo funkciją (53). Haneef ir kt. pastarųjų metų tyrimai taip pat pademonstravo, kad kamieninių ląstelių elektrostimuliacija yra saugus ir veiksmingas metodas ląstelių išgyvenamumo ir diferenciacijos į širdies ląsteles požiūriu (54). Kalbant apie KEAV vaidmenį, Spadaccio ir bendradarbiai atskleidė, kad nuolatinė elektrinė stimuliacija miokardo infarkto *in vivo* modelyje pagerino miokardo funkciją ir tuo pačiu pagerėjo angiogenezė dėl endotelio progenitorinių ląstelių migracijos ir KEAV gamybos (43). Šie nauji tyrimai skatina miokardo audinių inžinerijos tyrimus, orientuotus į naujus išeminės širdies ligos gydymo metodus. Mūsų tyrimas visiškai

atitinka minėtų tyrimų rezultatus. Be to, naujų širdies lygų gydymo metodų prasme, tikėtina, kad galimybė atlikti IES yra saugesnė, siekiant išvengti pašalinio poveikio, tokio kaip aritmija.

Su autoimunine sistema susijusių angiogeninių signalų perdavimas yra aprašytas, pavyzdžiui, *lupus erythematodes systemicus* arba *myasthenia gravis* atvejais (55, 56). Pastaruoju atveju, autoriai nustatė pacientų, sergančių *myasthenia gravis*, santykinę teigiamą KEAV koncentracijos koreliaciją, lyginant su sveikais kontrolinės grupės tiriamaisiais. Be to, atliekant vėžio tyrimus, žinoma, kad proangiogeniniai ir antiangiogeniniai veiksniai daug lemia auglių progresavimui ir ligos plitimui (57, 58). KEAV yra pagrindinis stiprus angiogeninis veiksnys, kuris atsako už naujų kraujagyslių formavimąsi. KEAV indukcija vėžio ląstelėse iki šiol nėra visiškai aiški. Lombardi su kolegomis įtikinamai nustatė, kad krūties vėžio IgG padidino KEAV ekspresiją auglio ląstelėse. Tokį atsaką paskatino muskarininių receptorių siunčiami signalai. Šie rezultatai ir mūsų tyrimo rezultatai akivaizdžiai patvirtina, kad antikūnai yra stiprūs augimo veiksnių, ypač KEAV, induktoriai įvairių ligų ir patologinių aplinkybių atvejais (59).

KEAV ir du jo receptoriai KEAVR-1 ir KEAVR-2 vaidiną pagrindinį vaidmenį angiogenezėje fiziologinėmis ar patologinėmis aplinkybėmis ir dalyvauja neuronų ir miokardo vystymesi bei morfogenezėje (60). Tyrimai su gyvūnų modeliais atskleidė, kad net ir nedideli KEAV koncentracijos sutrikimai neigiamai veikia širdies kraujagyslių vystymąsi ir embrionų išgyvenamumą (61,62). Nenormalios KEAV būsenos gali sukelti kardiomiocitų hipertrofiją, dėl kurios gali prasidėti ar progresuoti širdies remodeliacija (11,12). Be to, labai svarbu yra tai, kad kardiomiocitų išskiriamas KEAV parakrininiu būdu skatina širdies fibroblastų proliferaciją (13). Patologinė fibroblastų proliferacija gali paskatinti miokardo fibrozės progresavimą. Dėl mikrocirkuliacijos sutrikimų sumažėja deguonies slėgis ir tokiu būdu vėl skatinama KEAV indukcija. Šis ratas gali sąlygoti nevaldomos ir nefiziologinės KEAV gamybos sukeltos patologinės remodeliacijos progresavimą. Tokias prielaidas patvirtina ir tai, kad nustatyta, jog mechaninė perkrova, kuri yra plačiai paplitęs DKMP stimulas, taip pat padidina kardiomiocitų KEAV raišką, kurią autoantikūnai toliau didina.

Šis tyrimas praplečia žinias apie galimą sąryšį tarp autoimuninių signalų DKMP atveju ir KEAV indukcijos kardiomiocituose. Šis metodas gali padėti kuriant naujus farmakologinius metodus miokardo remodeliacijai DKMP atvejais moduliuoti molekuliniu pagrindu.

NAV ne tik yra pagrindinis simpatinės sistemos neuronų išgyvenamumo ir diferenciacijos veiksnys, bet taip pat atlieka ir kardiomiocitų išgyvenamumą stiprinančio veiksnio, kuris reguliuoja apoptozę, funkciją (63) bei yra labai svarbus širdies funkcijos atsistatymui po miokardo infarkto (64). NAV reguliacija taip pat yra susijusi su nervinių ataugų augimu ir staigia širdies mirtimi (65). Nors NAV neabejotinai dalyvauja širdies neurofiziologijoje, mechanizmas, kaip širdies NAV raiška padidėja ar sumažėja, kol kas yra sunkiai paaiškinama. Taip pat žinoma, kad mechaninis tempimas, universalus širdies audinio stimulas, skatina hipertrofiją ir NAV sumažėjimą kardiomiocituose Cn(kalcineurino)/NFAT (aktyvuotų T ląstelių branduolių faktorių) keliu (66).

Tempimas širdies ir kraujagyslių sistemoje vykdomas skirtingomis formomis ir laipsniais (cikliškas tempimas, statinis pradinio lygio tempimas ir tempimas dėl didėjančios šlyties jėgos).

MI ar arterijų hipertenzijos metu intrakardinės ląstelės vis labiau tempiamos, lyginant su pradiniu lygiu. Kalbant apie su tempimu susijusią neurotrofinę reguliaciją, kardiomiocitai NAV raišką mažina, o SSN atsakas į tempimą yra įvairių neurotrofinų, įskaitant NAV, kiekio padidėjimas (40,41). Šiame tyrime mes nustatėme, kad SSN tempimas vyksta kartu su KEAV indukcija ir priklauso nuo laiko ir dozės. Todėl NAV signalai vaidina svarbų vaidmenį, tuo tarpu neurotrofinių veiksnių NT-3 ir GDNF įtakos reikšmingumą neuronų KEAV indukcijai galima atmesti. Be to, ŽVAEL gyvybingumo bandymai patvirtino, kad mechaninio tempimo sąlygomis išskiriama neuronų KEAV koncentracija yra biologiškai aktyvi. Vienas kardiomiocitas atsako į tempimą padidėjusiu KEAV kiekiu ir tai yra vienas ląstelių šaltinis (29). Kadangi širdies autonominė nervų sistema dalyvauja reguliuojant įvairius fiziologinius ir patologinius reiškinius, žinojimas, kad SSN taip pat gali gaminti ir išskirti KEAV yra neabejotinai svarbus (34).

Simpatinės nervų sistemos reguliavimo mechanizmai veikia širdies ir kraujagyslių sistemą per postganglinius simpatinės sistemos neuronus, kurie inervuoja kraujagysles ir širdį. Kraujagyslių simpatinės inervacijos poveikis kraujo spaudimui ir kraujotakai yra labai didelis. Todėl kraujagyslių simpatinės inervacijos pokyčiai siejami su širdies ir kraujagyslių ligų išsivystymu ir buvimu (67). Teigiama, kad KEAV ir jo receptoriai skatina kraujagyslių simpatinę inervaciją (68, 69). Taigi, KEAV ne tik reguliuoja kolateralinių kraujagyslių, kaip stipraus angiogeninio veiksnio hipoksijos ar kitų biocheminių ir biomechaninių jėgų prasme, susidarymą, bet ir daro didelę įtaką širdies ir kraujagyslių reguliavimo sistemų fiziologijai.

NAV yra pagrindinis neurotrofinis faktorius, reguliuojantis SSN išgyvenamumą ir diferenciaciją (70). Pastarųjų metų tyrimai nustatė, kad NAV taip pat yra kardiomiocitų išgyvenamumą gerinantis veiksnys (71). Be to, NAV skatina angiogenezę išeminėse užpakalinėse galūnėse (10) ir gerina širdies funkciją po MI (39). Tikėtina, kad šie pastebėjimai susiję su kryžminiu NAV ir KEAV reguliacijos poveikiu. MI metu intrakardinės ląstelės vis labiau tempiamos. Iš vienos pusės, tempimas slopina NAV ekspresiją kardiomiocituose (41), o SSN atsakas į tempimą yra įvairių neurotrofinų, įskaitant NAV ir CNTF, kiekio padidėjimas (40).

Apibendrinant, dabartiniai tyrimai rodo, kad KEAV daro tiesioginį poveikį nervų sistemai neuronų augimo, išgyvenimo ir neuroapsaugos aspektais, taip pat nustatyta, kad NAV, kuris yra neurotrofinas, yra nauja angiogeninė molekulė, sukelianti įvairų poveikį endotelio ląstelėms. Šį kryžminį KEAV neurotrofinį poveikį ir NAV angiogeninį poveikį išsamiai nagrinėjo Lazarovici ir kt. (72).

6. Išvados

Stimuliuojant ikislenkstine didelio dažnio elektrine stimuliacija, kardiomiocitai geba sintezuoti angiogeninio veiksnio KEAV kiekius, kas teigiamai veikia ŽVAEL proliferaciją *in vitro*. Tai daug žadantis žingsnis IŠL gydyme. IES sukelta angiogenezė gali būti išeminės širdies ligos gydymo alternatyva.

IA terapija sumažina pacientų su DKM padidėjusią KEAV koncentraciją. DKMP-IgG sukelia KEAV ekspresiją išaugintuose ŽNKM. Tempimo indukuotą KEAV gamybą ir toliau skatina antikūnai, o dėl DKMP-IgG sukeltos beta1-adrenerginės stimuliacijos padidėjusio susitraukimų dažnio poveikis KEAV indukcijai nenustatytas.

Kardiomiocitai kontroliuoja savo NAV aukšto dažnio arba nereguliarios stimuliacijos sąlygomis.

SSN reaguoja į tempimą padidėjusia KEAV ekspresija ir sekrecija. Neuronų KEAV padidėjimas dėl tempimo priklauso nuo laiko ir dozės.

7. Tyrimo apribojimai

Nebuvo tirti mechanizmai, kaip IES indukuotas KEAV padidėjimas sukėlė KEAV iRNR sumažėjimą (neigiamas grįžtamasis poveikis). Šiuos mechanizmus galėtų paaiškinti tolimesni tyrimai, kurių metu būtų naudojami KDR receptorių antikūnai IES metu ir analizuojamos tarpląstelinių signalų perdavimo kaskados.

Tyrimai atlikti pasitelkiant žiurkių naujagimių dirbtinai išaugintų kardiomiocitų *in vitro* modelį. KEAV raiška gali reikšmingai skirtis tarp naujagimių ir suaugusių kardiomiocitų. Taip pat neanalizavome IES poveikio esant hipoksijai, kuri stebima esant miokardo infarktui.

Terapinė angiogenezė po miokardo infarkto išlieka daug žadanti, bet sudėtinga širdies ir kraujagyslių medicinos užduotis. Jos svarbą lemia aukštas sergamumas ir mirtingumas ūminių koronarinių sindromų ir jų komplikacijų, tokių kaip aritmija ir širdies nepakankamumas. Kai kurie bandymai indukuoti angiogenezę jau yra ištirti (pvz., su kamieninėmis ląstelėmis, vietiškai skiriant rekombinantinių angiogeninių augimo veiksnių (fibroblastų augimo veiksnių, KEAV).

Šis tyrimas rodo, kad IES gali padidinti endogeninę KEAV raišką skilvelio miocituose, tuo pačiu skatinant endotelio ląstelių proliferaciją, t.y. galimai skatinant angiogenezę. IES gali būti atliekama su stimuliavimo kateteriais, kurie įvedami KS endokardo plote arba per stimuliavimo vielas, įvedamas į vainikinę arteriją (pvz., po revaskuliarizacijos arba net ir į neužsikimšusias smulkiąsias kraujagysles, kurioms negali būti taikoma PKI) arba į venų šakas (vainikinius sinusus). Šis metodas gali būti patrauklus atliekant kitus išeminės širdies ligos *in vivo* tyrimus su gyvūnais modelius, kadangi taikant šį metodą nereikia ląstelių ar baltymų injekcijų ar infuzijų į miokardo taikinius, o stimuliacija viena elektrodų pora apima didelį plotą.

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11. List of publications

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Aleksejus Zorinas

MITRALINIO VOŽTUVO PARAVALVULINIŲ FISTULIŲ SUSIDARYMO PRIEŽASČIŲ NUSTATYMAS, JŲ GYDYMO METODŲ PALYGINIMAS

INCIDENCE AND RISK FACTORS FOR MITRAL PARAVALVULAR LEAK AND COMPARISON OF THE TREATMENT MODALITIES

Biomedicinos mokslai, medicina (06 B)
Biomedical Sciences, Medicine (06 B)

Santrumpos

3D – trimatis

AKJ – aortovainikinių jungčių operacija

EuroSCORE – Europos širdies operacijos rizikos įvertinimo sistema

KS – kairysis skilvelis

KSIF – kairiojo skilvelio išstūmimo frakcija

MAZE – prieširdžių virpėjimo chirurginės operacijos pavadinimas (angl. MAZE – labirintas, tokiu principu sukeliama chirurginių randų pažaida prieširdžiuose)

MRT – magnetinio rezonanso tomografija

MV – mitralinis vožtuvas

MVP – mitralinio vožtuvo protezavimas

NYHA – Niujorko širdies asociacijos širdies nepakankamumo klasifikacijos sistema

PA – plaučių arterija

PVF – paravalvulinė fistulė

STS – Krūtinės ląstos chirurgų draugijos (angl. STS – Society of Thoracic Surgeons) rizikos įvertinimo sistema

TEE – perstemplinis ultragarsinis širdies ištyrimas

TV – triburis vožtuvas

„Turi eiti ir bendrauti su žmonėmis, plėsti savo akiratį, užduoti klausimus, kitaip netobulėsi."

PROF. VYTAUTAS SIRVYDIS

1. Įvadas

1.1. Tiriamoji problema ir darbo aktualumas

Mitralinio vožtuvo ligų reikšmingumas yra gerai žinomas ir nenuginčijamas, tai yra antros pagal paplitimą kliniškai reikšmingos suaugusiųjų širdies vožtuvų ydos (Seeburger J, 2011). Apie 3 % žmonių išsivysčiusių ekonomikų šalyse turi mitralinio vožtuvo ydų, paplitimas tarp besivystančių šalių gyventojų yra dar didesnis (Freed LA, 1999; Devereux RB, 2001).

Senstant populiacijai, mitralinio vožtuvo patologija paliečia kas dešimtą senjorą, vyresnį negu 75 metų (Nkomo VT, 2006). Pasaulyje per metus mitralinis vožtuvas protezuojamas apie keletui šimtų tūkstančių žmonių (Butany J, 2005), o per visą širdies chirurgijos istoriją tokių ligonių yra milijonai (Piéchaud J, 2003). Mitralinio vožtuvo ydų chirurginio gydymo tikslas yra sumažinti arba panaikinti simptomus, pagerinti gyvenimo kokybę, sumažinti pakartotinių hospitalizavimų dažnį ir tikėtinai pailginti išgyvenamumą, palyginti su medikamentiniu gydymu (Asgar AW, 2015). Deja, nuolatinis chirurginio gydymo palydovas yra įvairios komplikacijos. Jos sunkina ligonių sveikimą, psichosocialinę būklę ir gyvenimo kokybę, taip pat yra psichosocialinė našta gydytojams ir pareikalauja papildomų sveikatos apsaugos sistemos išteklių (Pinto A, 2013, 2016; Patel AS, 2013; Iribarne A, 2012). Paravalvu-

linė fistulė yra išsamiai literatūroje aprašyta komplikacija po mitralinio vožtuvo protezavimo. Kliniškai ji pasireiškia širdies nepakankamumo simptomais, hemolizine anemija, kartais abiejų sindromų deriniu (Ruiz CE, 2011). Literatūroje nurodoma, kad jos dažnis kinta nuo 2 % iki 17 %, o kai kurių studijų duomenimis gali siekti net 32 % ankstyvuoju laikotarpiu po operacijos (Hammermeister K, 2000; Ionescu A, 2003; Genoni M, 2000; O'Rourke DJ, 2001). Įvairių šalių tyrėjai seniai pastebėjo sąsajas tarp mitralinių PVF ir tokių rizikos veiksnių kaip neišgydyta infekcija sergant infekciniu endokarditu, biologinio vožtuvo protezavimas, siuvimas ištisine siūle, mitralinio vožtuvo žiedo kalkėjimas ir daugybė kitų (Mullany CJ, 1995; D'Agostino RS, 1985; Dhasmana JP, 1983; Bonnefoy E, 1995). Deja, galutinio sutarimo šiuo klausimu literatūroje nerasta.

Konservatyvus PVF komplikacijos gydymas laikinai pagerina būklę (Kliger C, 2013). Daugybę metų pakartotinė širdies operacija su dirbtine kraujo apytaka buvo vienintelis efektyvus gydymo būdas, nors lydima didelio mirštamumo ir sergamumo (Echevarria J, 1991; Emery RW, 2010).

Pastaruoju metu spartus kateterinių gydymo metodų vystymasis, noras sumažinti ligonių pooperacinį mirštamumą ir sergamumą gydant mitralinio vožtuvo PVF, medikų profesionalų bendruomenei kartu su sveikatos apsaugos industrija pavyko įdiegti į klinikinę praktiką mažiau invazyvų gydymo metodą (Thourani VH, 2012; Calvert PA, 2016; Wells IV JA, 2017) – kateterinę PVF užkimšimo metodiką.

Per pastaruosius dešimt metų šis gydymo metodas pasaulyje labai išplito, o kai kur tapo pirmo pasirinkimo gydymo taktika. Nepaisant to, PVF komplikaciją patyrusių ligonių gydymo rezultatai išsamiai neištirti, literatūroje labai stinga šių duomenų dėl to, kad nėra vienodai apibrėžtų ligos reikšmingumo, gydymo saugumo ir efektyvumo kriterijų (Millan X, 2017). Lietuvos širdies chirurgijos praktikoje ir literatūroje taip pat yra daugybė neatsakytų klausimų, sietinų su mitralinio vožtuvo paraprotezine fistule. Šiuo tyrimu siekiama nustatyti, koks yra mitralinio vožtuvo PVF paplitimas tarp ligonių, kuriems buvo protezuotas mitralinis vožtuvas, išsiaiškinti reikšmingiausius rizikos veiksnius, sietinus su mitralinio vožtuvo PVF, o svarbiausiai – palyginti naują kateterinį fistulės užkimšimo per širdies viršūnę metodą naudojant tam skirtą prietaisą su įprastine pakartotine širdies operacija gydant ligonius, kuriems išsivystė mitralinio vožtuvo protezo PVF.

1.2. Darbo mokslinis naujumas ir praktinė reikšmė

Ankstyvieji ir vėlyvieji gydymo rezultatai po mitralinio vožtuvo protezavimo, PVF dažnis ir rizikos veiksniai, sietini su jų atsiradimu, niekada nebuvo tirti Lietuvos populiacijoje. Šis tyrimas padės nustatyti ankstyvuosius ir vėlyvuosius rezultatus, PVF dažnį ir jų atsiradimo rizikos veiksnius po izoliuoto mitralinio vožtuvo protezavimo didelės praktinės patirties širdies chirurgijos centre.

Literatūroje minimi šie mitralinio vožtuvo PVF rizikos veiksniai: infekcinis endokarditas, ištisinė protezo implantavimo siūlė, mitralinio vožtuvo žiedo kalkėjimas ir kiti (Hammermeister K, 2000; Mullany CJ, 1995; D'Agostino RS, 1985; Dhasmana JP, 1983; Bonnefoy E, 1995). Laiku ir tinkamai (atsižvelgiant į įrodymais pagrįstos medicinos rekomendacijas) valdant rizikos veiksnius, susijusius su liga ir gydymu, PVF dažnis gali būti sumažintas, o gydymo rezultatai pagerinti. Literatūroje minimi PVF rizikos veiksniai gali būti valdomi skiriant adekvatų antimikrobinį gydymą, tobulinant chirurginius įgūdžius ir techniką, kaip reikalauja įrodymais grįsta medicinos praktika. Mes iškėlėme hipotezę, kad keičiantis chirurginei klinikinių sprendimų priėmimo praktikai ir išvengiant klaidų galima užkirsti kelią įvairioms komplikacijoms, įskaitant mitralinio vožtuvo protezo PVF, arba sumažinti jų dažnį. Todėl pirmiausia turi būti atsakyta, kurie veiksniai turi didžiausią įtaką mitralinio vožtuvo protezo PVF atsirasti. Šiame tyrime buvo nutarta išplėsti tiriamų rizikos veiksnių grupę, įtraukiant ne tik su liga ir gretutine patologija, bet ir su gydymu susijusius kintamuosius, tokius kaip chirurgas, atlikęs mitralinio vožtuvo protezavimą. Literatūros duomenimis, anksčiau to niekas nėra daręs.

Kita šio tiriamojo darbo naujovė yra skirtingų mitralinio vožtuvo protezo PVF gydymo metodų palyginimas, tai yra – palyginti naują kateterinį fistulės užkimšimo per širdies viršūnę metodą naudojant tam skirtą prietaisą su įprastine pakartotine širdies operacija. Iki šiol tik pavieniuose literatūros šaltiniuose buvo lyginami skirtingi mitralinio vožtuvo protezo PVF gydymo metodai, todėl duomenų šiuo klausimu labai stinga. Iki šiol neaišku, kuris gydymo metodas yra efektyvesnis ir saugesnis. Taip yra įvykę dėl to, kad plačiai naudojami prietaisai, neskirti PVF gydyti, ir nėra gairių, kaip nustatyti ligos reikšmingumą bei apibrėžti gydymo saugumą ir efektyvumą. Literatūroje pristatytų publikacijų duomenys pasižymi heterogeniškumu, nes tame pačiame straipsnyje nagrinėjamos skirtingų vožtuvų PVF, buvo naudojamos skirtingos prieigos vietos kateteriams įstatyti, taip pat didelė įvairovė prietaisų PVF užkimšti (Calvert PA, 2016; Lampropoulos K, 2016; Smolka G, 2016). Klinikinių paravalvulinių fistulių tyrimų rekomendacijos, apibrėžiančios pagrindinius diagnozės ir gydymo tikslų principus, publikuotos tik prieš metus (Ruiz CE, 2017). Savo ruožtu prietaisas, specialiai skirtas PVF užkimšti, klinikinėje praktikoje taip pat naudojamas visai neseniai – tik keletą metų (Goktekin O, 2014). Šiame tyrime mes lyginame dviejų skirtingų mitralinio vožtuvo PVF gydymo metodų rezultatus. Vieną grupę sudarė ligoniai, kuriems atlikta pakartotinė širdies operaciją su dirbtine kraujo apytaka, kitą grupę – ligoniai, kurie gydyti taikant kateterinę metodiką per širdies viršūnę naudojant prietaisą, skirtą tik PVF gydyti. Apžvelgus literatūros duomenis teigtina, kad tokia homogeniška grupė ligonių, kuriems taikyta mitralinio vožtuvo protezo PVF kateterinė gydymo metodika, niekada nebuvo palyginta su įprastiniu chirurginio gydymo metodu.

Praktinė šio tyrimo reikšmė yra įrodyti, kad gydant ligonius, kuriems atsirado mitralinio vožtuvo PVF, naujas kateterinis metodas per širdies viršūnę naudojant prietaisą, skirtą paravalvulinėms fistulėms gydyti, yra ne tik efektyvus, bet ir saugesnis ankstyvuoju pooperaciniu laikotarpiu, palyginti su pakartotine širdies operacija. Be to, šis tyrimas padės įdiegti naują kateterinę gydymo metodiką į kasdienę klinikinę praktiką.

1.3. Tyrimo tikslas

Šio tyrimo tikslas yra nustatyti chirurginio ir kateterinio metodų saugumą ir efektyvumą gydant ligonius, kuriems susidarė PVF po mitralinio vožtuvo protezavimo.

1.4. Tyrimo uždaviniai

1.4.1 Nustatyti PVF dažnį po mitralinio vožtuvų protezavimo operacijų.

1.4.2 Nustatyti, kokius rizikos veiksnius sieja reikšmingiausios sąsajos su PVF susidarymu po mitralinio vožtuvo protezavimo operacijų.

1.4.3 Nustatyti ankstyvojo ir vėlyvojo mirštamumo ir sergamumo dažnius po mitralinio vožtuvo protezavimo operacijų.

1.4.4 Palyginti PVF po mitralinio vožtuvo protezavimo chirurginio ir kateterinio gydymo metodų saugumą ir efektyvumą.

1.5. Ginamieji teiginiai

1.5.1 Mitralinio vožtuvo PVF dažnis tarp ligonių, gydytų tretinio lygio gydymo įstaigoje nuo 2005 iki 2017 m., nesiskiria nuo literatūroje pateikiamų duomenų.

1.5.2 Atsiradusios mitralinio vožtuvo PVF yra susijusios su šio vožtuvo ydos etiologija ir perioperacinio gydymo kintamaisiais.

1.5.3 Chirurginis kateterinis mitralinio vožtuvo PVF gydymas per širdies viršūnę naudojant tam skirtą prietaisą efektyvumu nenusileidžia pakartotinei širdies operacijai.

1.5.4 Chirurginis kateterinis mitralinio vožtuvo PVF gydymas per širdies viršūnę, naudojant tam skirtą prietaisą, ankstyvuoju pooperaciniu laikotarpiu yra saugesnis už pakartotinę širdies operaciją.

2. Bendrosios žinios

2.1. Mitralinio vožtuvo protezavimas

Nuo pat širdies vožtuvų ydų chirurgijos pradžios iki šiol širdies vožtuvų chirurgijos įvairios metodikos natūralią širdies vožtuvų ligų eigą pakeitė iš esmės. Ligoniams, sergantiems mitralinio vožtuvo ydomis, gali būti taikomas konservatyvus medikamentinis gydymas, tačiau tai yra laikina ligonio būklę pagerinanti priemonė, tik laikinai atitolinanti chirurginį gydymą, bet ir galinti sukelti medikamentiniam gydymui atsparų širdies nepakankamumą. Tokių ligonių gydymas kelia didelį klinikinį iššūkį, o mirtis arba chirurginis gydymas po 10 metų nuo diagnozės nustatymo tampa neišvengiami (Rossi A, 2011; Ling LH, 1996). Nepaisant vožtuvo ydos etiologijos ir mechanizmo, mitralinio vožtuvo protezavimas buvo aukso vertės standartas daugybę metų (Remadi JP, 1998). Netgi besivystant mitralinio vožtuvo taisymo metodikoms, degeneracinę šio vožtuvo ydą turintiems ligoniams jo protezavimas išlieka vienu iš pasirinktinių gydymo metodų (Carabello BA, 2008; Bonow RO, 2008; Gillinov AM, 2001). Deja, nepaisant nuolatinio širdies vožtuvo protezų technologinio progreso ir chirurginių metodikų tobulėjimo pasitaiko įvairių komplikacijų ankstyvuoju ir vėlyvuoju laikotarpiu po operacijos (Genoni M, 2000; Fernandez J, 1994).

2.2. Komplikacijos po mitralinio vožtuvo protezavimo

Po mitralinio vožtuvo protezavimo gali įvykti įvairių su vožtuvu susijusių komplikacijų, o tai reikšmingai didina ligonių sergamumą ir mirštamumą (Fernandez J, 1994). Remiantis literatūra, sergamumą nulemia struktūrinis vožtuvo pakitimas, nestruktūrinė disfunkcija, vožtuvo trombozė, embolijos, kraujavimo įvykiai, protezinis infekcinis endokarditas ir pakartotinės intervencijos dėl protezuoto vožtuvo (Akins CW, 2008). Mitralinio vožtuvo PVF yra viena iš tokių komplikacijų ir priklauso nestruktūrinei disfunkcijai.

2.3. Mitralinio vožtuvo protezo PVF

Mitralinio vožtuvo protezo PVF gali susidaryti tiek ankstyvuoju, ties vėlyvuoju pooperaciniu laikotarpiu ir nulemti reikšmingus klinikinius iššūkius, susijusius su dideliu sergamumu ir mirštamumu (Hwang HY, 2015; Ionescu A, 2003; Genoni M, 2000). Mitralinio vožtuvo protezo PVF suformuoja patologinę atrioventrikulinę jungtį kairėje tarp mitralino vožtuvo protezo manžetės ir natyvinių širdies audinių (Smolka G, 2010; Sampaio RO, 2009). Ši patologinė jungtis lemia sistolinę kraujo grįžtamąją srovę iš kairiojo skilvelio (KS) į kairįjį prieširdį (Pierard LA, 2010). Mitralinė PVF gali būti nedidelė ir išlikti kliniškai nebyli, tokiu atveju klinikinė eiga yra

teigiama, o didesnės fistulės lemia prastesnę klinikinę eigą (Kim MS, 2009), kuriai būdinga širdies nepakankamumas, hemolizinės kilmės mažakraujystė arba šių dviejų sindromų derinys.

Žinoma keletas rizikos kintamųjų, kurie didina PVF susidarymą po mitralinio vožtuvo protezavimo operacijų: mitralinio vožtuvo žiedo kalkėjimas, infekcinis endokarditas, vožtuvo protezo įsiuvimo metodika ir keletas kitų (Skoularigis J, 1993; Pate GE, 2006). Priklausomai nuo literatūros šaltinio, tiriamųjų imties dydžio, publikacijos senumo, mitralinio vožtuvo PVF dažnis kinta nuo 2,8 % iki 32 % (Hassanin N, 2017; O'Rourke DJ, 2001; Ionescu A, 2003; Genoni M, 2000; Mullany CJ, 1995).

2.4. Mitralinio vožtuvo PVF diagnostika ir gydymo indikacijos

MV PVF galima įtarti įvertinus ligonio klinikinę būklę. Daugeliui simptominių ligonių būdingas stazinis širdies nepakankamumas dėl širdies perkrovos tūriu arba hemolizinė anemija dėl mechaninės eritrocitų pažaidos kraujui tekant PVF (Kliger C, 2013).

Siekiant nustatyti mitralinio vožtuvo PVF chirurginio arba kateterinio gydymo indikacijas, būtina objektyviais duomenimis patvirtinti mitralinę PVF kaip širdies nepakankamumo arba hemolizinės anemijos priežastį.

Dauguma ligonių, kuriems dėl mitralinio vožtuvo PVF išsivysto širdies nepakankamumas, priklauso III arba IV funkcinei klasei pagal Niujorko širdies asociacijos (angl. New York Heart Association – NYHA) klasifikaciją (Ponikowski P, 2016). Dėl paprastumo NYHA funkcinė klasifikacija yra plačiai naudojama klinikiniuose tyrimuose, gydymo gairėse ir kasdienėje klinikinėje praktikoje (Castel MA, 2010). Visgi dėl skirtingos gydytojų ir ligonių interpretacijos ši klasifikacija gali būti subjektyvi ir neaiški (Yap J, 2015).

Siekiant objektyviai įvertinti ligonio širdies nepakankamumo sunkumą, aukso vertės standartu laikomas kardiopulmoninio pajėgumo mėginys. Šis mėginys leidžia objektyviai įvertinti ligonio fizinį pajėgumą. Tačiau norint atlikti šį mėginį reikia daug laiko, specialios įrangos, tai lemia dideles šio tyrimo išlaidas (Balady GJ, 2010). Klinikinėje praktikoje dažnai naudojama alternatyva yra 6 minučių ėjimo testas – paprastas, lengvai atliekamas, praktiškai nemokamas tyrimo metodas, kuriuo galima patikimai ir objektyviai įvertinti širdies nepakankamumo sunkumą (Nordanstig J, 2014). Nustatyta, jog stiprus koreliacinis ryšys sieja NYHA III ir IV funkcinės klasės požymius ir atstumą, nueitą atliekant 6 minučių ėjimo testą (Yap J, 2015).

Norint patvirtinti hemolizinės anemijos diagnozę, būtina atlikti detalų laboratorinį ligonio kraujo tyrimą (Barcellini W, 2015). Įprastinėje klinikinėje praktikoje dažniausiai nustatoma hemoglobino koncentracija, retikulocitų skaičius kraujyje,

laktato dehidrogenazės, haptoglobino koncentracija serume (Bain BJ, 2015; Liesveld JL, 1987; Shih AW, 2014; Kormoczi GF, 2006).

2.5. Hemolizinės anemijos mechanizmas esant mitralinio vožtuvo PVF

Esant normaliai funkcionuojančiam šiuolaikiniam vožtuvo protezui, ši komplikacija pasitaiko retai. Hemolizinę anemiją po vožtuvo protezavimo operacijos dažniau lemia nestruktūrinės protezo disfunkcijos, kurioms priklauso ir paraprotezinės fistulės susidarymas (Nicoloff DM, 1981). Intravaskulinė hemolizė yra neišvengiama paraprotezinės kraujotakos pasekmė (Rodgers BM, 1969). Skirtingų šaltinių duomenimis, kliniškai nereikšminga lengva hemolizė gali būti nustatoma 40–85 % ligonių, turinčių normaliai funkcionuojantį širdies vožtuvų protezą (Crexells C, 1972; Deviri E, 1985; Febres-Roman PER, 1980). Visgi iki 15 % tokių ligonių hemolizinė anemija gali būti gana sunki ir lemti ligonio priešlaikinę mirtį arba pakartotinę protezo keitimo operaciją (Okita Y, 1988; Kinsley RH, 1986).

Keletas mechanizmų gali lemti hemolizinės anemijos išsivystymą ligoniams po širdies vožtuvų protezavimo operacijos. Šie mechanizmai būdingi tiek normaliai funkcionuojant vožtuvo protezui, tiek sutrikus protezo funkcijai. Literatūroje nurodoma, jog hemolizinę anemiją gali lemti mechaninės eritrocitų pažaidos, kontaktas su svetimkūniu, turbulentinė kraujo tėkmė, spaudimo svyravimai ir eritrocitų membranos anomalijos (Garcia MJ, 1996).

2.6. Diagnozės patvirtinimas vaizdinimo metodais

Jau kurį laiką echokardiografija yra aukso standartas vertinant natyvinių širdies vožtuvų, taip pat širdies vožtuvų protezų funkciją (del Val Martín D, 2017). Gydytojas širdies chirurgas arba gydytojas kardiologas, įtaręs mitralinio vožtuvo PVF diagnozę, pirmiausia turi atlikti transtorakalinį echokardiografinį ligonio ištyrimą ir taip patvirtinti PVF susidarymą.

2.7. Transtorakalinis ultragarsinis širdies ištyrimas

Ligoniui, kuriam įtariama širdies vožtuvo protezo PVF, būtina atlikti detalų dvimatį echokardiografinį ištyrimą, ypač kreipiant dėmesį į protezinio vožtuvo funkciją, vožtuvo morfologiją, judrumą ir vožtuvo padėtį fiksavimo manžetėje. Regurgitacijos laipsnio nustatymas spalviniu tėkmės dopleriu gali būti netikslus dėl akustinių šešėlių, kuriuos sukelia ultragarsui nelaidus širdies vožtuvo protezas (del Val Martín D, 2017).

Labai svarbu pabrėžti, jog dėl labai mažos neigiamos prognozinės vertės neigiamas transtorakalinis ultragarsinis širdies ištyrimo rezultatas neleidžia patikimai atmesti mitralinio vožtuvo protezo PVF galimybės (Cortes M, 2008).

Jei kliniškai įtariama mitralinio vožtuvo protezo PVF, bet transtorakalinis echokardiografinis ištyrimas yra neinformatyvus, diagnozei patvirtinti arba paneigti būtina atlikti perstemplinį (TEE) ultragarsinį širdies ištyrimą.

2.8. Perstemplinis ultragarsinis širdies ištyrimas

TEE yra labai jautrus ir specifiškas tyrimo metodas, kuriuo galima aptikti ir detaliai įvertinti mitralinio vožtuvo protezo PVF. Šis tyrimo metodas, nors ir brangus bei invazyvus, yra esminis siekiant tiksliai įvertinti paravalvulinę mitralinio vožtuvo regurgitaciją (Cortes M, 2008; Alton ME, 1992; Hung J, 2007).

TEE širdies 4 kamerų, 2 kamerų ir ilgosios ašies vaizdas leidžia detaliai vizualizuoti kairįjį prieširdį ir mitralinio vožtuvo protezo siuvimo manžetę bei nustatyti paraprotezinius defektus (del Val Martín D, 2017).

Atliekant TEE spalvinio doplerio tyrimą, vožtuvo siuvimo manžetę būtina apžiūrėti keliomis kryptimis. Apžiūrėjus vožtuvą keliose projekcijose, galima nustatyti vožtuvo nesandarumą, lengviau diferencijuoti patologinį nesandarumą ir normalias „apiplovimo“ regurgitacijos sroves (Flachskampf FA, 1995). TEE spalvinio doplerio tyrimu nustačius PVF regurgitacinę srovę, būtina kiekybiškai diferencijuoti regurgitacijos laipsnį (Fischer CH, 1998). TEE ištyrimas yra būtinas siekiant patikslinti vožtuvo nesandarumo patofiziologinį mechanizmą ir kiekybiškai įvertinti nesandarumo laipsnį (Van den Brink RB, 2006).

Angiografiniu tyrimu vožtuvo nesandarumo laipsnis apibūdinamas keturiomis kategorijomis, o echokardiografinio ištyrimo metu, remiantis gairėmis, ekspertų sutarimais, vožtuvo nesandarumo laipsnis apibūdinamas trimis kategorijomis (lengvas, vidutinis, sunkus). Pagrindinis šių klasifikacijų trūkumas – negalima tiksliai apibūdinti tarpinių nesandarumo laipsnių (Michel PL, 1987 Croft CH, 1984). Siekiant išvengti šios problemos, pastaruoju metu paravalvulines regurgitacijas imta skirstyti naudojant penkių nesandarumo laipsnių klasifikaciją (Ruiz CE, 2017; Pibarot P, 2015). Detaliai ir tiksliai nustatyti regurgitacijos laipsnį yra ypač svarbu prognozuojant ligos eigą. Ligoniai, kuriems nustatomas vidutinis sunkus ir sunkus nesandarumas, turi didžiausią riziką per ateinančius dvejus metus būti hospitalizuoti arba mirti nuo širdies nepakankamumo (Ribeiro HB, 2016; Kodali S, 2016).

TEE tyrimas svarbus ne tik identifikuojant PVF buvimą ar nustatant regurgitacijos laipsnį, bet ir tiksliai įvertinant paravalvulinio defekto formą ir dydį (Biner S, 2010). Tai yra svarbu parenkant tolesnę gydymo taktiką konkrečiam ligoniui. Būtent defekto dydis ir forma lemia defekto kimšimo per kateterį ar pakartotinės atviros širdies operacijos pasirinkimą.

2.9. Trimatis (3D) perstemplinis ultragarsinis širdies ištyrimas

Pastaraisiais metais ypač tobulėjanti echokardiografijos sritis – 3D echokardiografija – lėmė smarkiai pagerėjusį diagnostinį širdies vožtuvų ydų tikslumą (Tsang W, 2011; Kort S, 2006).

Galimybė kompiuteriu atlikti 3D širdies vožtuvų rekonstrukciją naudojant TEE būdu gautus skirtingų projekcijų vaizdus leido echokardiografijos specialistams apžiūrėti ir įvertinti širdies vožtuvų anatomiją ir funkciją taip, kaip to nebuvo įmanoma atlikti dvimatės echokardioskopijos metodu (Sugeng L, 2008). Dėl nesudėtingo atlikimo, atkartojamumo ir greito duomenų surinkimo bei apdorojimo, taip pat galimybės vizualizuoti širdies vožtuvus realiu laiku trimatėje erdvėje 3D TEE labai paplito klinikinėje mitralinio vožtuvo PVF diagnostikos ir gydymo praktikoje. Šiuo metodu galima ne tik diagnozuoti mitralinio vožtuvo PVF, bet ir realiu laiku kontroliuoti gydymo procedūros efektyvumą (Sugeng L, 2008; Singh P, 2009). Metodo tikslumą diagnozuojant mitralinio vožtuvo PVF galima palyginti su vožtuvo įvertinimu atliekant atvirąją operaciją (del Val Martín D, 2017).

Būtina paminėti, jog 3D TEE leidžia įvertinti ne tik vožtuvo disfunkciją, bet ir vožtuvo anatomines ir funkcines savybes, paravalvulinės regurgitacijos etiologiją (Singh P, 2009; Kronzon I, 2009). Atliekant 3D TEE tyrimą ypač tiksliai nustatoma fistulės vieta, forma, dydis ir plotas (Kronzon I, 2009).

3D spalvinis tėkmės dopleris leidžia tiksliai nustatyti PVF angos vietą (Biner SE, 2010), fistulės angos anatomines charakteristikas, mitralinio vožtuvo regurgitacinės srovės parametrus ir fistulės angos ryšį su gretimomis anatominėmis struktūromis (Kronzon I, 2009; Perk G, 2009).

Nepaisant tikslaus ir detalaus net ir mažiausių širdies vožtuvo protezo disfunkcijų vaizdinimo, šis metodas turi ir trūkumų. Didžiausias trūkumas tas, kad norint gauti aukštos kokybės 3D rekonstrukcijas būtina surinkti aukštos kokybės echokardiografinius vaizdus, o tai gali atlikti tik eksperto lygio echokardiografijos specialistas. Eksperto lygiui pasiekti būtina pereiti ilgą ir sudėtingą mokymo procesą.

2.10. Širdies kompiuterinė tomografija

Nors dabartiniais laikais pagrindinis širdies vožtuvų anatomijos ir funkcijos vertinimo metodas yra echokardiografija, naujausi kompiuterinės tomografijos technologijos pasiekimai leidžia šiuo metodu adekvačiai vaizdinti ir įvertinti daugumą šiuolaikinių širdies vožtuvų protezų (Suh YJ, 2016; Sucha D, 2015; Newland JA, 2014).

Deja, kompiuterinės tomografijos angiografija turi ir trūkumų. Šiuo tyrimu gaunamus vaizdus gali iškraipyti artefaktai, kuriuos sukelia rentgeno spinduliams nelaidžios struktūros (protezų metalinės dalys, išplitęs kalkėjimas). Artefaktai gali

riboti PVF dydžio vertinimą. Atliekant šį tyrimą būtina naudoti kontrastinę medžiagą. Ligoniams, sergantiems inkstų funkcijos nepakankamumu, ši medžiaga gali pabloginti inkstų funkciją. Tyrimas yra susijęs ir su jonizuojančiąja ligonio apšvita. Šiuo metu kompiuterinė širdies tomografija dėl minėtų trūkumų yra labiau pagalbinis diagnostikos metodas, papildantis duomenis, gautus TEE tyrimo metodu. Dėl galimos rizikos ligoniui širdies KT tyrimas turėtų būti atliekamas tik tada, kai tyrimo nauda nusveria riziką ligoniui (del Val Martín D, 2017).

2.11. Širdies magnetinio rezonanso tomografija

Atliekant širdies magnetinio rezonanso tomografiją (MRT) galima ypač tiksliai kiekybiškai įvertinti regurgitacijos tūrį esant vožtuvo nesandarumui (Dulce MC, 1992). Dėl šios priežasties širdies MRT tyrimas gali tapti neinvazinių tyrimo metodų standartu, kai reikia kiekybiškai nustatyti regurgitacijos tūrį (Ruiz CE, 2017; Dulce MC, 1992). Šis tyrimas ypač naudingas tiriant tuos ligonius, kurių echokardiografinis ištyrimas yra neinformatyvus arba gauti echokardiografiniai duomenys nepaaiškina ligonio klinikinės būklės (Dulce MC, 1992). Dar vienas širdies MRT pranašumas yra tas, kad jį atliekant galima išmatuoti regurgitacijos tūrį nepriklausomai nuo regurgitacinių srovių skaičiaus ar morfologijos (Merten C, 2013).

Nepaisant širdies MRT pranašumų, galimybės atlikti tikslius ir atkartojamus matavimus, šis metodas, kaip ir kiti, turi tam tikrų trūkumų. Norint tiksliai atlikti matavimus, būtina gauti aukštos kokybės vaizdus, kuriuos įvertintų patyręs specialistas. Vaizdų kokybę gali bloginti įvairūs artefaktai, susiję su dirbtiniais protezais arba ligonio judesiais, širdies ritmo sutrikimais. Šie artefaktai gali bloginti matavimų tikslumą ir patikimumą. Taip pat reikia pabrėžti, jog MRT tyrimas yra brangus, jam atlikti reikia ne tik specialaus personalo, bet ir techninės bazės. Galima teigti, jog kol nebus įrodytas neginčijamas širdies MRT privalumas diagnozuojant mitralinio vožtuvo PVF šis metodas liks kaip papildomas arba pakaitinis tuo atveju, kai ligoniui nėra galimybių atlikti TEE (del Val Martín D, 2017; Michel PL, 1987).

2.12. Medikamentinis mitralinio vožtuvo PVF gydymas

Ligonių, turinčių simptominę mitralinio vožtuvo paravalvulinę regurgitaciją, gydymas medikamentais tėra paliatyvus (Kliger C, 2013). Dažnu atveju jis apsiriboja standartiniu širdies nepakankamumo gydymu. Ligoniams skiriami kilpiniai diuretikai, tikintis sumažinti pokrūvį ir taip palengvinti plautinės ir sisteminės veninės kraujotakos stazę.

Galima teigti, jog kliniškai reikšmingą mitralinio vožtuvo PVF turinčių ligonių gydymas vaistais padeda palengvinti simptomus ir stabilizuoti būklę, iki bus galima taikyti operacinį arba kateterinį gydymą (Kliger C, 2013; Taramasso M, 2015).

2.13. Pakartotinė operacija dėl mitralinio vožtuvo PVF

Įprastinis pakartotinis operacinis gydymas dėl įvairių širdies vožtuvo ydų taikomas nuo pat pirmosios mitralinio vožtuvo protezavimo operacijos (Boesch C, 1987; Mary DS, 1974).

Pagrindinis simptominių ligonių gydymo būdas ilgą laiką buvo įprastinė pakartotinė mitralinio vožtuvo operacija (Taramasso M, 2015).

Daugelyje pasaulio širdies ydų gydymo centrų MV fistulių kimšimas per kateterį tampa pirmo pasirinkimo procedūra. Nepaisant to, dar ir dabar dėl susiklosčiusių istorinių aplinkybių įprastinis pakartotinis operacinis gydymas laikomas pagrindiniu gydymo būdu, reikšmingai sumažinančiu ilgalaikį ligonių mirštamumą, palyginti su konservatyviais gydymo metodais (Millan X, 2015).

Galima teigti, kad dėl tokių kateterinio mitralinio vožtuvo PVF gydymo kontraindikacijų kaip širdies viršūnės aneurizma arba krešulys, negrįžtama plautinė hipertenzija, iširęs daugiau kaip trečdalis protezą laikančios siūlės, būtinybės atlikti širdies operaciją dėl kitos patologijos (vainikinių arterijų ligos, kitų vožtuvų ydų, aktyvaus infekcinio endokardito) konvencinis operacinis gydymas atliekant pakartotinę širdies operaciją išliks kaip vienas iš PVF turinčių ligonių gydymo metodų (Goel K, 2018; Pate GE, 2006; Cruz-Gonzalez I, 2016).

2.14. Kateterinis mitralinio vožtuvo PVF užkimšimas

Mitralinio vožtuvo PVF gali būti užkemšama keliais skirtingais būdais: perkutaniniu antegradiniu (defektas užkišamas iš kairiojo prieširdžio pusės įstūmus kimšimo prietaisą per prieširdžių pertvarą), perkutaniniu retrogradiniu transaortiniu (kai mitralinio vožtuvo PVF užkemšama iš KS pusės, kimšimo prietaisą prastumiant per aortos vožtuvą) ir retrogradiniu transapikaliniu (kai vožtuvo PVF užkemšama iš KS pusės kimšimo prietaisą į širdį įstumiant per širdies viršūnę, kuri gali būti pasiekiama atviru būdu arba perkutaniniu) (Smolka G, 2017; Nijenhuis VJ, 2014; Jelnin V, 2011). Kurį būdą pasirinkti, sprendžia procedūrą atliekantis gydytojas, remdamasis savo klinikine patirtimi, defekto lokalizacija, taip pat „širdies komandos“ pateiktomis išvadomis (Smolka G, 2017).

3. Metodologija

3.1. Tyrimo ir tiriamųjų ligonių aprašas

Šis tyrimas yra retrospektyvusis kohortinis stebimasis tyrimas. Jo atlikimą patvirtino Vilniaus regiono biomedicininės etikos komitetas ir Valstybinė duomenų apsaugos inspekcija. Šis tyrimas buvo atliktas dviem etapais. Visi tiriamieji, įtraukti

į abu šio tyrimo etapus, perskaitė ir pasirašė informuoto sutikimo formą. Į tyrimą įtraukti ligoniai, kuriems mūsų klinikoje atliktas pirminis mitralinio vožtuvo protezavimas, taip pat ligoniai, kuriems dėl susiformavusios mitralinio vožtuvo PVF taikytas pakartotinis operacinis arba kateterinis gydymas per širdies viršūnę.

3.1.1. Pirmasis tyrimo etapas: mitralinio vožtuvo PVF paplitimo ir rizikos veiksnių nustatymas

Šio tyrimo pirmajame etape buvo siekiam nustatyti kliniškai reikšmingų mitralinio vožtuvo PVF paplitimą ir rizikos veiksnius. Tam mes atrinkome ligonius, kuriems mūsų centre buvo atlikta izoliuoto mitralinio vožtuvo protezavimo operacija nuo 2005 m. sausio 1 d. iki 2017 m. gruodžio 1 d. Ligoniams identifikuoti buvo atlikta automatinė Vilniaus universiteto ligoninės Santaros klinikų Elektroninės ligos istorijos duomenų bazės analizė ieškant ligos istorijų, turinčių raktinius žodžius „mitralinio vožtuvo protezavimas“. Atlikus automatinę paiešką, duomenų bazėje rasta 1308 įrašai. Iš šios grupės buvo pašalinti tie ligoniai, kuriems tai buvo pakartotinė mitralinio vožtuvo protezavimo arba plastikos operacija, taip pat tie ligoniai, kuriems kartu su šio vožtuvo protezavimo operacija atlikta ir aortos vožtuvo operacija. Ligoniai, kuriems kartu su mitralinio vožtuvo protezavimo operacija atlikta triburio vožtuvo plastika, vainikinių arterijų jungčių suformavimo operacija arba širdies ritmą atkurianti operacija (labirinto procedūra), buvo įtraukti į tyrimą. Iš tyrimo pašalinti ir tie ligoniai, kurių ligos istorijose reikšmingai trūko duomenų. Į galutinį tyrimą įtraukta 551 ligonio kohorta. Šios kohortos duomenys buvo analizuojama remiantis tarptautinėmis gairėmis ir rekomendacijomis, kuriose nurodyta, kaip reikia pateikti ligonių mirštamumo ir sergamumo po širdies vožtuvų operacijų duomenis (Akins CW, 2008). Surinkti ligonių demografiniai duomenys, taip pat duomenys apie klinikinę būklę prieš operaciją, bendrieji ir specialieji operaciniai duomenys. Tyrime ankstyvasis pooperacinis laikotarpis yra 30 dienų arba laikas, iki ligonis išrašomas iš ligoninės. Ligonių mirštamumas suskirstytas į neatidėliotiną (iki 72 valandų po procedūros), ankstyvą (30 dienų po operacijos arba kol ligonis išrašomas iš ligoninės) ir bendrą (vėlyvuoju stebėjimo laikotarpiu). Mitralinio vožtuvo PVF susiformavimo rizikos veiksniai (priklausantys nuo ligonio, patologijos ir gydymo) buvo nustatyti atliekant vienmatė ir daugiamatė Kokso (*Cox*) regresijos analizę. Siekiant išvengti interesų konflikto, operavę chirurgai buvo užkoduoti dvigubai aklu metodu.

3.1.2. Antrasis tyrimo etapas: skirtingų mitralinių PVF gydymo metodų efektyvumo ir saugumo palyginimas

Antrajame šio tyrimo etape, siekiant palyginti skirtingų mitralinio vožtuvo PVF gydymo metodų efektyvumą ir saugumą, buvo atlikta retrospektyvioji analizė

ligonių, kuriems nuo 2005 m. sausio 1 d. iki 2017 m. gruodžio 1 d. dėl mitralinio vožtuvo PVF atlikta įprastinė pakartotinė širdies operacija arba kateterinis mitralinio vožtuvo PVF užkimšimas per širdies viršūnę. Tam buvo atlikta automatinė paieška Vilniaus universiteto ligoninės Santaros klinikų Elektroninės ligos istorijos operacijų protokolų duomenų bazėje naudojant raktinius žodžius operacijos pavadinime „mitralinė paravalvulinė fistulė". Pirminė paieška leido identifikuoti duomenų bazėje 86 įrašus. Iš šios ligonių kohortos pašalintas 1 ligonis, kuriam buvo nustatytas aktyvus infekcinis endokarditas, 1 ligonis, kuriam nustatytas daugiau kaip vieno trečdalio protezo perimetrą laikančios siūlės iširimas, ir 11 ligonių, kuriems atliktas kateterinis fistulės kimšimas per širdies viršūnę naudojant prietaisą / kamštį, neturintį patvirtintos indikacijos šiai patologijai gydyti. Į galutinę kohortą įtraukti 73 ligoniai. Siekiant įvertinti skirtingų gydymo metodų efektyvumą ir saugumą visi ligoniai buvo padalinti į dvi grupes ir iš analizuoti pagal „Klinikinių tyrimų principų ir baigčių apibrėžimų gydant paravalvulinį nesandarumą po protezavimo operacijų" rekomendacijas (Ruiz CE, 2017). Grupė ligonių, kuriems atliktas kateterinis mitralinio vožtuvo PVF užkimšimas per širdies viršūnę specialiu tam pritaikytu kamščiu, buvo pavadina kateterio grupe ir ją sudarė 24 ligoniai. Grupė ligonių, kuriems buvo atlikta įprastinė pakartotinė širdies operacija, buvo pavadinta chirurgijos grupe ir ją sudarė 49 ligoniai. Surinkti abiejų grupių ligonių demografiniai duomenys, taip pat priešoperaciniai klinikiniai duomenys, bendrieji ir specialieji operaciniai duomenys. Ligonių duomenys buvo analizuojami prieš operaciją, perioperaciniu laikotarpiu, išrašant, 6 mėn. po operacijos ir kasmet po operacijos. Ankstyvos pooperacinės charakteristikos / komplikacijos analizuotos 30 dienų laikotarpiu arba iki išrašant ligonį. Mirštamumas suskirstytas į neatidėliotiną, ankstyvą

30 dienų pooperacinį / hospitalinį ir vėlyvojo stebėjimo laikotarpio. Procedūros efektyvumas nustatytas vertinant pooperacinę vožtuvo protezo funkciją ir liekamosios regurgitacijos laipsnį išrašant ir kasmetinio stebėjimo laikotarpiu. Metodų saugumas vertintas analizuojant ankstyvąjį (hospitalinį) ir vėlyvąją sergamumą bei mirštamumą. Siekiant nustatyti reikšmingiausius su ligoniu, patologija ir gydymo metodu susijusius vėlyvojo laikotarpio mirštamumo rizikos veiksnius, atlikta vienmatė ir daugiamatė Kokso regresijos analizė.

3.2. Statistinė analizė

Statistinė duomenų analizė atlikta duomenų kaupimo ir apdorojimo paketu SPSS v. 20.0 (IBM Corp.m Armonk, NY, USA). Kiekybinių duomenų normalumas tikrintas naudojant histogramos bei Šapiro ir Vilko (*Shapiro–Wilk*) kriterijų. Duomenys yra pasiskirstę pagal normalųjį skirstinį tada, kai jų histograma atitinka normaliojo skirstinio histogramą ir Šapiro ir Vilko kriterijaus *p* reikšmė yra didesnė nei 0,05. Kiekybiniai duomenys, kurie pasiskirstę pagal normaliojo skirstinio dėsnį,

pateikiami kaip vidurkis ± standartinis nuokrypis, o kokybiniai duomenys, kurie neatitinka normaliojo skirstinio, pateikiami kaip mediana ir kvartiliai. Kategoriniai duomenys pateikiami procentine išraiška. Reikšmingų klinikinių įvykių atsiradimas ir išgyvenamumas įvertinti naudojant Kaplano ir Mejerio (*Kaplan–Meier*) metodą. Į cenzūruotus duomenis įtraukti tų ligonių duomenys, kurie nustojo lankytis mūsų klinikoje vėlyvuoju laikotarpiu. Skirtumai tarp duomenų laikomi statistiškai reikšmingais, kai *p* reikšmė yra mažiau 0,05.

3.3. Pirminės mitralinio vožtuvo protezavimo operacijos aprašymas

Krūtinės ląsta atveriama atlikus vidurinę išilginę sternotomiją. Perikardas atveriamas apverstos „T“ raidės pjūviu. Prieš aortos kaniuliaciją ligoniui į veną suleidžiama heparino (300 veikimo vienetų 1 kilogramui svorio), siektinas krešėjimo laikas turi būti daugiau nei 400 sekundžių. Tuomet į aortą įstatoma arterinės perfuzijos kaniulė. Veninis drenažas užtikrinamas kaniuliuojant apatinę ir viršutinę tuščiąsias venas. Miokardo apsaugos tirpalas skiriamas antegradiniu keliu į aortos šaknį arba retrogradiniu keliu per vainikinį antį. Pradėjus dirbtinę kraujo apytaką, perspaudžiama kylančioji aorta ir pradedama vainikinės kraujotakos perfuzija kardiopleginiu (miokardą apsaugančiu) tirpalu. Mitralinio vožtuvo vizualizacija užtikrinama per prieširdžių pertvarą, kai mitralinio vožtuvo vizualizacija nepakankama, pjūvis pratęsiamas į kairiojo prieširdžio stogą. Po mitralinio vožtuvo apžiūros patvirtinama jo patologija, vožtuvas iškerpamas visas arba tik priekinė mitralinio vožtuvo burė. Pašalinus vožtuvą, išmatuojama jo anga ir pasirenkamas atitinkamo dydžio protezas. Vožtuvo protezas gali būti įsiuvamas dviem būdais: ištisine siūle arba pavienėmis siūlėmis per lopinėlius. Kairiojo skilvelio oro šalinimo ir dekompresijos vamzdelis įkišamas į jo ertmę per įsiūto vožtuvo protezą. Tuomet užsiuvami tarpprieširdinės pertvaros ir dešiniojo prieširdžio pjūviai. Atliekama oro šalinimo iš širdies ertmių procedūra. Nuimamas aortą perspaudžiantis instrumentas. Sušildžius ligonį iki normalios kūno temperatūros, sustabdoma dirbtinė kraujo apytaka. Atliekama hemostazė nutraukiant heparino poveikį protamino sulfatu. Krūtinės ląsta užsiuvama.

3.4. Įprastinės pakartotinės mitralinio vožtuvo operacijos dėl PVF aprašymas

Atliekama per pakartotinę vidurinę išilginę sternotomiją. Širdies ir greta esančios anatominės struktūros atidalijamos iš sąaugų. Antikoaguliacija nesiskiria nuo pirminės operacijos, siektinas krešėjimo laikas turi būti daugiau nei 400 sekundžių. Dirbtinė kraujo apytaka ir miokardo apsauga užtikrinami tokiu pat principu, kaip

ir atliekant pirminę MVP operaciją. Chirurginiai veiksmai, užtikrinantys mitralinio vožtuvo protezo vizualizaciją, nesiskiria nuo pirminės MVP operacijos. Po mitralinio vožtuvo protezo apžiūros patvirtinamas PVF buvimas, įvertinamas jos dydis, forma ir kompleksiškumas. Defekto korekcijos metodiką dažniausiai lemia PVF dydis. Kai fistulė santykinai nedidelė, defektą galima užsiūti vienu ar keliais dygsniais. Kai fistulė didelė protezas pašalinamas ir keičiamas nauju. Užsiuvus PVF, tolesni chirurginiai veiksniai nesiskiria nuo pirminės MVP operacijos.

3.5. Kateterinio mitralinio vožtuvo PVF užkimšimo per širdies viršūnę procedūros aprašymas

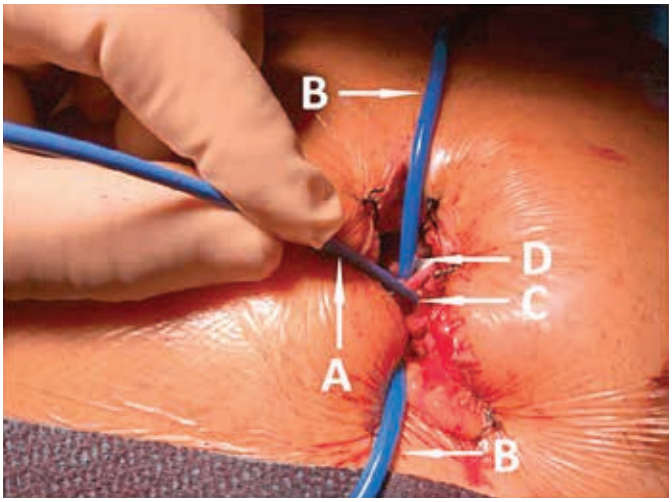
Sukėlus bendrąją nejautrą, atliekama maža šoninė torakotomija penktajame tarpšonkauliniame tarpe, širdies viršūnės projekcijoje (prieš pjūvį nustatoma echoskopijos būdu). Pjūviui išplėsti naudojamas Finochietto žaizdos plėstuvas, be to, tai palengvina chirurginės manipuliacijas (žr. 1 paveikslą).

1 PAV. Bendras operacinio lauko vaizdas su Finochietto plėstuvu žaizdoje. A – Finochietto plėstuvo kojytės plečia tarpšonkaulinį tarpą; B – turniketai, laikantys dvi „U“ formos siūles ties širdies viršūne; C – veltinio lopeliai siūlėms sutvirtinti.

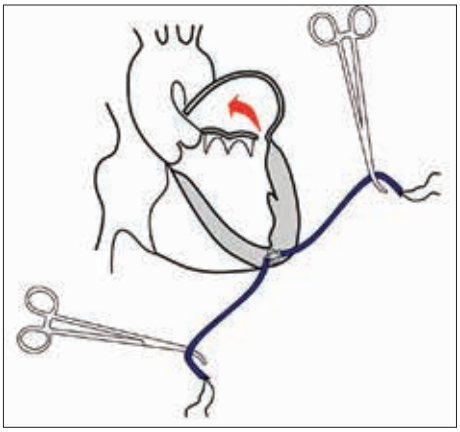
Identifikuojamas ir atveriamas perikardas. Jis gali būti tvirtai priaugęs prie epikardo, sąaugos turi būti atsargiai atidalintos. Perikardo kraštai pakeliami ant siūlių laikiklių ir tvirtinami prie odos po plėstuvu. Širdies viršūnė apsiuvama dviem „U“ formos dygsniais, sutvirtintais veltinio lopeliais, dygsniai turi būti skersi vienas kito atžvilgiu, tada fiksuojami turniketais. Apsiuvus širdies viršūnę, plėstuvas gali būti pašalinamas (žr. 2 ir 3 paveikslus).

Prieš pradedant kateterines manipuliacijas širdies ertmėse, ligoniui į veną suleidžiama heparino, siektinas aktyvaus krešėjimo laikas – tarp 250 ir 300 sekundžių. Adata tarp lopelių punktuojama KS ertmė, pro adatą į kairįjį skilvelį įkišama viela, o ant jos – introdiuseris.

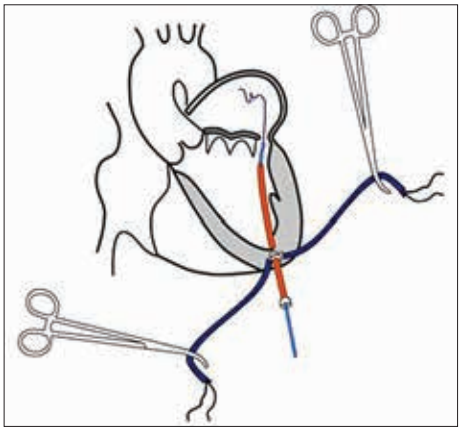
Įstačius introdiuserį, turniketai užveržiami. 3D TEE realiuoju laiku atliekama lygiagrečiai su chirurginiais veiksmais, šiuo tyrimu galutinai



2 PAV. Bendras operacinio lauko vaizdas iš žaizdos pašalinus Finochietto plėstuvą: A – kateteris, įstatytas į kairįjį skilvelį; B – turniketai, fiksuojantys „U“ formos dygsnius ant širdies viršūnės; C – kateterio įeigos į KS ertmę vieta; D – „U“ formos dygsniai ties širdies viršūne, skersi vienas kito atžvilgiu.

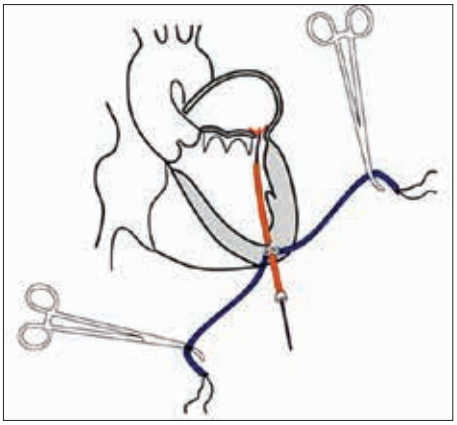


3 PAV. Scheminis pradinių procedūros veiksmų vaizdas. Du „U“ formos dygsniai su lopeliais, skersi vienas kito atžvilgiu ir fiksuoti turniketais širdies viršūnės projekcijoje. Paravalvulinė regurgitacija parodyta raudona rodykle.

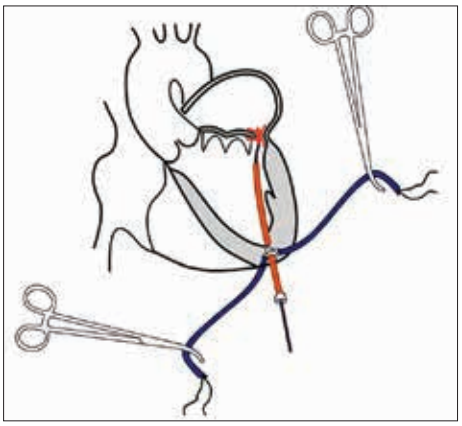


4 PAV. Hidrofiliškos vielos įstūmimas į kairiojo prieširdžio ertmę naudojant kreipiamąjį kateterį.

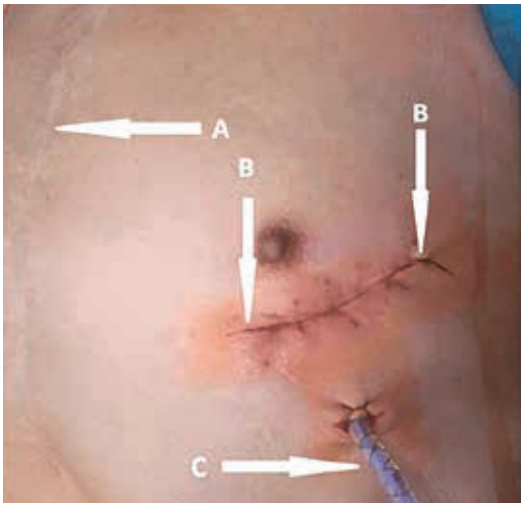
patvirtinama diagnozė, nustatoma fistulės dydis, forma ir lokalizacija. Pro defektą į kairiojo prieširdžio ertmę naudojant kreipiamąjį kateterį įstumiama hidrofiliška viela, veiksmai atliekami kontroliuojant procedūrą 3D TEE ir fluroskopijos būdu realiuoju laiku (žr. 4 paveikslą).



5 PAV. Prietaiso/kamščio (raudonas) įstūmimas į PVF, distalinis (kairiojo prieširdžio) diskas išskleistas iš kateterio.



6 PAV. Prietaiso / kamščio (raudonas) įstūmimas į PVF, proksimalinis (kairiojo skilvelio) diskas išskleistas iš kateterio.



7 PAV. Bendras pooperacinės žaizdos vaizdas: A – sternotomijos randas, B – torakotominis pjūvis užsiūtas, C – pleuros drenas.

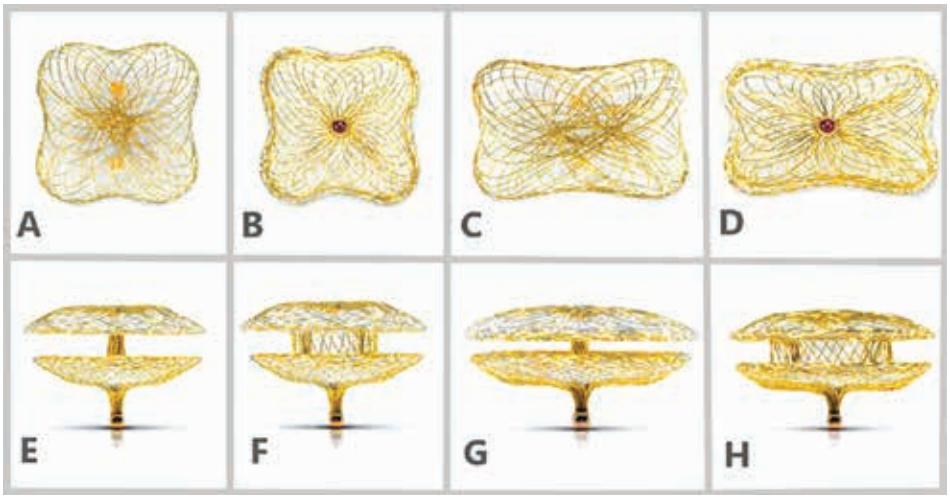
Kreipiamasi kateteris įstumiamas į kairiojo prieširdžio ertmę, tuomet hidrofiliška viela pakeičiama į kietą vielą. Kreipiamasis kateteris pašalinamas, ant kietos vielos į kairiojo prieširdžio ertmę įstumiamas kateteris, pro kurį – PVF užkemšantis prietaisas (kamštis). Kontroliuojant procedūrą 3D TEE ir fluroskopija realiu laiku, PVF užkemšantis prietaisas (kamštis) laipsniškai įstumiamas į PVF, iš pradžių išskleidžiamas distalinis (kairiojo prieširdžio) diskas. Kai disko tinkama pozicija patvirtinama 3D TEE, išskleidžiamas proksimalinis (kairiojo skilvelio) diskas. Po 3D TEE

patvirtinimo, kad prietaisas netrikdo mitralinio vožtuvo protezo funkcijos, kamštis atleidžiamas iš jį laikančios sistemos (žr. 5 ir 6 paveikslus).

Sėkmingai užkimšus PVF, kateteriai pašalinami iš kairiojo skilvelio, „U“ formos siūlės užrišamos. Heparino poveikis nutraukiamas skiriant į veną protamino sulfato. Perikardas užsiuvamas trimis pavienių siūlių dygsniais. Pleuros ertmė drenuojama vienu drenu. Žaizda užsiuvama.

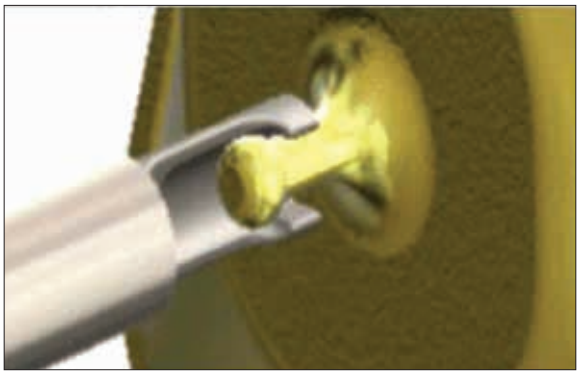
3.6. Prietaiso/kamštuko, skirto PVF gydyti, aprašymas

Prietaisas/kamštukas PVF gydyti pasirodė rinkoje 2014 metais. Kamštukas sudarytas iš dviejų tarpusavyje sujungtų diskų, pagamintas iš nitinolio lydinio vielos, supintos į reikiamą formą. Pagal disko formą gali būti dviejų tipų: kvadratinis ir keturkampis. Pagal diskus jungiančios kojytės formą gali būti Twist (angl.) ir Waist (angl.) tipo (žr. 8 paveikslą).



8 PAV. Prietaisas/kamštukas, skirtas PVF gydyti (Occlutech® PLD occluder). Vaizdai priklauso Occlutech International AB, visos teisės saugomos. A – kvadratinis, distalinio disko vaizdas; B – kvadratinis, proksimalinio disko vaizdas; C – keturkapis, distalinio disko vaizdas; D – keturkapis, proksimalinio disko vaizdas; E – kvadratinis su Twist tipo kojyte, vaizdas iš šono; F – kvadratinis su Waist tipo kojyte, vaizdas iš šono; G – keturkampis su Twist tipo kojyte, vaizdas iš šono; H – keturkampis su Waist tipo kojyte, vaizdas iš šono.

Kiekvieno disko viduje yra polietileno tereftalato lopinėliai, skirti greitam kraujo tėkmės sandarinimui. Taip pat viename iš diskų yra du aukso radiologiniai žymenys, padedantys orientuoti prietaisą defekte. Diskai tarpusavyje sujungti elipsės arba apskritimo formos kojyte. Prietaisas implantavimo sistemoje fiksuojamas dėl burbulo formos jungties (žr. 9 paveikslą).



9 PAV. Implantavimo sistemos prietaiso suėmimo mechanizmas. Vaizdai priklauso Occlutech International AB, visos teisės saugomos.

4. Rezultatai

4.1. Ligonių, kuriems atlikta MVP operacija, priešoperaciniai duomenys

Tiriamųjų kohortą sudarė 551 ligonis. Jų amžiaus mediana buvo 64 (55–71) metai. Du šimtai aštuoniolika ligonių (40 %) buvo vyrai. Didžioji dauguma operacijų, t.y. 549 (99,6 %), buvo atliktos planine tvarka. Išsamūs ligonių, kuriems atlikta MVP operacija, priešoperaciniai duomenys pateikiami 1 lentelėje.

1 LENTELĖ. Ligonių, kuriems atlikta MVP operacija, priešoperaciniai duomenys.

Rodiklis	N (%) / mediana[Q1–Q3]
Bendras ligonių skaičius	551 (100 %)
Amžius, metai	64 (55–71)
Kūno paviršiaus plotas, m^2	1,89 (1,74–2,04)
Lytis	
Vyrai	218(40 %)
Moterys	333 (60 %)
Operacijos atlikimo skubumas	
Planinė	549 (99,6 %)
Skubi	2 (0,4 %)
Mitralinio vožtuvo yda	
Infekcinio endokardito vegetacijos	47 (8,5 %)
Mitralinio vožtuvo stenozė	70 (12,7 %)
Mitralinio vožtuvo nesandarumas	251 (45,6 %)
Mitralinio vožtuvo nesandarumas ir stenozė	183 (33,2 %)
Mitralinio vožtuvo ydos etiologija	
Reumatas	283 (51,5 %)
Chordų plyšimas	72 (13,1 %)
Išeminės kilmės	53 (9,6 %)

Sklerodegeneraciniai pakitimai ir kalkėjimas	41 (7,4 %)
Infekcinis endokarditas	47 (8,5 %)
Degeneraciniai pakitimai	34 (6,2 %)
Dilatacinė kardiomiopatija	15 (2,7 %)
Įgimta patologija	5 (0,9 %)
Širdies nepakankamumo NYHA klasė	
II	86 (15,6 %)
III	439 (79,7 %)
IV	26 (4,7 %)
Gretutinės ligos	
Vainikinių arterijų liga	117 (21,2 %)
Persirgtas išeminis galvos smegenų kraujotakos sutrikimas	25 (4,5 %)
Anksčiau implantuotas širdies stimuliatorius	96 (17,4 %)
Prieširdžių virpėjimas	309 (56 %)
Hemoglobino koncentracija serume < 100 g/l	20 (3,6 %)
Kreatinino koncentracija serume, µmol/l	88 (72–102)
Kairiojo skilvelio sistolinė funkcija	
Labai sumažėjusi (KSIF < 30 %)	10 (2 %)
Vidutiniškai sumažėjusi (30 % ≤ KSIF < 45 %)	81 (15 %)
Nedaug sumažėjusi (45 % ≤ KSIF < 55 %)	194 (35 %)
Normali (KSIF ≥ 55 %)	266 (48 %)
PA sistolinis spaudimas >55 mmHg	106 (19 %)

Bendrieji MVP operacijų duomenys

Bendrieji operacijų duomenys išsamiai pateikiami 2 lentelėje. Siekiant išvengti šališkumo ir užtikrinti duomenų konfidencialumą, visi chirurgai buvo užkoduoti dvigubai aklu būdu, nepriklausomai nuo tyrėjų. Kreiptinas dėmesys į tai, kad beveik pusę 47,2 % visų operacijų atliko du chirurgai (chirurgas B ir chirurgas Z). Chirurgas B atliko trečdalį, t. y. 157 (28,5 %), visų kohortos tiriamųjų operacijų, Chirurgas Z – penktadalį, t. y. 103 (18,7 %).

2 LENTELĖ. Bendrieji MVP operacijų duomenys.

Rodiklis	N (%) / mediana [Q1–Q3]
Ligonių skaičius operavusiam chirurgui	
Chirurgas B	157 (28,5 %)
Chirurgas Z	103 (18,7 %)
Chirurgas P	67 (12,2 %)
Chirurgas F	45 (8,1 %)
Chirurgas L	39 (7,1 %)
Chirurgas K	25 (4,5 %)
Chirurgas D	115 (20,9 %)
Chirurginė procedūra	
Izoliuotas mitralinio vožtuvo protezavimas	53 (9,6 %)
Mitralinio vožtuvo protezavimas ir TV plastika	350 (63,5 %)
Mitralinio vožtuvo protezavimas ir AKJ	32 (5,8 %)
Mitralinio vožtuvo protezavimas su AKJ ir TV plastika	69 (12,5 %)
Mitralinio vožtuvo protezavimas ir MAZE procedūra	4 (0,4 %)
Mitralinio vožtuvo protezavimas su MAZE ir TV plastika	43 (7,8 %)

4.2. Specifiniai MVP operacijų duomenys

Specifiniai MVP operacijų duomenys išsamiai pateikiami 3 lentelėje. Pažymėtina, kad 371 (67 %) ligoniams buvo implantuoti mechaniniai vožtuvų protezai, o 180 (33 %) ligonių – biologiniai. Atkreiptinas dėmesys ir į tai, kad 355 (64 %) ligoniams mitralinio vožtuvo protezas įsiūtas naudojant pavienių siūlių metodiką, o 196 (35,6 %) – ištisinę siūlę.

3 LENTELĖ. Specifiniai operacijų duomenys.

Dirbtinės kraujo apytakos trukmė, min	N (%) / mediana [Q1–Q3]
≤ 60 min	2 (0 %)
≥ 61 min ≤ 120 min	208 (38 %)
≥ 120 min ≤ 180 min	242 (44 %)
≥ 181 min ≤ 240 min	68 (12 %)
≥ 241 min	31 (6 %)
Aortos perspaudimo / miokardo išemijos trukmė, min	
≤ 60 min	149 (27 %)
≥ 61 min ≤ 120 min	329 (60 %)
≥ 120 min ≤ 180 min	66 (12 %)
≥ 180 min	7 (1 %)
Bendra operacijos trukmė, min	
≥ 61 min ≤ 120 min	48 (9 %)
≥ 120 min ≤ 180	245 (44 %)
≥ 181 min ≤ 240 min	142 (26 %)
≥ 241 min ≤ 300 min	61 (11 %)
≥ 301 min	55 (10 %)
Neplanuotos intraoperacinės procedūros	
Kontrapulsacija aortos balionu	64 (12 %)
Ekstrakorporinė pagalbinė mechaninė kraujotaka	7 (1 %)
Reikšmingas intraoperacinis kraujavimas	15 (3 %)
Nesėkmė sustabdant dirbtinę kraujo apytaką pirmu bandymu	34 (6 %)
Vožtuvo protezo tipas	
Biologinis	180 (33 %)
Mechaninis	371 (67 %)
Vožtuvo protezo dydis	
25 mm	5 (1 %)
27 mm	116 (21 %)
28 mm	2 (0,4 %)
29 mm	347 (63 %)
31 mm	81 (15 %)
Vožtuvo protezo implantavimo (įsiuvimo) technika	
Ištisine siūle	196 (35,6 %)
Pavienėmis siūlėmis	355 (64,4 %)
Priciga prie mitralinio vožtuvo	
Pjūvis per prieširdžių pertvarą	150 (27 %)
Pjūvis per prieširdžių pertvarą ir kairiojo prieširdžio stogą	401 (73 %)

4.3. Ankstyvieji MVP pooperaciniai duomenys ir komplikacijos

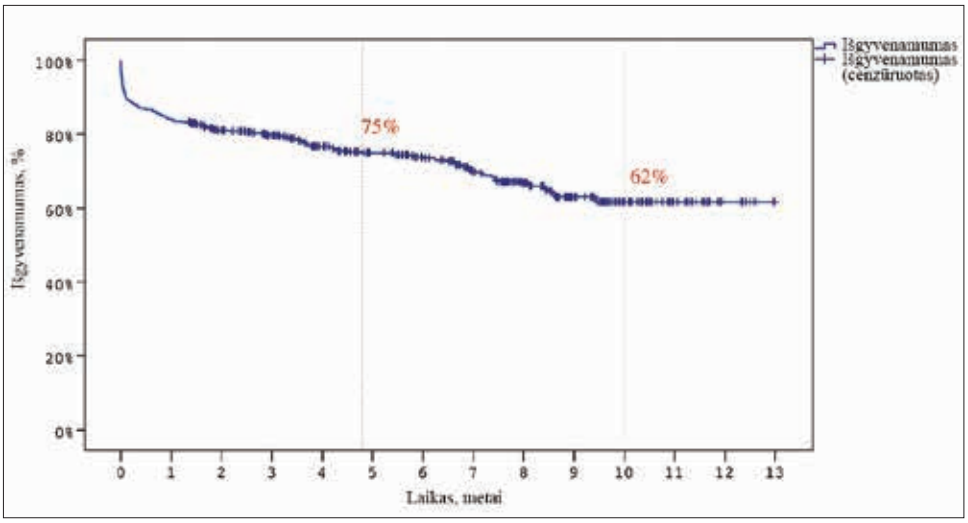
Labai ankstyvu pooperaciniu laikotarpiu mirė 18 (3,3 %) tiriamųjų imties ligonių. Penkiasdešimt devyni (10,7%) mirė per 30 dienų po MVP arba dar būdami ligoninėje po operacijos. Galvos kraujotakos sutrikimų ankstyvuoju pooperaciniu laikotarpiu patyrė 19 (3,4 %) ligonių. Ankstyvieji MVP pooperaciniai duomenys ir komplikacijos išsamiai aprašyti 4 lentelėje.

4 LENTELĖ. Ankstyvieji MVP pooperaciniai duomenys ir komplikacijos.

Rodiklis	N (%) / mediana [Q1–Q3]
Labai ankstyvas pooperacinis mirštamumas (iki 72 valandų)	18 (3,3 %)
Hospitalinis (iki 30 dienų po MVP) mirštamumas	59 (10,7 %)
Insultas	19 (3,4 %)
Pooperacinis širdies stimuliatoriaus implantavimas	61 (11,1 %)
Pooperacinė pakaitinė inkstų terapija	51 (9,3 %)
Resternotomija / revizija	67 (12,2 %)
Resternotomijos / revizijos priežastis	
Kraujavimas	40 (7 %)
Gili chirurginės žaizdos infekcija	5 (1 %)
Kardiopulmoninis gaivinimas	6 (1 %)
Ekstrakorporinės pagalbinės mechaninės kraujotakos poreikis	16 (3 %)
Nestabilus krūtinkaulis	6 (1 %)
Kraujo produktų perlipymas	
Raudonieji kraujo kūneliai	182 (33 %)
Šviežiai šaldyta plazma	145 (26,3 %)
Trombocitai	112 (20,3 %)
Kontrapulsacija aortos balionu	64 (11,6 %)
Ekstrakorporinės pagalbinės mechaninės kraujotakos poreikis	21 (3,8 %)
Gydymo reanimacijos skyriuje trukmė, dienos	3 (2–6)
Gydymo ligoninėje trukmė, dienos	14 (12–20)

4.4. Mitralinio vožtuvo PVF dažnis ir mirštamumas

Mūsų tirtoje 551 ligonio grupėje stebėjimo laikotarpio mediana buvo 5,5 metų, per šį laikotarpį mitralinio vožtuvo PVF nustatyta 38 (6,8 %) ligoniams, daugumai, t. y. 29 (77 %) ligoniams, – pirmaisiais po operacijos stebėjimo metais, likusiems 9 (23 %) – po vienerių metų nuo operacijos bet kuriuo stebėjimo laikotarpiu. Labai ankstyvos pooperacinės mirtys (iki 72 valandų po MVP) ištiko 18 (3,3 %) ligonių. Ligoninėje arba per 30 dienų po MVP mirė 59 (10,7 %) ligoniai (4 lentelė). Mirties tikimybė šioje ligonių kohortoje buvo ištirta pagal Kaplano ir Mejerio kreivę. Paaiškėjo, kad 5 metų išgyvenamumas yra 75 % (mirštamumas 25 %), o 10 metų išgyvenamumas – 62 % (mirštamumas 38 %) (žr. 10 paveikslą).



10 PAV. Ligonių po MVP Kaplano ir Mejerio išgyvenamumo kreivė.

4.5. Mitralinio vožtuvo PVF susidarymo rizikos veiksnių nustatymas

Vertinant veiksnius, turėjusius reikšmingų sąsajų su mitralinio vožtuvo PVF, iš pradžių atlikta paprastoji vienmatė analizė. Ji parodė, jog dažnesnį PVF lėmė vyriškoji lytis (p = 0,04); išeminė mitralinio vožtuvo pažeidimo etiologija (p = 0,013); MVP operaciją atlikęs chirurgas (p = 0,001); didesnis KS diastolinis matmuo (p = 0,013); ištisinė siūlė implantuojant protezą (p = 0,023).

Atlikus pusiau parametrinę proporcingos rizikos analizę Kokso metodu (5 lentelė) nustatyta, jog vyriškoji lytis (p = 0,043), kūno paviršiaus plotas ≥ 2 m2 (p =

5 LENTELĖ. Vienmatė analizė nustatant veiksnius, susijusius su mitralinio vožtuvo PVF susidarymu.

Rodiklis	Šansų santykis	95 % pasikliautinis intervalas	p reikšmė
Vyriškoji lytis	1,935	1,020–3,668	0,043
Kūno paviršiaus plotas, ≥2 m²	2,268	1,189–4,311	0,013
Ištisinės siūlės metodika	1,871	0,989–3,540	0,054
Chirurgas B	1,07	0,524–2,185	0,854
Chirurgas Z	2,989	1,565–5,709	0,001
Chirurgas P	0,268	0,048–1,51	0,136
Išeminės kilmės mitralinio vožtuvo yda	2,763	1,263–6,047	0,011
Kairiojo skilvelio diastolinis matmuo	1,679	1,139–2,476	0,009
B, P, Z – koduoti chirurgai			

0,013), ištisinės siūlės metodika (p = 0,054), MVP operaciją atlikęs chirurgas (p = 0,001), išeminės kilmės mitralinio vožtuvo yda (p = 0,011) ir KS diastolinis matmuo (p = 0,009) buvo reikšmingi veiksniai mitralinio vožtuvo PVF susidaryti.

Kadangi mitralinio vožtuvo PVF gali susiformuoti bet kuriuo metu po operacijos, rizikos veiksniams pritaikyta vienmatė Kokso regresijos analizė ir nustatyta, kad chirurgas, atlikęs MVP operaciją, yra reikšmingas rizikos veiksnys mitralinio vožtuvo PVF susidaryti (šansų santykis 2,631; 95 % pasikliautinis intervalas 1,311–5,279; p = 0,011) ir išeminės kilmės mitralinio vožtuvo yda (rizikos santykis 2,343; 95 % pasikliautinis intervalas 1,020–5,383; p = 0,045), pavaizduota 6 lentelėje.

6 LENTELĖ. Vienmatė Kokso regresinė analizė nustatant riziką mitralinio vožtuvo PVF susidaryti.

Rodiklis	Rizikos santykis	95 % pasikliautinis intervalas	p reikšmė
Vyriška lytis	1,235	0,557–2,736	0,603
Kūno paviršiaus plotas	2,969	0,625–14,097	0,171
Kūno paviršiaus plotas ≥2 m²	1,825	0,914–3,641	0,081
Chirurgas Z	2,631	1,311–5,279	0,006
Išeminės kilmės mitralinio vožtuvo yda	2,343	1,020–5,383	0,045
Kairiojo skilvelio galinis diastolinis matmuo	1,439	0,891–2,323	0,136
Z – koduotas chirurgas			

Vienmatės Kokso regresijos analizės rezultatus patvirtino daugiamatė Kokso regresijos analizė. Reikšmingi rizikos veiksniai PVF susidaryti buvo chirurgas, atlikęs MVP operaciją (rizikos santykis 3,327; 95 % patikimumo intervalas 1,733–6,389; p = 0,000305) ir išeminės kilmės mitralinio vožtuvo yda (rizikos santykis 2,343; 95 % patikimumo intervalas 1,166 – 2,575; p = 0,0071). Duomenys pateikiami 7 lentelėje.

7 LENTELĖ. Daugiamatė Kokso regresijos analizė nustatant veiksnius, susijusius su mitralinio vožtuvo PVF susidarymu.

Rodiklis	Šansų santykis	95 % pasikliautinis intervalas	p reikšmė
Chirurgas Z	3,327	1,733–6,389	0,000305
Išeminės kilmės mitralinio vožtuvo yda	2,343	1,166–2,575	0,0071
Z – koduotas chirurgas			

4.6. Ligonių, gydytų dėl PVF, priešoperaciniai duomenys

Jie išsamiai pateikiami 8 lentelėje. Iš viso ligonių buvo 73, jie gydyti dviem skirtingais metodais. Pagal taikytą gydymo metodą tiriamieji suskirstyti į dvi grupes. Atlikta arba įprastinė pakartotinė operacija ir šie ligoniai priskirti chirurgijos gru-

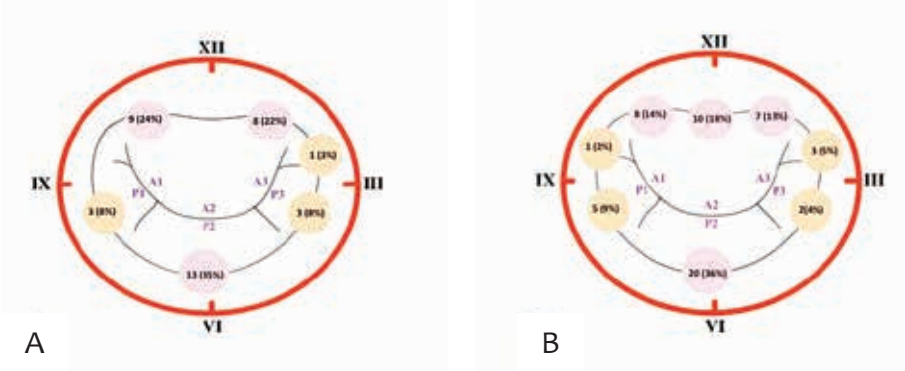
8 LENTELĖ. Ligonių, kuriems atlikta mitralinio vožtuvo PVF operacija, priešoperaciniaiduomenys.

Rodiklis	Visi ligoniai N (%) / mediana [Q1–Q3]	Kateteris N (%) / mediana [Q1–Q3]	Chirurgija N (%) / mediana [Q1–Q3]	*p*-reikšmė
Ligonių skaičius	73 (100 %)	24 (33 %)	49 (67 %)	
Amžius, metai	64 (58–68)	66 (61–69)	64 (57–67)	0,08
Lytis, vyrai	36 (49 %)	14 (58 %)	22 (45 %)	0,97
Operacijos atlikimo skubumas				
Planinė	69 (96 %)	23 (96 %)	46 (94 %)	0,73
Skubi	4 (5 %)	1 (4 %)	3 (6 %)	0,73
Laikas nuo MVP, mėnesiai	58 (13–177)	36 (13–177)	60 (14–179)	0,8
Ankstesnė operacija dėl PVF	9 (12 %)	4 (17 %)	5 (10 %)	0,43
NYHA				
II	8 (11 %)	5 (21 %)	3 (6 %)	0,06
III	54 (74 %)	16 (67 %)	38 (77 %)	0,32
IV	11 (15 %)	3 (12 %)	8 (16 %)	0,67
EuroSCORE II, %	7,4 (5,0–10,6)	6,0 (3,9–10,6)	8,0 (5,8–11,1)	0,07
STS mirštamumo rizika, %	1,95 (1,28–2,9)	1,8 (1,4–2,4)	1,98 (1,4–3,2)	0,32
Cukrinis diabetas	6 (8 %)	4 (17 %)	2 (4 %)	0,18
Hemolizė	28 (38 %)	13 (68 %)	15 (31 %)	0,05
Anemija, kai $Hb^c < 100$ g/l	19 (26 %)	9 (38 %)	10 (20 %)	0,12
Kairiojo skilvelio funkcija				
KSIF < 30%	3 (4 %)	1 (4 %)	2 (4 %)	0,97
30 % ≤ KSIF < 45 %	24 (33 %)	9 (38 %)	15 (31 %)	0,56
45 % ≤ KSIF < 55 %	28 (38 %)	6 (25 %)	22 (45 %)	0,1
KSIF ≥ 55%	18 (25 %)	8 (33 %)	10 (20 %)	0,23
PA spaudimas >55 mmHg	26 (36 %)	9 (38 %)	17 (35 %)	0,45
Vožtuvo protezo tipas				
Biologinis	9 (12 %)	5 (21 %)	4 (8 %)	0,12
Mechaninis	62 (85 %)	17 (71 %)	45 (92 %)	0,019
Anuloplastikos žiedas	2 (3 %)	2 (8 %)	0 (0 %)	0,04
Indikacijos gydyti PVF				
Hemolizinė mažakraujystė	3 (4 %)	2 (8 %)	1 (2 %)	0,2
Širdies nepakankamumas	45 (62 %)	11 (46 %)	34 (69 %)	0,05
Dviejų sindromų derinys	25 (34 %)	11 (46 %)	14 (29%)	0,144
MVP PVF skaičius ligoniui				
1 defektas	57 (78 %)	14 (58 %)	43 (88 %)	0,004
2 defektas	12 (16 %)	7 (29 %)	5 (10 %)	0,04
3 defektas	4 (5 %)	3 (13 %)	1 (2 %)	0,24
Paravalvulinės regurgitacijos laipsnis				
Vidutinio laipsnio	22 (30 %)	4 (17 %)	17 (35 %)	0,11
Didelio laipsnio	51 (70 %)	20 (83 %)	32 (65 %)	0,11

pei, arba kateterinis mitralinio vožtuvo PVF užkimšimas ir šie ligoniai priskirti kateterio grupei. Reikšmingo kintamųjų skirtumo tarp grupių nenustatyta.

4.7. Mitralinio vožtuvo PVF topografinė lokalizacija

Mitralinio vožtuvo PVF topografinė lokalizacija vaizduojama 11 paveiksle.



11 PAV. Mitralinio vožtuvo PVF topografinė lokalizacija, pavaizduota pagal adaptuotą laikrodžio schemą (De Cicco G, 2006): A – kateterio grupė; B – chirurgijos grupė. III, VI, IX ir XII – laikrodžio valandos; A1, A2, A3, P1, P2, P3 – mitralinio vožtuvo segmentai.

4.8. Mitralinio vožtuvo PVF gydymo ankstyvieji pooperaciniai duomenys ir komplikacijos

Labai ankstyvu laikotarpiu (per pirmąsias 72 valandas) po operacijos kateterio grupėje mirčių nebuvo, chirurgijos grupėje mirė 5 ligoniai (10 %); (p = 0,15). Hospitalinis mirštamumas (per 30 dienų arba ligoninėje) tarp grupių skyrėsi statistiškai reikšmingai: chirurgijos grupėje per minimą laikotarpį mirė 9 (12 %) ligoniai, o kateterio grupėje mirčių nebuvo (p = 0,025). Chirurgijos grupės 8 (16 %) ligoniai patyrė miokardo infarktą per pirmąsias 72 valandas po operacijos, šios komplikacijos kateterio grupės ligoniams nebuvo (p = 0,036). Ankstyvieji pooperaciniai duomenys išsamiai pateikiami 9 lentelėje.

9 LENTELĖ. Mitralinio vožtuvo PVF gydymo ankstyvieji pooperaciniai duomenys ir komplikacijos.

Rodiklis	Visi ligoniai N (%) / mediana [Q1–Q3]	Kateteris N (%) / mediana [Q1–Q3]	Chirurgija N (%) / mediana [Q1–Q3]	p -reikšmė
Ligonių skaičius	73 (100 %)	24 (33 %)	49 (67 %)	
Ankstyvas mirštamumas (< 72 h)	5 (7 %)	0 (0 %)	5 (10 %)	0,15
Hospitalinis mirštamumas (30 dienų)	9 (12 %)	0 (0 %)	9 (18 %)	0,025

Rodiklis	Visi ligoniai N (%) / mediana [Q1–Q3]	Kateteris N (%) / mediana [Q1–Q3]	Chirurgija N (%) / mediana [Q1–Q3]	*p* -reikšmė
Ankstyvas miokardo infarktas (≤72 h)	8 (11 %)	0 (0 %)	8 (16 %)	0,036
Miokardo infarktas (per 30 dienų)	9 (12 %)	0 (0 %)	9 (18 %)	0,025
Insultas (per 30 dienų arba ligoninėje)	3 (4 %)	0 (0 %)	3 (6 %)	0,22
Ūminis inkstų funkcijos nepakankamumas	19 (26 %)	5 (21 %)	14 (28 %)	0,48
1 stadija (SCr^c ↑ 1,5–2 kartus)	7 (10 %)	2 (8 %)	5 (10 %)	0,8
2 stadija (SCr^c ↑ 2–3 kartus)	4 (6 %)	3 (13 %)	1 (2 %)	0,07
3 stadija (SCr^c ↑ >3 kartus / IPT)	8 (11 %)	0 (0 %)	8 (16 %)	0,04
Kraujavimas pagal BARC	16 (22 %)	2 (8 %)	14 (28 %)	0,05
5, 3b ir 3c tipų	9 (12 %)	0 (0 %)	9 (18 %)	0,03
3a tipo	2 (3 %)	1 (4 %)	1 (2 %)	0,6
2 ar 3a tipo	5 (11 %)	1 (4 %)	4 (9 %)	0,52
Chirurginės prieigos komplikacijos				
Sunkios	12 (16 %)	1 (4 %)	11 (22 %)	0,05
Lengvos	5 (7 %)	0 (0 %)	5 (10 %)	0,11
Širdies ritmo ir laidumo sutrikimai				0,2
Naujas arba pablogėjęs sutrikimas	7 (10 %)	1 (4 %)	6 (12 %)	0,3
Elektrinio širdies stimuliatorius	4 (6 %)	0 (0 %)	4 (9 %)	0,025
Chirurginė revizija	6 (8 %)	1 (4 %)	5 (10 %)	0,4
Sepsis	7 (10 %)	0 (0 %)	7 (14 %)	0,05
Pooperacinis drenažas, ml/24 h	200 (100–500)	135 (100–250)	675 (600–1550)	0,001
Gydymo trukmė ligoninėje, dienos	13 (10–19)	10 (8–13)	15 (12–21)	0,001
Gydymo trukmė RITS, dienos	2 (1–4)	1 (1–1)	3 (2–8)	0,001

BARC 2 tipas – bet kuris kraujavimo požymis, kuris neatitinka 3, 4 ar 5 tipo kriterijų, bet reikalaujantis sveikatos priežiūros specialisto neplaninio mediciniinio įsikišimo; BARC 3a tipas – kraujavimas ir hemoglobino sumažėjimas nuo 3 iki 5 g /l; BARC 3b tipas – kraujavimas ir hemoglobino sumažėjimas ≥ 5 g/l arba širdies tamponada, arba kraujavimas, reikalaujantis chirurginės intervencijos kontrolės, arba kraujavimas, sukeliantis hemodinamikos nestabilumą ir simpatomimetikų poreikį; BARC 3c tipas – intrakranijinis kraujavimas arba intraokulinis kraujavimas, sutrikdantis regėjimą; BARC 5a tipas – gyvybei grėsmingas kraujavimas; BARC 5b tipas– mirtį sukėlęs kraujavimas; RITS - reanimacijos ir intensyviosios terapijos skyrius;
SCr^c – kreatinino koncentracija serume.

4.9. Mitralinio vožtuvo PVF gydymo rezultatai išrašant iš ligoninės

Iš viso 64 ligoniai iš 73-ijų imties išrašyti iš gydymo įstaigos. Išrašyti gyvi keturiasdešimt chirurgijos grupės ligonių (82 %) ir visi (100 %) kateterio grupės ligoniai (p = 0,025). PVF defektas visiškai panaikintas (be liekamosios regurgitacijos) 19 (79 %) kateterio grupės ligonių ir 36 (90 %) chirurgijos grupės, statistiškai reikšmingo skirtumo nenustatyta. Šie duomenys išsamiai pateikiami 10 lentelėje.

10 LENTELĖ. Paravalvulinė regurgitacija išrašant ligonius po mitralinio vožtuvo PVF gydymo

Rodiklis	Visi ligoniai N (%) / mediana [Q1–Q3]	Kateteris N (%) / mediana [Q1–Q3]	Chirurgija N (%) / mediana [Q1–Q3]	*p* reikšmė
Išrašytų ligonių skaičius	64 (88 %)	24 (100 %)	40 (82 %)	0,025
Paravalvulinės regurgitacijos laipsnis				
Nėra	55 (86 %)	19 (79 %)	36 (90 %)	0,23
Nedidelio laipsnio	5 (8 %)	4 (17 %)	1 (2,5 %)	0,04
Vidutinio laipsnio	1 (2 %)	0 (0 %)	1 (2,5 %)	0,43
Didelio laipsnio	3 (4 %)	1 (4 %)	2 (4 %)	0,88

4.10. Vėlyvieji mitralinio vožtuvo PVF gydymo rezultatai

Bendra visų ligonių stebėjimo laikotarpio trukmės mediana buvo 3 (1,5–8,5) metai, kateterio grupės ligonių – 1,9 (0,97–2,23) metų, chirurgijos grupės – 6 (2,87–9,3) metai ($p = 0,001$).

Bendras mirštamumas per stebėjimo laikotarpį buvo 29 %, kateterio grupės mirė 2 ligoniai (8 %), chirurgijos grupės – 19 (39 %) ($p = 0,007$). Reikšminga ar vidutinio laipsnio PVF regurgitacija atsinaujino keturiems (10 %) chirurgijos grupės ligoniams ir tik vienam (4 %) kateterio grupės ligoniui (11 lentelė).

11 LENTELĖ. Vėlyvieji mitralinio vožtuvo PVF gydymo rezultatai.

Rodiklis	Visi ligoniai N (%) / mediana [Q1–Q3]	Kateteris N (%) / mediana [Q1–Q3]	Chirurgija N (%) / mediana [Q1–Q3]	*p* reikšmė
Stebėjimo laikotarpis, metai	3 (1,5–8,5)	1,9 (0,97–2,23)	6 (2,87–9,3)	< 0,001
Mirštamumas	21 (29 %)	2 (8 %)	19 (39 %)	0,007
PVL atsinaujinimas	5 (8 %)	1 (4 %)	4 (10 %)	0,4
Vidutinis nesandarumas	1 (2 %)	0 (0 %)	1 (2,5 %)	0,43
Didelis nesandarumas	4 (6 %)	1 (4 %)	3 (7,5 %)	0,6

5. Rezultatų aptarimas

5.1. Mitralinio vožtuvo PVF dažnis

Natūrali mitralinio vožtuvo patologijos eiga buvo reikšmingai paveikta mitralinio vožtuvo protezavimo operacijų, kurių tikslas – pagerinti ligonių išgyvenamumą ir sumažinti ligos simptomus. Deja, po šių operacijų pasitaiko įvairių komplikacijų. Viena iš tokių yra mitralinio vožtuvo protezo PVF. Jei ši komplikacija tinkamai negydoma, ligonių simptomai progresuoja ir gali sukelti priešlaikinę mirtį. Nors literatūroje gausu šaltinių, nagrinėjančių mitralinio vožtuvo PVF dažnį ir jų atsiradimo priežastis, mes nutarėme išnagrinėti šiuos veiksnius ir mūsų tirtoje populiacijoje. Tiriamųjų skaičius mūsų atliktoje analizėje galėjo būti gausesnis, nes dėl tikslinių retrospektyviųjų duomenų stokos reikšmingas skaičius ligonių buvo pašalintas iš tyrimo. Apžvelgę literatūroje pateikiamas tiriamųjų kohortas, pastebėjome, kad jų dydžiai įvairuoja nuo 117 iki 1202 ligonių (Ionescu A, 2003; Genoni M, 2000, Dhasmana JP, 1983; Bonnefoy E, 1995; Hwang HY, 2015).

Atsižvelgiant į pasaulinius duomenis, mūsų tyrimo imties dydis yra pakankamas siekiant nustatyti mitralinio vožtuvo PVF dažnį, t. y. 551 ligonio grupė po mitralinio vožtuvo protezavimo operacijos.

Mitralinio vožtuvo PVF dažnis, nurodomas prieinamoje literatūroje, labai skiriasi ir tai priklauso nuo tokių kintamųjų kaip ligonių stebėjimo laikotarpis atskiros studijose, dešimtmetis, kai buvo atlikta studija, mitralinio vožtuvo siuvimo metodika ir kitų. Mitralinio vožtuvo PVF dažnis, įvairių autorių duomenimis, svyruoja nuo 2,8 % iki 31 % (Dhasmana JP, 1983; Ionescu A, 2003; Ionescu MI, 1982; Hammermeister K, 2000; Bonnefoy E, 1995; Hwang HY, 2015).

Mūsų nagrinėtoje 551 ligonio kohortoje mitralinio vožtuvo protezo PVF dažnis siekė 6,8 % per 5,5 metų stebėjimo laikotarpį ir šie duomenys nesiskiria nuo tų, kurie pateikiami literatūroje, o lyginant su kai kuriomis studijomis mūsų ligoniams mitralinio vožtuvo PVF pasitaikė rečiau.

5.2. Mitralinio vožtuvo PVF rizikos veiksniai

Pasaulio mokslinėje medicinos literatūroje galima aptikti įvairių veiksnių, kurie gali didinti mitralinio vožtuvo PVF susidarymo riziką. Iš jų pažymėtini mitralinio vožtuvo žiedo kalkėjimas, infekcija, siuvimo implantuojant protezą metodika, protezo dydis ir tipas (mechaninis arba biologinis), netgi ligonio rasė ir kitos (Hammermeister K, 2000; Ionescu A, 2003; Dhasmana JP, 1983; Hwang HY, 2015).

Mes išanalizavome visus veiksnius, galinčius didinti mitralinio vožtuvo PVF susidarymo riziką, ir juos išsamiai pateikėme 5 lentelėje. Daugiaveiksnė Kokso regresinė analizė parodė, kad mūsų populiacijoje svarbiausi rizikos veiksniai

buvo konkretus chirurgas, atlikęs MVP, ir išeminės kilmės mitralinio vožtuvo ydos etiologiją.

Norime atkreipti dėmesį, kad mums nepavyko rasti nė vieno šaltinio, kuriame išeminė mitralinio vožtuvo ydos etiologija ir chirurgas, atlikęs operaciją, būtų įvardijami kaip reikšmingi mitralinio vožtuvo PVF susidarymo rizikos veiksniai.

Aptardami išeminės kilmės mitralinio vožtuvo ydos etiologiją kaip priežastį PVF atsirasti, manome, kad nuolatinė neigiama KS remodeliacija su chirurginiu būdu fiksuotu protezu mitralinio vožtuvo pozicijoje gali sukelti natyvinio mitralinio vožtuvo žiedo atplyšimą nuo protezo, taip sudarydama sąlygas paravalvuliniam defektui susiformuoti.

Kalbant apie chirurgą kaip mitralinio vožtuvo PVF susidarymo rizikos veiksnį, reikia pasakyti, kad literatūroje gausu publikacijų, kuriose nagrinėjamos įvairios chirurgų charakteristikos, galinčios būti rizikos veiksniais įvairioms komplikacijoms atsirasti. Tarp šių charakteristikų minima patirtis, atliekamų procedūrų skaičius, rekomendacijų taikymas ir kitos. Siekiant sumažinti įvairių komplikacijų dažnį, rekomenduojama taikyti kokybės bei veiklos kontrolę ir tobulinti arba keisti gydymo metodikas (Bird SM, 2005; Bridgewater B, 2006; LaPar DJ, 2013; Schneider EC, 1996; Huckman RS, 2006; Lovegrove J, 1997). Tačiau išsami chirurgo kaip rizikos veiksnio analizė nėra mūsų tyrimo uždavinys ar tikslas, todėl gali būti kito, tęstinio, darbo tema, kuriame būtų galima nagrinėti tokius veiksnius kaip patirtis, chirurginiai metodai, kasmetis operacinis krūvis ir kt.

5.3. Ankstyvieji ir vėlyvieji MVP rezultatai

Mūsų tyrimo duomenimis, labai ankstyvas (iki 72 valandų po operacijos) procedūrinis mirštamumas po MVP buvo 3,3 %, o 30 dienų arba hospitalinis mirštamumas – 10,7 %. Ilgalaikio išgyvenamumo analizė, atlikta naudojant Kaplano ir Mejerio modelį, parodė, kad penkerių metų išgyvenamumas yra 75 %, o 10 metų – 62 %.

Lyginant mūsų tyrime nustatytus ankstyvuosius ir vėlyvuosius mirštamumo rezultatus su literatūroje prieinamas duomenimis, verta paminėti keletą tyrimų, kuriuose 30 dienų mirštamumas svyruoja nuo 2,7 % iki 9,1 % (Bakaeen FG, 2018; Badhwar V, 2016; Hammermeister KE,1987; Thourani VH, 2003; Miller DL, 1995).

Vienas iš mūsų tyrimo gautų rezultatų yra didelis mirštamumas po mitralinio vožtuvo protezavimo ankstyvuoju laikotarpiu po operacijos. Šiandienos palyginimas rodo, kad mūsų kohortoje 30 dienų mirštamumo rezultatai po mitralinio vožtuvo protezavimo yra prastesni, o ilgalaikio išgyvenamumo rezultatai yra panašūs į tuos, kurie randami literatūroje.

Siekiant nustatyti ankstyvo didelio pooperacinio mirštamumo priežastis, tikslinga išsamiai išnagrinėti reikšmingiausias pooperacines komplikacijas šioje ligonių kohortoje, bet tai – jau kito mokslinio darbo uždavinys.

Kadangi mūsų tiriamųjų ilgalaikio išgyvenamumo rezultatai yra panašūs į tuos, kurie skelbiami literatūroje, sumažinus pooperacinio (30 dienų) mirštamumo rodiklius, būtų galima tikėtis geresnio ligonių išgyvenamumo vėlyvuoju laikotarpiu po operacijos.

5.4. Kateterinis mitralinio vožtuvo PVF užkimšimas

Dar prieš dešimtmetį vienintelis veiksmingas ir visuotinai pripažintas mitralinio vožtuvo PVF gydymo būdas buvo pakartotinė širdies operacija su dirbtine kraujo apytaka (Miller DL, 1995). Yra žinoma, kad didelis pooperacinis sergamumas ir mirštamumas yra neatsiejami nuo pakartotinės mitralinio vožtuvo operacijos dėl PVF (Hammermeister K, 2000; Genoni M, 2000; Echevarria J, 1991). Siekis sumažinti šiuos didelio pooperacinio mirštamumo ir sergamumo rodiklius, taip pat spartus įvairių kateterinių širdies procedūrų vystymasis paskatino medikus diegti į klinikinę praktiką mažiau invazyvų ir tikėtinai saugesnį gydymo būdą – kateterinį PVF užkimšimą. Plintant šiai procedūrai pasauliniu mastu, iš pradžių paravalvuliniams defektams užkimšti buvo naudojami kamščiai, skirti kitokio pobūdžio patologijai gydyti, tai yra jie naudoti ne pagal paskirtį. Neseniai buvo sukurtas ir pradėtas klinikinėje praktikoje naudoti naujas prietaisas, skirtas būtent PVF užkimšti.

Iki šiol yra paskelbti tik keli darbai, kuriuose mitralinio vožtuvo PVF gydoma chirurginiu kateteriniu būdu per širdies viršūnę (Smolka G, 2013; Aydin U, 2017). Prieš keletą metų mūsų grupė į klinikinę praktiką pradėjo diegti mažai invaz yvų chirurginį kateterinį mitralinio vožtuvo PVF gydymą per širdies viršūnę. Literatūroje aptikti įrodymai paskatino mus pasirinkti chirurginę atvirą prieigą per širdies viršūnę, pro kurią įstumiamas kateteris PVF užkimšti. Yra šaltinių, įrodančių, kad tokia prieiga prie širdies viršūnės pasižymi retomis komplikacijomis; leidžia prieiti prie defektų visose anatominėse mitralinio protezavimo vietose; lemia mažą rentgeno spinduliuotės apšvitą ir procedūros trukmę (Nijenhuis VJ, 2014; Jelnin V, 2011; Calvert PA, 2016). Be to, mūsų centre tuo metu mitralinio vožtuvo nesandarumo gydymo metodika per širdies viršūnę jau buvo praktikuojama (Rucinskas K, 2014), tad ši patirtis buvo perimta mitralinio vožtuvo PVF užkimšti. Atviras chirurginis prieigos prie širdies viršūnės metodas taip pat buvo pasirinktas siekiant efektyviau kontroliuoti skilvelio punkcijos vietą ir išvengti vainikinių arterijų pažeidimo. Remdamiesi kitais tyrėjais ir savo pradine patirtimi, nustatėme, kad chirurginis prieigos prie širdies viršūnės metodas leidžia mitralinio vožtuvo PVF užkimšti visose mitralinio vožtuvo žiedo lokalizacijose.

5.5. Chirurgija *vs* kateteris gydant mitralinio vožtuvo PVF

Šios disertacijos rezultatai, taip pat mokslinėje literatūroje pateikti duomenys rodo, kad įprastinė pakartotinė širdies operacija naudojant dirbtinę kraujo apytaką gydyti ligoniams, kuriems nustatytas mitralinio vožtuvo paraprotezinis nesandarumas, susijusi su didesniu ankstyvu sergamumu ir nepriimtinai dideliu pooperaciniu mirštamumu, palyginti su kateterine intervencija per širdies viršūnę specialiu tam skirtu prietaisu. Panašūs rezultatai aptinkami ir kituose patikimuose literatūros šaltiniuose. Atlikę šį tyrimą, mes taip pat galime teigti, jog ligonių, kuriems nustatytas mitralinio vožtuvo paraprotezinis nesandarumas, gydymo kateteriniu būdu rezultatai nenusileidžia įprastinės pakartotinės širdies chirurgijos rezultatams.

5.6. Tyrimo ribotumai

Šis tyrimas turi keletą apribojimų.

Pirmiausia, abu šio tyrimo etapai atlikti remiantis retrospektyviaisiais vieno centro duomenimis, sukauptais gydant MV nesandarumą turinčius ligonius. Tik ligonių, specialiai įtrauktų į kateterinio gydymo grupę, duomenys gauti prospektyviuoju būdu.

Antra, ligonių skaičius pirmajame šio tyrimo etape galėjo būti gerokai didesnis, tačiau reikšminga atvejų dalis iš tyrimo buvo pašalinta dėl to, kad ligos istorijose labai stigo klinikinių duomenų. Ši priežastis lėmė, jog vykdydami tyrimą mes negalėjome pagal tarptautines rekomendacijas pateikti sergamumo ir mirštamumo po mitralinio vožtuvo protezavimo rodiklių. Nepaisant to, šio tyrimo pirmajame etape surinkti duomenys šiai specifinei problemai analizuoti šiandien yra gausiausi ir išsamiausi Lietuvoje.

Trečia, tarptautinių randomizuotų tyrimų duomenų trūkumas riboja mūsų galimybes palyginti savo duomenis su kitų tyrėjų grupių duomenimis, todėl pateiktos šios disertacijos išvados nėra tokios tvirtos, kokios galėtų būti.

Ketvirta, skirtingų gydymo būdų palyginimas atliktas naudojant palyginti mažą atvejų skaičių, ypač kateterinio gydymo grupėje, todėl, norint galutinai įrodyti arba paneigti vieno arba kito metodo pranašumą, būtina tęsti tyrimus įtraukiant daugiau ligonių.

Penkta, visuose literatūros šaltiniuose pateikiamos kateterinio mitralinio vožtuvo PVF gydymo būdo grupės nėra tokios homogeniškos, kaip mūsų tyrimo kateterinio gydymo grupė. Tai yra – daugumoje publikuotų tyrimų naudojami keli skirtingi kateterinio fistulės užkimšimo metodai, taip pat keli skirtingi prietaisai. Tai lėmė, kad mūsų duomenų palyginimas su literatūroje pateiktais duomenimis nėra tikslus.

6. Išvados

Mūsų tirtoje populiacijoje mitralinio vožtuvo PVF paplitimas siekia 6,8 %, esant ligonių stebėsenos po pirminės mitralinio vožtuvo protezavimo operacijos medianai 5,5 metų, ir tai atitinka literatūros šaltiniuose nurodytą mitralinio vožtuvo PVF paplitimą.

Reikšmingiausi mitralinio vožtuvo PVF susidarymo rizikos veiksniai mūsų tirtoje populiacijoje buvo ligonį operavęs chirurgas ir išeminė mitralinio vožtuvo pirminio pažeidimo etiologija.

Mūsų tirtos populiacijos labai ankstyvas mirštamumas po mitralinio vožtuvo protezavimo operacijos yra 3,3 %, hospitalinis arba 30 dienų pooperacinis mirštamumas – 10,7 %. Ligonių penkerių metų išgyvenamumas – 75 %, o 10 metų – 62 %.

Chirurginis kateterinis mitralinio vožtuvo PVF gydymas per širdies viršūnę naudojant tam skirtą prietaisą savo efektyvumu nenusileidžia įprastinei pakartotinei širdies operacijai siekiant panaikinant paravalvulinę regurgitaciją.

Chirurginis kateterinis mitralinio vožtuvo PVF gydymas per širdies viršūnę, naudojant tam skirtą prietaisą, yra saugesnis gydymo metodas, lyginant ankstyvuosius pooperacinius rezultatus su įprastinės pakartotinės širdies operacijos, naudojant dirbtinę kraujo apytaką, rezultatais.

7. Praktinės rekomendacijos

Šiuo metu ligonių, kuriems nustatyta mitralinio vožtuvo paravalvulinė fistulė, pirmo pasirinkimo gydymo būdas turėtų būti chirurginis kateterinis mitralinio vožtuvo PVF užkimšimas per širdies viršūnę, naudojant specialiai tam sukurtą prietaisą.

Įprastinė pakartotinė širdies operacija naudojant dirbtinę kraujo apytaką išlieka alternatyvus gydymo metodas tų ligonių, kuriems nepavyko defekto užkimšti kateteriniu būdu arba jiems kateterinis defekto užkišimas yra kontraindikuojamas.

Publikacijų darbo tema sąrašas

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2. Zorinas A, Janušauskas V, Davidavičius G, Šimakauskas R, Puodžiukaitė L, Zakarkaitė D, Bilkis V, Čypienė R, Samalavičius R, Onorato E, Aidietis A, Ručinskas K. Retrospective analysis of single-center early and midterm results of transapical catheter-based mitral paravalvular leak closure with a purpose-specific device. Advances in Interventional Cardiology/Postępy w Kardiologii Interwencyjnej. 2018; 14(2): 167–175. doi:10.5114/aic.2018.76408.

3. Onorato EM, Zorinas A, Janusauskas V, Davidavcius G, Zakarkaite D, Kramena R, Bilkis V, Rucinskas K, Samalavicius R, Aidietis A. Occlutech® Paravalvular Leak Device (PLD). In: Transcatheter Paravalvular Leak Closure. Singapore: Springer; 2017, p. 55–77.

Tarptautinių pranešimų darbo tema sąrašas

1. Zorinas A, Janusauskas V, Simkauskas R, Rucinskas K, Aidietis A. "Surgical vs. catheter-based paravalvular mitral valve leak closure (transapical approach). Early results: Single center experience". At the 25th Anniversary Congress of the World Society of Cardiothoracic Surgeons being hosted by the Royal College of Surgeons of Edinburgh, September 2015.

2. Zorinas A, Bilkis V, Simakauskas R, Janusauskas V, Rucinskas K, Davidavicius G, Zakarkaite D, Aidietis A. "Catheter-based mitral valve paravalvular leak closure. Early results". At the 16th International Society for Minimally Invasive Cardiothoracic Surgery (ISMICS) Annual Scientific Meeting, June 2016.

3. Zorinas A, Janusauskas V, Simkauskas R, Bilkis V, Rucinskas K, Aidietis A. "Mitral paravalvular leak. Retrospective comparison of surgical treatment modalities in 50 patients. Single centre experience". At the 26th Anniversary Congress of the World Society of Cardiothoracic Surgeons, September 2016.

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Audronė Mulevičienė

NUTRITIONAL ANEMIA RELATION TO CHANGES OF HEALTH INDICATORS AND FECAL MICROBIOTA IN INFANCY AND EARLY CHILDHOOD

KŪDIKIŲ IR MAŽŲ VAIKŲ MITYBINIŲ MAŽAKRAUJYSČIŲ RYŠYS SU SVEIKATOS RODIKLIŲ IR IŠMATŲ MIKROBIOTOS POKYČIAIS

Biomedical Sciences, Medicine 06B
Biomedicinos mokslai, medicina (06 B)

Abbreviations

BM	Breast milk
IDA	Iron deficiency anemia
WHO	World Health Organization
PHC	Primary healthcare
PCoA	Principal coordinates analysis
HC	Healthy controls

Definitions

Health indicators – anthropometric measurements (birthweight, weight by age in months) and nutritional peculiarities of infants and young children.

Exclusive breastfeeding – infants receive only breast milk (BM) (directly from the breast or expressed BM) without any food or drink, not even water; except for oral rehydration solutions, medicines and vitamins under medical indications [1].

Predominant breastfeeding – infants receive BM (directly from the breast or expressed BM) as the main food source along with other drinks (water, water-based liquids, juice), except for non-human milk, formula or solid, semi-solid, or soft food [1].

Complementary feeding – infants receive solid, semi-solid or soft food and any liquids along with BM [1].

Breastfeeding on demand – breastfeeding as often and as long as the infant or child wishes [2].

Continued breastfeeding – breastfeeding along with the food appropriate for the infant's or child's age [1].

Diet diversity – consumption of four or more food groups except for BM or infant formula: vegetables and fruit, cereals (including wholegrain bread), meat, fish, eggs and dairy products (including only unsweetened products of sour milk, cottage cheese and cheese) [1].

Products with added sugar – sweetened dairy products, canned juice, cookies, sweets and chocolates.

Low birthweight – birthweight that is less than 2500 g regardless of gestational age [3].

Introduction

Anemia as a serious child health problem

Anemia is diagnosed when the number of red cells and/or hemoglobin decreases and the supply of oxygen to the tissues is disturbed [4]. Children are the most vulnerable group due to the rapid growth and high needs of micronutrients and vitamins [5]. Even though the majority of anemic patients reside in developing countries, this problem is relevant to developed countries as well [6, 7].

Nutritional anemia develops when the dietary intake of iron, folic acid, vitamin B12, copper, etc. does not meet the demands of the human body and is common in infants and young children all over the world [5, 6]. Iron deficiency is the biggest concern as it is responsible for the development of anemia in more than 50% of the cases [6]. The majority of processes in the brain are known to be dependent on iron- containing proteins. Therefore, iron deficiency disturbs myelination, neurotransmitter synthesis and metabolism, dendritogenesis and synaptogenesis in the structures of the central nervous system, which are responsible for hearing and vision, speech comprehension and usage as well as for the higher cognitive functions [8, 9]. Even though timely therapy stabilizes the iron status, anemic infants achieve lower results in cognitive tests and this disparity increases with time if a child is raised in poor social conditions [10].

Moreover, iron is essential not only for the human body, but also for gut bacteria. Inadequate nutrition may negatively impact the composition of microbiota [11]. Recently it has been established that the interaction between the host and gut microbial community plays a crucial role in human health. Microbiota's changes are related to the higher risk of chronic non-communicable diseases: allergies, obesity, atherosclerosis, ischemic heart disease [12]. Therefore, appropriate prophylaxis of nutritional anemia is essential for improving the health of infants and young children [13].

ccording to the World Health Organization (WHO), the most effective measures to ensure optimal nutrition of infants and young children during the first two years of life are: 1) early initiation of breastfeeding, 2) exclusive breastfeeding for 6 months, 3) promotion, protection and support of the breastfeeding on demand until 2 years of age and beyond with an adequate complementary feeding, 4) micronutrient supplementation for low-birthweight infants [5, 13]. Health care providers have the greatest impact in adjusting nutritional habits and an evidence-based everyday practice in the primary healthcare (PHC) setting is crucial [13].

Relevance and scientific novelty

Nutritional anemia in infants and young children (up to 36 months old) is a relevant problem in Lithuania (Fig. 1). According to the data of Institute of Hygiene (Vilnius, Lithuania), the incidence of anemia has more than doubled in this group from 2010 to 2014: from 16.6 to 35.8 cases per 1,000 children [14].

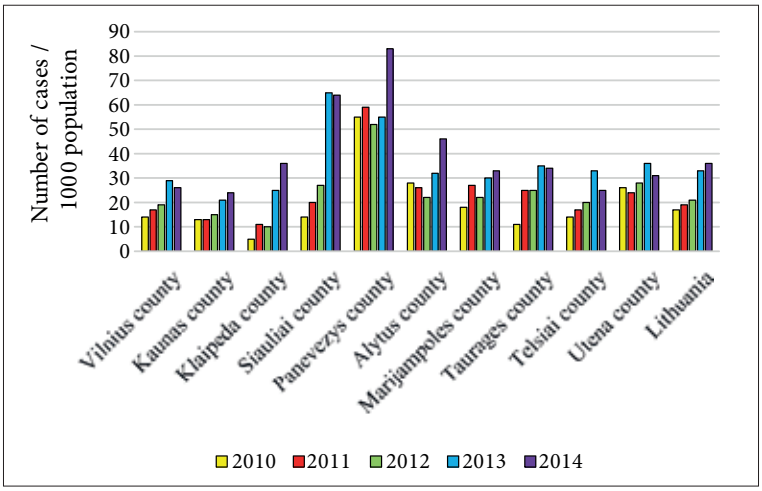

FIGURE 1. Incidence of nutritional anemia in infants and children under 3 years of age in Lithuania [14].

Nevertheless, previously the highest attention was paid to early diagnosis and treatment of anemia. Data on the risk factors associated with anemia in Lithuanian children is scarce [15, 16]. Therefore, this study focused on anthropometric measurements (birthweight, weight by age) and nutritional peculiarities of infants and young children diagnosed with nutritional anemia. An original, validated questionnaire was used for the first time in order to assess dietary habits of patients. This instrument could be implemented and used in the practice of primary healthcare physicians on a daily basis.

Moreover, a survey was conducted in order to assess the level of nutritional anemia prophylaxis performed by physicians in primary healthcare. The findings of the survey revealed gaps in providing infant breastfeeding and complementary feeding recommendations. It showed that nutritional anemia is a much more complex problem in Lithuania and there is a need for educating both parents and physicians. Based on the results of the study and literature, an algorithm of primary nutritional anemia prophylaxis was created. This instrument could also be implemented in the daily practice of primary healthcare physicians.

Next-generation sequencing of the V3-V4 hypervariable region of the 16S rRNA gene was used and the gut microbiome in Lithuanian infants and young children was analyzed for the first time. Next, nutritional iron deficiency anemia association with the changes of gut microbial community was identified. Worldwide, only a few studies have investigated the impact of iron deficiency and iron deficiency anemia on the intestinal microbiota in animal experiments [17, 18], *in vitro* colonic fermentation models [19, 20] and Indian women [21]. The association of mixed etiology anemia and intestinal microbiota changes in infants and school-aged children has been demonstrated in Africa [22, 23]. This study provides more insights into the development of the gut microbial community and reveals that anemia is a complex health problem. Primary nutritional anemia prophylaxis plays a crucial role in promoting the health of infants and young children.

The aim of the study

The aim of the study was to evaluate the relationship between nutritional anemia and changes of health indicators and fecal microbiota in infants and young children up to 36 months of age in Vilnius district.

Objectives

1. Compare anthropometric measurements of anemic and healthy infants and young children.
2. Compare nutritional peculiarities of anemic and healthy infants and young children.
3. Compare the gut microbiota profile of anemic and healthy infants and young children.
4. Assess nutritional anemia prophylaxis in the primary healthcare setting.

1. Materials and methods

The study was carried out in the Children's Hospital (Affiliate of Vilnius University Hospital, Santaros Klinikos) in 2016-2018. The study was approved by the Vilnius Regional Bioethical Committee (2015-10-06 No.158200-15-805-317) and the State Data Protection Inspectorate (2016-03-01 No.2R-1242(2.6-1.)).

The study was divided into three parts (Fig. 2).

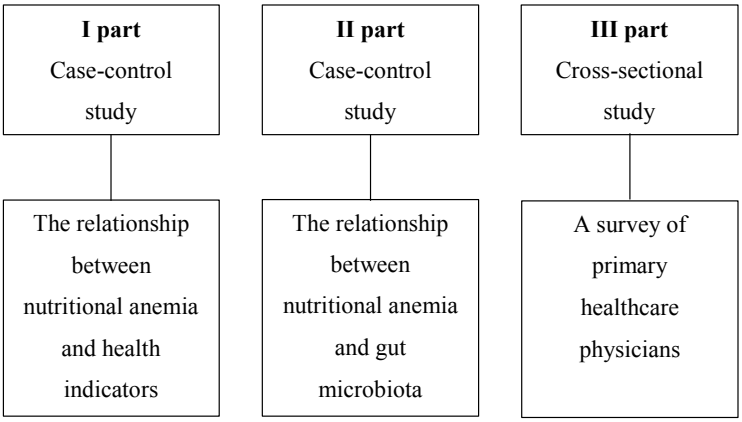

FIGURE 2. Parts of the study.

1.1 Part I: The relationship between nutritional anemia and health indicators

Infants and young children diagnosed with nutritional iron deficiency anemia (IDA) were enrolled in the study. Healthy children were recruited as a control group. All the subjects met the inclusion criteria: (1) up to 36 months old; (2) an otherwise healthy child and not having taken any medication for at least four weeks prior to the enrollment; (3) singleton pregnancy; (4) living in Vilnius district. The parents provided an informed consent. Detailed medical and nutritional data was collected by using a standard validated questionnaire. IDA group was divided into two subgroups according to age: <12 months (IDA-Inf) and between 12 and 36 months (IDA- Ch).

Based on the criteria of WHO, nutritional IDA was defined as: (1) serum hemoglobin <110 g/l; (2) ferritin level <12 μg/l and/or reticulocyte-hemoglobin equivalent <28 pg; and (3) after other etiological factors of iron deficiency excluded by pediatric hematologist [4, 24]. Mild, moderate and severe IDA was defined as hemoglobin concentration 109-100 g/l, 99-70 g/l and below 70 g/l respectively [4].

1.2 Part II: The relationship between nutritional anemia and gut microbiota changes

For the microbiota research, 10 IDA patients and 10 healthy controls were enrolled. Fresh stool samples were collected from diapers and immediately frozen at -80°C until the time of processing in the laboratory (Bologna, Italy).

Bacterial DNA from fecal samples was extracted using the repeated bead-beating plus column method [25]. Final DNA concentration was measured using

NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). The V3-V4 hypervariable region of the 16S rRNA gene was amplified using the 341F and 805R primers with added Illumina adapter overhang sequences [26]. Amplicons were purified with magnetic beads (Agencourt AMPure XP, Beckman Coulter, Brea, CA). Indexed libraries were prepared by limited-cycle PCR using Nextera technology and further cleaned up with magnetic beads (see above). Final library was pooled at equimolar concentrations (4 nM), denatured and diluted to a concentration of 6 pM. Sequencing was performed on Illumina MiSeq platform using a 2×250 bp paired-end protocol according to the manufacturer's instructions (Illumina, San Diego, CA).

Raw sequences were processed using a pipeline combining PANDAseq [27] and QIIME [28]. High-quality reads were clustered into Operational Taxonomic Units (OTUs) at 97% similarity using UCLUST [29]. Taxonomy was assigned using the RDP classifier against Greengenes database (May 2013 release). Singleton OTUs were discarded. Alpha diversity was measured by using observed OTUs, the Faith's phylogenetic diversity, Chao1 and Shannon index metrics [30]. Beta diversity was estimated by computing weighted and unweighted UniFrac distances.

1.3 Part III: A survey of primary health care physicians

An anonymous questionnaire-survey of primary healthcare physicians (family doctors and pediatricians) was conducted in Vilnius city and Vilnius district. Respondents were invited randomly and agreed to participate in the study voluntarily. All respondents were informed about the objectives of the study and the general use of the results only (preserving the anonymity and confidentiality of the respondents).

The questionnaire consisted of 19 closed, half-open and open questions to determine demographic indicators, feeding recommendations for infants and young children provided by physicians, specific IDA preventive measures applied and respondents' opinion on major obstacles for successful nutritional anemia prophylaxis.

1.4 Statistical analysis

Statistical analysis was performed using R software package (version 3.3.2). Continuous variables were provided as mean and standard deviation. Two samples were compared using Student's t-test or Wilcoxon rank-sum test based on normality. The Kruskal-Wallis rank sum test was used in order to compare three independent samples. Categorical variables were provided in absolute numbers and percentages. They were compared using Pearson's Chi-squared test.

When numbers of observations were lower than five, Fisher's exact test was applied.

For the microbiota analysis, UniFrac distances were plotted using the vegan package. Data separation in the Principal Coordinates Analysis (PCoA) was tested using a permutation test with pseudo-F ratios (function adonis). Alpha and beta diversity, taxon relative abundances were compared using Wilcoxon rank-sum test.

A p-value <0.05 was considered as statistically significant.

2. Results

2.1 Part I: The relationship between nutritional anemia and health indicators

During the study period 197 children (82 females (42%)) were examined by the pediatric hematologist due to the suspicion of nutritional anemia. Of these, 131 (66 %) had secondary anemia due to the co-morbidities and were not invited to participate in the study. Overall, the study included 36 IDA infants (IDA-Inf), 23 IDA children (IDA-Ch) and 32 healthy controls (HC) (Fig. 3).

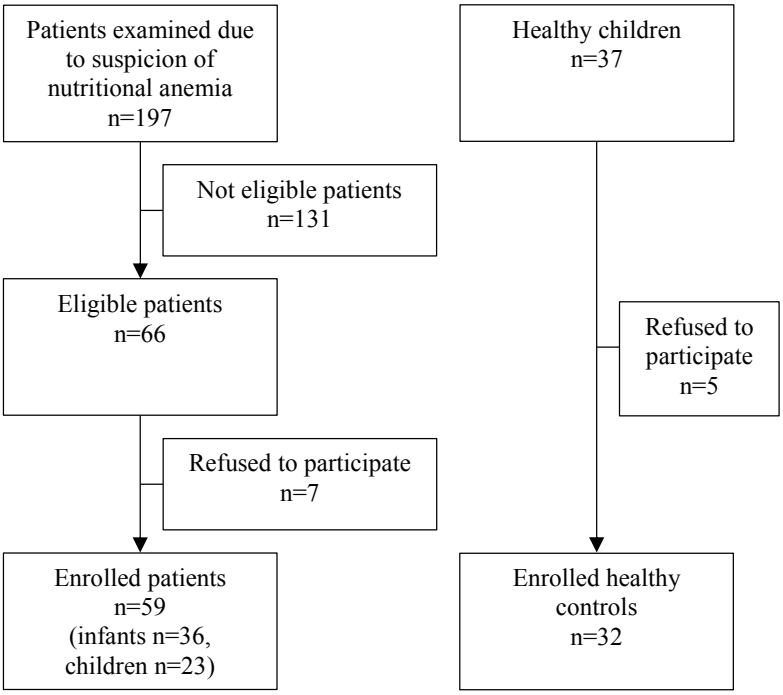

FIGURE 3. Flow chart of the study population.

Nutritional IDA was diagnosed at the average age of 8±2 months in the IDA-Inf group and 17±5 months in the IDA-Ch group. There were four infants (11%) whose diagnosis had been established at the age of 3-5 months. Basic characteristics of the study participants are given in Table 1. The parental age, education and a prevalence of anemia in pregnancy did not differ among the groups.

TABLE 1. Basic characteristics of the study population.

	IDA-Inf (n=36)	IDA-Ch (n=23)	HC (n=32)
Age	8 ± 2	17 ± 5	14 ± 8
Hemoglobin, g/l* Mild anemia Moderate anemia Severe anemia	96 ± 13 19 (52 %) 15 (42 %) 2 (6 %)	99 ± 9 14 (61 %) 8 (35 %) 1 (4 %)	120 ± 7
Male*	25 (70 %)	12 (52 %)	11 (34 %)
First child in a family	23 (64 %)	12 (52 %)	18 (56 %)
Vaginally delivered	28 (78 %)	16 (70 %)	24 (75 %)
Gestational age, weeks* Premature infants*	38 ± 3 11 (31 %)	39 ± 2 2 (9 %)	40 ± 1 1 (3 %)
Birthweight, g* Low birthweight newborns*	2844 ± 580 7 (22 %)	3417 ± 453 1 (5 %)	3621 ± 428 0
Age when birthweight was doubled, months*	3 ± 1	4 ± 1	6 ± 2
Received prophylactic iron supplementation	7 (21 %)	1 (5 %)	3 (12 %)

*Statistically significant with p<0.05

More than 70% of the study participants were delivered vaginally. Mothers reported that newborns were placed in a skin-to-skin contact (IDA-Inf n=20 (56%), IDA-Ch n=14 (61%), HC n=24 (75%), p>0.05), but only for a very short period of time. Skin-to-skin contact lasting more than 60 minutes was uncommon in the study cohort (IDA-Inf n=1 (5%), IDA-Ch n=2 (14%), HC n=2 (8%), p>0.05). About 50% of the mothers reported that the newborns had been put to the breast within one hour after the birth (IDA-Inf n=18 (50%), IDA-Ch n=12 (52%), HC n=22 (69%), p>0.05). There were more newborns separated from their mothers during day-time (and night-time as well) in the IDA-Inf group compared to the IDA-Ch and HC groups (IDA- Inf n=22 (61%), IDA-Ch n=8 (35%), HC n=8 (25%), p<0.05).

Healthy children were fed human milk directly from the breast more often than others during the stay in a postnatal ward, but these rates decreased when they were

discharged from hospital and returned home (Fig. 4). More than one third of the newborns were additionally given formula or glucose solution prior to the discharge from hospital (IDA-Inf n=15 (42%), IDA-Ch n=8 (35%), HC n=11 (34%), p>0.05). Nevertheless, only three mothers reported medical indications for supplementation. The most common reasons of this practice were "low milk production" and "an irritable newborn".

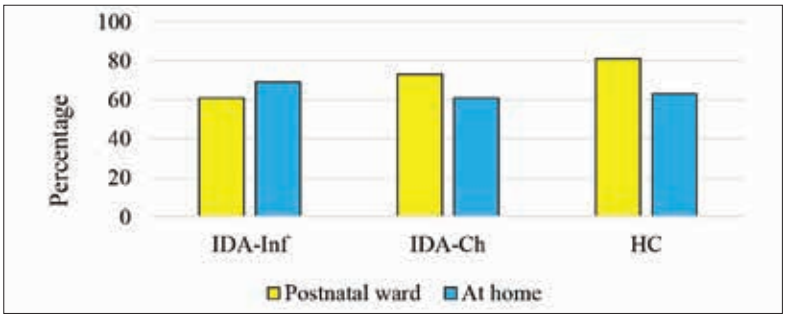

FIGURE 4. Distribution of the study participants based on the feeding directly from the breast during the stay in a postnatal ward and after returning home.

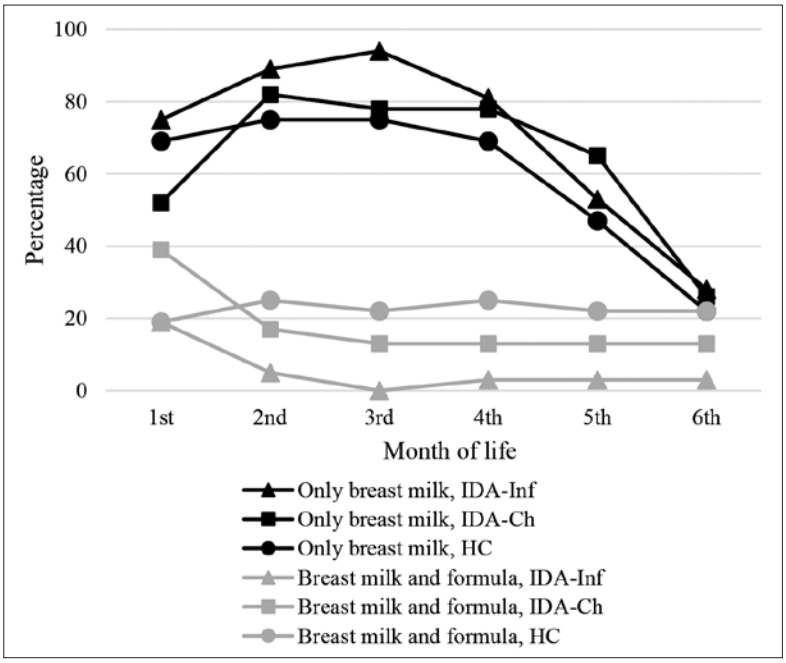

FIGURE 5. Distribution of the study participants based on exclusive breastfeeding during the first six months of life.

More than half of the mothers received gifts for artificial infant feeding in facilities providing maternity and newborn care, in PHC institutions (IDA-Inf n=23 (64%), IDA-Ch n=13 (57%), HC n=23 (72%), p>0.05). Parents of IDA infants and children used these gifts more often than the parents of healthy children (IDA-Inf n=16 (70%), IDA-Ch n=11 (85%), HC n=11 (48%), p>0.05).

Most of the children were breastfed on demand day and night (IDA-Inf n=26 (72%), IDA-Ch n=19 (83%), HC n=24 (75%), p>0.05). Nevertheless, the rates of exclusive breastfeeding in the first month of life were 50-75% (Fig. 5).

The frequency of exclusive breastfeeding increased in all the groups during the second and third months of life (most notably in the IDA-Inf group), but declined significantly later on. Only several infants were exclusively breastfed for the whole period of six months in a study cohort (IDA-Inf n=6 (18%), IDA-Ch n=3 (13%), HC n=5 (16%), p>0.05). The average duration of exclusive breastfeeding was 3±2 months and it did not differ among the groups.

About half of the mothers encountered breastfeeding problems (IDA-Inf n=13 (36%), IDA-Ch n=11 (48%), HC n=17 (53%), p>0.05). There were slightly more children receiving formula in the group of healthy controls (IDA-Inf n=1 (3%), IDA-Ch n=3 (13%), HC n=7 (22%), p=0.05) (Fig. 5).

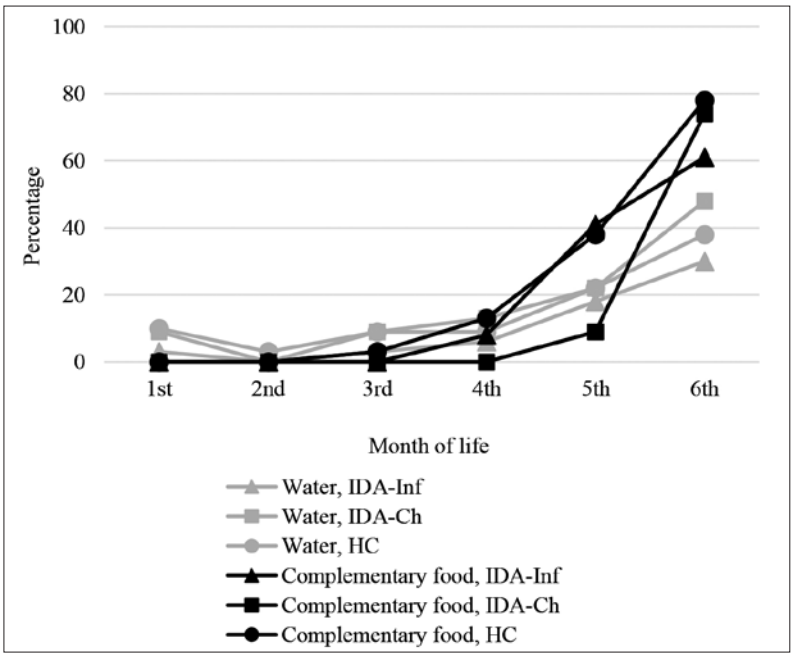

FIGURE 6. Distribution of the study participants based on the consumption of water and complementary food during the first six months of life.

At the beginning of the fifth month of life, water was introduced to the diet of increasingly more participants. More than one third of them were given water in the sixth month of life (IDA-Inf n=10 (30%), IDA- Ch n=11 (48%), HC n=12 (38%), p<0.05) (Fig. 6).

The consumption of complementary food significantly increased during the fifth month of life, especially in the IDA-Inf and HC groups (IDA-Inf n=14 (42%), IDA-Ch n=2 (9%), HC n=12 (38%), p<0.05). Nevertheless, the proportion of the study participants receiving complementary food at the age of six months did not differ among the groups (IDA-Inf n=20 (61%), IDA-Ch n=17 (74%), HC n=25 (78%), p>0.05) (Fig. 6).

Vegetables were introduced first and were followed by fruit and berries, cereals, meat, fish, eggs and dairy products. The IDA children were given meat later than the other study participants (IDA-Inf at 6±1 months, IDA-Ch at 7±1 months, HC at 6±1 months, p<0.05).

The consumption of unmodified cow's milk was uncommon in the cohort, but an increasing consumption of products with added sugar was noticed in the second half of infancy (Fig. 7). Every third participant consumed products with added sugar

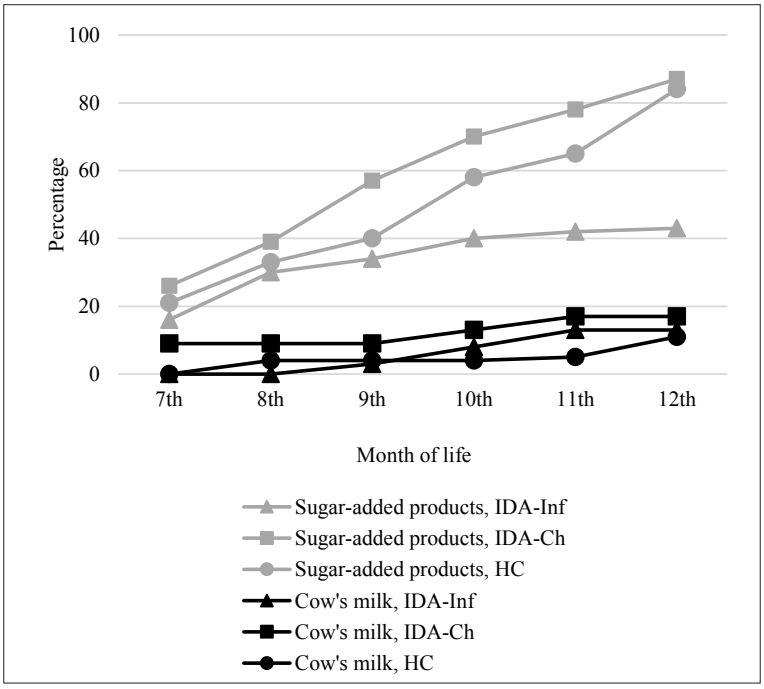

FIGURE 7. Distribution of the study participants based on the consumption of products with added sugar and cow's milk during the second six-month period of life.

in the seventh month of life and almost all the IDA and healthy children consumed them in the twelfth month. This was the least important problem in the IDA infants group and the difference was statistically significant in the twelfth month of life.

At the age of 6-11 months all the IDA infants continued to be breastfed, but three IDA children (13%) and four healthy controls (13%) were already weaned ($p<0.05$). Diet diversity increased during the second half-year period of life in all the groups, but was the lowest in the IDA infants, both at 6-8 and 9-11 months of age ($p<0.05$) (Fig. 8).

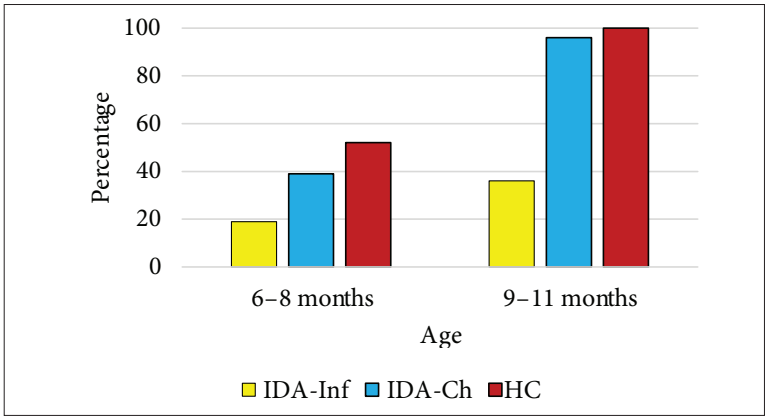

FIGURE 8. Distribution of the study participants based on the diversity of diet during the second six-month period of life.

When comparing the diet diversity of the IDA children and healthy controls in the second and third year of life, it was found that every third child consumed products from four or more food groups every day (IDA-Ch n=6 (28%), HC n=4 (24%), $p> 0.05$). Most children received vegetables, fruit and berries and cereals every day. About half of the study participants consumed meat and drank unmodified cow's milk daily. There were no statistically significant differences among the groups.

Although the consumption of cow's milk did not differ among the groups, there were two IDA children (9%) who consumed more than 400 ml of cow's milk (aged 13 and 23 months) and one IDA child (4%) who consumed about 2000 ml of cow's milk (aged 32 months).

2.2 Part II: The relationship of nutritional anemia and gut microbiota changes

The study included ten patients with nutritional iron deficiency anemia (IDA-gr) (6-32 months of age) and ten healthy children (HC-gr) (7-34 months of age).

The main characteristics of the participants are given in Table 3. The IDA group had a significantly lower level of hemoglobin and a lower birthweight. All subjects were vaginally delivered.

TABLE 3. Basic characteristics of the study population.

	IDA-gr (n=10)	HC-gr (n=10)
Hemoglobin, g/l*	102 ± 5	123 ± 8
Age, months	13 ± 8	16 ± 8
Gestational age, weeks	38 ± 2	40 ± 1
Birthweight, g*	3252 ± 613	3808 ± 309

*Statistically significant with p<0.05

All the study participants were exclusively breastfed for 4±2 months and introduced to plant-based products at 5±1 months of age, followed by animal-based products at 6±1 months of age with no differences among the groups. Most of the participants were still breastfed at the moment of enrollment (IDA-gr n=8 and HC-gr n=7, p>0.05). The proportion of children consuming meat (IDA-gr n=7 and HC-gr n=10, p=0.06) and fish (IDA-gr n=5 and HC-gr n=10, p<0.05) was lower in the IDA group.

Alpha diversity metrics did not significantly differ among the groups (Fig. 9). The Principal Coordinates Analysis (PCoA) based on the weighted and unweighted UniFrac distances showed distinct microbial signatures in the IDA patients and in the healthy controls (Fig. 10).

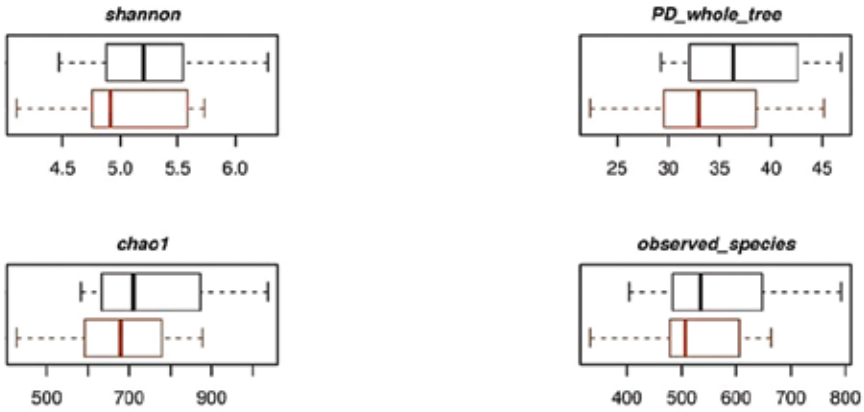

FIGURE 9. Microbiota alpha diversity metrics in the iron deficiency anemia patients (red) and in the healthy controls (black).

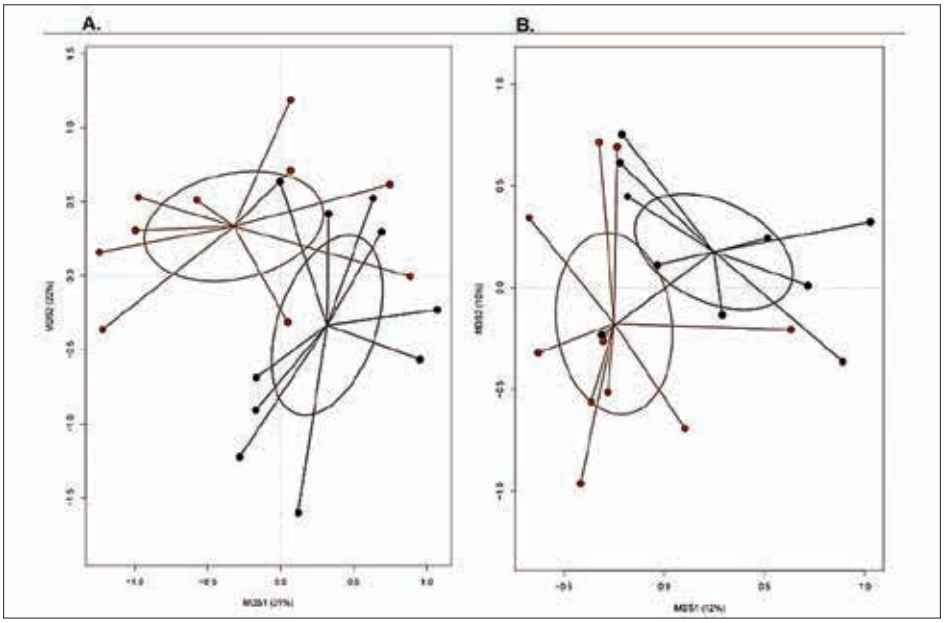

FIGURE 10. PCoA based on the unweighted (A) and weighted (B) UniFrac distances of the microbiota in the iron deficiency anemia patients (red) and in the healthy controls (black).

At the phylum-level, *Firmicutes* (mean relative abundance, 54.0%), *Actinobacteria* (23.4%) and *Bacteroidetes* (14.2%) dominated and accounted for about 90% of the gut microbiota community in the entire cohort. Although statistical significance was not achieved, microbial community of the IDA patients was enriched in *Bac-*

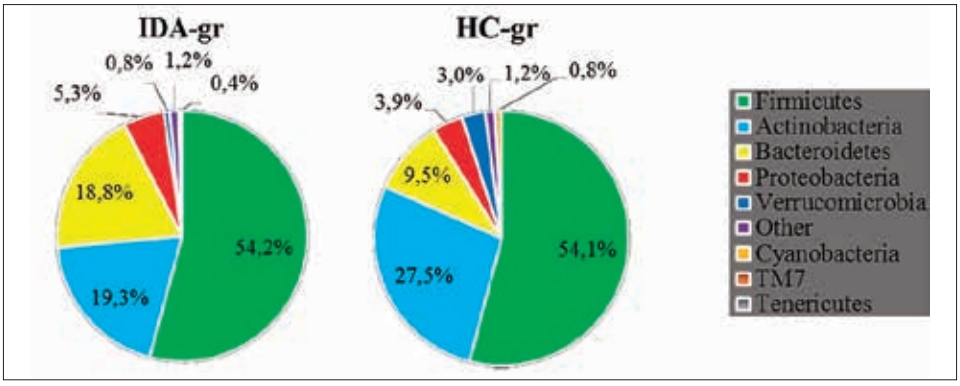

FIGURE 11. Relative abundances of phylum-level taxa in the iron deficiency anemia patients (IDA-gr) and in the healthy controls (HC-gr).

teroidetes (IDA-gr vs HC-gr, 18.8% vs 9.5%), *Proteobacteria* (5.3% vs 3.9%) and depleted in *Actinobacteria* (19.3% vs 27.5%), *Verrucomicrobia* (0.8% vs 3.0%) (Fig. 11).

At the family level (Fig. 12), a decreased relative abundance of *Coriobacteriaceae* was revealed in the IDA patients (IDA-gr vs HC- gr, 3.5% vs 8.8%, $p<0.05$). These changes were associated with the depletion of the *Collinsella* (*C. aerofaciens*) genus. Enrichment of *Veillonellaceae* (IDA-gr vs HC-gr, 13.7% vs 3.6%, $p<0.05$) was determined by the over-representation of *Veillonella* genus (*V. parvula* and *V. dis-*

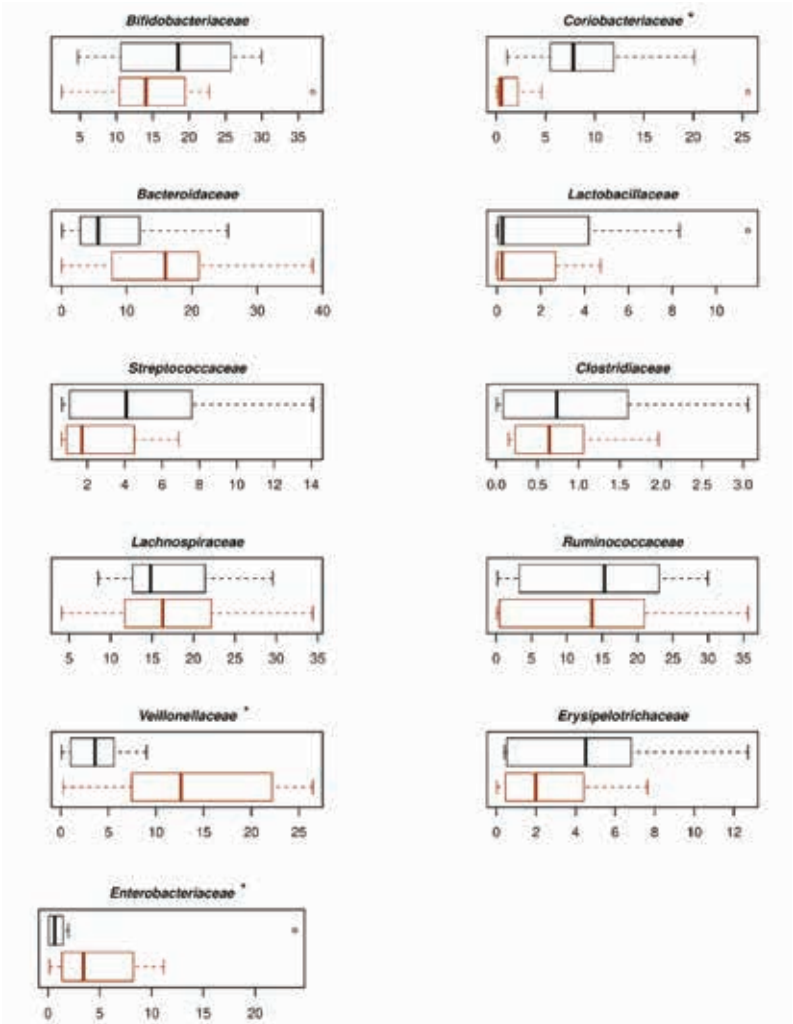

FIGURE 12. Relative abundances of the family-level taxa in the iron deficiency anemia patients (red) and in the healthy controls (black).
*Statistically significant with $p<0.05$

par species). Furthermore, bacterial community of the IDA patients was enriched in *Enterobacteriaceae* (IDA-gr vs HC- gr, 4.4% vs 3.0%, $p<0.05$), thus resulting in a significantly decreased *Bifidobacteriaceae* to *Enterobacteriaceae* ratio (IDA-gr vs HC-gr, 19.5 vs 93.3, $p<0.05$).

Decreased relative abundances of *Dorea* and *Dialister* bacteria were found in the IDA patients. *Clostridium neonatale* was detected in this group only.

2.3 Part III: A survey of primary health care physicians

Fifty-nine respondents participated in the survey, the majority were family doctors (n=43, 73%). The average age of the subjects was 37±10 years.

Fifty-six (95%) respondents answered the questions about the infant feeding recommendations they give to the families in the first six months after the child's birth. Although most of them would support exclusive breastfeeding for four or six months (n=51 (91%) and n=43 (77%) respectively), some of them would consider giving an infant water during illness or start complementary feeding when an insufficient weight gain or anemia is diagnosed. The remaining physicians (n=13, 23%) would always recommend mixed feeding or complementary feeding before the age of six months. Overall, there were 38 respondents who would consider starting complementary feeding before the age of six months. They would give vegetables (n=33, 87%), grains (n=17, 45%), fruit or berries (n=12, 32%) and meat (n=7, 18%).

After starting complementary feeding, the majority of the physicians would recommend continued on-demand breastfeeding (n=53, 90%), but breastfeeding until 24 months of age and beyond would be encouraged and supported by less than half of the respondents (n=20, 34 %). Therefore, exclusive breastfeeding until six months of age and continued on-demand breastfeeding until 24 months and beyond with adequate complementary food would be recommended only by some physicians (n=6, 10%).

Only 24 respondents (41%) would prescribe prophylactic iron supplements for premature infants, 6 (10%) – for the low birthweight infants and 11 (19%) – for the infants who get insufficient amount of iron with food.

Nevertheless, the respondents mentioned the following problems as major obstacles for the efficient IDA prophylaxis: the absence of reimbursable liquid iron supplements for infants and young children (n=30, 51%); too little time allocated for patient consultation (n=26, 44%); parents not following provided recommendations (n=24, 41%); lack of scientific information on infant and young children NA prophylaxis strategies for physicians (n=22, 37%).

3. Discussion

3.1 Part I: The relationship between nutritional anemia and health indicators

In Lithuania, this is the first study focusing on the anthropometric measurements of infants and young children, nutritional peculiarities from the first days of life and their link to nutritional IDA. By comparing three groups of subjects we defined the main risk factors associated with IDA in the first or in the second and third years of life.

The IDA infants group was significantly different from the other two groups, i.e. their gestational age and birthweight were lower compared to the rest of the subjects and they doubled their birthweight as early as being three months old. The body iron stores directly depend on the gestational age and birthweight; therefore, premature and low birthweight infants experience a greater IDA risk [31-34]. Nevertheless, patients in the IDA-infant group did not receive prophylactic iron supplementation and, in accordance with the studies conducted in other European countries, developed IDA anemia [32, 35, 36]. On the other hand, the IDA-infants doubled their birthweight earlier than the other subjects. Faster weight gain is known to increase the IDA risk [31, 37]. Thus, it can be concluded that the nutritional anemia in the IDA infants was associated with the lower body iron stores and the higher needs for rapid growth.

The IDA children group was less different from the healthy controls: all were born mature and had normal birthweight. Nevertheless, they grew faster and doubled their birthweight earlier than the healthy children. It was demonstrated once again that children who gain weight more rapidly have higher nutritional iron needs and are at a greater risk of developing IDA [31, 37].

In addition, there were more boys than girls among the children diagnosed with IDA. According to the literature, their iron status is more affected by growth velocity and nutritional habits due to hormonal differences [33]. Thus, boys experience a greater IDA risk [16, 37, 38].

According to the WHO, exclusive breastfeeding for the first six months should be protected, promoted and supported in order to ensure the optimal nutrition of the infant [39]. Implementation of the Baby-friendly Hospital Initiative is one of the most effective measures to increase exclusive and continued breastfeeding rates [40]. In accordance with the data of the World Breastfeeding Initiative Trends Report in Lithuania [41], it was found that the facilities providing maternity and neonatal care did not implement all the Baby-friendly Hospital Initiative practices. Only half of the newborns were placed in a skin-to-skin contact for a very short period of time and were breastfed within the first hour after the birth. Supplementation with formula or glucose solution without any medical indications was common in the

cohort. Moreover, violations of the International Code of Marketing of Breast-milk Substitutes were detected in the birth units and primary health care institutions. All these circumstances are known to negatively impact the success and duration of breastfeeding [40].

Indeed, only one third of the subjects were exclusively breastfed at the age of six months and only one fifth were exclusively breastfed for the entire six-month period. These results are in line with the national reports: only 35.4% of infants were exclusively breastfed until six months of age in 2016 [42]. Even worse rates of exclusive breastfeeding are noticed in the Latvia (16.4%) and Estonia (26.4%) [43, 44]. This is of particular concern as sub-optimal duration of exclusive breastfeeding increases the risk of infections and inflammation which may disturb iron homeostasis in the body [45-47]. On the other hand, no relationship was established between IDA and the duration or frequency of exclusive breastfeeding. This is in line with systematic reviews [45].

We noticed that more healthy controls were supplemented with formula during infancy compared to the IDA subjects. It is a known fact that formula fed infants have greater body iron stores and experience a lower IDA risk [33, 34, 48]. Unfortunately, excess iron intake in iron-replete infants is associated with decreased linear growth, increased incidence of infectious diseases and altered homeostasis of other microelements in the body [49].

Plain water and complementary food were introduced at the average age of four months in all the cohort. It was noticed that a smaller number of the IDA children consumed complementary food at this point of time, but there was no statistical difference at the age of six months. Taking into account that the first complementary food types are poor in iron, it remains doubtful whether this difference played a role in the development of anemia in this group [50]. It is possible that a later introduction of meat could be a stronger factor increasing the risk of IDA [51].

Consumption of unmodified cow's milk in the first year of life was uncommon in the cohort, but a sharp increase in the consumption of products with added sugar was found to be a new emerging problem. Usually these products are of high energy value, resulting in a higher weight gain and, in turn, in higher micronutrients needs. On the other hand, children that eat these products receive less iron, vitamin E, niacin, calcium and zinc [52, 53]. It may be speculated that the consumption of products with added sugar could increase the risk of nutritional anemia: compared to the healthy controls, the IDA children doubled their birthweight earlier (depleted body iron stores), received meat later (did not increase body iron stores) and their diet was complemented with non-nutritious snacks. One study that was conducted on preschool children in Lithuania found that almost 70% of children aged between12 and 36 months consume products with added sugar [54].

Furthermore, diet diversity was lowest in the IDA infant group (particularly at the age of 9-11 months) and contributed to the development of IDA. Nutritional needs increase during the second half of infancy, but infants are able to eat only small amounts of food [55]. First complementary food types are poor in microelements and vitamins and therefore only a diverse diet can meet the nutritional needs of a fast-growing body [50, 56]. On the other hand, the IDA infants had the lowest body iron stores at birth and it cannot be excluded that they were already iron deficient at the introduction of complementary food. Previously it was found that iron deficiency is associated with infant feeding difficulties [57, 58].

A low diet diversity was also found in the second and third years of life after assessing the diet of the IDA children and healthy controls. Although there were no statistically significant differences among the groups, these children were more vulnerable and any other risk factors might have a relatively higher significance in pathogenesis of nutritional anemia [5].

Consistent with other studies, consumption of unmodified cow's milk was associated with IDA development in the second or third year of life in our cohort [33, 59]. Cow's milk contains only small amounts of iron, inhibits iron absorption from other food types and often leads to occult bleeding from the intestine [60].

The major limitation of this study is a small sample size. Nevertheless, important gaps were revealed in making diagnosis of IDA in a primary healthcare setting. First, the diagnosis of IDA in some patients was rejected by pediatric hematologist after evaluating the complete blood count and additional blood tests. It may be speculated that complete blood test results were misinterpreted by primary healthcare physicians. Finally, we had only 197 patients with a confirmed diagnosis of anemia. Nevertheless, almost 70% of them had anemia due to co-morbidities and were not eligible for the study. Therefore, it cannot be excluded that the data provided by the Institute of Hygiene on the incidence of nutritional anemia in infants and young children is not accurate due to diagnostic errors and incorrect diagnosis coding according to the International Classification of Diseases. Detailed large-scale research is required in order to evaluate the problem of nutritional anemia in Lithuanian children. Only a detailed analysis of the results would help to effectively address this problem in Lithuania.

3.2 Part II: The relationship between nutritional anemia and gut microbiota changes

Worldwide, little is known about how iron deficiency and iron deficiency anemia impact the development of the gut microbial community.

First, it was found that the gut microbial community in the cohort consisted of *Firmicutes* (with the most abundant *Ruminococcaceae, Lachnospiraceae* and *Strep-*

tococcaceae families), *Actinobacteria* (dominated by *Bifidobacteriaceae*) and *Bacteroidetes* (dominated by *Bacteroidaceae*). These results were in line with the peculiarities of microbiota development in infants and children living in Europe [61-63], thus it was possible to continue further analysis.

The study included iron deficient anemic, but otherwise healthy children; nevertheless, significantly increased relative abundance of *Enterobacteriaceae* and decreased *Bifidobacteriaceae/ Enterobacteriaceae* ratio in the IDA patients was observed. Similar changes were observed not only *in vitro* and *in vivo* models [17, 19, 20], but also in populations of African infants and school-aged children with a high prevalence of infections and systemic inflammation [22, 23]. On the one hand, an increased abundance of *Enterobacteriaceae* is also associated with inflammatory bowel diseases. On the other hand, an enrichment in bifidobacteria and butyrate producers is observed in remission [64-66]. It cannot be ruled out that the unfavorable gut microbial community in our cohort could also induce local inflammatory response of the host and, in turn, interfere with iron absorption and homeostasis.

Furthermore, signs of a profound dysbiosis were observed in the *Veillonellaceae* family. The IDA children had an increased abundance of *V. parvula* and *V. dispar* and decreased levels of *Dialister*. Recently, over-representation of *Veillonellaceae* was associated with the increased bronchial asthma risk at five years of age and a deep ulceration in Crohn's disease [65, 67]. Moreover, an increased relative abundance of *Veillonella* genus was observed in feces of children with type 1 diabetes and *Dialister* genus was completely absent before the onset of type 1 diabetes in infants [68, 69]. Taking into account this data, we may speculate that the IDA children can experience a greater risk of autoimmune diseases in the future.

A significantly decreased abundance of *Coriobacteriaceae* family (*Collinsella aerofaciens* in particular) was found. These changes are also observed in inflammatory bowel diseases [66]. On the other hand, diminished level of *Bifidobacterium* and *Collinsella* at the age of six months is related to obesity at the age of 18 months [70]. Thus, IDA in infancy can increase the risk of metabolic diseases later in childhood.

Furthermore, a significantly decreased relative abundance of *Dorea* (butyrate producing bacteria) in the IDA children was revealed. Surprisingly, *Clostridium neonatale* was observed only in the IDA group. The latter bacteria was previously isolated only from newborn feces [71].

The findings of this study suggest that iron deficiency anemia is associated with a significant dysbiosis of the gut microbiota. In agreement with the previous studies, it was revealed that iron deficiency induced an overgrowth of *Enterobacteriaceae* family and, in turn, decreased the *Bifidobacteriaceae/Enterobacteriaceae* ratio [17-20, 22, 23]. Most significant changes associated with iron deficiency anemia in

infancy and early childhood were observed in the lactic acid producing bacterial community.

The study was conducted at a single point in time ant it remains unclear when exactly the changes in the gut microbiota occurred: did dysbiosis disrupt the host's ability to absorb iron from food and caused the anemia or, on the contrary, were the gut bacterial community changes induced by anemia? It is possible that unfavorable changes in the microbiota result in the competition for iron between the host and the bacteria. On the other hand, the most recent study shows that siderophores produced by the commensal *E. coli* are necessary for iron absorption by the host cells [72]. The present study provides more questions than answers and further research is needed to learn more about the role of the gut microbiota on the host's iron homeostasis.

Moreover, it was noticed that iron deficiency anemia is associated with unfavorable changes of the gut microbial community. It cannot be excluded that patients will experience a greater risk of autoimmune, allergic and metabolic diseases in the future. Further longitudinal research is needed in order to assess the relationship between iron deficiency anemia and these chronic non-communicable diseases.

3.3 Part III: A survey of primary health care physicians

Although exclusive breastfeeding for six months has been promoted in Lithuania since 2003, only 50% of the physicians recommended this practice in the previous decade [73, 74] and only 80% of the respondents in this study. This is alarming as there is a direct relationship between the practice of primary healthcare and the success of breastfeeding. According to a recent study, infants who attend a breastfeeding promoting clinic are breastfed six months longer [75]. Unfortunately, breastfeeding and infant feeding topics are covered poorly in the medical study programs and there is a lack of continued training services for physicians [41].

Although only several respondents would always recommend giving water to infants under six months of age, this practice was common in the previous decade and was promoted by almost 30-50% of physicians [73, 74, 76]. Nevertheless, it was noticed that some of the respondents would recommend giving water to the infant during illness or hot weather. This means that this practice could be more prevalent than it was observed in this study and reach even 30% as was published earlier [75].

About 25% of physicians would recommend complementary feeding before the infant reaches six months old. Another recent study also reveals significant differences in the clinical practice: early complementary feeding is promoted by 3-33% of physicians [75].

The majority of the respondents would recommend continued breastfeeding until the age of 12 months, but only one third would recommend it until the age of

24 months. These results are similar to the ones published in the past decade and may be related to healthcare professionals' insufficient knowledge of the significance and benefits of continued breastfeeding for children [73, 76].

Particular attention should be given to the fact that early complementary feeding is recommended for nutritional anemia prophylaxis in the cohort. However, first complementary food types are poor in vitamins and microelements and their bioavailability is lower than that in breast milk [50]. Thus, prophylactic supplementation with microelements is recommended for infants in high-risk groups (mainly, low birthweight infants) [5]. Unfortunately, less than 50% of the respondents would prescribe prophylactic iron supplements for premature infants; less than 10% – for low birthweight infants. Only 20% of the physicians would recommend iron supplementation for infants and children who get insufficient amounts of iron from food. This data reveals significant gaps in primary healthcare practice.

It is interesting to note that, according to the subjective assessment by the respondents, the greatest obstacles in the prophylaxis of nutritional anemia are external reasons, such as the absence of reimbursable liquid iron supplements for infants and small children, too little time allocated for patient consultation, parents not following provided recommendations. However, it was found that due to the lack of knowledge or other reasons, only few physicians adhere to the WHO recommendations, i.e. recommend exclusive breastfeeding until the age of six months, followed by frequent breastfeeding up to the age of 24 months or longer while feeding an age-appropriate complementary food. Most often it is argued that The European Society for Pediatric Gastroenterology, Hepatology and Nutrition recommends complementary feeding from four months of age.

However, this Society highlights that "exclusive breastfeeding until 6 months of age is a desirable goal" and complementary feeding should not be introduced before the age of four months or delayed beyond six months [77, 78].

In conclusion, our survey of parents and healthcare professionals revealed that no favorable conditions for proper nutrition of the infants and young children were created in healthcare facilities: principles of Baby-Friendly Hospital Initiative were not implemented or supervised; breastfeeding was not protected, promoted or supported in maternity units and parents were given inappropriate complementary feeding recommendations. Nevertheless, it was noticed that the physicians were very interested in breastfeeding and complementary feeding. This could serve as a basis for a fast and effective implementation of measures of evidence-based nutritional anemia prophylaxis.

Conclusions

1. Low birthweight and early doubling of the birthweight was associated with nutritional iron deficiency anemia.
2. Low diet diversity increased the risk of developing nutritional iron deficiency anemia in infancy and a later introduction of meat, consumption of unmodified cow's milk and products with added sugar increased the risk of developing nutritional iron deficiency anemia in second and third years of life.
3. Nutritional iron deficiency anemia is associated with unfavorable gut microbiota changes: a decreased *Bifidobacteriaceae/ Enterobacteriaceae* ratio and relative abundance of *Coriobacteriaceae,* increased *Veillonellaceae* relative abundance.
4. Primary healthcare physicians do not apply all nutritional anemia prophylactic measures in their practice and the greatest problems are a lack of breastfeeding promotion, protection and support; inappropriate complementary feeding recommendations and gaps in the use of specific prophylactic measures.

Recommendations

The algorithm of prophylaxis is provided in Figure 13. In order to implement these measures, it is necessary to educate primary healthcare physicians on lactation and breastfeeding and to update their knowledge on complementary infant feeding and iron deficiency anemia.

The results of the gut microbiota analysis support the need for further studies in order to determine whether the changes observed remain after treating iron deficiency anemia and whether the risk of chronic non-communicable diseases increases.

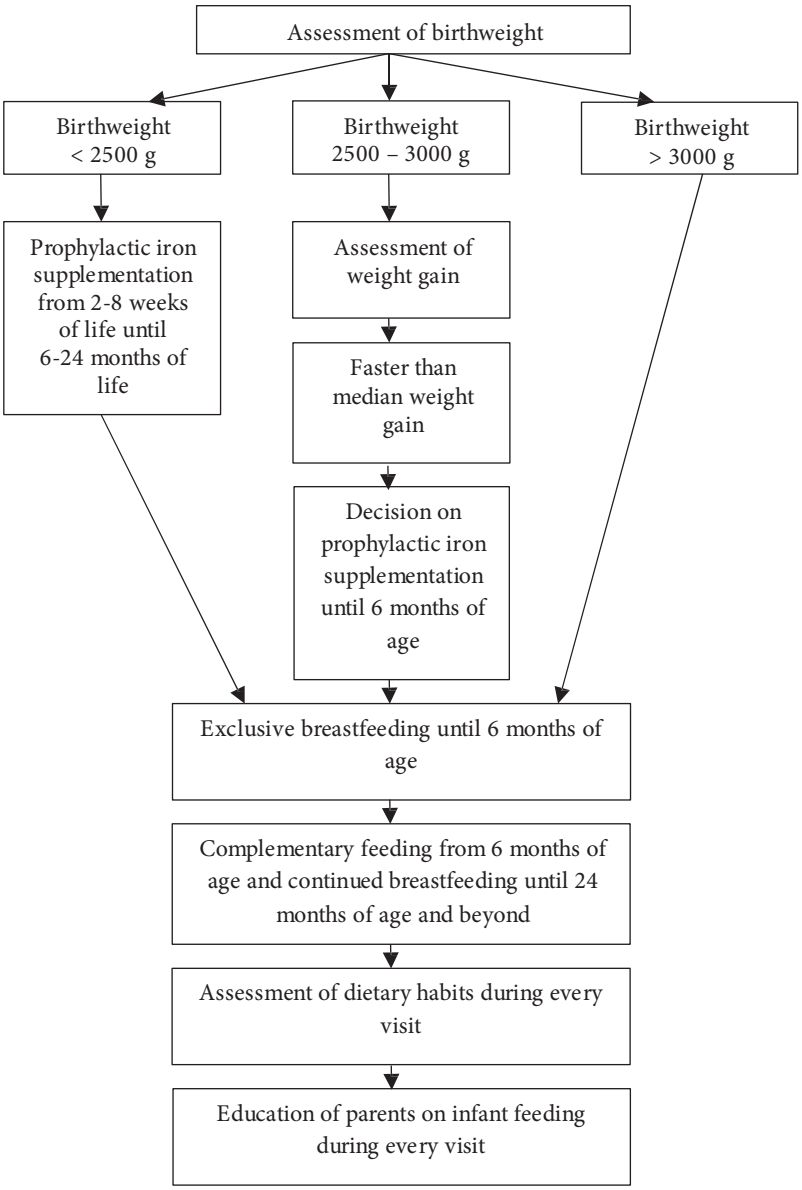

FIGURE 13. Algorithm for prophylaxis of nutritional iron deficiency anemia in infants and young children.

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Ieva Slivovskaja

THE EFFECTIVENESS OF INDIVIDUAL AEROBIC TRAINING IN SUBJECTS WITH METABOLIC SYNDROME

INDIVIDUALIOS AEROBINĖS FIZINĖS TRENIRUOTĖS VEIKSMINGUMAS ASMENIMS, KURIEMS NUSTATYTAS METABOLINIS SINDROMAS

Biomedical Sciences, Medicine 06B
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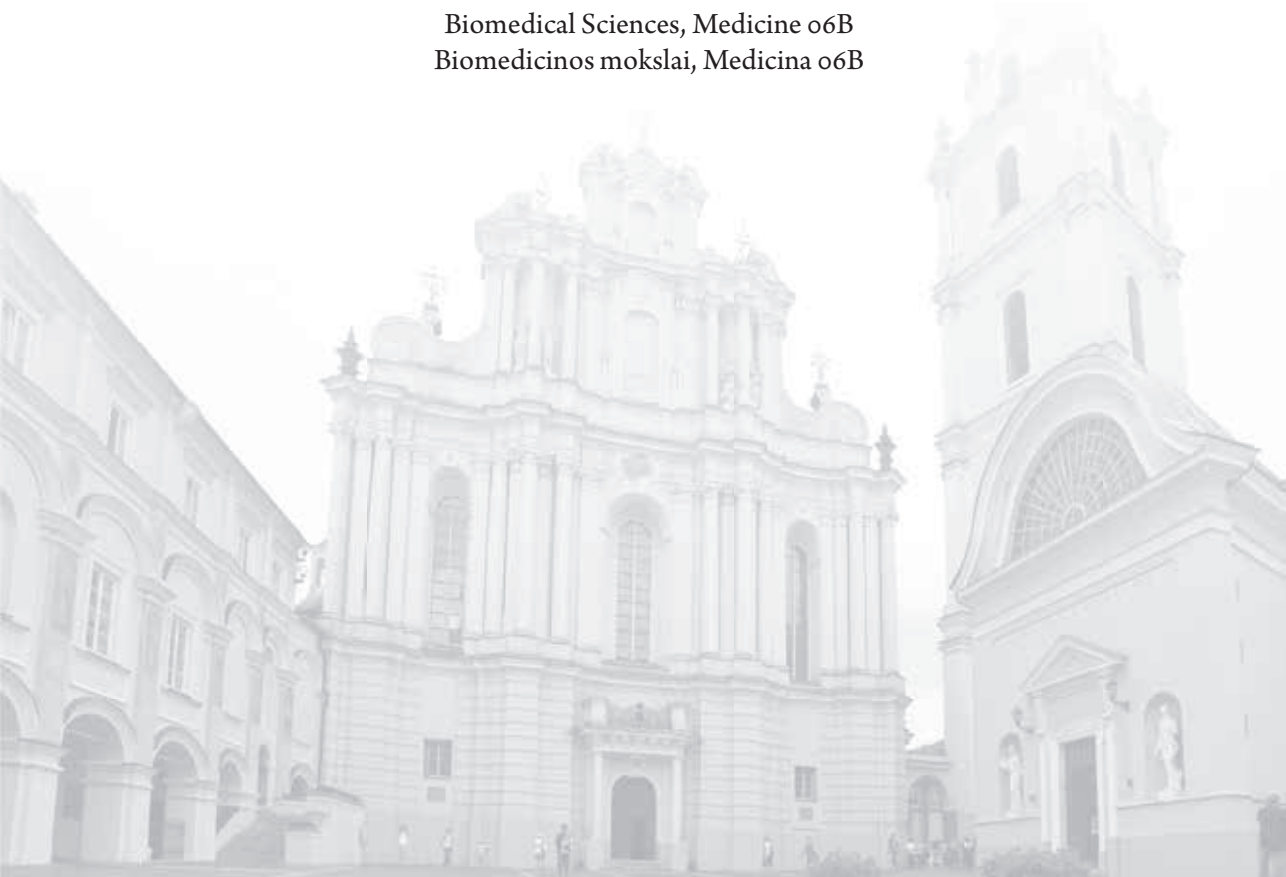

Abbreviations

AH – arterial hypertension
aPT – aerobic physical activity
AT – anaerobic threshold
BMI – body mass index
BP – blood pressure
CCA – common carotid artery
c-fPWV – carotid-femoral pulse wave velocity
CHD – coronary heart disease
CRF – cardiorespiratory fitness
c-rPWV – carotid-femoral pulse wave velocity
CV – cardiovascular
CVD – cardiovascular disease
dBP – diastolic blood pressure
EMI-2 – Exercise Motivation Inventory-2
HADS – Hospital Anxiety and Depression Screening
HDL-Chol – high density lipoprotein cholesterol
IMT – intima-media thickness
IPAQ – International Physical Activity Questionnaire
LDL-Chol – low density lipoprotein cholesterol
MBP – mean blood pressure
MET – metabolic equivalent of task
MetS – metabolic syndrome
MOS SF-36 – Medical Outcome Study Short Form Health Survey
NCEP ATP III – National Cholesterol Education Program Adult Treatment Panel III
PA – physical activity
PT – physical training
PWV – pulse wave velocity
QCS – Quality Carotid Stiffness
QOL – health related quality of life
ROC – receiver operating characteristic
sBP – systolic blood pressure
T-Chol – total cholesterol
TG – triglycerides
VO2max – maximal oxygen consumption
WC – waist circumference

1. Introduction

Relevance of the problem. Identification of high cardiovascular (CV) risk subjects without overt cardiovascular diseases (CVD) is essential for the effective primary prevention. Physical inactivity and obesity, in particular metabolic syndrome (MetS), are increasingly recognized as growing contributors to the global burden of cardiovascular diseases [1] and type 2 diabetes mellitus [1-3]. It is especially important in the face of the increasing incidence of MetS, which is predicted to reach 40 % by 2020 globally [4]. In some populations, the prevalence of MetS is well exceeding the global average, now reaching 43.8 % [5]. According to the Lithuanian High Cardiovascular Risk (LitHiR) primary prevention programme, the incidence of MetS among middle-aged subjects in Lithuania is 28.7 % [6]. Part of the increased CV risk in MetS is attributed to the changes in the arterial wall parameters, such as

arterial stiffness and increased carotid intima-media thickness [7]. These changes reflect subclinical atherosclerosis or early vascular aging [8], which eventually leads to an overt CVD, cardiac events and stroke. MetS, physical inactivity and central obesity contribute to early vascular aging, which leads to the increased CVD risk.

There is a number of publications regarding the evaluation of psycho-emotional condition in patients suffering from various diseases [9-10]. Some authors point out a link between MetS and depression, but not anxiety [11-14]. It is shown that the fear associated with obesity, arterial hypertension (AH), diabetes mellitus (DM) and CVD affects people with MetS by reducing their quality of life [15-19]. However, only a single publication was found, analysing positive aerobic exercise training and a diet effect on the quality of life in subjects with MetS [20]. Very few studies have investigated the influence of physical training on the symptoms of depression in low physical activity (PA) patients with type 2 DM, heart failure and coronary heart disease [21-25].

In 2016, European guidelines for CV prevention provided common recommendations on physical activity: at least 150 minutes of aerobic moderate intensity physical activity a week (by 30 minutes, 5 days a week) or 75 minutes of high intensity aerobic physical activity a week (by 15 minutes, 5 days a week) or their combination [26]. Joint recommendations of several scientific societies in the USA recommended a complex lifestyle intervention programme composed of nutrition, physical intervention and behaviour therapy in overweight and obese subjects [27]. Physical training (PT) could be planned as a programme that is structured, regular, dosed and controlled. This could be a method of treatment and prevention aimed at improving or maintaining one or several components of physical fitness as well as the prognosis of the disease and quality of life [28]. Aerobic physical training (aPT) is one of the most investigated modalities of training with a proven dose/response effect [29, 30]. Patient motivation is a key factor for daily PA and PT.

We hypothesized that the heart rate (HR) targeted aPT could not only contribute to the increase in physical fitness but also improve anthropometric, hemodynamic, metabolic and arterial wall parameters. Since the outcomes of CVD occur only in the long run, measuring arterial wall parameters during the PT programme could prove to be a useful tool for recurrent CV and metabolic risk assessment as well as positively affect patient's motivation for a physically active lifestyle. Our hypothesis is supported by a relatively small study, which has demonstrated a positive effect of HR targeted aPT on arterial parameters in MetS subjects [31]. Other comparable studies were limited in their scope as they analysed the effect of PT on arterial parameters in respect to a single risk factor response [32, 33]. There is limited data on the effect of individually HR targeted aPT in this population.

Primary purpose and tasks of the study. The primary purpose of this study was to evaluate the effectiveness of the 2-month duration individually heart

rate targeted aerobic physical training programme in subjects with metabolic syndrome by assessing their cardiovascular risk factors, metabolic syndrome components, arterial wall functional and structural parameters, psycho- emotional status, quality of life, physical activity, cardiorespiratory physical fitness and motivation for physical training.

The tasks of the study were as follows:

1. To estimate, evaluate and compare the effect of the 2-month duration aerobic physical training programme in the intervention group and in the control group of individually followed recommendations for physical activity by exploring the changes in cardiovascular risk factors, components of metabolic syndrome, arterial wall functional and structural parameters.
2. To estimate, evaluate and compare the effect of the 2-month duration exercise training programme in the intervention group and in the control group of individually followed recommendations for physical activity by assessing psycho-emotional status, quality of life, physical activity, cardiorespiratory physical fitness and motivation for physical training.
3. To estimate and evaluate a long-term effect of the exercise training programme in the intervention group after 8 months, by assessing the changes in cardiovascular risk factors, components of metabolic syndrome, arterial wall functional and structural parameters and physical activity, cardiorespiratory fitness, motivation for physical training and quality of life.

Principal statements for defence.

1. The individually heart rate targeted 2-month duration aerobic physical training programme in subjects with metabolic syndrome not only improves cardiorespiratory fitness and anthropometric parameters, but also positively affects cardiovascular risk parameters, components of metabolic syndrome, arterial wall functional and structural parameters as well as improves the psycho-emotional status of a subject, physical activity, motivation for physical training and quality of life.
2. The arterial wall response to aerobic physical training assessed by measuring pulse wave velocity and central mean aortic pressure enables to evaluate the effect of training on cardiovascular risk and metabolic syndrome and to prognosticate the preventive effect of physical training in the objective quantitative way.
3. The individually heart rate targeted 2-month duration aerobic physical training programme in subjects with metabolic syndrome has a long-term effect after 8 months on the improvement of cardiovascular risk, metabolic syndrome components, arterial wall parameters, improvement of quality of life, physical activity, cardiorespiratory fitness, and motivates

subjects more for further physical activity and training, while recommendations on physical activity provided only individually lack such effect or it is weaker.

Novelty of the research. For the first time, according to a published research available, a complex assessment of cardiovascular risk factors together with components of metabolic syndrome, physical activity, cardiorespiratory fitness, motivation for physical training and quality of life was conducted at baseline, after 2 months of the aerobic physical training programme, and the long-term effect assessed after 8 months. It was established that the individually heart rate targeted aerobic physical training programme in subjects with metabolic syndrome not only improves anthropometric parameters (body mass index, waist circumference), blood pressure, components of metabolic syndrome, blood lipids and cardiorespiratory fitness, but also positively affects arterial stiffness and central mean arterial pressure, the findings that are considered to be associated the with lower cardiovascular risk.

2. Study subjects

Study subjects were randomly recruited from the 45–64-year-old participants of the ongoing national LitHiR primary prevention programme [5] whose comprehensive baseline cardiovascular assessment was carried out at Vilnius University Hospital Santaros Klinikos within 2014–2017. The study included 170 individuals with MetS. The subjects were recruited in a random way using a 1:1 random sampling method, and were divided into the intervention aPT group of 85 subjects and the control group of 85 subjects, to whom only recommendations on PA were provided. Metabolic syndrome subjects in both groups were age and gender matched and followed the exact study protocol, except for the HR targeted aPT. The diagnosis of MetS was based on the updated NCEP ATP III criteria [34]. Patients with overt cardiovascular disease or type 2 DM were excluded from the study. All the study subjects received detailed recommendations on the reduction of CV risk factors, including PA and home based training. Subjects of the intervention group additionally participated in an individually supervised 2-month duration HR targeted aPT programme 5 days a week. Antihypertensive treatment was not changed during the whole study. Most of the subjects were statin naive. Subjects in both groups were investigated for 3 times: at baseline, after 2 months and after 8 months in order to evaluate the long-term effect of 2-month aPT (Fig.1).

All the participants were not physically active prior to the inclusion into our study: at the baseline, they indicated having less than 30 minutes of moderate intensity PT five times a week. Management of the risk factors by diet or drug therapy

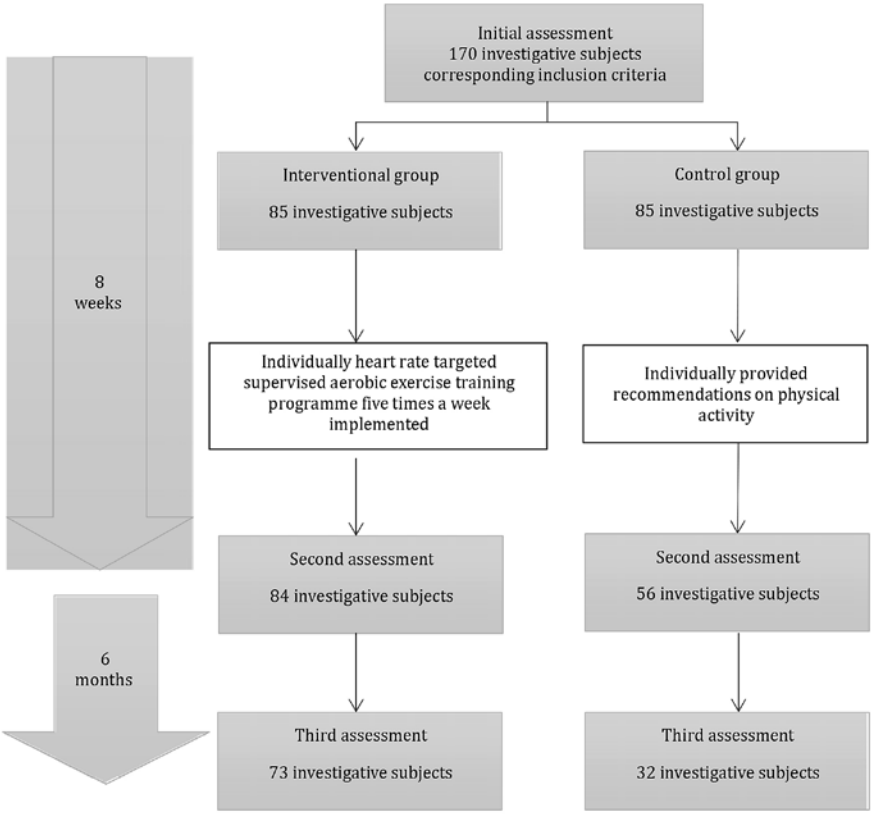

FIG. 1. Flowchart of the study

was not changed throughout the whole period of the study. The mean age of the subjects was 53.3 ± 6.9 years, in the intervention group – 53.9 ± 6.468 and in the control group – 52.1 ± 7.1 years. Gender distribution was 45 % vs. 55 % in the favour of female subjects. Central obesity and poor aerobic physical fitness were present in all the subjects. AH was present in 91.3 %, dyslipidaemia – in 97.6 %, and fasting dysglycemia – in 62.7 % of cases. Out of 170 individuals, 30 (21.4 %) of the subjects missed the second appointment: 1 from the intervention group and 29 – from the control group. Data of these subjects was not included into further analysis. So, after 2 months, 140 individuals were investigated – 84 from the intervention group and 56 – from the control group. Informed consent was obtained from all the participants included into the study. The study protocol was approved by the local ethics committee.

3. Methods

Anthropometric parameters, such as body mass index (BMI), waist circumference (WC), BP, venous blood sampling for blood lipid levels, fasting glucose, arterial wall functional and structural parameters, assessment of anxiety-depression, physical activity, cardiorespiratory fitness (CRF), QOL, motivation for physical training were obtained both in the study and control groups at baseline, after 2 and 8 months. All measurements were obtained at rest, between 7 AM and 12 PM after overnight fast and abstinence from caffeine and at least 2 hours after smoking. Comprehensive non-invasive assessment of arterial markers reflecting the subclinical atherosclerosis was carried out by measuring arterial stiffness parameters by several methods, as well as common carotid artery intima-media thickness.

Arterial stiffness and wave reflection measurement by applanation tonometry. Parameters of arterial stiffness and wave reflection were assessed by the applanation tonometry system (SphygmoCor v.8.0; AtCor Medical) with a high-fidelity micromanometer (Millar R; Millar Instruments, Houston, TX, USA), placed on the skin surface at the projection of radial, carotid and femoral arteries to obtain pulse pressure wave curves [4]. Brachial BP was recorded and the distance between the surface markings of the sternal notch and the femoral artery was measured. These data and the simultaneously recorded ECG allowed the system to use pulse wave curves to compute the main parameters of arterial stiffness: aortic (carotid–femoral pulse wave velocity, cfPWV), peripheral (carotid-radial pulse wave velocity, crPWV) and aortic augmentation index (AIxHR@75), mean blood pressure in the aorta (MBP in the aorta).

Common carotid artery wall assessment. Common carotid artery (CCA) intima media thickness (IMT), cross-sectional carotid artery distensibility (both calculated in μm), and non-dimensional index Quality Carotid Stiffness (QCS) of common carotid artery (CCA) [35] were measured using high-resolution echo-tracking technology (Art. Lab, Esaote Europe B.V.) at 1 cm proximal to the carotid bifurcation along the 4 cm arterial segment. Mean values of the right and the left side CCA measurements of IMT and QCS were used in the analysis.

Assessment of anxiety and depression. HAD (*Hospital Anxiety and Depression*) is a screening test for depression and anxiety that helps to evaluate the level of depression and anxiety in patients treated for other various diseases in general profile or specialized non-psychiatric medical institutions. The test is widely used because of its reliability and simplicity for both: a patient and an investigator. HADS does not require special knowledge of psychology or psychiatry [36], therefore it is appropriate for MetS subjects [11, 13, 37]. The HADS is a fourteen item scale in which seven of the items relate to anxiety and seven – to depression. Each item is

scored from 0 to 3. A person can score between 0 and 21 for either anxiety or depression: 0–7 score is considered to be normal, 8–10 points – mild, 11–14 – moderate, and 15–21 – severe anxiety or depression.

Assessment of physical activity and physical fitness. Physical activity of the subjects was assessed by using IPAQ (*International Physical Activity Questionnaire*) [38]. The IPAQ is a 27-item self-reported measure of physical activity to be used by individual adult patients aged from 15 to 69 years old. The IPAQ can be used clinically or for a population research study that compares physical activity levels between populations internationally.

Duration (minutes) and frequency (days) of physical activity in the last 7 days were measured in the domains of:

1) Job-related time;
2) Transportation;
3) Housework, house maintenance, caring for family members;
4) Recreation, sports, and leisure time;
5) Time spent while sitting.

The overall score was calculated using the responses to all the questions. Subscores were calculated for:

1) Walking;
2) Moderate-intensity activity;
3) Vigorous-intensity activity.

Physical activity was calculated by MET (Metabolic Equivalent of Task) minutes a week (MET-min/week); using the formula: MET × minutes a day × days a week.

Assessment of cardiorespiratory physical fitness and target HR for exercise training. Spiroergometric system Vmax® Encore 229, SensorMedics was used to test physical fitness by performing a progressive incremental exercise test on a cycle ergometer. The cycle ergometer was set to a ramp mode from 15 to 30 W per minute, and was chosen according to the anticipated physical capacity, gender, age, body mass, and a self-reported physical activity status. After 2 minutes of cycling without resistance, the load was constantly increased until voluntary or symptom-limited exercise termination occurred. The exercise protocol was designed to last approximately 8–12 minutes. Throughout the test, BP and constant electrocardiographic monitoring were maintained. The ventilator anaerobic threshold (AT) was estimated by the "V-slope" method (VCO2/VO2 ratio), the first curve inflection. Ventilatory equivalents for O2 (VE/VO2, where VE – maximal minute ventilation) and CO2 (VE/VCO2) were used as additional criteria for the establishment of AT: the start point of the increase in VE/VO2, while VE/VCO2 reaches a plateau or is still decreasing. HR registered at AT was taken as a recommended level

for initial training. Peak oxygen consumption (VO2peak = VOSmax in our case) was considered to be achieved if VO2 reached a plateau in the presence of a growing power output (W). If the subject was exhausted before the attainment of VO2peak, the VO2 value was accepted as a reasonable maximum and a respiratory quotient (RQ) > 1.00 was recorded. CRF was described by oxygen consumption (VO2max ml/kg/min) [39-43].

Assessment of Quality of life (QOL). SF-36 (MOS SF-36; *Medical Outcome Study Short Form Health Survey*) is a multidimensional questionnaire, which is designed to assess the condition of general health. This questionnaire is widely used globally and can be applied to patients suffering from various diseases [44, 45], including subjects with established MetS [46]. Moreover, the test is used to evaluate the effect of physical exercise [20, 47]. The questionnaire consists of 36 questions that reflect the eight domains of life: physical functioning, role limitations due to physical health, role limitations due to emotional problems, energy/fatigue, pain, assessment of general health, social functioning, and emotional well-being, and two integrated measurements of physical and mental health that combine these different domains. The questionnaire assists in the evaluation of well-being over the last 4 weeks. The participants filled out the SF-36 questionnaire on their own. The answers were evaluated in points and each domain of the questionnaire was assessed according to an established algorithm. The numerical value of each domain was from 0 to 100 (100 points show the best estimate).

Assessment of motivation for physical training. EMI-2 (*The Exercise Motivation Inventory-2)* was created in 1997 by D. Markland, D. K. Ingledew [48]. It was developed as a mean of assessing participation motives in order to examine such issues as the influence of motives on exercise participation. The questionnaire comprised 51 statement, fourteen scales and five groups of motives: revitalization and emotional (4 scales), social (3 scales), health (3 scales), appearance (2 scales) and development of physical power motives (2 scales). The questionnaire was adapted not only for physically active persons, but also for non-exercisers. In response to each statement, the participants had to choose an answer on a six-step scale from 0 points – "not at all true for me", to 5 points – "very true for me". The questionnaire was composed of motives for physical training and was divided into 14 scales and motive groups – recreational and emotional, social and health.

Heart rate targeted physical training programme in the study group. The aPT protocol was applied to investigate the influence of the 2-month exercise intervention on the anthropometric, metabolic, arterial wall parameters, CRF, psycho-emotional status, motivation for PT and QOL. HR at ventilatory anaerobic threshold was determined during cardiorespiratory testing and was taken as the recommended level for aPT (41). Specifically, the training intervention consist-

ed of the 2-month duration supervised aerobic training for 30–40 min a day, 5 days a week. The exercise intensity was gradually increased up to the target HR [49]. The ERS-2 Ergoline bicycle based cardiac rehabilitation system was used for the HR targeted PT. It allowed to control the exercise load in the way that HR was maintained at the constant level estimated individually by the cardiorespiratory testing [50].

The control group without targeted HR training. All the MetS subjects in the control group were informed about the necessary home-based physical activity and instructed to perform it 5 times a week for 30-minutes, aiming at the intensity of cardiac workout.

Statistical analysis

Statistical analysis was performed using STATISTICA 10, IBM SPSS 20.0, R package „*party*". Descriptive statistics were presented as means, standard deviations, and frequencies expressed as absolute numbers and percentages. The Shapiro-Wilk test was applied to check for Gaussian distribution of the data. Continuous variables between the groups were compared by the unpaired Student's t-test for normally distributed data and the Mann-Whitney U-test for non-normally distributed data. Categorical variables were analysed with the χ^2 test or the Fisher's exact test. Baseline and follow-up results were compared with the paired Student's t- test for normally distributed continuous data or the Wilcoxon signed-rank test for non-normally distributed continuous data, the McNemar test – for 2 × 2 categorical data, and the marginal homogeneity test – for categorical data with more than two categories. Correlation between variables was determined by calculating Pearson's or Spearman's correlation coefficients. Multiple linear regression analyses were conducted to determine factors associated with the reduction in aortic stiffness. Linear regression and the regression trees were used to evaluate associations between the changes in pulse wave and baseline values. According to the results, the decision tree was built. This allowed to determine possible baseline PWV cut-off values for the outcome prediction. The ROC analysis was used to find out the optimal cut-off value of the changes from baseline for the considered risk factors. Optimal change cut-off was set where the best accuracy to define patient groups were met. Accuracy were evaluated with sensitivity, specificity and Youden's index (J=Sensitivity+Specificity-1) calculated for all possible cut-off values. Cut-off value with maximum Youden's index was chosen as an optimal change from the baseline cut-off value. Additionally, odd ratios according to the optimal cut- off value were calculated for every risk factor. All p values were two-tailed and the level of significance was set to 0.05.

4. Results

Baseline data comparison in the intervention and the control groups.

Homogeneity of the intervention and control groups was checked including the age, gender, main anthropometric parameters, cardiovascular risk factors, components of metabolic syndrome, arterial wall parameters and cardiorespiratory capacity at the baseline (***Table 1***). At the baseline, T-Chol and LDL-Chol were higher in the intervention group. Elevated LDL-Chol was found in 87.95 % of the subjects in the intervention group and in 76.79 % of the subjects in the control group (p = 0.082). There were no significant differences of HDL-Chol and TG between the groups studied. Dyslipidaemia was found in all investigative subjects in the intervention group and in 95 % of subjects in the control group.

TABLE 1. Initial characteristics of the subjects in the investigative group

Signs	Intervention group (n=84)	Control group (n=56)	p value between the groups
Age (years), M ± SD	53.9 ± 6.4	52.1 ± 7.1	0.133
Gender:			0.188
• women (%)	59.52	48.21	
• men (%)	40.48	51.79	
Assessment of AH			
sBP (mm Hg), M ± SD	132.73 ± 13.01	133.78 ± 15.32	0.667
dBP (mm Hg), M ± SD	81.56 ± 9.23	82.71 ± 10.88	0.511
AH (in %)	94.05	87.50	0.222
Stages of BP:			0.225
• optimal BP (%)	13.25	19.64	
• normal BP (%)	21.69	17.86	
• high-normal BP (%)	30.12	17.86	
• I° elevation of BP (%)	19.28	14.29	
• II° elevation of BP (%)	4.82	8.93	
• Isolated systolic hypertension (%)	10.84	21.43	
Assessment of dyslipidaemia			
T-Chol (mmol/l), M ± SD	6.41 ± 1.40	5.86 ± 1.24	0.012
LDL-Chol (mmol/l). M ± SD	4.19 ± 1.17	3.80 ± 1.05	0.044
TG (mmol/l), M ± SD	2.35 ± 2.81	1.94 ± 0.91	0.231
HDL-Chol (mmol/l), M ± SD	1.19 ± 0.30	1.15 ± 0.29	0.410
Dyslipidaemia (in %)	100.00	94.64	0.062

Signs	Intervention group (n=84)	Control group (n=56)	p value between the groups
Smoking:			
• smoking (%)	11.90	19.64	0.209
• non smoking (%)	88.10	80.36	
Assessment of obesity			
Waist circumference (cm), M ± SD	102.18 ± 9.13	104.98 ± 9.10	0.082
Abdominal obesity (proc.)	81.9	80.4	0.816
BMI (kg/m^2), M ± SD	30.86 ± 3.96	31.05 ± 3.21	0.769
Obesity:			0.850
• no (%)	4.82	1.79	
• overweight (%)	38.55	35.71	
• I degree (%)	40.96	48.21	
• II degree (%)	14.46	14.29	
• III degree (%)	1.20	0.00	
Assessment of MetS components			
Waist circumference (cm), M ± SD	102.18 ± 9.13	104.98 ± 9.10	0.082
Abdominal obesity (%)	81.9	80.4	0.816
TG (mmol/l), M ± SD	2.35 ± 2.81	1.94 ± 0.91	0.231
Study subjects with elevated TG concentration (%)	63.1	53.6	0.261
HDL-Chol (mmol/l), M ± SD	1.19 ± 0.30	1.15 ± 0.29	0.410
Study subjects with the reduction of HDL-Chol (%)	51.2	53.6	0.782
sBP (mm Hg), M ± SD	132.73 ± 13.01	133.78 ± 15.32	0.667
dBP (mm Hg), M ± SD	81.56 ± 9.23	82.71 ± 10.88	0.511
Study subjects with elevated BP (%)	94.0	89.3	0.347
Fasting glucose in plasma (mmol/l), M ± SD	5.99 ± 0.83	5.80 ± 0.59	0.340
Study subjects with elevated glucose concentration (%)	64.3	61.8	0.768
Number of MetS components:			0.408
• 3 (%)	44.0	50.0	
• 4 (%)	36.9	39.3	
• 5 (%)	19.0	10.7	
Assessment of arterial wall functional and structural parameters			
Carotid-radial a. PWV (m/s), M ± SD	9.07 ± 1.18	5.2.4	0.614
Carotid-femoral a. PWV (m/s), M ± SD	8.53 ± 1.36	8.11 ± 1.36	0.066

Signs	Intervention group (n=84)	Control group (n=56)	p value between the groups
Carotid-femoral a. PWV:			0.370
• ≥10 m/s (%)	14.46	7.14	
• 9–9.9 m/s (%)	20.48	16.07	
• 8–8.9 m/s (%)	32.53	28.57	
• 7–7.9 m/s (%)	20.48	32.14	
• <7 m/s (%)	12.05	16.07	
AIxHR@75 (%), M ± SD	22.13 ± 12.59	21.41 ± 10.72	0.745
Aortic MBP (mm Hg), M ± SD	104.17 ± 9.49	103.54 ± 10.78	0.560
CCA IMT (μm), M ± SD	648.23 ± 104.70	636.12 ± 95.42	0.396
CCA stiffness, M ± SD	4.39 ± 1.68	4.14 ± 1.36	0.547
Assessment of anxiety and depression signs			
Anxiety (scores), M ± SD	5.21 ± 2.87	4.33 ± 3.34	0.151
Depression (scores), M ± SD	3.40 ± 2.92	2.65 ± 2.40	0.188
Anxiety			0.158
• 0–7 scores (% .)	84.6	75.5	
• 8–10 scores (%)	10.3	20.7	
• 11–14 scores (%)	5.1	1.9	
• 15–21 scores (%)	0	1.9	
Depression			0.111
• 0–7 scores (%)	88.5	98.1	
• 8–10 scores (%)	10.2	1.9	
• 11–14 scores (%)	1.3	0	
• 15–21 scores (%)	0	0	
Assessment of physical activity			
Total Physical Activity (MET-minutes/week), M ± SD	5316.41 ± 4409.31	4653.28 ± 4200.04	0.497
Physical activity at work (MET-minutes/week), M ± SD	1496.85 ± 3802.33	1244.98 ± 2566.95	0.529
Physical activity for transportation (MET-minutes/week), M ± SD	994.58 ± 1057.19	665.58 ± 1154.72	0.077
Physical activity in a domestic setting and gardening (MET-minutes/week), M± SD	1912.67 ± 2652.40	1397.40 ± 2256.32	0.182
Physical activity in leisure time (MET-minutes/week), M ± SD	912.32 ± 1144.34	1345.32 ± 2645.06	0.699
Walking physical activity (MET-minutes/week), M ± SD	1916.85 ± 2124.19	1939.08 ± 2735.24	0.469

Signs	Intervention group (n=84)	Control group (n=56)	p value between the groups
Moderate physical activity (MET-minutes/week), M ± SD	2512.67 ± 2616.01	1850.20 ± 2263.90	0.107
Vigorous physical activity (MET-minutes/week), M ± SD	886.90 ± 2186.70	864.00 ± 1492.38	0.725
Sitting (minutes/week), M ± SD	1927.30 ± 877.00	1620.29 ± 989.90	0.105
Assessment of cardiorespirative physical capacity			
VO2max (ml/kg/min), M ± SD	22.28 ± 5.47	22.51 ± 4.61	0.637
AT (%), M ± SD	76.22 ± 17.63	77.98 ± 17.55	0.541
Heart rate at rest (times/min), M ± SD	63.57 ± 7.96	61.30 ± 8.04	0.173
Assessment of cardiorespirative physical capacity:			1.000
• bad (%)	97.6	100	
• satisfactory (%)	1.2	0	
• good (%)	1.2	0	
• very good (%)	0	0	
• excellent (%)	0	0	
Assessment of health-related quality of life			
Physical Health: Summary	73.57 ± 17.43	73.69 ± 15.29	0.707
Physical Functioning	81.58 ± 17.10	85.99 ± 12.40	0.185
Role: Physical	77.53 ± 33.87	76.83 ± 34.64	0.925
Bodily Pain	76.79 ± 21.44	70.73 ± 20.30	0.112
General Health	58.45 ± 15.07	61.22 ± 14.00	0.330
Mental Health: Summary	74.43 ± 15.21	76.41 ± 16.12	0.325
Vitality	62.37 ± 18.21	64.38 ± 15.57	0.693
Social Functioning	84.97 ± 17.26	86.89 ± 15.80	0.622
Role: Emotional	78.06 ± 31.53	83.74 ± 31.73	0.257
Mental Health	72.67 ± 14.51	70.50 ± 14.57	0.445
Change in Reported Health	42.53 ± 19.89	44.23 ± 17.64	0.726
Assessment of motivation for physical training			
Enjoyment and Revitalisation Motive	2.97 ± 1.26	2.98 ± 1.13	0.958
Social Engagement Motive	1.85 ± 1.47	2.16 ± 1.33	0.122
Health motive	4.09 ± 0.78	3.53 ± 1.53	0.166
Appearance / Weight Motive	3.76 ± 1.00	3.91 ± 1.05	0.229
Fitness Motive	3.63 ± 1.13	3.63 ± 1.01	0.756

Abbreviations and explanations: M ± SD – mean ± standard deviation, AH – arterial hypertension, BP – blood pressure, sBP – systolic blood pressure, dBP – diastolic blood pressure, BMI – body mass index, T-Chol – total cholesterol, HDL-Chol – high density lipoprotein cholesterol, LDL-Chol – low density lipoprotein cholesterol, TG – triglycerides, MetS – metabolic syndrome, PWV – pulse wave velocity, MBP – mean blood pressure, CCA – common carotid artery, IMT – intima–media thickness, MET – metabolic equivalent, VO2max – maximal oxygen consumption, AT – anaerobic threshold, HR – heart rate.

Changes in cardiovascular risk factors, metabolic syndrome components, arterial wall parameters, psycho-emotional status, physical activity, cardiorespiratory fitness, quality of life, and motivation for physical training after 2 months.

Arterial hypertension. Statistically significant improvement in BP was found in the intervention group: systolic blood pressure (sBP) decreased in average for 4.09 mm Hg (3.08 %, p=0.006), diastolic blood pressure (dBP) – in average for 2.51 mm Hg (3.08 %, p=0.003) (Fig. 2). There were no significant changes of BP after two months in the control group.

Lipid profile. Statistically significant improvement in some lipid parameters, such as decreasing T-Chol, on average, by 0.52 mmol/l (8 %, p=0.001) and LDL-

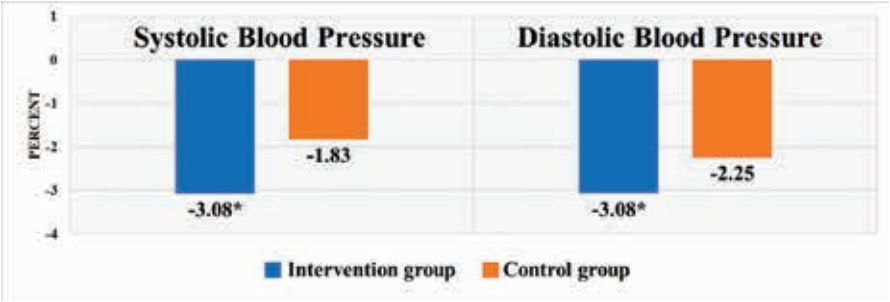

FIG. 2. Changes of blood pressure parameters
in the intervention and control groups after 2 months
Abbreviations and explanations: BP – blood pressure; * – statistically significant difference (p<0.05).

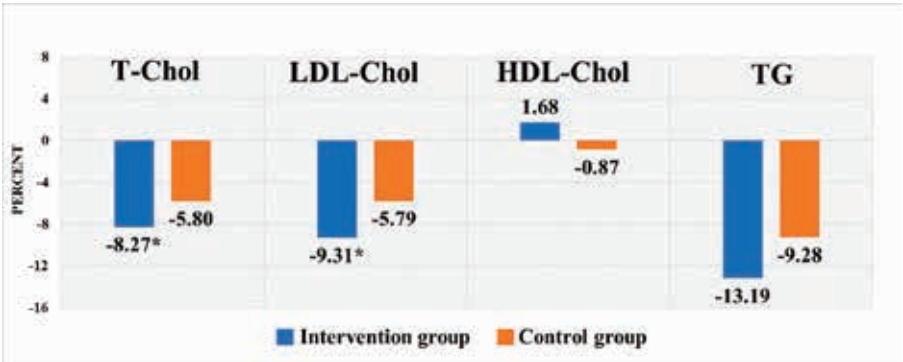

FIG. 3. Changes of lipid parameters in the intervention and control groups after 2 months
Abbreviations and explanations: T-Chol – total cholesterol, HDL-Chol – high density lipoprotein cholesterol, LDL-Chol – low density lipoprotein cholesterol, TG – triglyceride; * – statistically significant difference (p<0.05).

Chol – by 0.39 mmol/l (9 %, p=0.002) in the intervention group but not in the control group was found after 2 months. There were no statistically significant changes in TG and HDL-Chol, although in the intervention group there was a trend of HDL-Chol increase and reduction of TG. (Fig. 3).

Obesity. Statistically significant reduction in BMI was found in both groups: in the intervention group – on average, by 0.37 kg/m^2 (1.2 %, p<0.001), in the control group – on average, by 0.34 kg/m^2 (1.1 %, p=0.011). WC significantly decreased only in the intervention group, on average, by 1.34 cm (1.31 %, p<0.001) (Fig. 4).

Components of metabolic syndrome. The baseline evaluation of MetS components revealed that abdominal obesity (WC in men ≥102 cm and in women ≥88

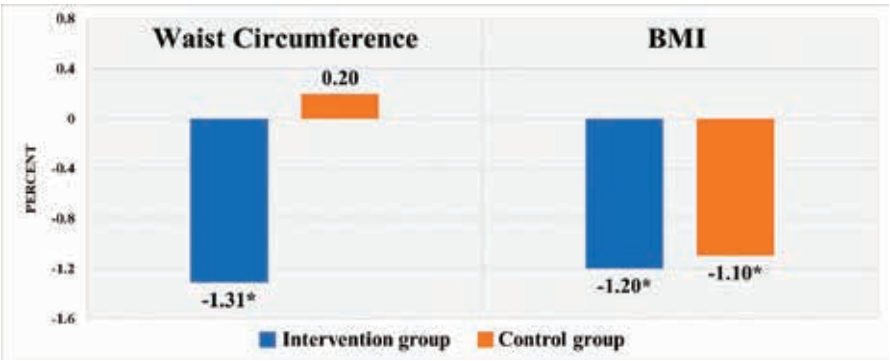

FIG. 4. Changes of waist circumference
and body mass index in the intervention and control groups after two months
Abbreviations and explanations: BMI – body mass index; * – statistically significant difference (p<0.05).

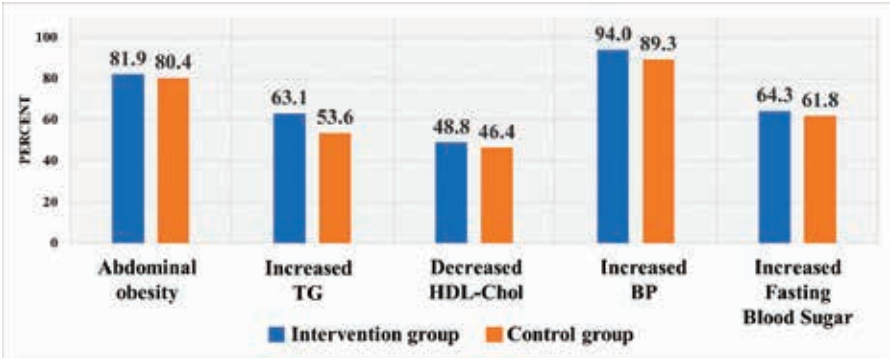

FIG. 5. The distribution of the metabolic syndrome components in both groups at baseline
Abbreviations and explanations: HDL-Chol – high density lipoprotein cholesterol,
BP – blood pressure.

cm) was present in 81.3 % of the subjects, BP ≥130/85 mm Hg – in 92.1 %, fasting glucose ≥5.6 mmol/l – in 63.3 %, elevated TG ≥1.7 mmol – in 59.3 %, reduced HDL-Chol (<1.03 mmol/l – in men and <1.3 mmol/l – in women) in 47.9 % of all investigated subjects (Fig. 5). Three components of MetS were present in 46.4 %, four – in 37.9 %, all five components – in 15.7 % of all the investigated subjects (Fig. 6).

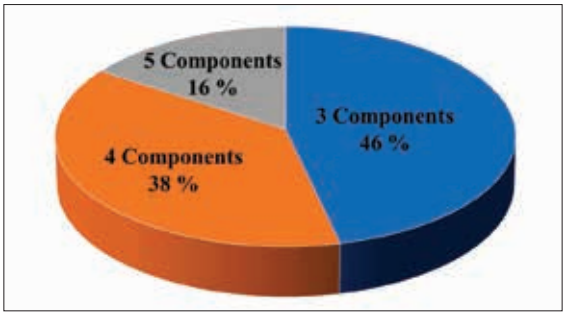

FIG. 6. The number of MetS components in both groups at baseline

After 2 months, statistically significantly improved values in 2 of 5 components – waist circumference, that decreased, on average, by 1.34 cm (1.31 %, p<0.001), and blood pressure – sBP 4.09 mm Hg (3.08 %, p=0.006) and dBP 2.51 mm Hg (3.08 %, p=0.003) were found in the intervention group. There were no significant changes in the values of MetS components in the control group after 2 months (Fig. 7 and Table 2).

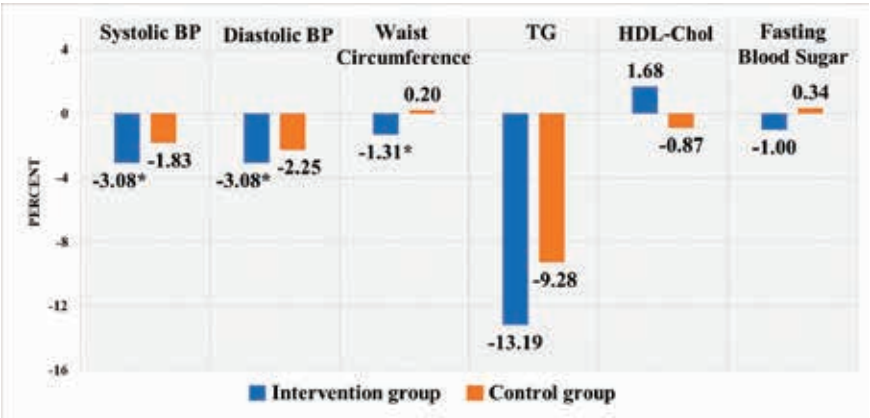

FIG. 7. Changes in the values of the MetS components after 2 months
Abbreviations and explanations: BP – blood pressure, TG – triglyceride, HDL-Chol – high density lipoprotein cholesterol; * – statistically significant difference (p<0.05).

TABLE 2. Changes in the values of the MetS components in the intervention and control groups after 2 months

Intervention group				p value between the groups	Control group			
At baseline	After 2 months	Change	p value		p value	Change	At baseline	After 2 months
Waist circumference (cm), M ± SD					Waist circumference (cm), M ± SD			
102.18 ± 9.13	100.84 ± 9.15	-1.34 ± 3.89	**<0.001**	0.086	0.705	0.20 ± 3.94	104.98 ± 9.10	105.19 ± 9.88
TG (mmol/l), M ± SD					TG (mmol/l), M ± SD			
2.35 ± 2.81	2.04 ± 1.31	-0.31 ± 2.85	0.302	0.635	0.082	-0.17 ± 0.73	1.94 ± 0.91	1.76 ± 0.71
HDL-Chol (mmol/l), M ± SD					HDL-Chol (mmol/l), M ± SD			
1.19 ± 0.30	1.21 ± 0.28	0.01 ± 0.18	0.455	0.068	0.545	-0.02 ± 0.19	1.15 ± 0.29	1.14 ± 0.29
sBP (mm Hg), M ± SD					sBP (mm Hg), M ± SD			
132.73 ± 13.01	128.64 ± 11.58	-4.09 ± 12.99	**0.006**	0.465	0.146	-2.45 ± 12.33	133.78 ± 15.32	131.33 ± 12.67
dBP (mm Hg), M ± SD					dBP (mm Hg), M ± SD			
81.56 ± 9.23	79.05 ± 8.02	-2.51 ± 7.26	**0.003**	0.687	0.151	-1.85 ± 9.44	82.71 ± 10.88	80.85 ± 10.39
Fasting glucose in plasma (mmol/l), M ± SD					Fasting glucose in plasma (mmol/l), M ± SD			
5.99 ± 0.83	5.93 ± 0.81	-0.06 ± 0.53	0.226	0.409	0.740	0.02 ± 0.43	5.80 ± 0.59	5.82 ± 0.60

Abbreviations and explanations: M+SD – mean plus minus standard deviation, TG – triglyceride, HDL-Chol – high density lipoprotein cholesterol, sBP – systolic blood pressure, dBP – diastolic blood pressure.

The number of MetS components after 2 months statistically significantly decreased in both intervention and control groups (Fig. 8) from the average of 3.75 components in the intervention group and 3.61 – in the control group at baseline to 3.38 (decrease of 0.38, p=0.001) and 3.31 (decrease of 0.29, p=0.016), respectively. After 2 months, 3 of 5 MetS components were no more present in 18.8 % of the intervention group subjects and in 16.7 % – in the control group subjects.

Arterial wall functional and structural parameters. After 2 months, we have observed the decrease of aortic stiffness in the reduction of c-f PWV, on average, by 0.54 m/s (6.33 %, p<0.001) in the intervention group. The significant difference of the change of c-f PWV between the groups (p=0.034) was revealed. The reduction of MBP in the aorta, on average, by 3.73 mm Hg (3.58 %, p=0.001) was found. No reduction of the parameters was found in the control group. (Fig. 9 and 10).

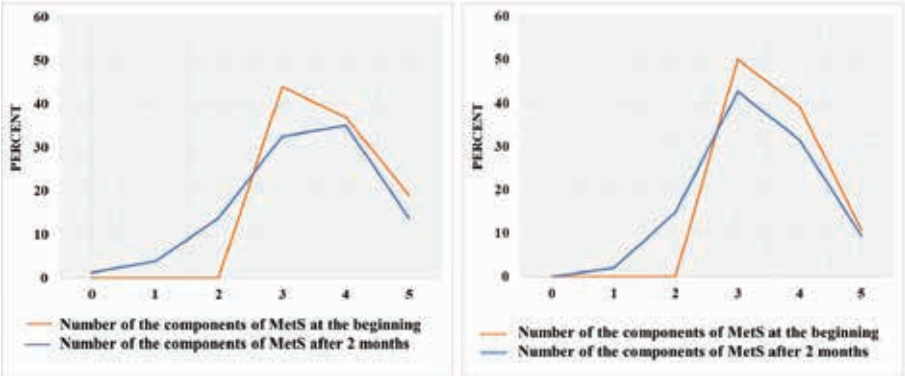

FIG. 8. Change in the number of MetS components after 2 months – *p=0.001* (in the intervention group) and *p=0.016* (in the control group)
Abbreviations and explanations: MetS – metabolic syndrome.

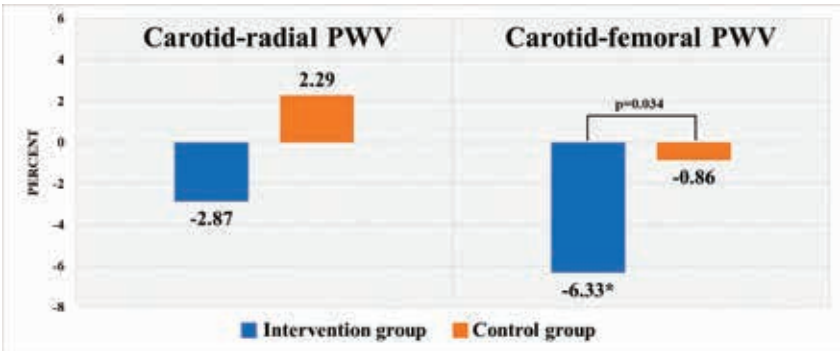

FIG. 9. Carotid-femoral and carotid-radial pulse wave velocity changes after 2 months
Abbreviations and explanations: PWV – pulse wave velocity; * – statistically significant difference (p<0.05).

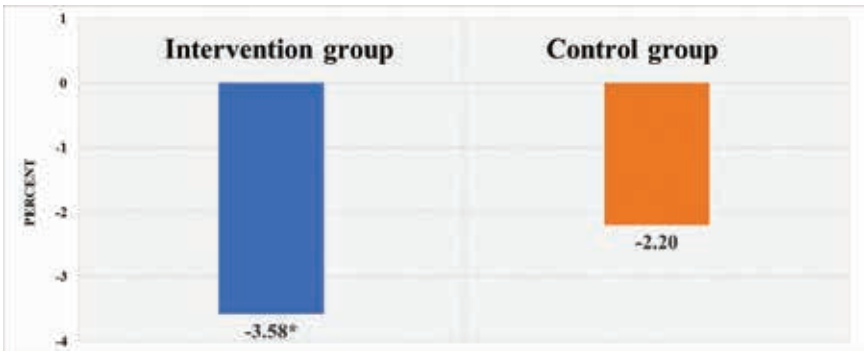

FIG. 10. Changes of mean blood pressure in the aorta after 2 months
Abbreviations and explanations: * – statistically significant difference (p<0.05).

TABLE 3. Changes in arterial wall functional and structural parameters after 2 months

Intervention group				p value between the groups	Control group			
At base-line	After 2 months	Change	p value		p value	Change	At baseline	After 2 months
M ± SD	M ± SD	M ± SD				M ± SD	M ± SD	M ± SD
Carotid-radial PWV (m/s)					Carotid-radial PWV (m/s)			
9.07 ± 1.18	8.81 ± 1.20	-0.26 ± 1.40	0.137	0.105	**0.384**	0.21 ± 1.69	9.18 ± 1.22	9.39 ± 1.59
Carotid-femoral PWV (m/s)					Carotid-femoral PWV (m/s)			
8.53 ± 1.36	7.99 ± 1.04	-0.54 ± 1.32	**<0.001**	0.034	0.533	-0.07 ± 1.17	8.11 ± 1.36	8.04 ± 1.19
AIxHR@75 (%)					AIxHR@75 (%)			
22.13 ± 12.59	20.84 ± 12.86	-1.30 ± 11.73	0.368	0.595	0.299	-1.18 ± 7.88	21.4 ± 10.72	20.22 ± 11.26
MBP in aorta (mm Hg)					MBP in aorta (mm Hg)			
104.17 ± 9.49	100.44 ± 9.99	-3.73 ± 10.11	**0.001**	0.439	0.146	-2.28 ± 11.35	103.54 ± 10.78	101.26 ± 12.75
IMT of CCA (μm)					IMT of CCA (μm)			
648.23 ± 104.70	648.88 ± 94.00	0.65 ± 60.69	0.923	0.088	**0.032**	21.90 ± 83.96	636.12 ± 95.42	658.02 ± 100.14
Stiffness of CCA					Stiffness of CCA			
4.39 ± 1.68	4.06 ± 1.35	-0.33 ± 1.33	0.079	**0.022**	0.121	0.26 ± 1.39	4.14 ± 1.36	4.40 ± 1.69

Abbreviations and explanations: M ± SD – mean ± standard deviation, PWV – pulse wave velocity, AIxHR@75 – augmentation index, normalized by 75 b/min heart rate, MBP – mean blood pressure, CCA – common carotid artery, IMT – intima-media thickness.

TABLE 4. Changes of carotid-femoral pulse wave velocity after 2 months depending on the baseline pulse wave velocity.

Parameter	Intervention group			Control group		
	At baseline	After 2 months	p value	At baseline	After 2 months	p value
Carotid-femoral PWV:			**0.001**			0.491
• ≥ 10 m/s (%)	14.46	4.88		7.14	5.36	
• 9–9.9 m/s (%)	20.48	10.98		16.07	16.07	
• 8–8.9 m/s (%)	32.53	34.15		28.57	26.79	
• 7–7.9 m/s (%)	20.48	34.15		32.14	32.14	
• < 7 m/s (%)	12.05	15.85		16.07	19.64	

Abbreviations and explanations: PWV – pulse wave velocity.

After 2 months, no statistically significant reduction of CCA stiffness and carotid IMT was observed in either intervention or control group. However, there was a statistically significant difference between the magnitude of stiffness of CCA change between the groups (p=0.022).

Changes in the arterial wall functional and structural parameters after 2 months are presented in Tables 3 and 4.

Anxiety-depression parameters. We did not observe the symptoms of anxiety in 84.6 % of the intervention group subjects. Light symptoms were observed in 10.3 % and symptoms of moderate severity in 5.1 % of subjects. In the control group, 75.5 % of the subjects had no symptoms of anxiety, 20.7 % had light, 1.9 % moderate and 1.9 % severe symptoms (Table 1). There were no symptoms of anxiety in 88.5 % of the intervention group subjects, in 10.2 % light and in 1.3 % moderate. In both groups there were no symptoms of severe depression. After 2 months, there were no statistically significant changes in the level of anxiety and only in the intervention group the reduction of depression was observed in 11 % of the subjects (p=0.021) (Table 5).

TABLE 5. Changes of anxiety and depression levels after 2 months

Sign	Intervention group	p value	Control group	p value
Anxiety		0.109		0.796
Improved (%)	2.8		10.0	
Not changed (%)	84.9		82.5	
Worsened (%)	12.3		7.5	
Depression		**0.021**		1.000
Improved (%)	10.9		1.9	
Not changed (%)	87.7		98.1	
Worsened (%)	1.4		0	

Physical activity. After 2 months, the analysis of physical activity, focusing on the domain characters and levels, revealed statistically significant differences between the intervention, and control group. In the former, we observed a significantly greater improvement in performing moderate and vigorous physical activity during leisure time: working in the garden and yard, recreation, sports and other leisure activities (Fig. 11).

Likewise, we observed a statistically significant difference in the change of the sitting time between the intervention and control group (Fig. 12).

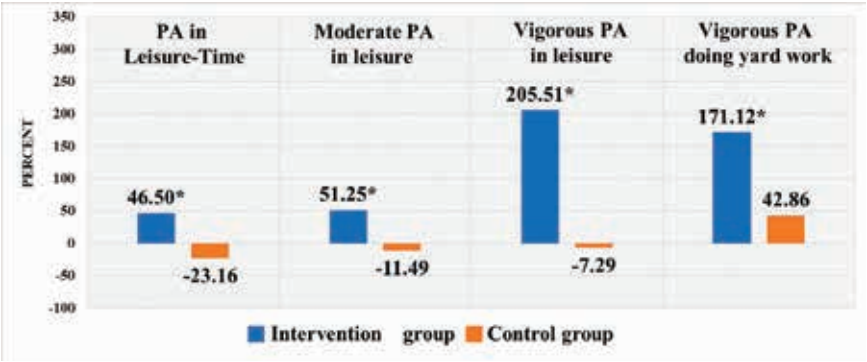

FIG. 11. Changes of physical activity according to the domains after 2 months
Abbreviations and explanations: PA – physical activity, * – statistically significant difference (p<0.05).

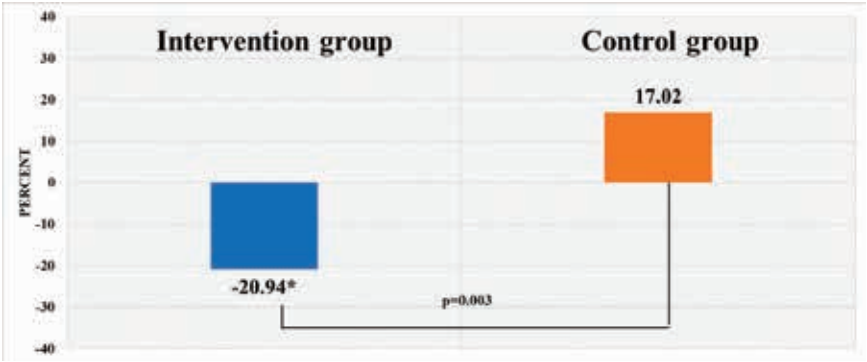

FIG. 12. Change of the sitting time after 2 months.
Abbreviations and explanations: * – statistically significant difference (p<0.05).

There were no significant changes of any intensity physical activity and the sitting time in the control group (Tab. 6).

TAB. 6. Changes of physical activity after 2 months according to the domain and character.

Intervention group				p value between the groups	Control group			
At baseline	After 2 months	Change	p value		p value	Change	At baseline	After 2 months
M ± SD	M ± SD	M ± SD				M ± SD	M ± SD	M ± SD
Physical activity at work (MET-minutes/ week)					Physical activity at work (MET-minutes/ week)			
1496.85 ± 3802.33	1750.71 ± 2907.98	253.86 ± 4366.86	0.242	0.648	0.164	195.48 ± 2000.52	1244.98 ± 2566.95	1440.46 ± 2116.50

Intervention group				p value between the groups	Control group			
At baseline	After 2 months	Change	p value		p value	Change	At baseline	After 2 months
M ± SD	M ± SD	M ± SD				M ± SD	M ± SD	M ± SD
Walking at work (MET-minutes/week)					Walking at work (MET-minutes/week)			
414.78 ± 1250.78	407.95 ± 853.22	-6.83 ± 1018.04	0.728	0.445	0.199	368.28 ± 1487.07	417.78 ± 1045.38	786.06 ± 1489.69
Vigorous physical activity at work (MET-minutes/week)					Vigorous physical activity at work (MET-minutes/week)			
699.31 ± 2191.94	717.93 ± 1702.68	18.62 ± 2857.20	0.602	0.710	0.813	-182.40 ± 1941.48	556.80 ± 1437.87	374.40 ± 1125.87
Moderate physical activity at work (MET-minutes/week)					Moderate physical activity at work (MET-minutes/week)			
382.76 ± 953.37	624.83 ± 1744.22	242.07 ± 1912.89	0.932	0.613	0.767	9.60 ± 534.45	270.40 ± 689.28	280.00 ± 581.03
Physical activity for transportation (MET-minutes/week)					Physical activity for transportation (MET-minutes/week)			
994.58 ± 1057.19	1059.41 ± 1851.12	64.84 ± 2025.68	0.866	0.396	0.168	205.32 ± 1258.98	665.58 ± 1154.72	870.90 ± 862.12
Walk (MET-minutes/week)					Walk (MET-minutes/week)			
929.41 ± 1028.44	695.28 ± 839.28	-234.13 ± 1174.05	0.523	0.309	0.224	120.12 ± 1230.83	622.38 ± 1147.88	742.50 ± 817.87
Cycle (MET-minutes/week)					Cycle (MET-minutes/week)			
65.17 ± 209.75	364.14 ± 1694.18	298.97 ± 1707.94	0.221	0.800	0.345	85.20 ± 324.69	43.20 ± 182.09	128.40 ± 338.69
Physical activity in a domestic setting and gardening (MET-minutes/week)					Physical activity in a domestic setting and gardening (MET-minutes/week)			
1912.67 ± 2652.40	2457.59 ± 4130.29	544.91 ± 4309.16	0.733	0.996	0.753	-163.80 ± 2063.91	1397.40 ± 2256.32	1233.60 ± 1402.46
Vigorous physical activity doing yard work (MET-minutes/week)					Vigorous physical activity doing yard work (MET-minutes/week)			
354.66 ± 1017.35	961.55 ± 2166.41	606.90 ± 2205.30	0.037	0.762	0.394	178.20 ± 1296.52	415.80 ± 945.60	594.00 ± 1056.52
Moderate physical activity doing yard work (MET-minutes/week)					Moderate physical activity doing yard work (MET-minutes/week)			
637.59 ± 978.25	749.66 ± 1575.65	112.07 ± 1646.30	0.701	0.789	0.723	-172.80 ± 847.96	456.00 ± 872.93	283.20 ± 321.23
Moderate physical activity doing inside chores (MET-minutes/week)					Moderate physical activity doing inside chores (MET-minutes/week)			
920.43 ± 1588.00	746.38 ± 1116.55	-174.05 ± 1594.97	0.700	0.620	0.313	-169.20 ± 628.46	525.60 ± 711.82	356.40 ± 525.74

Intervention group				p value between the groups	Control group			
At baseline	After 2 months	Change	p value		p value	Change	At baseline	After 2 months
M ± SD	M ± SD	M ± SD				M ± SD	M ± SD	M ± SD
Physical activity in leisure time (MET-minutes/week)					Physical activity in leisure time (MET-minutes/week)			
912.32 ± 1144.34	1336.51 ± 1508.95	424.19 ± 1544.89	0.032	0.166	0.578	-311.64 ± 1795.18	1345.32 ± 2645.06	1033.68 ± 1242.14
Walking physical activity in leisure (MET-minutes/week)					Walking physical activity in leisure (MET-minutes/week)			
572.66 ± 881.99	533.41 ± 749.06	-39.26 ± 812.10	0.825	0.666	0.560	-273.24 ± 1742.39	898.92 ± 2070.85	625.68 ± 833.62
Moderate physical activity in leisure (MET-minutes/week)					Moderate physical activity in leisure (MET-minutes/week)			
152.07 ± 389.04	230.00 ± 365.71	77.93 ± 481.29	**0.042**	0.463	0.161	-16.00 ± 414.25	139.20 ± 418.11	123.20 ± 233.79
Vigorous physical activity in leisure (MET-minutes/week)					Vigorous physical activity in leisure (MET-minutes/week)			
187.59 ± 439.85	573.10 ± 1032.07	385.52 ± 1027.47	0.004	0.246	0.722	-22.40 ± 524.35	307.20 ± 648.15	284.80 ± 661.34
Walking physical activity (MET-minutes/week)					Walking physical activity (MET-minutes/week)			
1916.85 ± 2124.19	1636.63 ± 1646.77	-280.22 ± 2122.50	0.708	0.688	0.587	215.16 ± 3034.06	1939.08 ± 2735.24	2154.24 ± 2198.61
Moderate physical activity (MET-minutes/week)					Moderate physical activity (MET-minutes/week)			
2512.67 ± 2616.01	3676.55 ± 4966.91	1163.88 ± 5292.23	0.430	0.751	0.841	-85.00 ± 2354.09	1850.20 ± 2263.90	1765.20 ± 1654.82
Vigorous physical activity (MET-minutes/week)					Vigorous physical activity (MET-minutes/week)			
886.90 ± 2186.70	1291.03 ± 2319.43	404.14 ± 3175.93	0.077	0.119	0.576	-204.80 ± 2031.50	864.00 ± 1492.38	659.20 ± 1296.00
Sitting (minutes/week)					Sitting (minutes/week)			
1927.30 ± 877.00	1523.72 ± 867.59	-403.58 ± 835.85	0.000	0.003	0.201	275.71 ± 1250.81	1620.29 ± 989.90	1896.00 ± 940.55
Total Physical Activity (MET-minutes/week)					Total Physical Activity (MET-minutes/week)			
5316.41 ± 4409.31	6604.22 ± 5384.76	1287.80 ± 7056.06	0.163	0.391	0.819	-74.64 ± 3668.28	4653.28 ± 4200.04	4578.64 ± 2655.55

Abbreviations and explanations: M ± SD – mean ± standard deviation, MET – metabolic equivalent of the task.

Cardiorespiratory fitness. The mean values of the VO2max parameter at baseline were as follows: 22.28 ml/kg/min in the intervention group and 22.51 ml/kg/min in the control group. AT in the intervention group was 76.2 % and in the control group 77.98 % (of the predicted value). In the intervention group, mean HR at rest interventionwas 63.57 b/min, whereasin control group 61.30 b/min. We assessed CRF according to the guidelines of the *Cooper* aerobic investigations' institute and determined that it was very poor almost in all the study subjects (in 97.6 % of the intervention group subjects and in 100 % of the control group subjects). After 2 months, statistically significant improvement in CRF was found in both groups, but in the intervention group these changes were pronounced more statistically significantly (Fig.16). VO2max increased, on average, by 2.22 ml/kg/min (9.96 %, p<0.001) in the intervention group and by 0.94 ml/kg/min (4.18 %, p=0.005) in the control group, with a statistically significant difference between the groups (p=0.005). AT assessed by the percentage of estimated VO2 in the intervention group increased, on average, by 9.05 (11.87 %, p<0.001), whereas in the control group it decreased, on average, by 3.64 (4.67 %, p=0.018), the change difference between the groups being statistically significant (p<0.01). HR at rest significantly decreased only in the intervention group: on average, by 2.78 b/min (4.37 %, p=0.018).

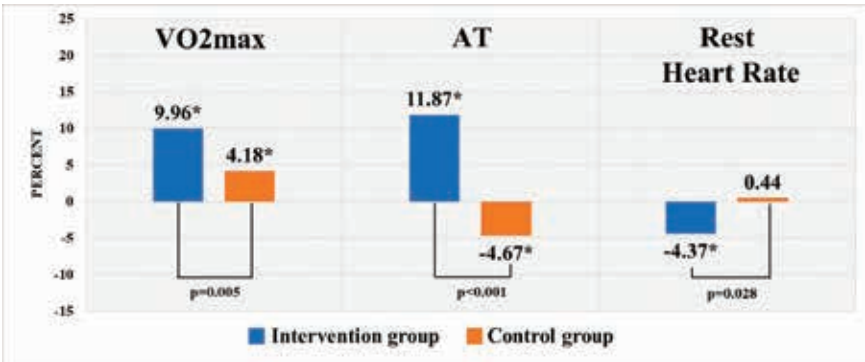

FIG. 13. The values of cardiorespiratory fitness parameters after 2 months
Abbreviations and explanations: VO2max – maximal consumption of oxygen, AT – anaerobic threshold, VO2 – consumption of oxygen, HR – heart rate; * – statistically significant difference (p<0.05).

The parameters of cardiorespiratory fitness are presented in Table 7.

TABLE 7. The changes of cardiorespiratory fitness parameters.

Intervention group				p value between the groups	Control group			
At base-line	After 2 months	Change	p value		p value	Change	At baseline	After 2 months
M ± SD	M ± SD	M ± SD				M ± SD	M ± SD	M ± SD
VO2max (ml/kg/min)					VO2max (ml/kg/min)			
22.28 ± 5.47	24.50 ± 5.24	2.22 ± 2.76	**<0.001**	**0.005**	**0.005**	0.94 ± 2.39	22.51 ± 4.61	23.45 ± 5.03
AT (%)					AT (%)			
76.22 ± 17.63	85.27 ± 16.54	9.05 ± 13.27	**<0.001**	**<0.001**	**0.018**	-3.64 ± 11.15	77.98 ± 17.55	74.34 ± 19.33
HR at rest (b/min), M ± SD					HR at rest (b/min), M ± SD			
63.57 ± 7.96	60.79 ± 8.38	-2.78 ± 8.28	**0.003**	**0.028**	0.778	0.27 ± 7.06	61.30 ± 8.04	61.57 ± 7.81

Abbreviations and explanations: M ± SD – mean and standard deviation; VO2max – maximal consumption of oxygen, AT – anaerobic threshold, VO2 – consumption of oxygen, HR – heart rate.

Quality of life. Subjects of both groups rated the health changes during the last year, general health condition, energy and vitality status as the worst. Social functions, physical activity and limitations due to emotional disorders were rated with top scores. (Fig. 14).

Based on the analysis of how various aspects of QOL were evaluated by the subjects of different genders, age groups and obesity levels, we conclude that men rated their physical activity better than women, (p = 0.002 in the intervention group and p = 0.007 in the control group), and the younger subjects better than older (p = 0.009 in the intervention group and p = 0.012 in the control group). In the intervention group, pain more affected older subjects (p = 0.009), and the social functions were

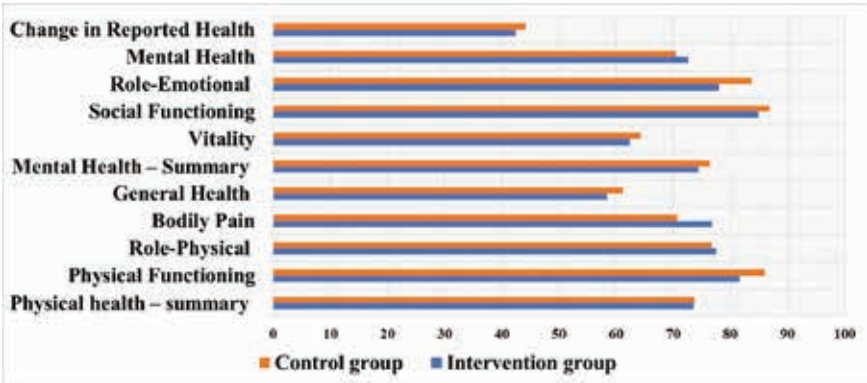

FIG. 14. Initial health-related evaluations of quality of life

rated worse by women as well as by the older (p = 0.003 and 0.029, respectfully). By assessing the changes of QOL after 2 months it was estimated that, in the intervention group, physical activity increased. Limitation of activity decreased, most likely due to emotional disorders and changes in the generalized score of mental health. The overall score of physical activity, scores of basic health assessment, energy, vitality, emotional status and changes of health statistically significantly improved in both groups, and the score of pain increased (that means decreased bodily pain) only in the control group (Fig. 15).

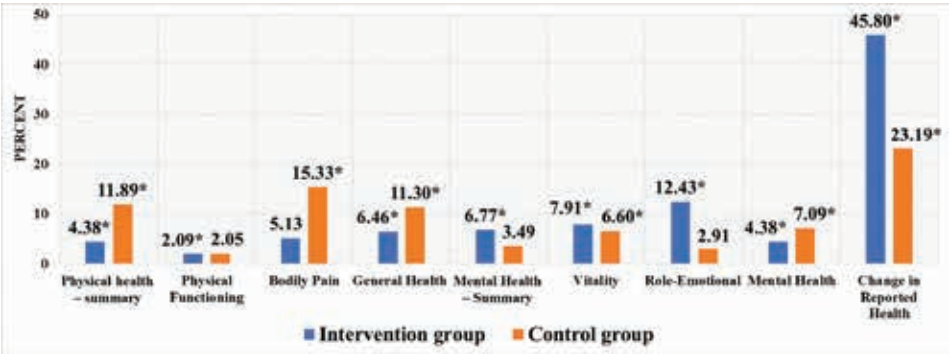

FIG. 15. Changes of quality of life scores after 2 months
Abbreviations and explanations: * – statistically significant difference (p<0.05).

Changes after 2 months of QOL are presented in Table 8.

TABLE 8. Changes of health-related quality of life after 2 months

Intervention group				p value between groups	Control group			
At baseline	After 2 months	Change	p value		p value	Change	At baseline	After 2 months
M ± SD	M ± SD	M ± SD				M ± SD	M ± SD	M ± SD
Physical health – summary					Physical health – summary			
73.57 ± 17.43	76.79 ± 17.43	3.22 ± 11.68	**0.020**	0.051	**<0.001**	8.76 ± 15.19	73.69 ± 15.29	82.45 ± 14.23
Physical Functioning					Physical Functioning			
81.58 ± 17.10	83.29 ± 17.10	1.71 ± 12.05	**0.010**	0.279	0.265	1.76 ± 8.12	85.99 ± 12.40	87.75 ± 9.40
Role-Physical					Role-Physical			
77.53 ± 33.87	80.91 ± 31.36	3.38 ± 28.54	0.373	0.364	0.051	15.24 ± 53.00	76.83 ± 34.64	92.07 ± 38.91
Bodily Pain					Bodily Pain			
76.79 ± 21.44	80.73 ± 27.08	3.94 ± 25.16	0.211	0.052	**0.004**	10.84 ± 21.01	70.73 ± 0.30	81.57 ± 18.36

Intervention group				p value between groups	Control group			
At baseline	After 2 months	Change	p value		p value	Change	At baseline	After 2 months
M ± SD	M ± SD	M ± SD				M ± SD	M ± SD	M ± SD
General Health					General Health			
58.45 ± 15.07	62.23 ± 16.06	3.78 ± 12.13	**0.006**	0.934	**0.014**	6.92 ± 22.76	61.22 ± 14.00	68.14 ± 22.01
Mental Health – Summary					Mental Health – Summary			
74.43 ± 15.21	79.47 ± 13.73	5.03 ± 12.03	**<0.001**	0.295	0.108	2.67 ± 11.03	76.41 ± 16.12	79.08 ± 13.81
Vitality					Vitality			
62.37 ± 18.21	67.31 ± 14.43	4.94 ± 14.27	**0.002**	0.932	**0.036**	4.25 ± 12.38	64.38 ± 15.57	68.63 ± 15.02
Social Functioning					Social Functioning			
84.97 ± 17.26	86.23 ± 16.09	1.27 ± 16.94	0.496	0.719	0.779	0.00 ± 13.11	86.89 ± 15.80	86.89 ± 15.80
Role-Emotional					Role-Emotional			
78.06 ± 31.53	87.76 ± 23.37	9.70 ± 29.31	**0.009**	0.138	0.724	2.44 ± 28.27	83.74 ± 31.73	86.18 ± 26.85
Mental Health					Mental Health			
72.67 ± 14.51	75.85 ± 13.31	3.18 ± 11.66	**0.009**	0.365	**0.011**	5.00 ± 11.77	70.50 ± 14.57	75.50 ± 14.86
Change in Reported Health					Change in Reported Health			
42.53 ± 19.89	62.01 ± 21.69	19.48 ± 25.20	**0.0001**	0.101	0.003	10.26 ± 16.93	44.23 ± 1 7.64	54.49 ± 19.76

Abbreviations and explanations: M ± SD – mean ± standard deviation.

In sum, we conclude that, after 2 months, the scores of QOL have improved in both intervention and control groups. However, in the intervention group, physical activity, limitation of activity due to emotional disorders and the generalized assessment of mental health scores improved more.

Motivation for physical training. In both groups, the dominant motives for being physically active were health, appearance, fitness, whereas the least frequently mentioned motives were social engagement, recreative and emotional (Fig. 16).

After 2 months, in both groups recreation and emotional motives increased and only in the intervention group social and physical fitness motives increased. Statistically significant improvement was also observed in the motive for stress management, recreation joy, challenge, social engagement, affiliation and competition scores, whereas social engagement and competition remained the weakest among the motivation for physical training factors. In the control group, appearance and weight management motives decreased the most. (Fig. 17 and Table 9).

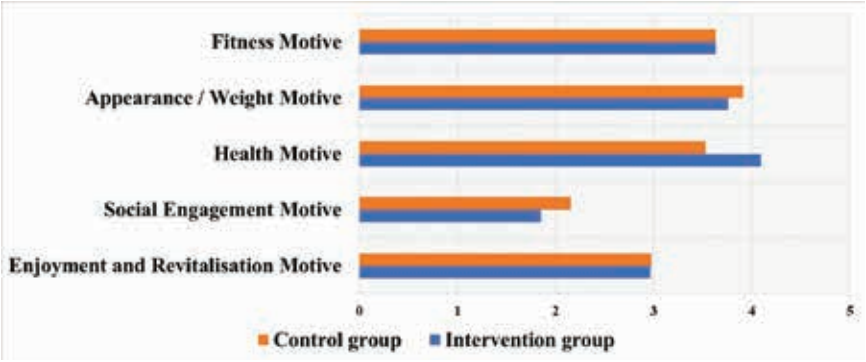

FIG. 16. Initial scores of motives for physical training

FIG. 17. Changes in scores of motives for physical training after 2 months
Abbreviations and explanations: * – statistically significant difference (p<0.05).

TABLE 9. Changes in scores of motives for physical training after 2 months

Intervention group				p value between the groups	Control group			
At baseline	After 2 months	Change	p value		p value	Change	At base-line	After 2 months
M ± SD	M ± SD	M ± SD				M ± SD	M ± SD	M ± SD
Enjoyment and Revitalisation Motive					Enjoyment and Revitalisation Motive			
2.97 ± 1.26	3.34 ± 1.12	0.37 ± 0.81	**<0.001**	**0.048**	**0.848**	0.03 ± 0.83	2.98 ± 1.13	3.01 ± 1.20
Stress Management					Stress Management			
2.83 ± 1.37	3.14 ± 1.22	0.32 ± 0.98	**0.002**	0.115	0.743	0.04 ± 0.82	3.01 ± 1.20	3.05 ± 1.25
Revitalisation					Revitalisation			
3.92 ± 1.11	4.26 ± 0.89	0.34 ± 0.85	**0.001**	0.215	0.254	0.08 ± 0.86	3.78 ± 0.94	3.86 ± 1.03

Intervention group				p value between the groups	Control group			
At baseline	After 2 months	Change	p value		p value	Change	At base-line	After 2 months
M ± SD	M ± SD	M ± SD				M ± SD	M ± SD	M ± SD
Enjoyment					Enjoyment			
2.85 ± 1.47	3.32 ± 1.25	0.47 ± 1.11	**0.001**	0.069	0.864	-0.03 ± 1.13	2.80 ± 1.35	2.77 ± 1.33
Challenge					Challenge			
2.36 ± 1.49	2.75 ± 1.43	0.38 ± 0.93	**0.001**	0.328	0.177	0.19 ± 0.87	2.49 ± 1.41	2.68 ± 1.46
Social Engagement Motive					Social Engagement Motive			
1.85 ± 1.47	2.22 ± 1.41	0.37 ± 0.94	**0.003**	0.146	0.568	0.08 ± 0.94	2.16 ± 1.33	2.24 ± 1.46
Social Recognition					Social Recognition			
1.80 ± 1.56	2.17 ± 1.50	0.37 ± 1.06	**0.004**	0.548	0.300	0.19 ± 0.87	2.18 ± 1.49	2.39 ± 1.41
Affiliation					Affiliation			
1.88 ± 1.56	2.30 ± 1.45	0.41 ± 1.19	0.006	0.060	0.756	-0.01 ± 1.03	2.26 ± 1.37	2.25 ± 1.51
Competition					Competition			
1.75 ± 1.48	2.09 ± 1.44	0.35 ± 1.01	0.004	0.228	0.359	0.12 ± 0.82	2.00 ± 1.50	2.12 ± 1.61
Health Motive					Health Motive			
4.09 ± 0.78	4.15 ± 0.81	0.06 ± 0.73	0.177	0.870	0.421	0.39 ± 1.53	3.53 ± 1.53	3.92 ± 0.91
Health Pressures					Health Pressures			
3.39 ± 1.31	3.43 ± 1.30	0.04 ± 1.20	0.566	0.537	**0.806**	-0.05 ± 1.09	3.40 ± 1.39	3.35 ± 1.29
Ill-Health Avoidance					Ill-Health Avoidance			
4.46 ± 0.78	4.47 ± 0.83	0.01 ± 0.95	0.581	0.928	0.443	0.40 ± 1.61	3.75 ± 1.57	4.15 ± 0.86
Positive Health					Positive Health			
4.41 ± 0.72	4.46 ± 0.76	0.05 ± 0.81	0.470	0.468	0.667	-0.05 ± 0.78	4.26 ± 0.84	4.21 ± 0.80
Appearance / Weight Motive					Appearance / Weight Motive			
3.76 ± 1.00	3.80 ± 1.15	0.04 ± 0.82	0.222	0.001	**0.002**	-0.93 ± 1.78	3.91 ± 1.05	2.98 ± 1.65
Weight Management					Weight Management			
4.27 ± 1.01	4.14 ± 1.15	-0.13 ± 0.88	0.564	0.088	**0.010**	-0.96 ± 1.92	4.23 ± 0.95	3.27 ± 1.76
Appearance					Appearance			
3.22 ± 1.30	3.39 ± 1.34	0.17 ± 1.05	0.251	0.077	0.185	-0.15 ± 0.93	3.43 ± 1.36	3.28 ± 1.20

Intervention group				p value between the groups	Control group			
At base-line	After 2 months	Change	p value		p value	Change	At baseline	After 2 months
M ± SD	M ± SD	M ± SD				M ± SD	M ± SD	M ± SD
Fitness Motive					Fitness Motive			
3.63 ± 1.13	3.89 ± 1.01	0.26 ± 0.94	**0.023**	0.162	0.986	-0.05 ± 0.84	3.63 ± 1.01	3.59 ± 1.07
Strength & Endurance					Strength & Endurance			
3.71 ± 1.22	4.05 ± 0.93	0.35 ± 0.99	0.006	**0.049**	0.558	-0.13 ± 0.98	3.75 ± 0.93	3.62 ± 1.06
Nimbleness					Nimbleness			
3.57 ± 1.24	3.65 ± 1.26	0.08 ± 1.10	0.435	0.540	0.539	0.07 ± 0.91	3.48 ± 1.29	3.55 ± 1.22

Abbreviations and explanations: M ± SD – mean ± standard deviation.

Optimal changes of cardiovascular risk factors in the intervention and control groups in 2 months. In order to assess the effect of the 2-month individually HR controlled supervised aPT programme, the ROC (*Receiver Operating Characteristic*) curve analysis was performed and the threshold values were estimated. Using the ROC curves we estimated the optimal threshold values of changes in waist circumference, HDL-Chol, c-f PWV, rest HR, VO2max, AT (in percent of VO2) and HR during AT. These threshold values separated the groups with the highest prognostic accuracy (the highest sensitivity and specificity). Then the odds ratio for the groups to achieve the optimal effect of the change of a risk factor were determined. *Waist circumference*. The optimal threshold value for the change of the waist circumference is 0 cm, (sensitivity 0.657 and specificity 0.541) (Fig. 18). The odds ratio for the intervention group to lower waist circumference more than 0 cm or equal after 2 months was 1.5 [1.02–2.20] (p=0.039) as compared to the control group.

HDL-Chol. The optimal threshold value for the change of the HDL-Chol was 0 cm, (sensitivity 0.772 and specificity 0.529) (Fig. 19). The odds ratio for the intervention group to increase HDL-Chol more than 0 cm or equal after 2 months was 1.71 [1.2–2.43] (p=0.003) as compared to the control group.

Carotid-femoral PWV. The optimal threshold value for the decreasing of c-f PWV was ≥ 1.4 m/s (sensitivity 0.786 and specificity 0.459) (Fig. 20). The odds ratio for the intervention group to lower c-f PWV for more than 1.4 m/s or equal after 2 months was 1.76 [1.08–2.87] (p = 0.023) as compared to the control group.

*HR at rest***.** The optimal threshold value of the decreasing of the HR value at rest was ≥ 3 b/min (sensitivity 0.672 and specificity 0.5) (Fig. 21). The odds ratio for the intervention group to lower HR at rest more than 3 b/min or equal after 2 months was 1.43 [1.0–2.05] (p = 0.048) as compared to the control group.

VO2max. The optimal threshold value of the increasing of VO2max was ≥ 0.3 ml/min/kg (sensitivity at this point was 0.691 and specificity – 0.619) (Fig. 22). The odds ratio for the intervention group to increase VO2max more than 0.3 ml/min/kg or equal after 2 months was 1.91 [1.3–2.78] (p=0.001) as compared to the control group.

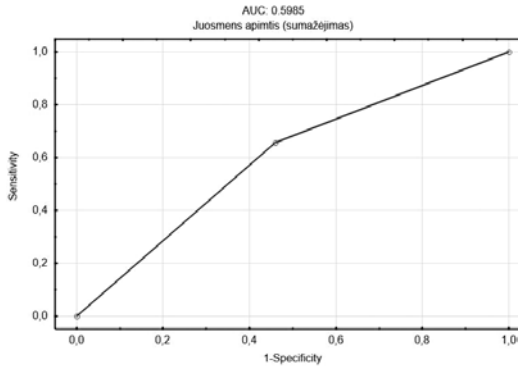

FIG. 18. ROC curve for the optimal threshold value 0 cm for the change of the waist circumference.

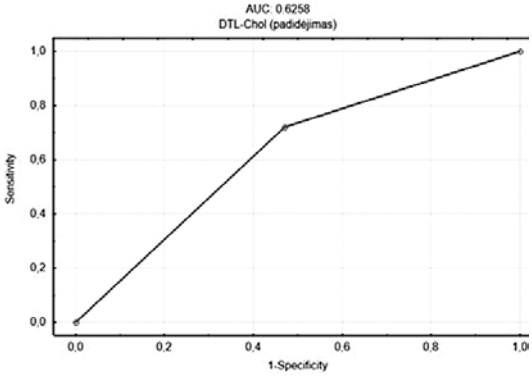

FIG. 19. ROC curve for the optimal threshold value 0 mmol/l for the change of the high density lipoprotein cholesterol

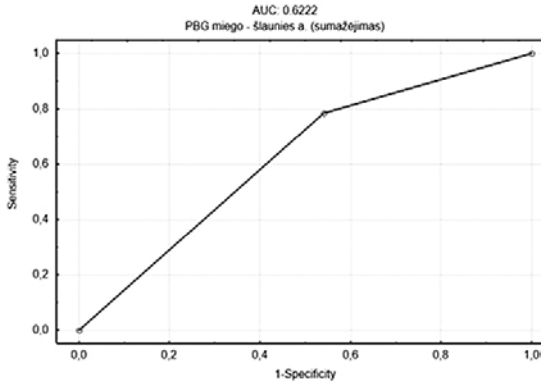

FIG. 20. ROC curve for the optimal threshold value 1.4 m/s for the decreasing of the carotid femoral pulse wave velocity

Anaerobic threshold (percent of VO2). The optimal threshold value of the increasing AT of predicted VO2 was ≥ 8 % (sensitivity 0.86 and specificity – 0.585) (Fig. 23). The odds ratio for the intervention group to increase AT more than or equal 8 % of predicted VO2 after 2 months was 2.94 [1.91–4.54] (p<0.001) as compared to the control group.

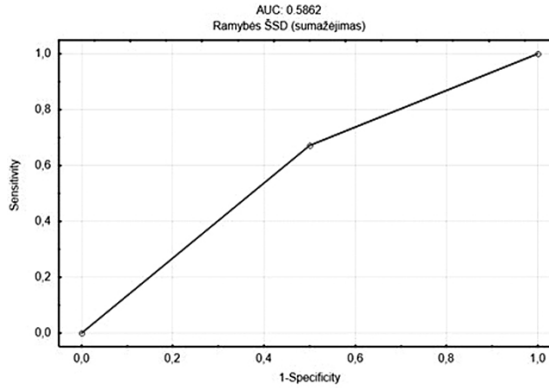

FIG. 21. ROC curve for the optimal threshold value 3 b/min for the decreasing of the heart rate at rest

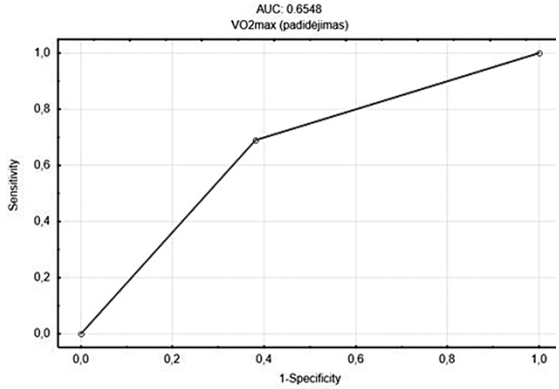

FIG. 22. ROC curve for the optimal threshold value 0.3 ml/min/kg for the increasing of the maximal consumption of oxygen

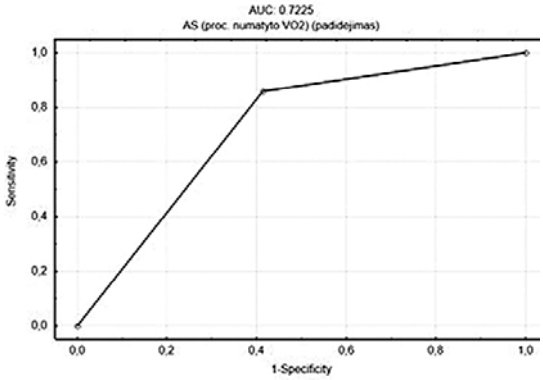

FIG. 23. ROC curve for the optimal threshold value 8 % for the increasing of the of anaerobic threshold (the percent of predicted oxygen consumption)
Abbreviations and explanations:
AUC – area under the curve.

Interdependence analysis of the observed changes in the intervention and control groups. After the estimation of the statistically significant changes in the CV risk factors, MetS components, scores of QOL and motivation for physical training after 2 months of physical training in the intervention group, we estimated their interdependence.(Fig 24 and 25).

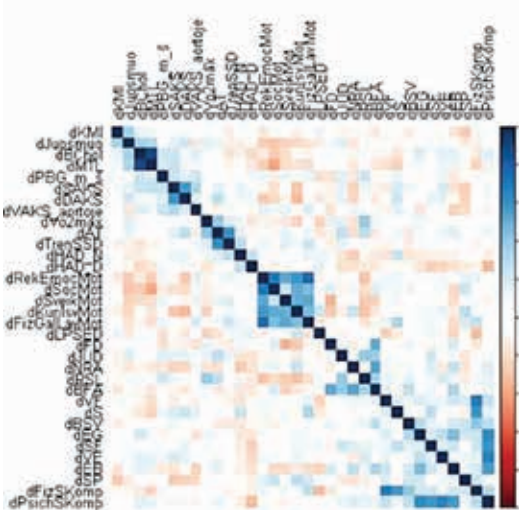

FIG. 24. Correlation between cardiovascular risk factors, components of metabolic syndrome, arterial wall functional and structural parameters, cardiorespiratory fitness, quality of life and motivation for physical training in the intervention group.

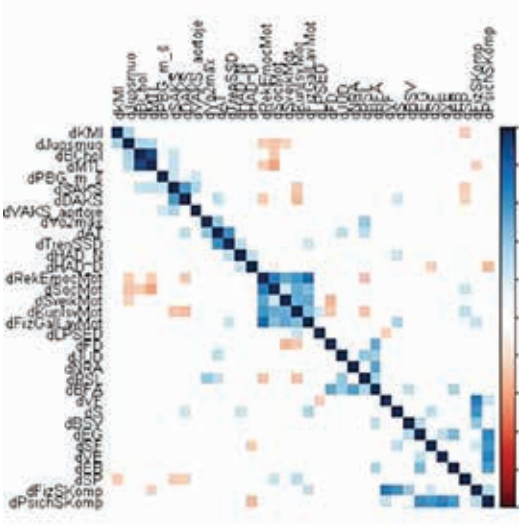

FIG. 25. Statistically significant correlation of cardiovascular risk factors, components of metabolic syndrome, arterial wall functional and structural parameters, cardiorespiratory fitness, quality of life and motivation for physical training in the intervention group.
Abbreviations and explanations: Correlation coefficient is presented in colours.

Statistically significant correlation between the CV risk factors, components of MetS, cardiorespiratory fitness and signs of physical activity after 2 months was estimated in the intervention group (Table 10).

TABLE 10. Statistically significant correlations between cardiovascular risk factors, components of MetS, cardiorespiratory fitness and signs of physical activity after 2 months in the intervention group

Parameters	r	p
Δ Waist circumference and Δ T-Chol	0.35	**0.001**
Δ Waist circumference and Δ LDL-Chol	0.31	**0.005**
Δ Body mass and Δ Waist circumference	0.27	**0.014**
Δ sBP and Δ T-Chol	0.24	**0.031**
Δ SBP and Δ LDL-Chol	0.26	**0.020**
Δ sBP ir Δ MBP in aorta	0.32	**0.005**
Δ Carotid-femoral PWV and Δ sBP	0.24	**0.033**
Δ Carotid-femoral PWV and Δ MBP in aorta	0.23	**0.042**
Δ VO2max and Δ Physical activity in leisure-time	0.39	**0.003**

Abbreviations and explanations: Δ – change, T-Chol – total cholesterol, LDL-Chol – low density lipoprotein cholesterol, sBP – systolic blood pressure, MBP – mean blood pressure, PWV – pulse wave velocity, VO2max – maximal oxygen consumption.

In the intervention group, the decrease of c-f PWV positively correlated with the decrease in BP parameters: sBP and MBP in the aorta. The correlation between the decrease of WC and the reduction of T-Chol and LDL-Chol was also observed. We also observed a significant correlation between the decrease of sBP and the reduction of T-Chol, LDL-Chol, MBP in the aorta, elevation of VO2 max and increased recreation, sports and leisure activity. In order to further investigate the dependence of the significant c–f PWV change on other parameters in the intervention group, we performed a univariate linear regression analysis of the change of c-f PWV. Regression models were constructed to investigate whether the reduction in c-f PWV in the intervention group is dependent on the change in sBP, MBP in the aorta, WC, T-Chol and LDL-Chol, increase of VOmax and increased recreation, sports and leisure activity. The univariate linear regression analysis demonstrated that the change of c-f PWV in the intervention group was significantly associated only with the decrease in sBP and MBP in the aorta (Table 11). However, the analy-

sis of multiple linear regression that included sBP and MBP in the aorta was not dependent on the influence of any of these parameters on c- f PWV after 2 months of aPT (Table 12).

TABLE 11. Univariate linear regression models for the change in carotid-femoral pulse wave velocity (as a dependent variable) in the intervention group

Independent variables	Unstandardized coefficients		95 % CI for B		Standardized coefficients		95 % CI for Beta		p	R^2	Adj. R^2
	B	SE	Lower limit	Upper limit	Beta	SE	Lower limit	Upper limit			
Δ sBP	0.024	0.011	0.002	0.047	0.242	0.111	0.020	0.463	**0.033**	0.058	0.046
Δ MBP in aorta	0.030	0.014	0.001	0.058	0.230	0.111	0.009	0.451	**0.042**	0.053	0.041
Δ Waist circumference	-0.020	0.041	-0.101	0.060	-0.056	0.113	-0.281	0.169	0.619	0.003	-0.010
Δ T-Chol	0.173	0.104	-0.033	0.379	0.184	0.111	-0.036	0.404	0.099	0.034	0.022
Δ LDL-Chol	0.257	0.130	-0.003	0.517	0.218	0.111	-0.002	0.438	0.052	0.047	0.035
Δ Vo2max	0.020	0.051	-0.082	0.122	0.044	0.113	-0.181	0.269	0.698	0.002	-0.011
Δ PALT	0.000	0.000	0.000	0.000	0.209	0.133	-0.058	0.476	0.122	0.044	0.026

Abbreviations and explanations: Δ – change, CI – confidence interval, sBP – systolic blood pressure, MBP – mean blood pressure, T-Chol – total cholesterol, LDL-Chol – low density lipoprotein cholesterol, PALT – physical activity in leisure time, R^2 – coefficient of determination, Adj. R^2 – adjusted coefficient of determination; B – unstandardized regression coefficient, Beta – standardized regression coefficient, SE – standard error.

TABLE 12. Multiple regression model for change of carotid-femoral PWV (as a dependent variable) in the intervention group.

Model	Unstandardized coefficients		95 % CI for B		Standardized coefficients		95 % CI for Beta		p	R^2	Adj. R^2
	B	SE	Lower limit	Upper limit	Beta	SE	Lower limit	Upper limit			
Intercept	-0.400	0.161	-0.720	-0.080					**0.015**	0.090	0.065
Δ sBP	0.022	0.012	-0.002	0.046	0.216	0.118	-0.019	0.451	0.071		
Δ MBP in aorta	0.019	0.015	-0.011	0.049	0.150	0.118	-0.085	0.385	0.207		

Abbreviations and explanations: Δ – change, CI – confidence interval, sBP – systolic blood pressure, MBP – mean blood pressure, R^2 - coefficient of determination, Adj. R^2 – adjusted coefficient of determination; B – unstandardized regression coefficient, Beta – standardized regression coefficient, SE – standard error.

We performed the linear regression analysis of the c-f PWV change as a dependent variable and the study group as an independent variable adjusted for age and gender and determined the association between the reduction of c-f PWV and the intervention group (Table 13).

TABLE 13. Linear regression analysis for change of carotid-femoral PWV as a dependent variable and the study group as an independent variable adjusted for age and gender

Model	Unstandardized coefficients		95 % CI for B		Standardized coefficients		95 % CI for Beta		p	R^2	Adj. R^2
	B	SE	Lower limit	Upper limit	Beta	SE	Lower limit	Upper limit			
Intercept	0.744	1.393	-2.012	3.499					0.594	0.038	0.016
Intervention group	-0.223	0.111	-0.443	-0.004	-0.173	0.086	-0.343	-0.003	**0.046**		
Male gender	-0.065	0.179	-0.420	0.290	-0.051	0.140	-0.328	0.226	0.717		
Age	-0.020	0.026	-0.072	0.032	-0.106	0.141	-0.384	0.172	0.453		

Abbreviations and explanations: CI – confidence interval, R^2 – coefficient of determination, Adj. R^2 – adjusted coefficient of determination; B – unstandardized regression coefficient, Beta – standardized regression coefficient, SE – standard error.

Interrelationships of the change of carotid-femoral pulse wave velocity with baseline carotid femoral pulse wave velocity. Comparing the dynamics of arterial stiffness among the study subjects divided into groups according to the quartiles of the baseline c-f PWV, we found that, in the intervention group, c-f PWV change after 2 months of aPT was dependant on the initial magnitude of c-f PWV: the stiffer aorta was at the inclusion, the greater reduction of c- f PWV was achieved.. These differences remained statistically significant even after the correction for the sBP change . The reduction of c-f PWV after 2 months of aPT occurred when c-f PWV at baseline was in the third quartile (≥ 8.2 m/s) (Table 14).

TABLE 14. The dependence of change in carotid-femoral pulse wave velocity on the magnitude of the baseline values.

Quartiles	Baseline carotid-femoral PWV (m/s)	Change of carotid-femoral PWV (m/s) M ± SD	p value	p value (adjusted for the change in sBP)
I	<7.4	0.73 ± 0.95	<0.001	<0.001
II	7.4–8.19	0.005 ± 0.98		
III	8.2–9.09	-0.61 ± 0.92		
IV	>=9.1	-1.75 ± 1.03		

Abbreviations and explanations: M ± SD – mean ± standard deviation, PWV – pulse wave velocity, sBP – systolic blood pressure

The same dynamic was found in correlation analysis: we observed a statistically significant negative correlation between c-f PWV after 2 months of aPT and baseline c-f PWV (Fig. 26): the higher was the baseline c-f PWV, the lower c-f PWV after the intervention– correlation coefficient (r) equals 0.656 (a medium strength relationship, *p<0.001*).

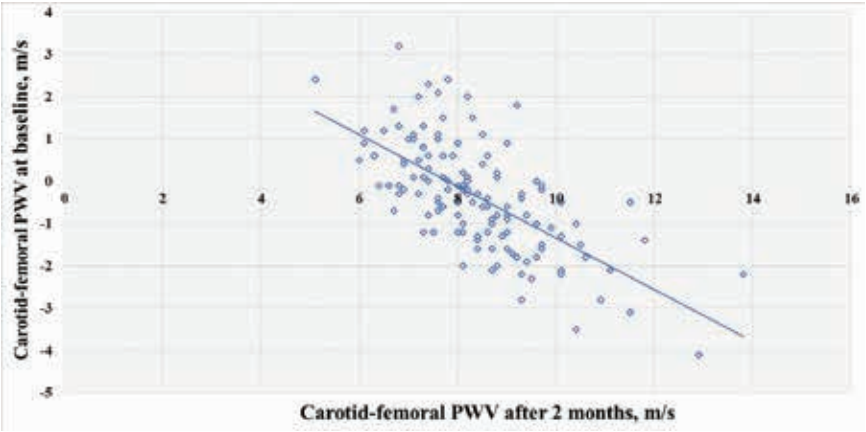

FIG. 26. Scatter plot for the interdependence between carotid-femoral pulse wave velocity at baseline and after 2 months. Abbreviations and explanations: PWV – pulse wave velocity.

Estimation of the threshold carotid-femoral pulse wave velocity value at baseline. *ROC curve analysis*. In order to estimate the value of c-f PWV at baseline with the highest prognostic accuracy (the highest sensitivity and specificity) that enables to predicted the improvement of c-f PWV after 2 months of aPT, we conducted the ROC curve analysis. The threshold value of baseline c-f PWV was found to be 8.1 m/s, with the prediction sensitivity 0.778 and specificity 0.704, and the area under the curve 0.8175 (see Fig. 27).

Regression Tree method. For a more accurate estimation of the relationship between c-f PWV at baseline and its change after 2-month of aPT, we used Regression Tree method. The application of it allowed to construct the decision tree that enabled to assess which threshold values of baseline c-f PWV might be used to predict lowering or further growing of c-f PWV after 2 month of aPT (Fig. 28). We found that the highest improvement after training was achieved when c-f PWV was > 10.1 m/s (-2.31 ± 1.15 m/s, M ± SD), moderate – in the interval 8.7–10.1 m/s (-1.22 ± 0.86 m/s). No changes were observed in the interval 7.4–8.6 m/s (-0.10 ± 0.94 m/s), and a minor worsening in the interval ≤ 7.3 m/s (0.73 ± 0.95 m/s).

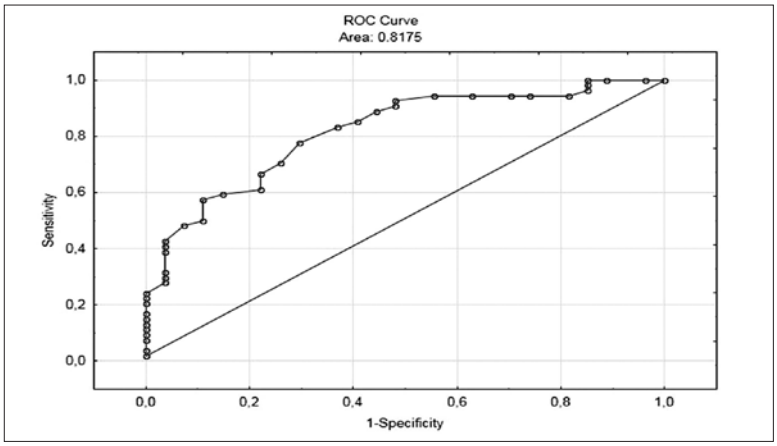

FIG. 27. ROC curve for estimating the threshold value of baseline carotid-femoral pulse wave velocity predictive of the interventation-related arterial stiffness reduction.

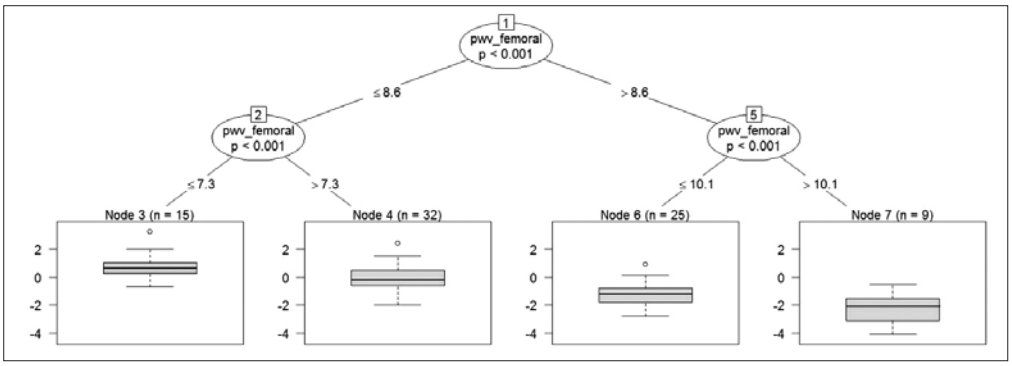

FIG. 28. Regression tree of the change of carotid-femoral pulse wave velocity
Abbreviations and explanations: c-f PWV – carotid-femoral pulse wave velocity.

Long-term assessment of the 2-month duration aerobic training programme after 8 months.

In order to assess what had happened with the CV risk factors, cardiorespiratory fitness, anxiety and depression, QOL in the long-term period, after 8 months, all subjects who arrived for a control visit were reassessed. Out of 140 subjects who participated in the investigation after 2 months, this third visit was missed by 35 (25 %) subjects: 11 (13.1 %) in the intervention group and 24 (42.9 %) in the control group. The data of these subjects were not included into the 8-month analysis. During the third visit, 105 subjects were investigated,:73 (68 %) in the intervention group and 32 (22 %) in the control group.

Cardiovascular risk factors, metabolic syndrome components after 8 months. After 8 months, the changes in BP from baseline were assessed in both groups, and the results were compared with those obtained at baseline. The analysis of the results in the intervention group after 8 months as well as after 2 months revealed the same tendencies of the BP change: sBP decreased in average by 4.46 mm Hg (3.35 %, p=0.003) (after 2 months the decrease was 4.09 mm Hg, 3.08 %), dBP decreased in average by 4.46 mm Hg (3.35 %, p=0.003) (after 2 months it decreased by 4.09 mm Hg, 3.08 %). In the control group, neither BP change after 2 months nor after 8 months was statistically significant (Fig. 29).

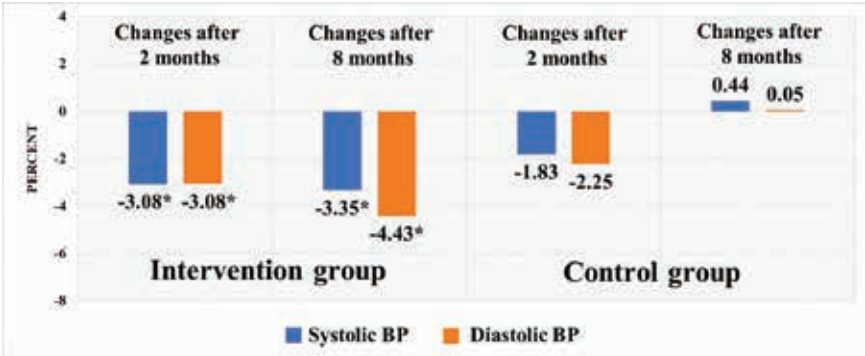

FIG. 29. Blood pressure changes after 2 and 8 months
Abbreviations and explanations: BP – blood pressure; * – statistically significant difference (p<0.05).

In the intervention group, the overall incidence of AH after 8 months statistically significantly decreased (10.47 %, p=0.021), and the severity of hypertension has improved.intervention:Namely, the number of subjects with grade I and II hypertension has decreased, whereas the number of subjects with pre-hypertension and normal BP increased. Likewise, after 8 months there were less subjects with isolated systolic AH. The incidence of AH and the distribution of the subjects according to the hypertension grade did not change in the control group after 8 months (Table 15).

TABLE 15. Changes of the degree of BP and the incidence of AH after 8 months

Parameter	Intervention group				Control group			
	First visit	Third visit	Difference	p	First visit	Third visit	Difference	p
Systolic BP (mm Hg), M ± SD	132.98 ± 13.26	128.52 ± 11.03	-4.46 ± 11.58	**0.003**	135.23 ± 14.28	135.82 ± 16.33	0.59 ± 11.65	0.814

Parameter	Intervention group				Control group			
	First visit	Third visit	Difference	p	First visit	Third visit	Difference	p
Diastolic BP (mm Hg), M ± SD	81.94 ± 9.55	78.31 ± 7.81	-3.63 ± 7.67	**<0.001**	84.14 ± 10.04	84.18 ± 11.25	0.05 ± 7.67	0.978
Arterial hypertension (%)	94.05	83.58	-10.47	**0.021**	87.50	95.45	7.95	1.000
Degrees of BP:				**0.014**				0.558
- optimal BP (%)	13.25	19.70	6.44		19.64	13.64	-6.01	
- normal BP (%)	21.69	27.27	5.59		17.86	18.18	0.32	
- high-normal BP (%)	30.12	36.36	6.24		17.86	22.73	4.87	
- I° elevation of BP (%)	19.28	7.58	-11.70		14.29	27.27	12.99	
- II° elevation BP (%)	4.82	1.52	-3.30		8.93	9.09	0.16	
- Isolated systolic hypertension (%)	10.84	7.58	-3.27		21.43	9.09	-12.34	

Abbreviations and explanations: BP – blood pressure, M ± SD – mean ± standard deviation.

After 8 months, as well as after 2 months, T-Chol (p<0.001) and LDL-Chol statistically significantly decreased in the intervention group. T-Chol decreased in average by 0.82 mmol/l (13 %) while after 2 months it decreased by 0.52 mmol/l (8 %). LDL-Chol after 8 months decreased in average by 0.66 mmol/l (15 %), while after 2 months by 0.39 mmol/l (15 %). Statistically significant decrease in TG – in average by 0.42 mmol/l (17 %, p=0.027) – was found in the intervention group

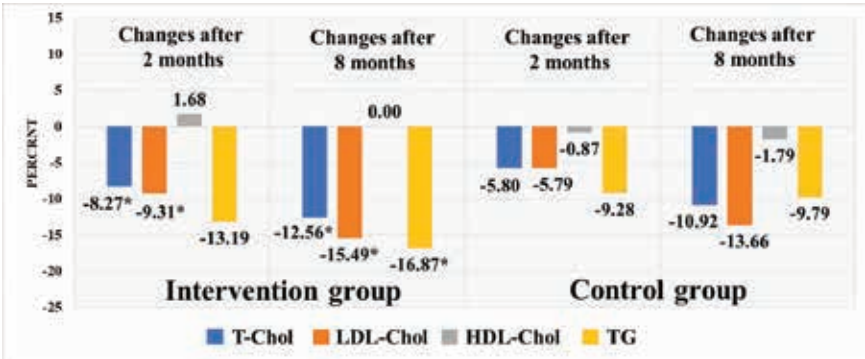

FIG. 30. Changes in the lipid parameters after 2 and 8 months
Abbreviations and explanations: T-Chol – total cholesterol, MTL-Chol – low density lipoprotein cholesterol, HDL-Chol – high density lipoprotein cholesterol, TG – triglycerides, * – statistically significant difference (p<0.05).

after 8 months. In the intervention group, this parameter did not reach statistical significance after 2 monthsintervention. In the control group there was no significant change in TG both after 8 and 2 months. There were no statistically significant changes in HDL-Chol both in the intervention and control groups after 8 months as well as after 2 months (Fig. 30).

Although the values of lipid parameters changed in the intervention group, the incidence of dyslipidaemia did not change neither after 8 months nor 2 months (Table 16).

TABLE 16. Changes of the lipid parameters after 8 months

Parameter	Intervention group				Control group			
	First visit	Third visit	Difference	p	First visit	Third visit	Difference	p
T-Chol (mmol/l), M ± SD	6.53 ± 1.43	5.71 ± 1.25	-0.82 ± 1.45	**<0.001**	5.68 ± 1.33	5.06 ± 1.11	-0.62 ± 1.76	0.123
LDL-Chol (mmol/l), M ± SD	4.26 ± 1.17	3.60 ± 1.18	-0.66 ± 1.09	**<0.001**	3.66 ± 1.12	3.16 ± 0.99	-0.50 ± 1.54	0.140
HDL-Chol (mmol/l), M ± SD	1.19 ± 0.31	1.19 ± 0.31	0.01 ± 0.15	0.939	1.12 ± 0.29	1.10 ± 0.23	-0.03 ± 0.17	0.441
TG (mmol/l), V ± SN	2.49 ± 3.10	2.07 ± 1.59	-0.42 ± 3.08	**0.027**	1.94 ± 0.63	1.75 ± 0.84	-0.19 ± 0.84	0.140
Dyslipidaemia (%)	100.0	93.2	-6.8	-	94.6	100.0	5.4	-

Abbreviations and explanations: M ± SD – mean ± standard deviation, T-Chol – total cholesterol, MTL-Chol – low density lipoprotein cholesterol, HDL-Chol – high density lipoprotein cholesterol, TG – triglycerides.

Both after 8 and 2 months, the decrease of BMI in the intervention group remained statistically significant – on average, by 0.53 kg/m^2 (1.69 %, p<0.001). After 2 months it was 0.37 kg/m^2 (1.2 %). In the control group, the reduction in BMI did not reach statistical significance after 8 months, and was only 0.14 kg/m^2 (0.41 %), although after 2 months it was 0.34 kg/m^2 (1.1 %, p=0.011). After 8 months, WC remained statistically significantly reduced, on average, by 1.07 cm (1.06 %, p=0.038), whereas after 2 months by 1.34 cm (1.31 %). In the control group, after 8 months this parameter even increased, on average, by 3.05 cm, p=0.002). After 2 months, it has also increased, on average, by 0.2 cm, however the increase wasnot statistically significant (Fig. 31).

The distribution of subjects according to the degree of obesity improved statistically significantly in the intervention group – the number of the subjects with BMI < 25 kg/m^2 increased up to 6.94 %, thus the number of overweight and obesity

FIG. 31. Changes of waist circumference and body mass index after 2 and 8 months
Abbreviations and explanations: BMI – body mass index, * – statistically significant difference (p<0.05).

containing subjects decreased (p=0.029). There was no such change in the control group neither after 2 nor 8 months. The changes of waist circumference, BMI, the incidence of abdominal obesity and obesity degree after 8 months are presented in Table 17.

Table 17. Changes of waist circumference, body mass index, the incidence of abdominal obesity and obesity degree after 8 months

Parameter	Intervention group				Control group			
	First visit	Third visit	Differ-enc e	p	First visit	Third visit	Differ-enc e	p
Waist circumference (cm), M ± SD	102.09 ± 9.51	101.01 ± 9.22	-1.07 ± 4.16	**0.038**	106.38 ± 10.26	109.43 ± 12.13	3.05 ± 3.83	0.002
Abdominal obesity (%)	81.9	79.1	-2.8	0.289	80.4	90.5	10.1	1.000
Body mass index (kg/m^2), M ± SD	30.81 ± 4.06	30.29 ± 4.03	-0.53 ± 1.10	**<0.001**	31.41 ± 3.75	31.28 ± 3.79	-0.14 ± 0.89	0.469
Obesity:				**0.029**				0.317
no (%)	4.82	11.76	6.94		1.79	4.55	2.76	
overweight (%)	38.55	35.29	-3.26		35.71	27.27	-8.44	
I degree (%)	40.96	39.71	-1.25		48.21	45.45	-2.76	
II degree (%)	14.46	13.24	-1.22		14.29	22.73	8.44	
III degree (%)	1.20	0.00	-1.2		0.00	0.00	0	

Abbreviations and explanations: M ± SD – mean ± standard deviation

Changes of the MetS components were assessed 8 months after the evaluation at baseline. It was ascertained that in the intervention group after 8 months 3 of 5 MetS components improved statistically significantly. After 2 months, the improvement of only 2 components was observed in the intervention group. The decrease of WC, BP and TG was estimated after 8 months. WC statistically significantly decreased after 8 months as well as 2 months, respectively by1.07 cm (1.06 %, p=0.038) and 1.34 cm (1.31 %). In the control group, WC even increased by 3.05 cm (2.87 %, p=0.002) after 8 months, whereas its increase by 0.2 cm after 2 months was not sig-

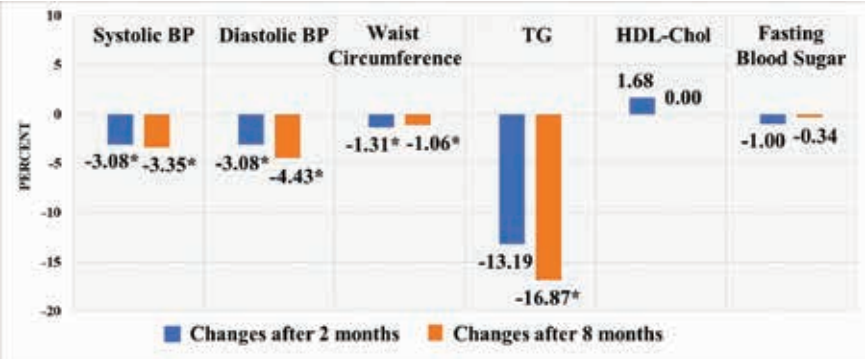

FIG. 32. Changes in the metabolic syndrome components after 2 and 8 months in the intervention group
Abbreviations and explanations: BP – blood pressure, TG – triglycerides, HDL-Chol – high density lipoprotein cholesterol, * – statistically significant difference (p<0.05).

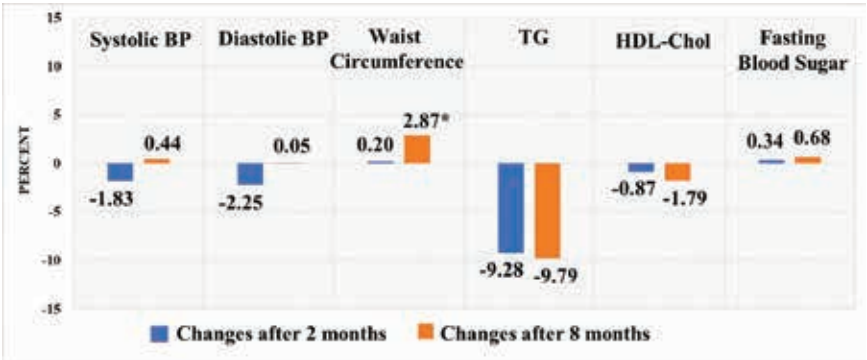

FIG. 33. Changes in the metabolic syndrome components after 2 and 8 months in the control group
Abbreviations and explanations: BP – blood pressure, TG – triglycerides, HDL-Chol – high density lipoprotein cholesterol, * – statistically significant difference (p<0.05).

nificant. Systolic BP in the intervention group after 8 months decreased, on average, by 4.46 mm Hg (3.35 %, p=0.003), after 2 months it also decreased, on average, by 4.09 mm Hg (3.08 %). Diastolic BP in the intervention group after 8 months decreased, on average, by 3.63 mm Hg (4.43 %, p<0,001). After 2 months it was also significantly decreased, on average, by 2.51 mm Hg (3.08 %). TG in the intervention group after 8 months statistically significantly decreased, on average, by 0.42 mmol/l (16.87 %, p=0.027), while after 2 months the decrease was not significant. There were no statistically significant changes in the MetS components in the control group, with the exception of the increased waist circumference (Fig. 32 and 33).

After 8 months, the number of MetS components in the intervention group statistically significantly decreased:– from 3.75 components at baseline to 3.29 (decrease by 0.46, p<0.001). There were no significant changes in the number of MetS components in the control group after 8 months, while after 2 months the decrease was observed in both groups (Fig. 34). After 8 months, 25.3 % of the subjects in the intervention group and 14.3 % of subjects in the control group had less than 3 out of 5 MetS components.

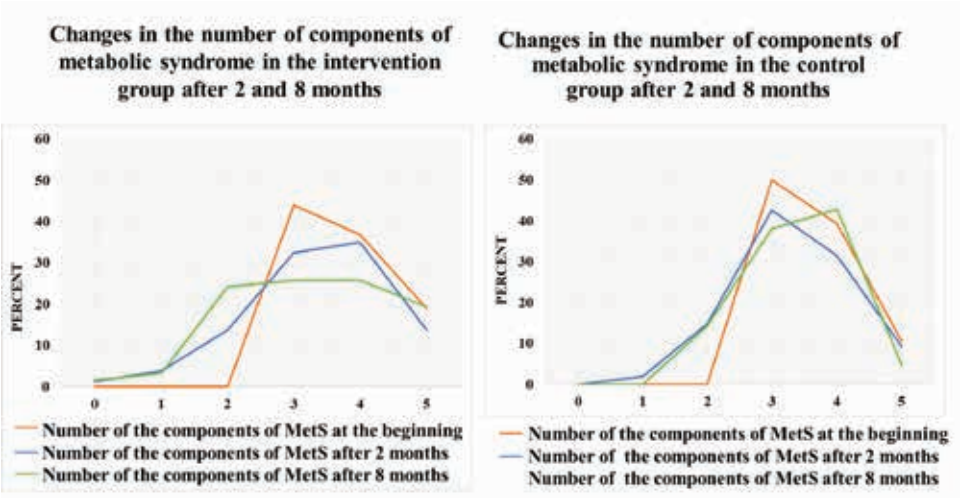

FIG. 34. Changes in the number of metabolic syndrome components after 2 and 8 months

After 2 months, p=0.001 After 2 months, p=0.016
After 8 months, p<0.001 After 8 months, p=0.109
Abbreviations and explanations: MetS – metabolic syndrome.

Changes in the MetS components after 8 months are presented in Table 18.

TABLE 18. Changes in theMetS components after 8 months

Parameter	Intervention group				Control group			
	First visit	Third visit	Difference	p	First visit	Third visit	Difference	p
sBP (mm Hg), M ± SD	132.98 ± 13.26	128.52 ± 11.03	-4.46 ± 11.58	**0.003**	135.23 ± 14.28	135.82 ± 16.33	0.59 ± 11.65	0.814
dBP (mm Hg), M ± SD	81.94 ± 9.55	78.31 ± 7.81	-3.63 ± 7.67	**<0.001**	84.14 ± 10.04	84.18 ± 11.25	0.05 ± 7.67	0.978
Waist circumference (cm), M ± SD	102.09 ± 9.51	101.01 ± 9.22	-1.07 ± 4.16	**0.038**	106.38 ± 10.26	109.43 ± 12.13	3.05 ± 3.83	0.002
TG (mmol/l), M ± SD	2.49 ± 3.10	2.07 ± 1.59	-0.42 ± 3.08	**0.027**	1.94 ± 0.63	1.75 ± 0.84	-0.19 ± 0.84	0.140
HDL-Chol (mmol/l), M ± SD	1.19 ± 0.31	1.19 ± 0.31	0.01 ± 0.15	0.939	1.12 ± 0.29	1.10 ± 0.23	-0.03 ± 0.17	0.441
Fasting glucose in plasma (mmol/l), M ± SD	5.97 ± 0.86	5.95 ± 0.97	-0.02 ± 0.55	0.736	5.86 ± 0.60	5.90 ± 0.54	0.04 ± 0.44	0.654
Number of MetS components:				**<0.001**				0.109
0 (%)	0.0	1.6	1.6		0.0	0.0	0	
1 (%)	0.0	3.2	3.2		0.0	0.0	0	
2 (%)	0.0	24.2	24.2		0.0	14.3	14.3	
3 (%)	44.0	25.8	-18.2		50.0	38.1	-11.9	
4 (%)	36.9	25.8	-11.1		39.3	42.9	3.6	
5 (%)	19.0	19.4	0.4		10.7	4.8	-5.9	

Abbreviations and explanations: M ± SD – mean ± standard deviation, sBP – systolic blood pressure, dBP – diastolic blood pressure, TG – triglycerides, HDL-Chol – high density lipoprotein cholesterol, MetS – metabolic syndrome.

Functional and structural parameters of the arterial wall after 8 months. After 8 months, as well as after 2 months, the decrease of c-f PWV, on average, by 0.74 m/s (8.6 %, p<0.001) remained statistically significant in the intervention group. After 2 months, the reduction was 0.54 m/s (6.33 %) (Fig. 35).

After 8 months, MBP in the aorta was decreased, on average, by 8.44 mm Hg (7.99 %, p=0.001) (after 2 months the decrease was by 3.73 mm Hg, 3.58 %) in the intervention group. We did not observe such changes in the control group neither after 2 nor after 8 months (Fig. 36).

The analysis according to the categories of c-f PWV in the intervention group demonstrated that, from the first to the third visit, the number of subjects with high-

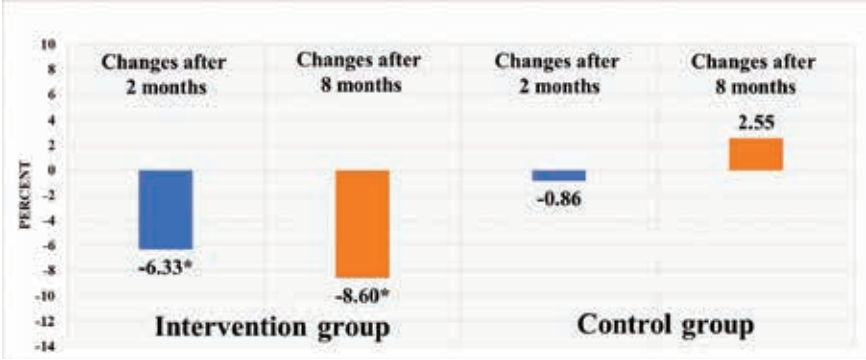

FIG. 35. Change of carotid-femoral pulse wave velocity after 2 and 8 months
Abbreviations and explanations: * – statistically significant difference (p<0.05).

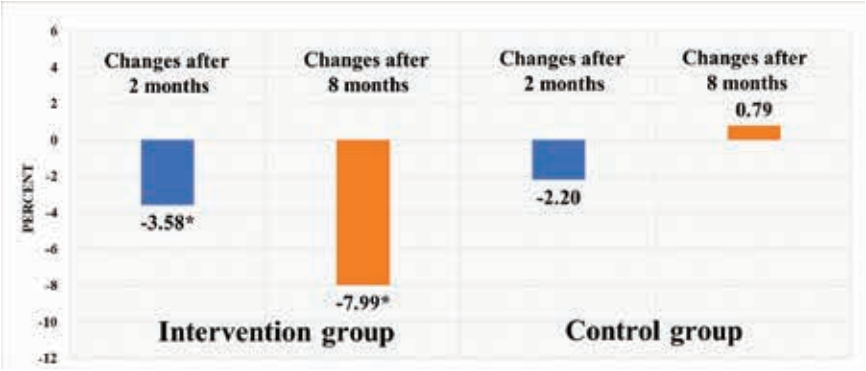

FIG. 36. Change of mean blood pressure in the aorta after 2 and 8 months
Abbreviations and explanations: * – statistically significant difference (p<0.05).

er c-f PVW values (>8 m/s) statistically significantly decreased on the account of the number of subjects with lower c-f PVW values increasing. We did not observe the changes of c-f PWV in the control group. The changes in the functional and structural parameters of the arterial wall after 8 months are presented in Table 19.

TABLE 19. Changes in the functional and structural parameters of the arterial wall after 8 months

Parameters	Intervention group				Control group			
	First visit	Third visit	Difference	p	First visit	Third visit	Difference	p
c-r PWV (m/s), M ± SD	9.13 ± 1.20	10.67 ± 13.67	1.55 ± 13.74	0.220	8.96 ± 0.85	13.57 ± 17.68	4.61 ± 17.75	0.062

Parameters	Intervention group				Control group			
	First visit	Third visit	Difference	p	First visit	Third visit	Difference	p
c-f PWV (m/s), M ± SD	8.60 ± 1.30	7.86 ± 1.42	-0.74 ± 1.42	**<0.001**	8.25 ± 0.83	8.46 ± 1.65	0.20 ± 1.37	0.490
c-f PWV:				**<0.001**				0.67
• -≥10 m/s (%)	14.46	6.15	-8.30		7.14	13.64	6.49	
• 9-9.9 m/s (%)	20.48	12.31	-8.17		16.07	18.18	2.11	
• 8-8.9 m/s (%)	32.53	24.62	-7.91		28.57	27.27	-1.30	
• 7-7.9 m/s (%)	20.48	27.69	7.21		32.14	31.82	-0.32	
• <7 m/s (%)	12.05	29.23	17.18		16.07	9.09	-6.98	
MBP in aorta (mmHg), M ± SD	105.67 ± 8.93	97.23 ± 15.62	-8.44 ± 15.06	**<0.001**	104.32 ± 11.67	105.14 ± 18.21	0.82 ± 19.30	0.844
CCA IMT (μm), M ± SD	653.65 ± 109.24	658.38 ± 99.20	4.73 ± 75.72	0.614	646.84 ± 97.23	628.55 ± 87.47	-18.30 ± 74.28	0.261
CCA stiffness, M ± SD	4.41 ± 1.73	4.42 ± 1.38	0.01 ± 1.30	0.835	4.23 ± 1.66	4.47 ± 1.97	0.24 ± 1.45	0.385

Abbreviations and explanations: M ± SD – mean ± standard deviation, c-r PWV – carotid–radial pulse wave velocity, c-f PWV – carotid–femoral pulse wave velocity, MBP – mean blood pressure, CCA – common carotid artery, IMT – intima-media thickness.

Anxiety and depression. There were no statistically significant changes in the scores of anxiety and depression after 8 months, as well as after 2 months.

Physical activity. Positive changes of recreation, activity in sports and leisure, particularly performing moderate and vigorous physical activity during leisure time remained statistically significant in the intervention group after 8 months, as well as it was after 2 months. Moderate physical activity during leisure time increased, on average, by 217.24 METmin/week (p=0.046), while after 2 months the increase was by 77.93 METmin/week. Vigorous physical activity during leisure time increased, on average, by 696.55 METmin/week (p=0.001), while after 2 months it was 385.52 METmin/week. In the control group, we observed the increased walking activity, on average, by 1298.79 METmin/week (p=0.048) (Fig. 37 and Table 20).

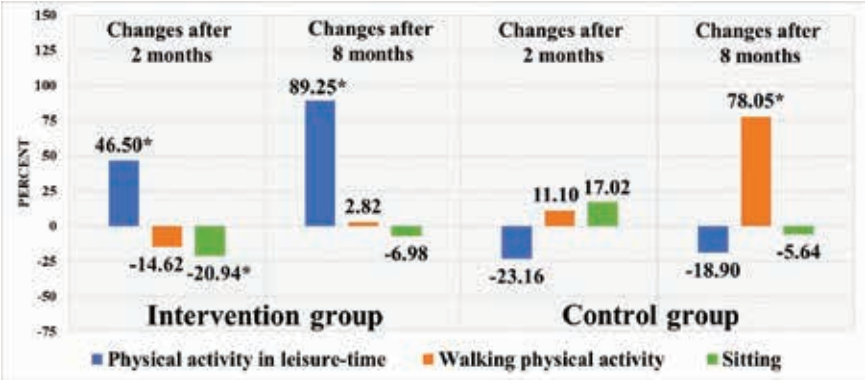

FIG. 37. Changes in physical activity according to the domains after 2 and 8 months
Abbreviations and explanations: * – statistically significant difference (p<0.05).

TABLE 20. Changes in physical activity according to the domains and the character after 8 months

Intervention group				p value between groups	Control group			
At baseline	After 8 months	Change	p value		p value	Change	At baseline	After 8 months
M ± SD	M ± SD	M ± SD				M ± SD	M ± SD	M ± SD
Walking at work (MET-minutes/week)					Walking at work (MET-minutes/week)			
457.45 ± 1358.50	862.55 ± 1537.49	405.10 ± 1867.59	0.102	0.174	**0.021**	1072.50 ± 1803.53	218.04 ± 531.33	1290.54 ± 1698.21
Walk (MET-minutes/week)					Walk (MET-minutes/week)			
984.31 ± 1066.15	718.03 ± 909.26	-266.28 ± 1411.01	0.611	0.066	**0.041**	221.57 ± 967.85	894.54 ± 1462.55	1116.11 ± 809.87
Physical activity in leisure time (MET-minutes/week)					Physical activity in leisure time (MET-minutes/week)			
932.71 ± 1281.64	1765.14 ± 1790.25	832.43 ± 1821.85	**0.005**	**0.045**	0.594	-318.14 ± 1105.28	1683.00 ± 2152.58	1364.86 ± 2003.34
Moderate physical activity in leisure (MET-minutes/week)					Moderate physical activity in leisure (MET-minutes/week)			
195.17 ± 480.88	412.41 ± 557.76	217.24 ± 779.48	**0.046**	0.105	0.834	-111.43 ± 561.79	205.71 ± 522.92	94.29 ± 164.26
Vigorous physical activity in leisure (MET-minutes/week)					Vigorous physical activity in leisure (MET-minutes/week)			
140.69 ± 358.83	837.24 ± 1172.83	696.55 ± 978.02	**0.001**	**0.007**	0.225	-211.43 ± 574.84	925.71 ± 1933.47	714.29 ± 1915.50

Intervention group				p value between groups	Control group			
At baseline	After 8 months	Change	p value		p value	Change	At baseline	After 8 months
M ± SD	M ± SD	M ± SD				M ± SD	M ± SD	M ± SD
Walking physical activity (MET-minutes/week)					Walking physical activity (MET-minutes/week)			
2038.60 ± 1934.70	2096.07 ± 2286.47	57.47 ± 2561.25	0.991	0.144	**0.048**	1298.79 ± 2564.39	1664.14 ± 2206.43	2962.93 ± 2012.70
Sitting (minutes/week)					**Sitting** (minutes/week)			
1713.08 ± 821.42	1593.46 ± 914.02	-119.62 ± 734.33	0.315	0.873	0.445	-102.35 ± 539.07	1814.12 ± 754.05	1711.77 ± 662.08

Abbreviations and explanations: M ± SD – mean ± standard deviation, MET – metabolic equivalent of task.

Cardiorespiratory fitness parameters after 8 months. Statistically significant improvement in VO2max (ml/kg/min) and the rest HR parameters persisted after 8 months in the intervention group. VO2max in the intervention group increased, on average, by 1.62 ml/kg/min (7.38 %, p<0.001), while after 2 months it increased by 2.22 ml/kg/min, 9.96 %. After 8 months, HR at rest decreased, on average, by 3.10 b/min (4.85 %, p<0.001), while after 2 months the decrease was 2.78 b/min (4.37 %). In the control group, there was no such improvements after 8 months, although after 2 months there was an improvement of VO2max (Fig. 38).

Changes of the CRF parameters after 8 months are presented in Table 21.

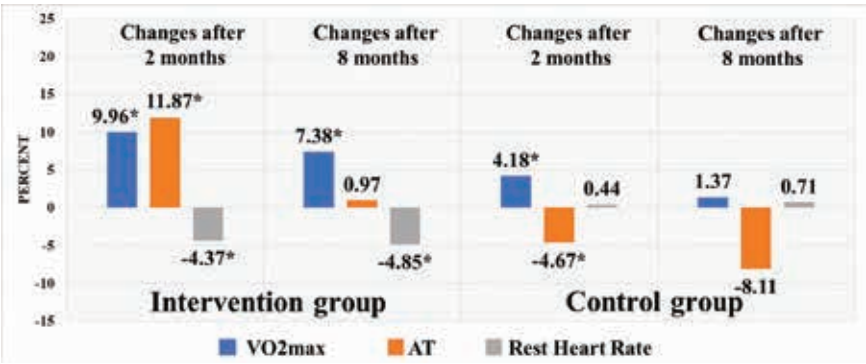

FIG. 38. Changes of the cardiorespiratory fitness parameters after 2 and 8 months
Abbreviations and explanations: * – statistically significant difference (p<0.05).

TABLE 21. Changes of the cardiorespiratory fitness parameters after 8 months

Parameter	Intervention group				Control group			
	First visit	Third visit	Difference	p	First visit	Third visit	Difference	p
VO2max (ml/kg/min), M ± SD	21.95 ± 5.20	23.57 ± 4.85	1.62 ± 3.57	**<0.001**	21.91 ± 5.13	22.21 ± 5.64	0.31 ± 2.62	0.628
AT (in % from predicted VO2), M ± SD	76.24 ± 17.81	76.98 ± 18.02	0.74 ± 16.48	0.539	80.89 ± 20.08	74.33 ± 26.27	-6.56 ± 13.66	0.058
Rest HR (b/min), M ± SD	63.94 ± 7.88	60.84 ± 7.55	-3.10 ± 7.42	**<0.001**	63.14 ± 7.68	63.59 ± 8.88	0.45 ± 7.79	0.732

Abbreviations and explanations: M ± SD – mean ± standard deviation, VO2max – maximal consumption of oxygen, AT – anaerobic threshold, HR – heart rate.

Quality of life after 8 months. The analysis of various aspects of QOL changes after 8 months, as well as after 2 months, revealed that in the intervention group the

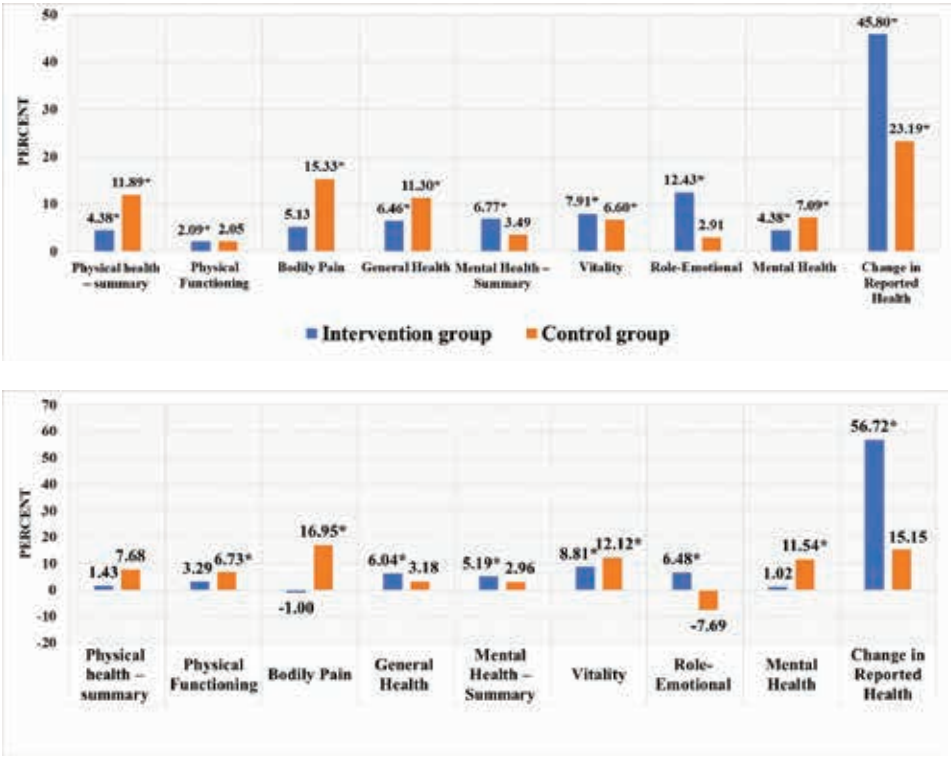

FIG. 39. Changes of the quality of life scores
after 2 months (upper figure) and after 8 months (lower figure)
Abbreviations and explanations: * – statistically significant difference (p<0.05).

scores of general health, vitality, role-emotional and the change in reported health remained statistically significantly improved. Bodily pain, vitality, mental health scores improved in the control group also (Fig. 39, Table 22).

TABLE 22. Changes of quality of life after 8 months

Intervention group				p value between the groups	Control group			
At baseline	After 2 months	Change	p value		p value	Change	At baseline	After 2 months
M ± SD	M ± SD	M ± SD				M ± SD	M ± SD	M ± SD
Physical Functioning					Physical Functioning			
82.89 ± 19.07	85.61 ± 14.88	2.72 ± 13.96	0.155	0.365	**0.045**	5.83 ± 11.15	86.67 ± 14.55	92.50 ± 8.27
Bodily Pain					Bodily Pain			
77.52 ± 21.89	76.74 ± 25.29	-0.78 ± 21.60	0.943	0.114	**0.033**	12.35 ± 23.15	72.84 ± 22.94	85.19 ± 17.46
General Health					General Health			
57.73 ± 13.13	61.22 ± 15.83	3.49 ± 11.24	**0.048**	0.660	0.456	2.08 ± 11.58	65.56 ± 14.44	67.64 ± 11.83
Mental Health – Summary					Mental Health – Summary			
73.49 ± 14.63	77.31 ± 15.72	3.81 ± 10.37	**0.019**	0.621	0.372	2.41 ± 9.98	81.62 ± 10.34	84.03 ± 11.23
Vitality					Vitality			
59.32 ± 14.89	64.55 ± 16.38	5.23 ± 12.20	**0.012**	0.377	**0.006**	8.24 ± 10.74	67.94 ± 13.93	76.18 ± 7.81
Role-Emotional					Role-Emotional			
83.72 ± 25.59	89.15 ± 25.94	5.43 ± 22.92	**0.038**	0.233	0.201	-7.41 ± 24.40	96.30 ± 10.78	88.89 ± 28.01
Mental Health					Mental Health			
70.79 ± 13.45	71.51 ± 14.43	0.72 ± 13.50	0.604	**0.049**	**0.028**	8.21 ± 13.56	71.16 ± 13.24	79.37 ± 9.26
Changes in Reported Health					Changes in Reported Health			
41.88 ± 19.10	65.63 ± 22.42	23.75 ± 24.64	**<0.001**	**0.030**	0.161	6.94 ± 18.80	45.83 ± 17.68	52.78 ± 11.79

Abbreviations and explanations: M ± SD – mean ± standard deviation.

5. Discussion

The influence of physical training on cardiovascular risk factors and metabolic syndrome components

Regular physical activity decreases CV mortality by 20 % [51]. We have demonstrated that regular 2-month duration aerobic HR targeted PT has a positive effect on cardiovascular risk factors: AH, dyslipidaemia, and the components of metabolic syndrome. Most of these effects persisted even after 8 months. We did not find such changes in the control group where only recommendations for PA and PT were provided to the subjects.

After 2 months of aPT, statistically significant decrease in sBP and dBP was found and these changes persisted even after 8 months. It is well known that even a small decrease in BP by 2–3 mm Hg due to PT lowers the risk of coronary heart disease by 5–9 %, the risk of stroke by 8–14 % and general mortality by 4 % [52].

Good physical fitness at baseline and its long-term maintenance protects against the development of dyslipidaemia [53-56]. In meta-analysis performed the lack of physical training programmes that improve lipid profile in subjects with MetS is stated [57]. The European Society of Cardiology concluded that it has not yet been established how much exercise is required in order to improve the lipid profile and reduce cardiovascular risk [58]. High fitness at baseline and its maintenance over time protects against the development of atherogenic dyslipidaemia [53]. The lack of evidence for PT programs that optimally improve lipid profile in MetS subjects was indicated in the meta-analysis [57]. It was shown that moderate aerobic exercise increases HDL-Chol and reduces TG level without prominent effect on LDL-Chol [56]. In our study, after 2 months of aPT, we observed a statistically significant decrease in LDL-Chol (from 4.19 mmol/l up to 3.8 mmol/l) without a significant decrease in TG or change in HDL-Chol. After 8 months, the lowering of LDL-Chol persisted and a statistically significant decrease in TG was observed in the interventiona group. These changes were not present in the control group. Other authors had pointed out, that in subjects with elevated LDL-Chol at baseline, intensive PT lowered LDL-Chol [59, 60]. In our study, baseline levels of LDL-Chol were elevated:4.19 in the intervention group and 3.8 mmol/l in the control group. Thus, our MetS subjects, additionally to the MetS components, had an elevated LDL- Chol at baseline, which decreased significantly after aPT. Similar results were found in the study by Dunn et al. [61], in which 24 week-long training (20–60 minutes of aPT, 3 times a week) was accompanied by a significantly decreased LDL-Chol without the increase in HDL. Significant decrease of LDL-Chol, non-significant decrease of TG and the increase of HDL- Chol has been reportedin premenopausal women after

intensive walking intervention program [62]. Scholars agree that over months, not weeks, endurance exercise involving minimum expenditure of about 1200 kcal per week results in the increase of HDL-Chol [56].

Overweight and obesity incidence is growing dramatically all over the world. It is related to CV, metabolic, gastroenterological and oncological diseases. In the early stages of overweight, the prevention of obesity becomes very important, and physical activity is a major means to achieve it [63]. Measurement of BMI and WC are the recognised methods to assess this disorder and are related to the risk of CVD and DM [64]. In our study, we estimated that in the intervention aPT group statistically significant decrease of WC took place and persisted for 8 months, while in control group it even increased after 8 months in average by 3.05 cm. BMI decreased after 2 months in both groups, the intervention group and control group. After 8 months, statistically significant decrease of BMI was observed only in the intervention aPT group. So, according to our results, measurement of WC was a more specific parameter than BMI for the assessment of the effect of aPT.

The growing rates of abdominal obesity and MetS in Europe and the USA are increasingly recognized as the predictors of CVD[2] and T2DM [65-69]. The incidence of MetS in the middle-aged subjects in Lithuania is 28.7 %, in women 31.5 %, in men 25.1 % [6]. In our study, MetS was diagnosed according to NCEP/APT III criteria[34, 70]. In our MetS cohort,AH was present in 92.1 %, fasting glucose ≥5.6 mmol/l in 63.3% , elevated TG in 59.3% and decreased HDL-Chol in 47.9% of subjects. An important characteristic of our MetS subjects was the high frequency of an elevated LDL-Chol,which additionally rises the CV risk of these subjects. At baseline, 3 out of 5 MetS components were present in 46.4 %, 4 out of 5 in 37.9 %, and all 5 in 15.7 % of the study subjects. In all individuals low level physical activity was observed at baseline.

Heretofore, there is no consensus on the optimal type and intensity of PT for CV risk lowering [58] in MetS subjects. Various types of PT are applied – aerobic, interval and endurance [71-73]. In our study, we used individually HR targeted aerobic physical training. We demonstrated a statistically significant decrease in BP and WC following aPT intervention, both after 2 intervention and 8 months. Statistically significant decrease in TG in the aPT group was observed only after 8 months; after 2 months, it did not reach statistical significance. All these changes were absent in the control group. According to other studies, an individually adapted PT programme also improved most of the cardiometabolic parameters: BMI, WC, TG, and HDL-Chol. Only fasting glucose did not change statistically significantly [57, 72, 74, 75]. Similarly, we also did not observe a statistically significant change in plasma glucose either in the intervention or control group. After 2 months of aPT intervention, 18.8 % of our subjects had less that 3 components of MetS, and this percentage was

even higher after 8 months (25.3 %). interventionOne other study has reported the decrease of MetS components after PT from 22.3 % to 13.5 % [74]. Another study has demonstrated that, due to PT intervention, 30.5 % of subjects were no longer classified as having MetS [75].

The effect of aerobic physical training on the functional and structural parameters of the arterial wall.

Early vascular aging is a relatively new definition of biological and vascular age mismatch, which increases the cardiovascular risk [8]. Vascular age can be assessed by obtaining arterial wall parameters. It was estimated, that 1 m/s increase in aortic pulse wave velocity corresponds to an age-, sex-, and risk factor-adjusted risk increase of 15 % in CVD and all-cause mortality [76]. Oxidative stress and inflammation are the main mechanisms causing stiffening of the arteries [77, 78]. Furthermore, a 0.1 mm increase in CCA IMT is associated with an 18 % and 15 % increased risk for a stroke and myocardial infarction [79]. In spite of the relatively rich cross-sectional data on vascular aging in the MetS subjects without overt CVD, the data on dynamics of the arterial wall parameters after the supervised HR targeted aPT is very limited. Our study is one of the first to assess the influence of such modality of training not only on CV risk factors and the components of MetS, but also on the arterial wall parameters reflecting the aging of the arteries. Improvement of the arterial wall parameters after PT has been shown in several previously reported studies. After training performed 3 times a week for 4 weeks, resting arterial compliance was elevated by ~30 % [80]. In the group of 21 middle-aged overweight and obese men, the 12-week walking or jogging for 40–60 minutes 3 days a week significantly increased carotid arterial compliance and reduced the stiffness index, assessed by post-study image analysis software, which was a relatively inaccurate method of the direct clipper measurements from ultrasound images [32]. In another study, arterial pulse wave velocities and augmentation index were measured immediately before and within 5–10 min after an incremental shuttle walk test [81]. Reduction of augmentation index after acute exercise but not decrease in aortic stiffness (c-f PWV) was observed. We did not measure the acute effect of training on augmentation index. According to the systematic review and meta-analysis of the large number of randomized controlled trials performed in various subjects, which did not specify the method used for dosing the exercise load, the reduction of brachial-ankle PWV was more significant than c-f PWV [82]. The authors also stated that higher intensity aerobic PT was associated with larger reductions in augmentation index, and aPT had more effect on brachial-ankle PWV rather than on central c- f PWV. The latter observation was not confirmed in our study, which analysed arterial wall parameters in a uniform group of the middle-aged MetS subjects under supervised

HR targeted aPT. In our study, after 2 months of aPT in the intervention group there was statistically significant decrease in c-f PWV indicating the improvement of aortic compliance (decrease of aortic stiffness) and decrease of mean BP in the aorta. These changes persisted in the intervention group even after 8 months. In the control group, where only recommendations for PA and PT were provided, we did not observe such changes. We think that this result is of major importance and shows that HR targeted aPT can contribute not only to physical status of the MetS subject, but also to the reduction of early preclinical arterial damage.

It has been shown that cardiorespiratory exercise testing prior to exercise training is very important for the choice of the optimal exercise load [40]. In this respect, the assessment of VO2max during the exercise testing and the oxygen consumption at the AT are of great significance for the adjustment of the exercise load during training [40]. In our study, the training at the AT was chosen as the most effective way to enhance the exercise effectiveness for CVD risk reduction. Hence, our study demonstrates that by decreasing the c-f PWV by HR targeted aPT, we can, firstly, positively influence the mean BP in the aorta and, secondarily, affect the indices of the early "vascular aging" in the middle-aged subjects with MetS, and, possibly, delay the development of type 2 DM or CVD.

Previously conducted studies revealed that arterial stiffness independently of BP can predict an elevated risk of stroke and CHD [83-86]. A single study on the effect of aerobic training on arterial stiffness, comparable to ours, was published by Donley DA et al. in 2014 [31]. The study assessed arterial stiffness parameters before and after 8 weeks of supervised aerobic exercise training by deploying a ramp exercise protocol in a relatively small group of 22 individuals with metabolic syndrome. The findings revealed the improvement of the aortic and peripheral arteries stiffness parameters after exercising without the positive effect on lipid parameters. The publication concludes that the study needs to be continued in the bigger cohort of subjects. In our study we raised the hypothesis that HR targeted dosed supervised APT can reduce arterial and aortic stiffness as well as CCA stiffness and IMT and this will reduce the CV risk of our subjects.

We have analysed much more numerous cohort of the middle-aged subjects than Donley et al.: 170 subjects were divided into the intervention aPT group (85 subjects) and the control group (85 subjects). Arterial and aortic stiffness in our study was measured using the golden standard method, applanation tonometry (*Sphygmocor, AtCor Medical*) [87, 88]. After the analysis of the arterial wall functional parameters by the categories was carried out, we observed the statistically significantly increased number of subjects in whom c-f PWV was <9 m/s. There was no such result achieved in the control group. intervention We estimated that, after 2 months, the intervention group showed the improvement of aortic stiffness:

a statistically significant reduction in c-f PWV. The same result was obtained while measuring another parameter, MBP in the aorta. These effects persisted even after 8 months. There were no statistically significant changes in these parameters in the control group. The ROC curve analysis in the intervention group showed that the cutoff point, when the effect of aPT is optimal, occurred when the baseline c-f PWV value was > 8.1 m/s. According to the regression tree method, the improvement started when it was >8.6 m/s, and the maximal effect was reached at c-f PWV >10.1 m/s. In the multiple regression analysis, we also demonstrated that intervention was an independent predictor of the improvement in arterial compliance.intervention.

One of the gratest methodological assets of our study was that, as compared to other studies, we analysed a relatively larger group of MetS subjects. The other methodologocal advantage was that we applied a HR targeted and thus individually adjusted aPT. Likewise, we used the most accurate assessment of carotid stiffness and IMT currently available:CCA measurement by a highly accurate echotracking system (*Art. Lab, Esaote Europe B.V.*) [35]. The CCA measurements were based on the analysis of the radiofrequency signals and were obtained by high-resolution echotracking technology [35]. Hence, the fact that we did not observe any improvement in carotid distensibility or reduction of IMT after training is the more credible.. We are confident that the findings of our more numerous and accurate study are highly reliable. It is possible that longer intervention is needed to impact these parameters. The results of our study showed that HR targeted aPT reduces some of the arterial parameters which are which are surrogate markers of early atherosclerosis. The aerobic training-induced changes of anthropometric, metabolic and arterial markers can contribute to the reduction of the CV risk in subjects with metabolic syndrome and increase patient's motivation for further physical training, as the residual effect of aPT takes place even after 8 months.

The effect of aerobic physical training on anxiety and depression, physical activity, cardiorespiratory fitness, quality of life and motivation for physical training.

The data on how MetS is associated with depression and anxiety is limited. Some studies have observed that MetS is associated with depression, but not anxiety [11-13]. Other researchers emphasise that some of the MetS components, including obesity, impaired glucose tolerance or insulin resistance, and subclinical signs of atherosclerosis are linked with symptoms of depression or anxiety [9, 10, 14]. There are very few studies analysing the effect of the PT programme on subjects with MetS and their levels of anxiety and depression. There are some studies which demonstrated a positive or even compromising effect of PT on depression symptoms in patients suffering from chronic diseases, including type 2 DM or CHD, and those

with decreased PA [22-25]. Our study have also evaluated the levels of anxiety and depression in subjects with MetS before and after HR targeted 2-month duration aPT in the intervention group and compared their results with the results of the subjects who were just provided with the recommendations for PA and PT in the control group. The results were reassessed after 8 months. There were no statistically significant differences between the subjects in different age, BMI and physical capacity according to depression and anxiety estimates.

The lack of PA has an adverse effect on the prevalence of CVD and type 2 DM (3, 61]. According to the national LitHiR programme, sedentary living was estimated in 49.9 % (54.4 % of women and in 43.1 % of men) of middle-aged subjects in Lithuania (89). Sedentary living is a prevalent risk factor in subjects with MetS [90-93]. The total PA duration and intensity inversly correlate with the risk of MetS and the freqency of its components. Conversely, the time spent in a sitting position directly correlates with the presence of MetS [94]. Sitting time is a very important living style factor affecting total mortality and is related to MetS [95], arterial stiffness [96-100] and risk of atherosclerosis [101]. That is why diminishing of sitting time is a very important means of MetS prevention [92]. In our study, sitting time at baseline in average was 1927.3 min/week (4.5 h/d) in the intervention group and 1620.29 min/week (almost 4 h/d) in the control group. After 2 months, sitting time statistically significantly diminished in both groups: in the intervention group by 403.58 min/week ($p<0.0001$) and in the control group by 275.71 ($p<0.003$) min/week. After 8 months, such changes were no longer observed . After 2 and 8 months, in the intervention group statistically significant improvement in leisure time, moderate and vigorous physical activity was observed. There were no such changes in the control group subjects. However, after 8 months, walking PA significantly improved.

The low level of cardiorespiratory fitness is a well-known factor of CV mortality [102, 103]. Better CRF means the lower risk of CVD, MetS, AH and dyslipidaemia. There is a positive relation between CRF and PA, and negative between PA and body fat amount [104]. In MetS subjects, there is an obvious relation between CRF and CVD mortality [105]. High CRF in subjects with elevated CV, MetS, glycemic disorders, type 2 DM means better prognosis in these subjects [106]. More precisely, CRF can be assessed by performing a cardiorespiratory stress test and assessing VO-2max, AT, ventilation threshold and other parameters [107]. This is how we assessed CRF in our study subjects when comparing the intervention and control groups after 2 and 8 months. After 2 months, we found statistically significant improvement in VO2max and AT both in the intervention and the control groups, while HR at rest significantly decreased only in the intervention group. Similar results were presented in two other studies where the improvement of VO2max was found after PT in MetS subjects [20, 57]. When assessing CRF after 8 months we were able to find

the improvement of VO2max and HR at rest only in the intervention group. That means a higher motivation of the subjects after 2-month duration aPT for further PT during next months.

It is known that patients with MetS syndrome are at increased risk to develop diabetes and cardiovascular diseases [15] which are associated with worse QOL [16, 17]. The evaluation of life quality disorders is especially relevant for this type of population, in which the adverse results of treatment, such as poor response to treatment, rapid disease progression and high mortality, are seen more often [17-19]. Some of the MetS components, including obesity, insulin resistance and arterial hypertension, are related to worse QOL [108-111]. This suggests that MetS itself may be the cause of life quality disorders. Several studies have confirmed this conclusion [112-116]. Our study revealed how quality of life in terms of QOL domains, measured with the SF-36 questionnaire is affected in MetS subjects. At the baseline, during the initial examination, both study groups had highest scores in the categories of social and physical functioning, and activity limitation due to emotional problems, and the worst in the change of reported health, general health assessment and vitality. After 2 months, in the intervention aPT group statistically significant improvement was achieved in those life quality domains that had been previously evaluated by the worst scores: changes in the reported health and emotional state, and activity limitation due to emotional problems. Changes in reported health, general health and physical health improved in the control group of PA and PT recommendations after 2 months. In the intervention group, the scores of general health, vitality, role-emotional, and the change in reported health, improved statistically significantly both after 2 and 8 months.intervention. Bodily pain, vitality, and mental health scores improved in the control group. Recently, Jahangiry L *et al*. have investigated HR QOL of MS subjects in comparison with subjects without overt MetS [117]. Similarly to the results of our study, it was observed that the most impaired domains of QOL in subjects having MetS were physical condition, energy and psycho-emotional condition [117]. In addition, some studies have also demonstrated that aerobic exercises can significantly improve QOL in subjects with obesity, or patients suffering from type 2 diabetes [21, 22, 118]. In contrast to previous studies, Vetter ML *et al*. reported that MetS was not associated with decreased QOL [119]. It was suggested that not MetS itself, but the components of MetS, such as obesity, depression and greater disease burden may significantly affect QOL in this population [119].

The role of motives for physical activity have been analysed in various studies [120-127], but most of them were based on self-determination theory perspective and examined general population. Since we used a standardized questionnaire (Exercise Motivations Inventory v. 2) to investigate MetS subjects and their motives

for physical activity, our study provides a more accurate estimate. We obtained that the most dominant motives to be physically active were health motives, especially ill-health avoidance and positive health. The participants of our study were the least motivated by social engagement and enjoyment, and revitalization motives, and the most motivated by health, fitness and appearance/weight motives measured at baseline. Self-reported evaluation of motives for physical activity did not differ between the patients of different genders, age groups, BMI and physical capacity categories. After 2 months, in both groups the health related motive improved most.In the intervention group, the best improvement was observed in recreation and emotional, social and physical fitness motives. After 2 months, in the control group appearance and weight management reasoning decreased the most. To our best knowledge, there are no previous studies that assessing MetS subjects' motivation for physical exercise. Only a few studies analysed motivation for exercise in patients with type 2 diabetes [128, 129]. Korkiakangas E *et al*. highlighted gender differences and demonstrated that women were more likely to indicate the importance of the positive body image for engaging in physical activity, whereas men more often indicated the importance of health-promoting behaviours [129]. Ferrand C *et al*. showed that motivation to physical activity in subjects with type 2 diabetes were mostly related to weight management, social relationships, physical and mental well-being [106]. According to Murer M *et al*., subjects having MetS are usually motivated to improve their physical activity and are familiar with its positive effect [130]. However, patients have to be better supported and advised how to get physically active [130].

Lastly**,** we investigated how individually tailored aerobic physical training by the heart rate targeting had affected MetS patients' quality of life, motives for physical activity and psycho-emotional state. Although BMI and oxygen uptake at peak load after the physical training programme statistically significantly improved, there was no statistically significant relation between the changes of these parameters and the changes of QOL domains, motives for physical activity, estimates of anxiety and depression.

Conclusions

1. Our complex assessment of cardiovascular risk factors, components of metabolic syndrome, arterial wall functional and structural parameters, cardiorespiratory fitness, motivation for physical training, and quality of life before and after the training programme in metabolic syndrome subjects has demonstrated the efficacy of two- month long heart rate targeted supervised aerobic physical training:

1.1. Only in the intervention group statistically significant positive effect on blood pressure indices, total cholesterol, low density lipoprotein cholesterol and waist circumference was obtained.
1.2. Both in the intervention and control groups the reduction of components of metabolic syndrome was observed.
1.3. Only in the intervention group there was an improvement of arterial wall parameters: a decrease of carotid-femoral pulse wave velocity and mean blood pressure in the aorta. At the baseline measured carotid-femoral pulse wave velocity > 8.6 m/s was associated with greater improvement.
1.4. Only in the intervention group sitting time decreased.
1.5. Both in the intervention and control groups cardiorespiratory fitness improved after 2 months. That suggests that even in the control group some subjects followed the recommendations for physical activity and training.

2. In the intervention group statistically significant improvement in motivation for physical training was found. Both in the intervention and control groups quality of life has improved.
3. The evaluation of the residual effect after 8 months of the 2-month duration aerobic physical training programme in the intervention group and in the control subjects who only followed standard recommendations for physical activity and training, demonstrated statistically significant residual positive effect of training only in the intervention group. Supervised PT had a positive impact on blood pressure indices, total cholesterol, low density lipoprotein cholesterol and triglycerides, waist circumference, components of metabolic syndrome, aortic stiffness, and cardiorespiratory fitness.

6. Practical recommendations

1. In subjects with metabolic syndrome, supervised aerobic physical training not only improves the quality of life, appearance and physical fitness but also reduces the risk of development of cardiovascular disease and/or diabetes mellitus by reducing cardiovascular and metabolic risk and development of atherosclerosis.
2. Heart rate controlled aerobic physical training can be reccomened as an integral part of primary prevention programmes in metabolic syndrome subjects. Such training is the means for improving health quality, physical activity, cardiorespiratory fitness, vascular health and motivation for further physical training, and reduces cardiovascular risk and number of metabolic syndrome components.

3. Based on this study, a proposal to the government could be prepared for funding aerobic physical training activity once a year as a part of the ongoing Lithuanian High Cardiovascular Risk Programme.
4. From among the many parameters that might be considered for the monitoring of aerobic physical training effect, which we analyzed in this study, aortic stiffness and mean blood pressure in the aorta, measured by applanation tonometry, could be recommended for clinical practice.

7. List of publications and presentations

Publications

1. Ieva Slivovskaja, Ligita Ryliskyte, Pranas Serpytis, Rokas Navickas, Jolita Badarienė, Jelena Celutkiene, Roma Puronaite, Kristina Ryliskiene, Alma Cypiene, Egidija Rinkuniene, Vaida Sileikiene, Birute Petrauskiene, Alvydas Juocevicius, Aleksandras Laucevicius. Aerobic Training Effect on Arterial Stiffness in Metabolic Syndrome. American Journal of Medicine. Publication stage: In Press Accepted Manuscript. Published online: August 29, 2017: <http://www.amjmed.com/article/S0002-9343(17)30839-2/abstract>.

2. Ieva Slivovskaja, Jurgita Buzinskaitė, Ligita Ryliškytė, Jūratė Balsytė, Rokas Navickas, Roma Puronaitė, Agnė Jucevičienė, Alvydas Juocevičius, Aleksandras Laucevičius. Positive impact of a 4-week duration supervised aerobic training on anthropometric, metabolic, hemodynamic and arterial wall parameters in metabolic syndrome subjects. Seminars in Cardiovascular Medicine. 2017; 23: 11-16.

3. Ieva Slivovskaja, Alvydas Juocevičius, Galina Kargina, Aurelija Meškaitė. Fizinio aktyvumo vaidmuo mažinant metabolinio sindromo sukeltą širdies ir kraujagyslių ligų bei diabeto riziką. Sveikatos mokslai. 2012; 22(6):5-10.

Presentations:

1. Slivovskaja Ieva, Ryliskyte Ligita, Badariene Jolita, Cypiene Alma, Rinkuniene Egidija, Juocevicius Alvydas, Laucevicius Aleksandras. Immediate effect of aerobic training on arterial stiffness – novel marker for monitoring of cardiovascular fitness. 20th European Congress of Physical and Rehabilitation Medicine, 2016.04.23–28, Estoril–Lisbon, Portugal.

2. Slivovskaja, L. Ryliskyte, J. Badariene, A. Cypiene, E. Rinkuniene, J. Buzinskaite, J. Balsyte, A. Juocevicius and A. Laucevicius. Aerobic training improves arterial stiffness in metabolic syndrome subjects. EuroPRevent 2016 The leading international congress from the European Association for Cardiovascular Prevention & Rehabilitation, 2016.05.05–07, Sophia Antipolis, France.

3. Ieva Slivovskaja, Jurgita Buzinskaite. Effectiveness of different physiotherapy methods in patients with increased cardiometabolic risk. 4th Baltic and North Sea Conference on Physical and Rehabilitation Medicine, 2015.09.16–18, Riga, Latvia.

4. Ieva Slivovskaja, Jolita Badarienė, Rokas Navickas, Alvydas Juocevičius. Estimation of cardiorespiratory fitness in middle-age adults with metabolic syndrome and correction of physical activity: evidence, rationale and organization. 7th Baltic Rehabilitation Association Conference on Physical and Rehabilitation Medicine, November 30–December 1, 2012, Vilnius, Lithuania.

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SANRAUKA

Įvadas

Širdies ir kraujagyslių ligos (ŠKL) Lietuvoje, kaip ir visame pasaulyje buvo ir tebėra pagrindinė mirtingumo priežastis. Lietuvoje nuo ŠKL miršta beveik dvigubai daugiau gyventojų negu vidutiniškai Europos Sąjungos (ES) šalyse senbuvėse – Lietuvos vyrų ir moterų standartizuoti mirtingumo nuo kraujotakos sistemos ligų rodikliai yra vieni didžiausių ES.. Rūkymas, dislipidemija, cukrinis diabetas (CD), arterinė hipertenzija (AH), nepakankamas fizinis aktyvumas, netaisyklinga mityba, nutukimas, ypač pilvinis, psichosocialiniai veiksniai ir nesaikingas alkoholio vartojimas visame pasaulyje sudaro didžiąją dalį rizikos susirgti koronarine širdies liga (KŠL) ir miokardo infarktu (MI). Europos 2016 metų širdies ir kraujagyslių ligų prevencijos gairėse nurodoma bendrinė fizinio aktyvumo rekomendacija: bent 150 minučių aerobinio vidutinio intensyvumo fizinio aktyvumo (FA) per savaitę (po 30 minučių 5 dienas per savaitę) arba 75 minutės aerobinio didelio intensyvumo fizinio aktyvumo per savaitę (po 15 minučių 5 dienas per savaitę) arba jų derinys. Tam gali pagelbėti ir fizinės treniruotės pagal iš anksto sudarytą programą. Aerobinės fizinės treniruotės (aFT) labiausiai ištirtos, žinomas šių pratimų dozės ir atsako efektas.

Darbo tikslas ir uždaviniai

DARBO TIKSLAS – nustatyti individualiai pagal širdies susitraukimų dažnį (ŠSD) dozuojamų prižiūrimų dviejų mėnesių trukmės aFT programos veiksmingumą asmenims, kuriems nustatytas metabolinis sindromas (MetS), vertinant jų ŠKL rizikos veiksnių, MetS komponentų, arterijų sienelės funkcinių ir struktūrinių parametrų, kardiorespiracinio fizinio pajėgumo (KRFP), fizinių treniruočių motyvacijos bei gyvenimo kokybės pokyčius.

DARBO UŽDAVINIAI:

1. Nustatyti, įvertinti ir palyginti fizinių treniruočių programos ir savarankiško fizinio aktyvumo rekomendacijų laikymosi poveikį širdies ir kraujagyslių ligų rizikos veiksniams, MetS komponentams, arterijų sienelės funkciniams ir struktūriniams parametrams bei KRFP.
2. Nustatyti, įvertinti ir palyginti aFT programos ir savarankiško fizinio aktyvumo rekomendacijų laikymosi poveikį fizinių treniruočių motyvacijai ir gyvenimo kokybei.
3. Nustatyti, įvertinti ir palyginti liekamąjį 2 mėnesių trukmės aFT ir savarankiško fizinio aktyvumo rekomendacijų laikymosi poveikį po 8 mėnesių ŠKL rizikos veiksniams, MetS komponentams, arterijų sienelės funkciniams ir struktūriniams parametrams, KRFP, taip pat fizinių treniruočių motyvacijai ir gyvenimo kokybei.

Tiriamųjų kontingentas

Į tyrimą įtraukta 45–64 metų amžiaus 170 asmenų. Tiriamiesiems buvo nustatytas MetS, jie nesirgo CD, gavo optimalų AH gydymą vaistais bei sutiko dalyvauti tyrime. MetS

buvo diagnozuotas pagal 2005 metais NCEP/ATP III modifikuotus kriterijus. Atrinkti asmenys, taikant 1:1 atsitiktinės atrankos metodą, suskirstyti į intervencinę (n=85) ir kontrolinę (n=85) grupes. Intervencinės grupės tiriamiesiems, be įprastinių aktyvios ŠKL rizikos veiksnių mažinimo – dietos, atsisakymo rūkyti, FA rekomendacijų, buvo taikomos individualiai pagal spiroergometrijos (SEM) metu nustatytą tikslinį ŠSD dažnį dozuotos prižiūrimos aFT 8 savaites 5 kartus per savaitę. Kontrolinės grupės tiriamiesiems buvo suteiktos dietos, atsisakymo rūkyti ir FA rekomendacijos. Abiejų grupių tiriamieji ištirti 3 kartus – tyrimo pradžioje, po 2 mėnesių bei vėlyvuoju laikotarpiu, praėjus 8 mėnesiams, siekiant įvertinti ilgalaikį taikytų treniruočių efektyvumą.

Tyrimo metodai

Atlikti antropometriniai matavimai – matuotas tiriamųjų svoris, ūgis, juosmens apimtis (JA), apskaičiuotas kūno masės indeksas (KMI), matuotas arterinis kraujo spaudimas (AKS), atlikta lipidograma, gliukozė nevalgius.

Atliktas arterijų sienelės parametrų vertinimas: pulsinės bangos greičio (PBG) ir vidutinio arterinio kraujo spaudimo (VAKS) aortoje matavimas aplanacinės tonometrijos metodu, miego arterijų intimos ir medijos sluoksnio storio ir miego arterijų dilatacijos bei aterosklerozinių plokštelių miego arterijose nustatymas ultragarsiniu metodu.

HAD skale vertinti nerimas ir depresija, atliktas kasdienio fizinio aktyvumo vertinimas naudojant tarptautinį fizinio aktyvumo klausimyną IPAQ, atliekant spiroergometriją vertintas KRFP, MOS SF-36 klausimynu vertinta su sveikata susijusi gyvenimo kokybė (SSGK), EMI-2 fizinių treniruočių aprašu vertinta fizinių treniruočių motyvacija.

Rezultatai ir jų aptarimas

Vertindami AKS pokyčius mūsų atliktame tyrime praėjus 2 mėnesiams po pirminio ištyrimo, radome, kad intervencinėje aFT grupėje statistiškai reikšmingai sumažėjo sistolinis ir diastolinis AKS. Tirdami po 8 mėnesių tik intervencinėje aFT grupėje nustatėme sistolinio ir diastolinio AKS sumažėjimą. Net nedidelis AKS sumažėjimas (2–3 mmHg) po fizinių treniruočių sumažina KŠL riziką nuo 5 iki 9 proc., insulto riziką – nuo 8 iki 14 proc., o bendrąjį mirtingumą – 4 proc.. Kontrolinėje FA grupėje statistiškai reikšmingų skirtumų tarp AKS rodiklių tyrimo pradžioje ir praėjus 2 mėnesiams nebuvo rasta.

Mūsų atliktame tyrime nustatytas statistiškai reikšmingas mažo tankio lipoproteinų cholesterolio (MTL-Chol) lygio sumažėjimas intervencinėje grupėje ir tokio sumažėjimo nekonstatavome kontrolinėje grupėje. Buvo matoma trigliceridų (TG) mažėjimo tendencija abiejose grupėse bei didelio tankio lipoproteinų (DTL-Chol) didėjimo tendencija intervencinėje grupėje, tačiau šie pokyčiai nebuvo statistiškai reikšmingi. Tirdami po 8 mėnesių tik intervencinėje grupėje nustatėme teigiamą aFT poveikį mažinantį bendrą cholesterolį ir MTL-Chol. Taip pat tik intervencinėje grupėje po 8 mėnesių atsirado prieš 2 mėnesius dar neišryškėjęs TG mažėjimo statistinis reikšmingumas.

Nutukimui vertinti atlikome KMI ir JA matavimus. KMI ir JA apimtis yra panašiai svarbūs ir stipriai susiję su ŠKL ir antro tipo CD rizika. Mūsų atliktame tyrime po 2 mėnesių JA statistiškai reikšmingai sumažėjo tik intervencinėje aFT grupėje, o kontrolinėje grupėje ji net padidėjo. Nustatėme, kad KMI po 2 mėnesių statistiškai reikšmingai sumažėjo abiejose grupėse. Po 8 mėnesių, kaip ir po 2 mėnesių, nustatytas statistiškai reikšmingas KMI sumažėjimas intervencinėje grupėje, kontrolinėje grupėje po 8 mėnesių KMI sumažėjimas nesiekė statistinio reikšmingumo. Intervencinėje grupėje po 8 mėnesių, kaip ir po 2 mėnesių, nustatyta statistiškai reikšmingai sumažėjusi JA, kontrolinėje grupėje šis rodiklis po 8 mėnesių net padidėjo. Taigi, mūsų duomenimis, JA mažėjimas buvo labiau specifinis aFT charakterizuojantis parametras negu KMI. Nustatėme, kad intervencinėje grupėje po 2 mėnesių aFT iš penkių MetS komponentų statistiškai reikšmingai pagerėjo dviejų – JA ir AKS, o po 8 mėnesių pagerėjo trijų – JA, AKS bei TG rodikliai. Kontrolinėje grupėje statistiškai reikšmingų MetS komponentų rodiklių skirtumų, išskyrus net padidėjusią JA po 8 mėnesių, nekonstatuota.

Savo darbe kėlėme hipotezę, kad pagal ŠSD dozuojamos aFT gali padėti sumažinti arterijų standumą ir vidutinį AKS aortoje, kas yra siejama su mažesne ŠKL rizika. Kadangi ŠKL baigtys pasireiškia tik laikui bėgant, arterijų standumo parametrų matavimas gali suteikti galimybę dinaminiam ŠKL rizikos vertinimui, o po aFT sumažėjusi rizika gali labai padidinti pacientų motyvaciją būti fiziškai aktyviems. Atlikta analizė parodė, kad pagal ŠSD dozuojamos aFT statistiškai reikšmingai sumažina aortos standumą (miego-šlaunies a. PBG) ir VAKS aortoje pacientams, turintiems MetS. Šio poveikio nerasta kontrolinėje grupėje, kuriai nebuvo taikomos individualios aFT, o tik suteiktos rekomendacijos dėl FA ir treniruočių. Periferinių arterijų standumas (miego-stipininės a. PBG) sumažėjimas intervencinėje nepasiekė statistinio reikšmingumo. Mūsų atliktame darbe, siekiant nustatyti pradinio miego-šlaunies a. PBG slenkstinę reikšmę, leidžiančią su didžiausiu prognoziniu tikslumu numatyti PBG pagerėjimą po individualiai pagal ŠSD dozuojamo fizinio krūvio 2 mėnesių trukmės aFT programos, buvo atlikta ROC kreivės analizė ir nustatyta pradinio miego-šlaunies a. PBG slenkstinė reikšmė – 8,1 m/s. Regresijos medžio metodu ši reikšmė buvo patikslinta - 8,6 m/s. Šie mūsų gauti duomenys yra visiškai originalūs. Svarbu ir tai, kad po 8 mėnesių intervencinėje, bet ne kontrolinėje grupėje, išliko ir aortos standumo (miego-stipininės a. PBG) nei VAKS sumažėjimo statistinis reikšmingumas. Tai rodo liekamąjį 2 mėnesių pagal ŠSD dozuojamų prižiūrimų aFT poveikį ŠKL rizikos mažėjimui. Mūsų atlikto tyrimo radiniai tiriant arterijų funkciją ir struktūrą leidžia manyti, kad aortos standumo parametrų ir VAKS aortoje pagerėjimas po mūsų taikytos aFT programos prisideda prie galimų subklinikinių arterijų pokyčių prevencijos ir aterosklerozės vystymosi MetS turintiems pacientams perspėjimo.

Pritaikius HAD skalę, statistiškai reikšmingų nerimo įvertinimo pokyčio skirtumų nerasta abiejose grupėse, o intervencinėje grupėje statistiškai reikšmingai pagerėjo depresijos įvertinimas. Įvertinus fizinį aktyvumą Tarptautiniu fizinio aktyvumo klausimynu, po 2 mėnesių tik intervencinėje grupėje rastas statistiškai reikšmingas pokytis atliekant labai intensyvią fizinę veiklą sode arba kieme, vidutiniškai ir labai intensyvią fizinę veiklą laisvalaikiu bei sėdėjimo laiko sumažėjimas. Konstatuotas statistiškai reikšmingas visų KRFP rodiklių

pagerėjimas tiek intervencinėje, tiek ir kontrolinėje grupėje. Pažymėtina, kad intervencinėje grupėje pagerėjimas buvo statistiškai reikšmingai didesnis ir tai liudija apie geresnį dozuojamų prižiūrimų aFT poveikį ŠKL rizikai mažinti. Tirdami KRFP po 8 mėnesių, tik intervencinėje grupėje konstatavome statistiškai reikšmingą VO_2max (ml/kg/min) ir ramybės ŠSD rodiklių pagerėjimą. Tai rodo, jog šie asmenys buvo pakankamai motyvuoti ir tęsė fizines treniruotes savarankiškai, pasibaigus 2 mėnesių pagal ŠSD dozuojamų aFT laikotarpiui.

Mūsų tyrimas parodė, kaip MetS veikia pacientų SSGK, vertinant ją SF-36 klausimynu. Šio tyrimo pradžioje abiejų grupių tiriamieji blogiausiai vertino sveikatos pasikeitimą per metus, bendrą sveikatos būklę bei energingumą ir gyvybingumą, o geriausiai – socialinę funkciją, fizinį aktyvumą ir veiklos apribojimą dėl emocinių sutrikimų. Po 2 mėnesių aFT intervencinėje grupėje statistiškai reikšmingai padidėjo fizinio aktyvumo, veiklos apribojimo dėl emocinių sutrikimų sričių įverčiai ir apibendrintas psichikos sveikatos įvertis. Apibendrintas fizinės sveikatos, bendrojo sveikatos vertinimo, energingumo ir gyvybingumo, emocinės būklės bei sveikatos pasikeitimo sričių įverčiai ir apibendrintas fizinės sveikatos įvertis statistiškai reikšmingai padidėjo abiejose grupėse. Tai rodo labai subjektyvų tokių vertinimų pobūdį, nesutampantį su objektyviu aFT sukeliamų pamatuojamų rodiklių dinamika.

Mūsų duomenimis, vertinant aFT motyvacijos vaidmenį labiausiai dominuojantys motyvai buvo sveikatos, kūno išvaizdos ir fizinių galių lavinimo, o mažiausiai tiriamuosius motyvuoja socialiniai bei rekreacijos ir emociniai motyvai. Po 2 mėnesių tik intervencinėje aFT grupėje statistiškai reikšmingai pagerėjo vidinės motyvacijos: patiriamo malonumo, iššūkio, fizinių galių lavinimo ir afiliacijos aspektai. Taigi, 2 mėnesių individualiai pagal tikslinį ŠSD dozuota prižiūrima aFT programa neabejotinai pagerina ilgalaikio fizinių treniruočių atlikimo motyvaciją. Originali mūsų atlikto tyrimo idėja – įvertinti 2 mėnesių aFT programos liekamąjį poveikį praėjus 8 mėnesiams.

Svarbu pažymėti, kad iš 140 asmenų, dalyvavusių antrajame ištyrime, į trečią etapą po 6 mėnesių atvyko 86,9 proc. intervencinės ir tik 57,1 proc. kontrolinės grupės asmenų, o tai rodo pakankamą intervencinės grupės dalyvių motyvaciją tęsti pradėtą FT programą ir motyvacijos stoką kontrolinėje grupėje.

Išvados

1. Dviejų mėnesių trukmės pagal širdies susitraukimų dažnį dozuojamų prižiūrimų aerobinių fizinių treniruočių programos taikymas asmenims, kuriems nustatytas metabolinis sindromas, yra veiksmingas. Tai rodo teigiami palyginamieji rezultatai, gauti prieš treniruotes ir po jų kompleksiškai vertinant širdies ir kraujagyslių ligų rizikos veiksnių ir metabolinio sindromo komponentų, arterijų funkcijos parametrų, fizinio pajėgumo, fizinių treniruočių motyvacijos ir gyvenimo kokybės įverčius:
   1.1. Tik intervencinėje grupėje matomas statistiškai reikšmingas teigiamas poveikis širdies ir kraujagyslių ligų rizikos veiksniams – sistoliniam ir diastoliniam arteriniam kraujo spaudimui, bendro cholesterolio, mažo tankio lipoproteinų cholesterolio koncentracijai, juosmens apimčiai.

1.2. Intervencinėje ir kontrolinėje grupėse buvo rastas statistiškai reikšmingas metabolinio sindromo komponentų skaičiaus sumažėjimas.
1.3. Tik intervencinėje grupėje arterijų sienelės parametrų dinamika buvo teigiama: sumažėjo miego-šlaunies arterijų pulsinės bangos greitis ir vidutinis arterinis kraujo spaudimas aortoje. Nustatytas statistiškai reikšmingas skirtumas tarp pokyčių grupėse. Kai miego- šlaunies arterijų pulsinės bangos greitis viršija 8,6 m/s, šio parametro informatyvumas ima didėti.
1.4. Tik intervencinėje grupėje statistiškai reikšmingai sumažėjo laikas, praleidžiamas sėdint. Nustatytas statistiškai reikšmingas skirtumas tarp pokyčių grupėse.
1.5. Intervencinėje ir kontrolinėje grupėse buvo konstatuotas statistiškai reikšmingas kardiorespiracinio fizinio pajėgumo pagerėjimas, rodantis, kad dalis kontrolinės grupės asmenų vykdė fizinio aktyvumo rekomendacijas. Nustatytas statistiškai reikšmingas skirtumas tarp pokyčių grupėse, pokyčiai intervencinėje grupėje buvo didesni.

2. Intervencinėje grupėje nustatytas statistiškai reikšmingas fizinių treniruočių motyvacijos pagerėjimas, vertinant įverčių, priskiriamų vidinei motyvacijai, kuri skatina ilgalaikį fizinių treniruočių atlikimą, pokyčius – pagerėjo rekreacijos ir emocinių, socialinių ir fizinių galių lavinimo motyvų grupių įverčiai ir atskirų motyvų skalių įverčiai: streso įveikos / valdymo, rekreacijos, džiaugsmo, iššūkio, socialinio pripažinimo, afiliacijos bei varžymosi. Gyvenimo kokybė pagerėjo ir intervencinėje, ir kontrolinėje grupėse. Intervencinėje grupėje statistiškai reikšmingai pagerėjo abu apibendrinti (fizinės ir psichikos sveikatos) bei penkių iš aštuonių sričių įverčiai, kontrolinėje grupėje – apibendrintas fizinės sveikatos bei keturių iš aštuonių sričių įverčiai.
3. Įvertinus dviejų mėnesių trukmės aerobinių fizinių treniruočių programos ir savarankiško fizinio aktyvumo rekomendacijų laikymosi liekamąjį poveikį po aštuonių mėnesių, tik intervencinėje grupėje konstatuotas statistiškai reikšmingas poveikis arteriniam kraujo spaudimui, bendro cholesterolio, mažo tankio lipoproteinų cholesterolio ir trigliceridų koncentracijai, juosmens apimčiai, metabolinio sindromo komponentų skaičiui, arterijų standumui bei kardiorespiraciniam fiziniam pajėgumui.

Praktinės rekomendacijos

1. Skiriant fizines treniruotes asmenims, turintiems metabolinį sindromą, siekiama ne tik pagerinti jų gyvenimo kokybę, išvaizdą ir fizinį pajėgumą, bet ir sumažinti riziką susirgti širdies ir kraujagyslių ligomis ir (ar) diabetu, kuriam savo ruožtu būdingos kardiovaskulinės komplikacijos.
2. Kaip vieną efektyviausių priemonių vykdant pirminę širdies ir kraujagyslių ligų prevenciją metabolinį sindromą turintiems asmenims rekomenduojame individualiai pagal širdies susitraukimų dažnį dozuojamas prižiūrimas fizines treniruotes,

kurios reikšmingai gerina kardiorespiracinį fizinį pajėgumą, turi teigiamą poveikį mažinant širdies ir kraujagyslių ligų rizikos veiksnius, mažina metabolinio sindromo išreikštumą, pagerina psichinę ir emocinę būklę, gyvenimo kokybę bei fizinių treniruočių motyvaciją, taip pat turi liekamąjį poveikį ir geriau motyvuoja asmenis būti fiziškai aktyvius.

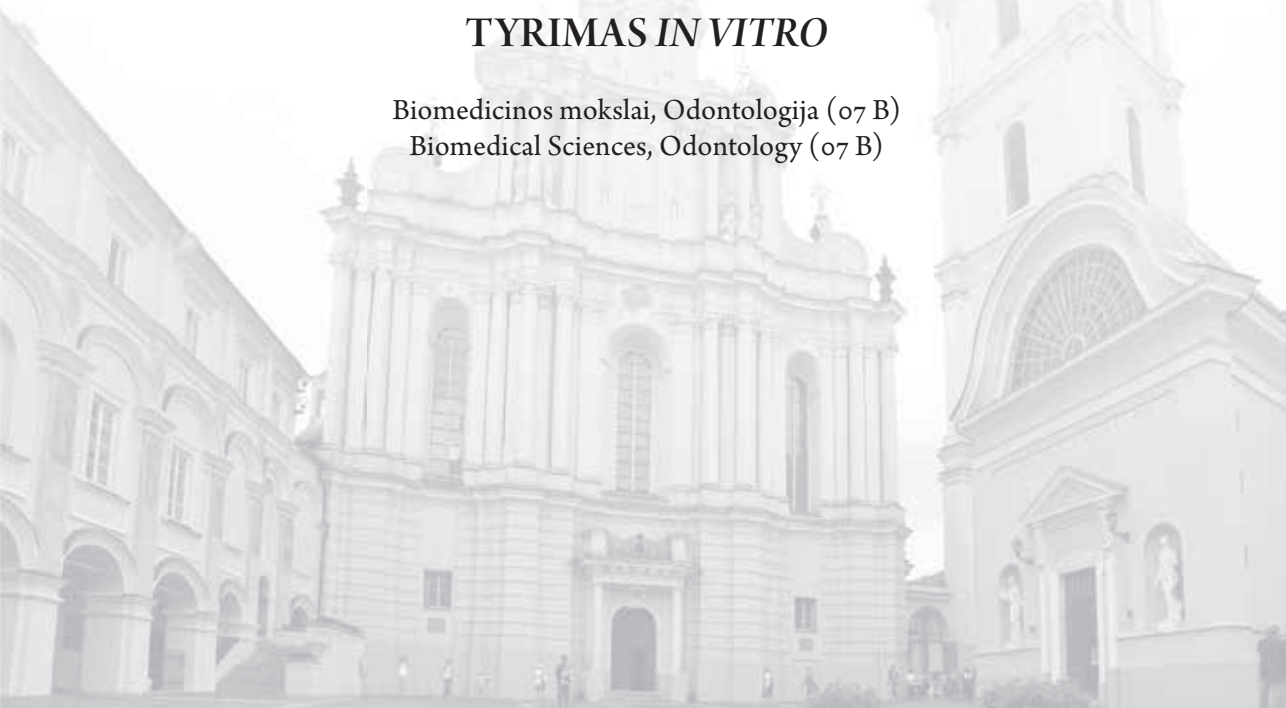
3. Remiantis šiuo darbu būtų galima plėsti Lietuvoje vykstančios „Aukštos kardiovaskulinės rizikos pirminės prevencijos programos“ rėmus ir rekomenduoti Lietuvos Respublikos sveikatos apsaugos ministerijai pradėti bent kartą per metus kompensuoti šią prevencijos priemonę metabolinį sindromą turintiems asmenims, siekiant ne tik pagerinti tokių asmenų fizinį pajėgumą ir treniruotumą, bet ir sumažinti jų kardiovaskulinę riziką bei užkirsti kelią aterosklerozės progresavimui. Siekiant tolesnių „Aukštos kardiovaskulinės rizikos prevencijos programos“ rezultatų, reikia ne tik vykdyti arterinės hipertenzijos ir lipidų kontrolę, bet ir ieškoti būdų modifikuoti metabolinį sindromą turinčių vidutinio amžiaus asmenų gyvensenai. Šis darbas vienas pirmų, kuriuo remiantis galėtų būti kuriama valstybės remiama parama fiziniam aktyvumui skatinti. Dviejų mėnesių aktyvių fizinių treniruočių programą, skiriamą bent kartą per metus, galėtų paremti valstybė ir darbdaviai.
4. Iš daugelio parametrų, kuriais šiame darbe buvo vertintas fizinio aktyvumo poveikis kraujagyslėms, tinkamiausi ir jautriausi yra aortos standumas ir vidutinis arterinis kraujo spaudimas aortoje, todėl juos rekomenduojama plačiai naudoti klinikinėje praktikoje.

Eglė Nedzinskienė

PECULIARITIES OF ULTRASTRUCTURE OF TOOTH ROOT CANALS TREATED WITH RESORCINOL FORMALDEHYDE RESIN, EVALUATION OF INFLUENCE OF ENDODONTIC RETREATMENT PROCEDURES AND RESISTANCE TO FRACTURE. STUDY *IN VITRO*

REZORCINO FORMALINU GYDYTŲ DANTŲ ŠAKNŲ KANALŲ ULTRASTRUKTŪROS YPATUMAI, ENDODONTINIO PERGYDYMO PROCEDŪRŲ POVEIKIO IR ATSPARUMO SKILIMUI VERTINIMAS. TYRIMAS *IN VITRO*

Biomedicinos mokslai, Odontologija (07 B)
Biomedical Sciences, Odontology (07 B)



Abreviations

BL – buccolingual direction
EN – Eglė Nedzinskienė
MD – mesiodistal direction
mm – millimeter
N – newtons
NiTi – nickel-titanium
RF – resorcinol-formaldehyde
s – second
SEM – scanning electron microscope
VRF – vertical root fracture
w/o – without

1. Introduction

1.1 Research question and its relevance

In China, India, France and all the Eastern Europe countries, resorcinol-formaldehyde (RF) paste has been used for root canal treatment for many years (1, 2). In Lithuania this method was widely applied almost until 1994. Consequently, dentists are still struggling to resolve endodontic retreatment problems related to the use of this material in clinical practice. Based on clinical experience, the retreatment of teeth that previously were treated with RF paste has unpredictable outcomes. Until today there have been no studies carried out proving the impact of the treatment procedures on the dentin surface of such teeth and its mechanical properties. Still, the results of other studies show that teeth after endodontic treatment are more prone to fracture (3).

During the last couple of decades the attitude towards the principles of root canal mechanical preparation has changed dramatically. This change is thought to be related to the implementation of new alloys, such as nickel-titanium (NiTi), into the manufacturing of endodontic instruments. A huge impact was also made by machine-driven instruments that started to be used for root canal preparation and significantly reduced the time of the procedure as well as the rate of the most common complications (4, 5, 6). As all new technologies, apart from the discussed benefits, this one also has certain drawbacks. One of them is an increased probability of root dentin surface cracks. As regards the main principles of mechanical root fracture, it has already been established that dentin surface defects, such as cracks or unevens in the canal wall, have influence on the decrease of tooth resistance to fracture (4, 10). In addition, in the instance of functional load, it can proliferate and become the reason for vertical root fracture (VRF) (11, 12, 13, 14)

Apart from the impact on the mechanical resistance of a tooth, cracks have one more important influence on the outcomes of endodontic treatment. They become a

niche for accumulation of bacterial colonies which resist treatment and may become the main reason of unsuccessful treatment (11).

The probability of dentin cracks during endodontic retreatment procedures is another important factor determining the prognosis of the treatment. First of all, it is related to a possibly harmful impact on the structures of the tooth which may be caused by the following factors: the instruments and materials that were used during the initial endodontic treatment, vertical force pressure used by the dentist, the way and quality of tooth crown restoration, occlusion forces load, and finally, the usage of aggressive instruments during retreatment procedures.

No studies have been carried out on the physical properties of RF paste as well as its impact on tooth tissue and ultrastructure of dentin and, therefore, the impact of the treatment using RF paste on tooth tissue is still unknown. Based on individual experience of dentists, it has been attested that such teeth are more brittle. The present study *in vitro* was aimed to evaluate the impact of endodontic retreatment procedures on teeth previously treated with RF paste.

1.2 The aim and objectives

The aim of the present study is to evaluate the peculiarities of the dentin surface ultrastructure in teeth previously treated with resorcinol-formaldehyde paste, test the reliability of diagnostic methods that are used in endodontology, and compare the influence of endodontic retreatment procedures on dentin and its resistance to fracture *in vitro*.

To achieve the aim of this study, the following objectives were set:

1. To evaluate the number, types, localization of dentin cracks after endodontic retreatment procedures have been performed.
2. To investigate the effectiveness of dye and different levels of microscopic magnification in order to detect dentin defects cracks of teeth previously treated with resorcinol-formaldehyde paste.
3. To investigate the resistance to fracture of root dentin in teeth previously treated with resorcinol-formaldehyde and pulp-vital teeth.
4. To evaluate the resistance to fracture of teeth previously treated with resorcinol-formaldehyde after endodontic retreatment procedures were performed, taking into account whether there are or no micro-cracks in the dentin.
5. To investigate the root canal surface and dentin ultrastructure of teeth previously treated with resorcinol-formaldehyde containing different tissue discoloration.

1.3 Scientific novelty and relevance

In the scientific literature there are only a few studies discussing the problems related to the treatment of teeth previously filled with RF paste. This may be related to the rare usage of this material type in dental practice. Even though this material has been used in Lithuania for a long time, there seem to be no studies carried out on its impact on the properties of the tooth tissue. In modern dentistry such teeth are considered to be in need of endodontic retreatment as well as coronal restoration procedures. In order to evaluate a possible impact of retreatment procedures on root dentin it was decided to carry out this study.

Rapid advance in technologies offers dentists more possibilities of using different combinations of instruments during treatment. The most common one is the combination of ultrasound devices and machine-driven instruments. Because of its wide application, this particular combination was chosen in the study to evaluate its impact on the root dentin surface of teeth previously treated with RF paste. The present study gives an in-depth analysis of the overall impact of the combination of both machine-driven and ultrasound instruments on root dentin. In addition, the reliability of diagnostic methods (i.e. dyeing with methylene blue and inspection under microscope), which are commonly used in dental clinical practice in order to detect dentin cracks in teeth previously treated with RF paste when their colour differs from the neutral colour of the tooth tissue, have been tested in the study.

Moreover, a study of resistance to fracture was carried out not only to evaluate the impact of load on root dentin after the endodontic retreatment but also to assess the level of the impact that dentin surface defects resulting from the retreatment can have on the tooth strength.

The results of the present study *in vitro* are difficult to be interpreted and directly related to the real clinical situation. Still, the results allow to make certain assumptions when it comes to the prognosis of endodontic retreatment of such teeth.

2. Material and methods

The institutional Ethics committee, Vilnius University hospital Zalgirio clinic, approved the study.

2.1 The impact of endodontic retreatment procedures on root dentin surface of teeth previously treated with resorcinol-formaldehyde paste

2.1.1 Preparation of tooth roots

Eighty permanent first mandibular molars with mature apices previously filled with resorcinol-formaldehyde paste were chosen. After extraction, all teeth were gently cleaned with a gauze and immediately placed into distilled water to avoid dehydration and were kept in it during the study. Such categories as patient age, sex and the reasons of extraction were unknown.

Distal roots were sectioned from the tooth crown using a water-cooled diamond bur *(MASTERtorque LUX M9000L, KaVo Dental GmbH, Bismarcking 39, D-88400 Biberach, Germany*) and only roots with a length of 12 mm were used in this study. Only roots with one canal (I type) (15) and without cracks were included. External surface and coronal section of the roots were observed with a microscope at the x 16 magnifications (*Zeiss Stemi SV6, Carl Zeiss, Jena*, *Germany*). The number of canals was evaluated radiographically. Radiographs were taken in the buccolingual and mesiodistal directions with a 3 cm distance between the X-ray tubuse and the root with a 0.08 ms exposition (*Planmeca Only, Helsinkii, Finland*). Roots were coded and immersed in the contents accordingly coded to roots.

2.1.2 Preparation of root specimens

2 mm of root coronal parts were fixed in resin for stability and then sectioned horizontally at the 3 mm and 9 mm distances from the anatomical apexes using a water-cooled low-speed saw (*Leica SP 1600*, *Wetzlar, Germany*). The surfaces of apical and coronal sections of the specimens were evaluated and photographs were taken under the x 10 and x 16 magnifications (*Zeiss Stemi SV6, Carl Zeiss, Jena*, *Germany*) using the same distance between the specimens and microscope object-glass. Two observers (endodontists) independently inspected all specimens in order to exclude the ones with cracks prior to retreatment procedures. Images of the specimens prior to the procedures (no cracks) served as the controls.

Mesiodistal (MD) and buccolingual (BL) diameters of the apical and coronal sections were measured three times and an average estimate was calculated in oder

to avoid deviations of root diameters (range of variations: MD 3.62 – 3.98 mm BL 6.34 – 6.69 mm, MD 2.99 – 3.14 mm BL 4.61 – 4.83 mm). The specimens were divided into two groups according to the hardness of resorcinol-formaldehyde paste in the root canals: soft filling material (Group 1) and hard filling material (Group 2). If a root canal patency could be gained with a #15 K file (*Dentsplay Maillefer, Bellaigues, Switzerland*), the filling material was considered soft and, if no patency was gained, the filling material was considered hard.

2.1.3 Chemomechanical preparation of specimen root canals

In order to simulate periodontal ligament space and mimic the mechanisms of stress distribution, a silicon impression material (*Panasil Putty Soft, Kettenbach, Gmbh and Co, Germany*) was used as matrix around roots during their canal preparation procedures.

Group 1 contained 40 specimens where the filling material was soft, i.e. a canal patency was easy to gain with a #15 K file (*Densplay Maillefer, Ballaigues, Switzerland*). The mechanical preparation of the root canals was done with Pro Taper rotary system using a full sequence of rotary files (SX, S1, S2, F1, F2, F3, F4, F5) (*Densplay Maillefer, Ballaigues, Switzerland*) following the manufacturer's recommendations. For this root canal preparation, an electric motor (*Densplay Maillefer, Ballaigues, Switzerland*) with a torque control at a constant speed of 300 rpm and gentle in-and-out motions were employed.

Group 2 contained 40 specimens where the filling material was hard and the *ProUltra Endo* tip # 4 (*Densplay Maillefer, Ballaigues, Switzerland*) and Master Piezon Scaler (*EMS SA, CH-1260 Nyon, Switzerland*) were used to remove the filling material. Subsequently, the Pro Taper instruments were used to enlarge all the canals as it was done for the specimens in Group 1.

A 2 ml 2% sodium hypochlorite rinse prior to each subsequent instrument was used and after the completion of procedures, the canals were rinsed with 2 ml of distilled water. All the treatment procedures were completed by the same operator (EN).

2.1.4 Evaluation of flat surfaces of root specimens

After the preparation of the root canal specimens, the images of apical and coronal flat surfaces of both group 1 and group 2 were taken under the x10 and x16 levels of magnification: (*Zeiss Stemi SV6, Carl Zeiss, Jena, Germany*). In order to evaluate the use of dye as an aid for the detection of dentin cracks in teeth previously treated with resorcinol-formaldehyde paste, the coronal and apical flat surfaces of slices of root dentin were stained for two minutes with Vista-Blue 2% methylene

blue dye (*Inter-Med/Vista Dental Products, Racine, JAV*) and subsequently rinsed with water. Similarly to the undyed specimens, the stained images were also examined under x10 and x16 levels of magnification: (*Zeiss Stemi SV6, Carl Zeiss, Jena, Germany*).

Three observers (endodontists) independently evaluated the microphotographs twice, with a two week interval between these evaluations. The number and type of dentin defects observed after the retreatment and dyeing were compared with the same specimen prior to the procedures (control condition). In cases of discrepancy among the three examiners, the images were re-inspected and a consensus was reached.

The following scheme for recording root defects was prepared, where the number of dentin defects in each specimen surface was recorded (if there were no defects – 0; if some defects were present, numbers 1, 2, 3, etc. were ascribed). Four types of defects were singled out for the evaluation. No defect – root dentin devoid of any cracks or lines (Figure 1). In complete fractures, the line extended from an inner root canal wall to an outer root surface (Figure 2). In incomplete (partial)

FIGURE 1. No defect

FIGURE 3. Incomplete fracture

FIGURE 2. Complete fracture

FIGURE 4. Intradental fracture

fractures, the line started from an outer or inner root surface but it did not extend throughout the whole dentin surface (Figure 3). In intradental fractures, the line was localized inside the dentin without reaching the outer or inner surface of the root (Figure 4).

2.2 Investigation of root dentin resistance to fracture

2.2.1 Preparation and selection of teeth

One hundred twenty permanent first mandibular molars with mature apices previously filled with resorcinol-formaldehyde paste (where a filling material was soft, i.e. a canal patency was easy to gain with a #15 K file (*Densplay Maillefer, Ballaigues, Switzerland*)) were chosen. For the negative control group, 40 permanent pulp-vital first mandibular molars with mature apices were chosen. The roots were prepared as it was done in 2.1.1 part., coded and immersed in the contents accordingly coded to roots.

2.2.2 Preparation of specimens

A total of 160 standardized roots were used to form four study groups (C1, E1, E2, and E3), each comprising 40 roots. 80 distal roots previously treated with RF paste were chosen randomly and root canal preparation was performed the way it was done in 2.1.3 part. The preparation and evaluation of root specimens were performed as described above in 2.1.2 part. The negative control group (C1) included the roots with intact pulp. The first experimental group (E1) included roots without micro-cracks which were previously treated with resorcinol-formaldehyde. No further treatments for the E1 group were initiated. The second experimental group (E2) included roots without micro-cracks which had previously been treated with RF paste and were retreated. The third experimental group (E3) included roots with micro-cracks which were previously treated with RF and received a similar retreatment as the roots from the E2 group.

2.2.3 The loading test

Prior to the loading, each root was embedded in a silicon impression material block (*Panasil Putty Soft, Kettenbach, Gmbh and Co, Germany*) to simulate a periodontal ligament space by mimicking its mechanism of stress distribution. The root width was measured at the buccolingual and mesiodistal directions for the apical and coronal sections of the specimens. The specimens were placed between the jaws of a universal testing machine (*Toni Technik GmbH-Gustav-Meyer-Allee 25-D-13355, Berlin, Germany*) and the loading forces were applied from the coronal end of a speci-

men to a slowly increasing vertical direction with the point of application being centered in the root canal. The loading speed of 250 N/s was applied until the specimen fractured. The "fracturing moment" was determined when a sudden drop in the force occurred that was observed in the display of the testing machine. The maximum force (F) required to fracture each specimen was recorded in Newtons (N).

2.2.4 Calculations of dentin strength index

An image of an apical surface of a specimen was evaluated in the graphic AutoCAD program. Using the buccolingual and mesiodistal diameters as landmarks, the synapse was drawn as indicated by the red line and subsequently an area of a synapse $\boldsymbol{A_0}$ was calculated (Figure 5). The green line corresponds to an apical outline of a specimen and the $\boldsymbol{A_{real}}$ is calculated automatically by the AutoCAD program. Considering the discrepancy (error) between the A_0 and A_{real} measurements, formulas were used to calculate the apical areas using the synapse (oval), the area of which was calculated from the BL (a) and MD (b) diameter via:

$$A_0 = \frac{\pi \cdot a \cdot b}{4}$$

The fracture to resistance was calculated using the following formula:

$$\sigma_{fr} = \frac{F_u}{A_0}$$

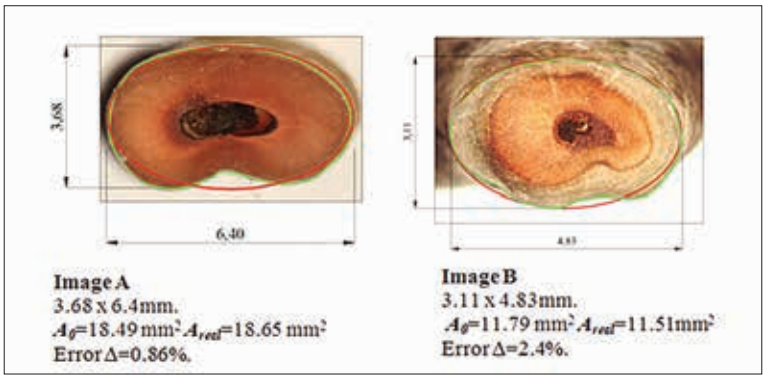


FIGURE 5. Calculation of the adjusted areas in the cross-sectional sections of specimens

2.3 SEM investigation of root canal surface of teeth previously treated with resorcinol-formaldehyde paste

Twenty extracted mandibular molars with mature apices previously treated with RF paste were used in this study. The removal of distal roots and observation for cracks were done as it was described above (2.1.1 part). Two groups according to the colour of the root were formed: group A–10 roots with discoloration of brownish-dark or red and group B–10 roots with conventional yellowish color. To facilitate vertical root fracture into two separate parts, longitudinal grooves on buccal and lingual surfaces of the root with a small fissure diamond bur were made avoiding penetration into the root canal space. The roots were splinted with a small chisel into two parts. These specimens were dehydrated in a graded series of ethanol solutions, attached to coded stubs, vacuum dried and observed with a scanning electron microscope (*ZEISS 1550VP, Zeiss, Germany*) in a back-scattered electron (BSE) mode. Photomicrographs at the apical, middle and coronal third of the root canals were taken at x200, x1000 and x2000 magnification.

Statistical analyses

The SPSS 21.0 software was used for all statistical analyses with a threshold for statistical significance set at $P<0.05$. For the comparison of defects identified after employing the two different methods (ProTaper versus ProTaper+Ultrasound) and for the evaluation of effectiveness of magnification to aid the crack identification, Chi-square or Fisher's exact tests were used. For the assessment of Odds Ratios having fractures after the two different preparation methods (ProTaper vs. ProTaper+Ultrasound), logistic regression was employed.

The univariate analysis (Shapiro-Wilk test) tested the data for normality in preparation for the inferential analyses (bivariate and multivariate).

The bivariate analysis (one-way ANOVA with a post hoc Dunnett adjustment) compared the resistance to fracture among the four study groups. The assumption regarding the homogeneity of variance was tested employing the Levene test. The multivariate analysis (linear multiple regression with three contrasts) was used to test the three study hypotheses. The absence of collinearity was considered if the tolerance values were below 0.500 and the Variance Inflation Factor (VIF) values were below 2.0.

The assumption of normality was fulfilled as indicated by a non-significant value according to the Shapiro-Wilk test ($P=0.549$). According to Levene's test, the assumption of the homogeneity of variance was also fulfilled ($P=0.565$).

3. Results

3.1 The impact of endodontic retreatment procedures on root dentin surface of teeth previously treated with resorcinol-formaldehyde paste

The percentage distribution of cracks in coronal third cross-section of the root is shown in figure 6. There were statistically significantly more complete fractures (p=0.05) and incomplete fractures from the outer root surface (p=0.01) in cases where methylene blue was used.

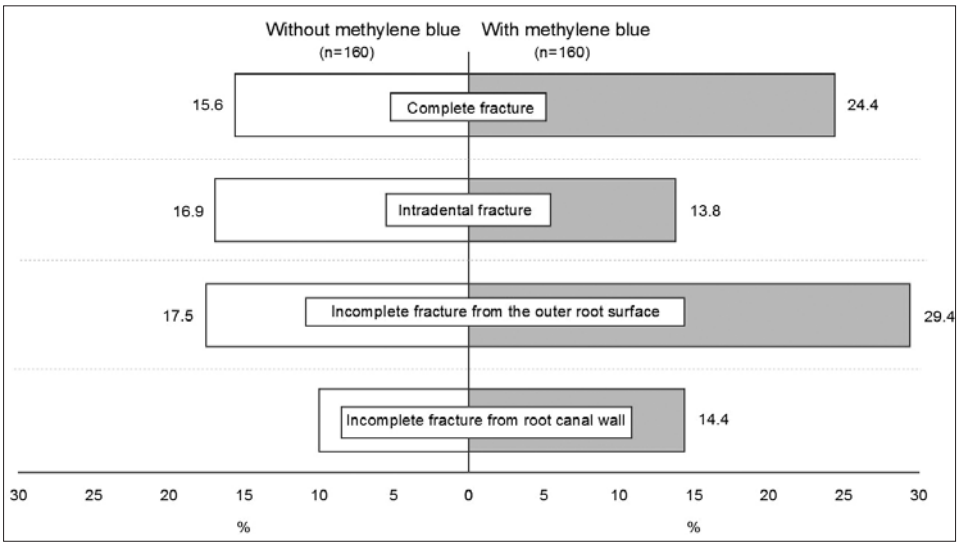


FIGURE 6. The percentage of cracks of root coronal surface

Table 1 compares the total number of root fractures of coronal cross-sectional surfaces after the root canal preparation with NiTi rotary ProTaper instruments (Table 1). There were statistically significantly more cracks (p=0.008) using x10 magnification after dyeing with methylene blue than in the cases when dye was not used.

Also more statistically significant (p<0.001) cracks were found after dyeing using x16 magnification.

An overall statistically significant trend was that more cracks were observed in the coronal surface as compared to the apical surface of the root. These findings were consistent with and without dyeing with methylene blue (Table 1).

TABLE 1. A total of root fractures of the first group (ProTaper), using x10 and x16 magnification, with and without dye

RESULTS: Any Root Fractures			Magnification x10#		
			No (%)	Yes (%)	p value#
ProTaper	Coronal	Without Methylene	32 (80.0)	8 (20.0)	0.008
		With Methylene	25 (62.5)	15 (37.5)	
	Apical	Without Methylene	40 (100.0)	0	1.000
		With Methylene	40 (100.0)	0	
COMPARISONS		**ProTaper: Coronal vs. Apical:** w/o Methylene (P=0.003) with Methylene (P<0.001)			
Magnification x16#					
ProTaper	Coronal	Without Methylene	35 (87.5)	5 (12.5)	<0.001
		With Methylene	24 (60.0)	16 (40.0)	
	Apical	Without Methylene	40 (100.0)	0	0.314
		With Methylene	39 (97.5)	1 (2.5)	
COMPARISONS		**ProTaper: Coronal vs. Apical:** w/o Methylene (P=0.021) with Methylene (P=0<0.001)			

Chi Square Test or Fisher Exact Test

The use of dye under x10 magnification did not significantly aid crack identification at any of root location in the second group, where the combination of ProTaper and Ultrasound instruments was used for root canal retreatment (Table 2). The use of dye statistically significanly (p=0.025) help to identify more cracks at the apical sross-sectional surface under x16 magnification.

Concomitantly, there were detected more cracks at coronal surface between two magnification levels in the ProTaper+Ultrasound group as it was in the ProTaper group (Table 2).

TABLE 2. A total of root fractures of the second group (ProTaper+Ultrasound), using x10 and x16 magnification, with and without dye

RESULTS: Any Root Fractures			Magnification x10#		
			No (%)	Yes (%)	p value#
ProTaper+ Ultrasound	Coronal	Without Methylene	21 (52.5)	19 (47.5)	0.655
		With Methylene	19 (47.5)	21 (52.5)	
	Apical	Without Methylene	37 (92.5)	3 (7.5)	0.456
		With Methylene	35 (87.5)	5 (12.5)	

RESULTS: Any Root Fractures			Magnification x10#		
			No (%)	Yes (%)	p value#
COMPARISONS		**ProTaper+Ultrasound: Coronal vs. Apical** with Methylene (P<0.001 w/o Methylene (P<0.001)			
Magnification x16#					
ProTaper+ Ultrasound	Coronal	Without Methylene	20 (50.0)	20 (50.0)	0.654
		With Methylene	18 (45.0)	22 (55.0)	
	Apical	Without Methylene	39 (97.5)	1 (2.5)	0.025
		With Methylene	33 (82.5)	7 (17.5)	
COMPARISONS		**ProTaper+Ultrasound: Coronal vs. Apical** with Methylene (P=0.001) w/o Methylene (P<0.001)			

Chi Square Test or Fisher Exact Test

Comparing the total number of root fractures after the two different root canal retreatment methods, in both specimens groups there were found more cracks at the coronal cross-sectional surfaces of roots.

The use of dye was effective in crack detection in the first (ProTaper) group, however in the second specimen group (ProTaper+Ultrasound) statistically significantly more cracks were identified without the use of methylene blue (Table 3).

When comparing the occurrence of cracks at the apical surfaces of the root between the groups, with or without the use of dye, more cracks were detected after ProTaper+Ultrasound preparation (Table 3), but there were no significant differences. However, the use of dye for crack identification at the apical surface of the root was statistically significant (x10 - p=0.021, x16 – p=0.025).

TABLE 3. A total of root fractures – comparison between the ProTaper and ProTaper+Ultrasound methods (x10 and x16 magnification, with and without dye)

RESULTS: Any Root Fractures		MAGNIFICATION x10#		
		1 group ProTaper N (%)	2 group ProTaper+Ultrasound N (%)	p value#
Coronal surface	Without Methylene	5 (12.5)	19 (47.5)	0.009
	With Methylene	16 (40.0)	21 (52.5)	0.178
Apical surface	Without Methylene	0 (0.0)	3 (7.5)	0.078
	With Methylene	1 (2.5)	5 (12.5)	0.021

RESULTS: Any Root Fractures		MAGNIFICATION x10#		
		1 group ProTaper N (%)	2 group ProTaper+Ultrasound N (%)	p value#
MAGNIFICATION x16#				
Coronal surface	Without Methylene	5 (12.5)	20 (50.0)	<0.001
	With Methylene	16 (40.0)	22 (55.0)	0.179
Apical surface	Without Methylene	0 (0.0)	1 (2.5)	0.315
	With Methylene	1 (2.5)	7 (17.5)	0.025

Chi Square Test or Fisher Exact Test

Comparing the different types of root fracture after the two different preparation techniques between the groups, with or without methylene blue under both magnifications, there were statistically significantly more complete fractures in the second specimen group (Table 4).

TABLE 4. Complete fractures – a comparison between the ProTaper and ProTaper+Ultrasound methods (x10 and x16 magnification, with and without dye)

RESULTS: Complete fractures			MAGNIFICATION x10#		
			1 group ProTaper N (%)	2 group ProTaper+Ultrasound N (%)	p value#
	Without Methylene	No	39 (58.2)	28 (41.8)	p=0.001
		Yes	1 (7.7)	12 (92.3)	
	With Methylene	No	35 (57.4)	26 (42.6)	p=0.020
		Yes	5 (26.3)	14 (73.7)	
p value#			p=0.090	p=0.633	
MAGNIFICATION x16#					
	Without Methylene	No	40 (58.8)	28 (41.2)	p <0.001
		Yes	0 (0.0)	12 (100.0)	
	With Methylene	No	35 (58.3)	25 (41.7)	p=0.010
		Yes	5 (25.0)	15 (75.0)	
p value#			p=0.021	p=0.478	

Chi Square Test or Fisher Exact Test

When comparing the occurrence of intradental cracks, in both specimen groups with or without dye and under both magnifications, there were statistically significantly more intradental fractures in the ProTaper+Ultrasound group (Table 5).

TABLE 5. Intradental fractures – a comparison between the ProTaper and ProTaper+Ultrasound methods (x10 and x16 magnification, with and without dye)

RESULTS: Intradental fractures			MAGNIFICATION x10#		
			1 group ProTaper N (%)	2 group ProTaper+Ultrasound N (%)	p value#
	Without Methylene	No	40 (58.8)	28 (41.2)	p<0.001
		Yes	0 (0.0)	12 (100.0)	
	With Methylene	No	40 (55.6)	32 (44.4)	p=0.003
		Yes	0 (0.0)	8 (100.0)	
p value#			p=1.000	p=0.302	
MAGNIFICATION x16#					
	Without Methylene	No	40 (61.5)	25 (38.5)	p<0.001
		Yes	0 (0.0)	15 (100.0)	
	With Methylene	No	40 (60.6)	26 (39.4)	p<0.001
		Yes	0 (0.0)	14 (100.0)	
p value#			p=1.000	p=0.816	

Chi Square Test or Fisher Exact Test

Similarly, although statistically non-significant findings were indicated when incomplete fractures from the outer root surface were compared between the ProTaper and the ProTaper+Ultrasound groups, using dye or not under both magnifications, there were more cracks detected in the ProTaper+Ultrasound group (Table 6). Identifications of incomplete fractures from the outer root surface were better when the dye was not used in both specimen groups.

TABLE 6. Incomplete fractures from the outer root surface – a comparison between the ProTaper and ProTaper+Ultrasound methods (x10 and x16 magnification, with and without dye)

RESULTS: Incomplete fractures from the outer root surface			MAGNIFICATION x10#		
			1 group ProTaper N (%)	2 group ProTaper+Ultrasound N (%)	p value#
	Without Methylene	No	35 (54.7)	29 (45.3)	p=0.093
		Yes	5 (31.3)	11 (68.8)	
	With Methylene	No	30 (54.5)	25 (45.5)	p=0.228
		Yes	10 (40.0)	15 (60.0)	
	p value#		p=0.152	p=0.340	

RESULTS: Incomplete fractures from the outer root surface			MAGNIFICATION x10#		
			1 group ProTaper N (%)	2 group ProTaper+Ultrasound N (%)	p value#
MAGNIFICATION x16#					
	Without Methylene	No	38 (55.9)	30 (44.1)	p=0.012
		Yes	2 (16.7)	10 (83.3)	
	With Methylene	No	32 (55.2)	26 (44.8)	p=0.133
		Yes	8 (36.4)	14 (63.6)	
	p value#		p=0.043	p=0.329	

Chi Square Test or Fisher Exact Test

Concomitantly, there were identified more incomplete fractures from the root canal in the ProTaper+Ultrasound group when comparisons were made between the two specimen groups with or without dye under both magnifications, but findings were not statistically significant (Table 7).

TABLE 7. Incomplete fractures from the root canal – a comparison between the ProTaper and ProTaper+Ultrasound methods (x10 and x16 magnification, with and without dye)

RESULTS: Incomplete fractures from the root canal		MAGNIFICATION x10#		
		1 group ProTaper N (%)	2 group ProTaper+Ultrasound N (%)	p value#
Without Methylene	No	38 (53.5)	33 (46.5)	p=0.077
	Yes	2 (22.2)	7 (77.8)	
With Methylene	No	37 (52.9)	33 (47.1)	p=0.176
	Yes	3 (30.0)	7 (70.0)	
p value#		p=0.064	p=1.000	
MAGNIFICATION x16#				
Without Methylene	No	37 (50.7)	36 (49.3)	p=0.692
	Yes	3 (42.9)	4 (57.1)	
With Methylene	No	33 (49.3)	34 (50.7)	p=0.762
	Yes	7 (53.8)	6 (46.2)	
p value#		p=1.000	p=0.500	

Chi Square Test or Fisher Exact Test

Table 8 presents RR Risk Ratio for different types of fractures in the coronal third of roots comparisons between the two groups (ProTaper vs. ProTaper+Ultrasound) using methylene blue and without it. The risk ratio of complete fractures was in the ProTaper+Ultrasound group.

TABLE 8. The risk of crack type of root coronal surface – a comparison between two groups using x10 and x16 magnification

ProTaper vs. ProTaper+Ultrasound Location: Root's Coronal Third		MAGNIFICATION x10			MAGNIFICATION x16		
		RR*	P-value	(95% CI)	RR*	P-value	(95% CI)
Complete fractures	Without Methylene	1.3	0.033	(1.0; 1.7)	0.1	<0.001	(0.0; 0.6)
	With Methylene	0.2	0.011	(0.1; 0.5)	0.3	0.009	(0.1; 0.8)
Incomplete fractures from the outer root surface	Without Methylene	0.5	0.081	(0.2; 1.2)	0.2	0.018	(0.1; 0.9)
	With Methylene	0.7	0.167	(0.3; 1.3)	0.6	0.105	(0.3; 1.2)
Incomplete fractures from the root canal	Without Methylene	0.3	0.077	(0.1; 1.3)	0.8	0.499	(0.2; 3.1)
	With Methylene	0.4	0.155	(0.1; 1.5)	1.2	0.500	(0.4; 3.2)

Chi Square Test or Fisher Exact Test *RR Risk Ratio

3.2 Investigation of root dentin resistance to fracture

The results of this testing are presented in Table 9. As expected, the highest resistance to fracture was found for the teeth with an intact pulp (C1 group) and the lowest resistance to fracture was observed in the E3 group which contained the retreated RF roots with micro cracks. For the C1 group, the mean ± sd was 227.5 ± 29.6 N/mm^2, for the E1 group it was 174.7±41.5 N/mm^2, for the E2 group it was 169.7± 31.4 N/mm^2, and for the E3 it was 150.5±11.4 N/mm^2.

TABLE 9. Resistance to fracture (N/mm^2) – comparisons among the study groups

Groups	Mean±sd	Difference #	95% CI#	P values*
C1-Negative control	227.5±29.6			
E1-Resorcinol formaldehyde w/o cracks	174.7±41.5	-53.2	-69.4; -37.1	<0.001
E2-Re-treated resorcinol formaldehyde w/o cracks	169.7±31.4	-57.2	-73.3; -41.1	<0.001
E2-Re-treated resorcinol formaldehyde with cracks	150.5±11.4	-77.5	-93.9; -61.3	<0.001

Mean difference from the mean of the control;

*One Way ANOVA with a Post Hoc Dunnett adjustment: comparisons with the control group

The linear multiple regression (LMR) employing the three independent contrasts tested the three study hypotheses. The results of this testing are presented in Table 10. The collinearity diagnostics showed that the assumption for the independence among the three contrasts was fulfilled, as indicated by the relatively high tolerance values (>0.65) and by the low VIF values (<1.0). The summary of the LMR model indicates that an overall linear regression model was highly statistically significant (P<0.001) and that the three contrasts, namely 1) the treatment with RF, 2) the retreatment of RF teeth without micro cracks, and 3) the retreatment of RF teeth with micro cracks, jointly explained 47% of variance (adjusted R-squared=0.47) in the study outcome (root resistance to fracture). It is important to note that each of the three contrasts, namely the risks decreasing the resistance to fracture as postulated by the three study hypotheses, had an independent effect even when controlled for the other two contrasts. The constant in the LMR model represents the value of the first contrast indicating the magnitude of RFR's detrimental effect (β=-

TABLE 10. Resistance to fracture (N/mm²) – comparisons among experimental teeth fractured under different study conditions (Linear Multiple Regression Analysis)
Summary: Adjusted R-Squared 0.47; F=47.221 (df=3); P<0.001

Condition	B (se)#	β (95% CI) *	P value	Tolerance	VIF Factor^
Constant	227.9 (4.8)		<0.001		
Contrast 1: Effect of the treatment with resorcinol formaldehyde (RFR) (controlled for the resistance to fracture of teeth with an intact pulp)	-53.2 (6.8)	-0.56 (-66.7; -39.8)	<0.001	0.67	1.50
Contrast 2: Effect of the retreating the RFR teeth (controlled for the resistance to fracture of teeth with an intact pulp and for the treatment with RFR)	-14.1 (5.9	-0.17 (-25.7; -2.5)	0.018	0.68	1.5
Contrast 3: Effect of the cracks (controlled for the resistance to fracture of teeth with an intact pulp, for the treatment with RFR and for the re-treatment of RFR teeth)	-10.1 (3.4)	-0.17 (-16.9; -3.4)	0.003	1.00	1.0

B (se)# Unstandardized regression coefficient (standard error)

β (95% CI) * Standardized regression coefficient (95% Confidence Interval)

^ VIF Factor Variance Inflation Factor

0.56; p<0.001). The second contrast which represents the negative effect due to the retreatment of non-cracked RFR roots was also statistically significant (β=-0.17; P=0.0018). The third contrast or the detrimental effect due to the retreatment of cracked RFR roots was also statistically significant (β=-0.17; P=0.003).

More details about the resistance to fracture observed in different groups and their visual comparison are presented in Figure 8, where the box and whisker plots, one for each group, represent the distribution of the resistance to fracture dependent upon the experimental condition. There was a clear, increasing pattern of decreased resistance to fracture as more risks were involved. Roots in the negative control group (C1) were most resistant to fracture and this group had a substantially less intra-group variation as compared to the other groups. The least resistant group to fracture was the cracked roots of the RF teeth that were also retreated.

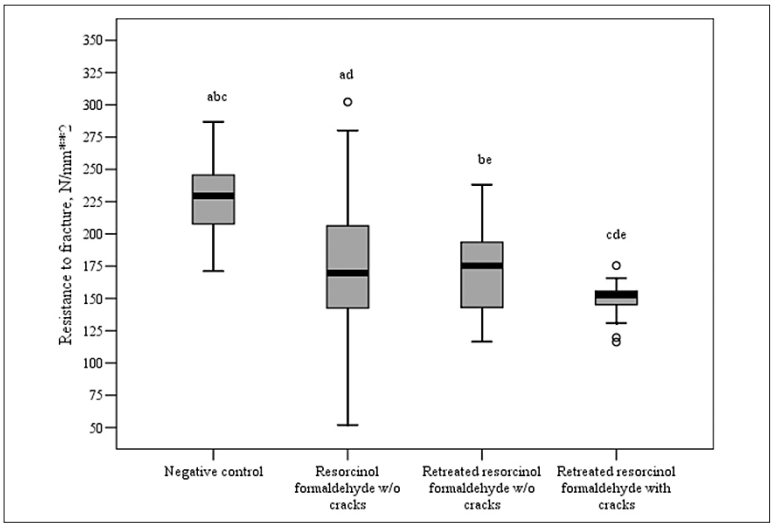
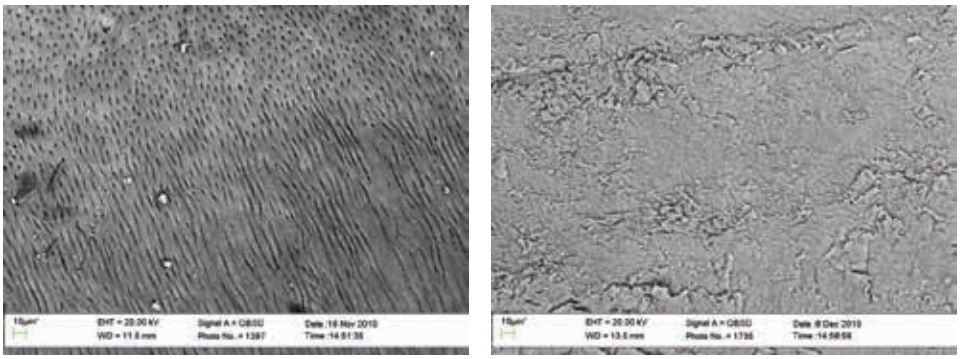


FIGURE 8. Study groups and their sequencing in the linear regression analysis

3.3 SEM investigation of root canal surface of teeth previously treated with resorcinol- formaldehyde paste

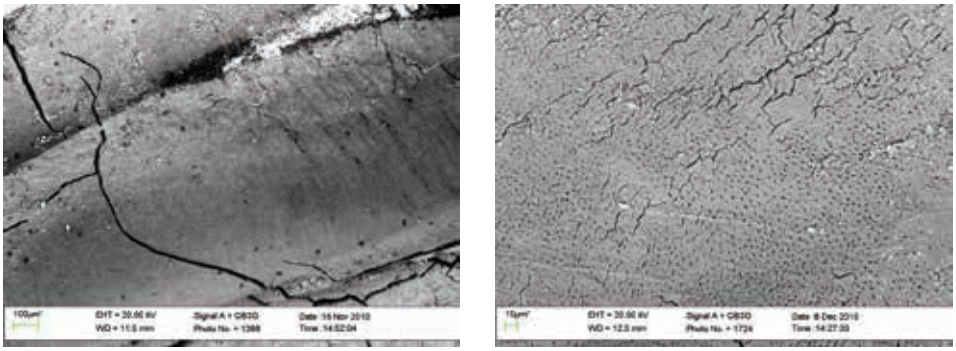
In the brownish-red root scanned specimens smear layer was not detected, but exhibited varying amounts of remaining debris and RF paste on the surface of the root canals. The fields without coverage with filling material were found in all the samples. In those areas the morphology of dentin was ordinary and open dentinal tubules were clearly seen (Fig.9a). While in the samples of non-stained roots the dentin profile was sufficiently unusual. In the coronal thirds just few open dentinal tubules were detected while in the middle and apical thirds no open dentinal tubules were observed and dentin profile was similar to sclerotic dentin (Fig. 9b).



a b

FIGURE 9. The appearance of root wall dentin of RF treated tooth. Ordinary dentin morphology of colored root sample at the middle third of the root canal (a). The apical third of root canal of non-colored sample: dentin profile similar to sclerotic dentin (b). Original magnification x1000

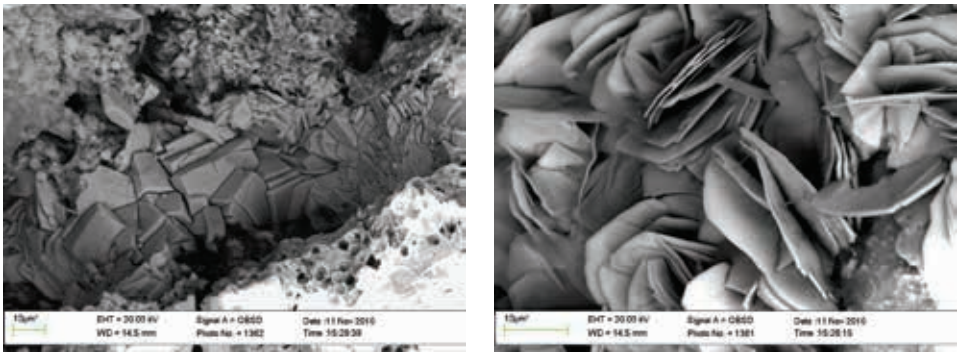
The multiple dentinal defects (infractions and cracks) were detected in all thirds of scanned specimens at all magnifications, however visually significantly more dentinal defects were detected in the brownish-red roots in comparison with the non-colored root samples (Fig. 10a,b).



a b

FIGURE 10. Multiple dentinal defects of discolored root dentin. Original magnification x200 (a) ir x1000 (b)

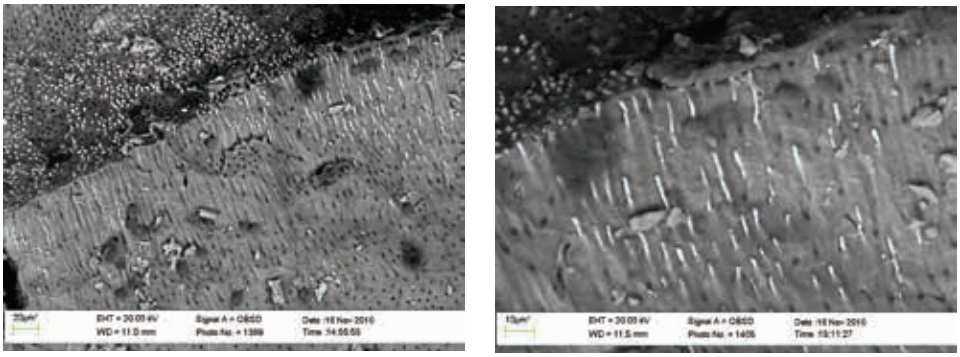
The mass of RF paste lying on the root canal surface was detected in all the specimens of colored roots, however usually just in the coronal and partially in the middle third of root canals (Fig. 11a). At a higher magnification the crystals of RF paste compounds were detected (Fig. 11b).



a b

FIGURE 11. The mass of RF paste on the root canal wall (a). Original magnification x1000. Crystals in RF based material (b). Original magnification x2000

Examination with SEM revealed good adherence of RF resin to the root canal dentin wall. The multiple solid and quite polymerized tags of RF paste deeply penetrated into the dentinal tubules in all root canal third of the brown or red root sample (Fig. 12a,b).



a b

FIGURE 12. Multiple tags of RF paste penetrated in dentinal tubules. Original magnification x1000 (a) and x2000 (b).

3. Discussion

Endodontic retreatment involves a number of procedures where different instruments and materials are used and all of them have different effect on the surface of the tooth root canal wall. During the mechanical root canal preparation for the initial endodontic treatment, the dentin surface is first exposed to direct mechanical

forces (vertical instrument pressure, friction that is created between the instrument and the root walls, etc.). In cases of retreatment the tooth is already experiencing repeated mechanical force as well as ultrasound vibration. The use of different combinations of instruments for treatment determines the accumulative effect of performed treatment on the root dentin surface (7, 16). Root canal filling with RF paste techniques differed not only in their specifics but also in the ability to change the tooth colour. When using this technique, modern chemomechanical standards of the root canal preparation were not applied (1, 2). Therefore it can be stated that, differently from many conventional endodontic treatments, the dentin of the tooth root canal has been affected by long-term chemical effect of RF paste.

Retreatment procedures typically make up a large portion of a dentist's daily work load. Despite the prohibition by both the European Union Directive (17) and the European Society of Endodontists (18), on the use of resorcinol-formaldehyde paste as root canal filling material, roots filled with this material still constitute a substantial part of retreatment cases in clinical dental practice.

In the present study, the machine-driven system ProTaper (*Densplay Maillefer, Ballaigues, Switzerland*) for mechanical preparation of the root canal was chosen because these instruments are used in many countries. The previous *in vitro* studies have reported that the use of rotary instruments may cause cracks in root dentin (19, 20). The machine-driven system has progressively varying taper and removes relatively more of the dentin coronally as compared to other systems (19). Thus, the taper preparation could be a contributing factor to the generation of dentin cracks (19). During canal preparation, the canal is shaped via contact between the instrument and the dentinal walls, creating a momentary stress concentration in the dentin which may lead to dentinal defects (19, 20).

Similar to other *in vitro* studies (13, 14, 16, 19), we employed the sectioning method that allows a direct inspection of root dentin, which is impossible in clinical practice. This method allowed us to evaluate the impact of the retreatment on root dentin of teeth previously treated RF paste.

The recent study attempted to mimic the technical procedure of the root canal mechanical preparation and load testing as it is done in a clinical setting. In order to simulate stress absorption, during the present experiment a silicon layer surrounding the specimen was used as it was done in the other studies (13, 20, 22-24).

It is also important to emphasize that irrigation with NaOCl can significantly decrease the elastic modulus and flexural strength of dentin (25-27). In order to mimic the clinical situation where 2% NaOCl is commonly used, the solution of the same concentration was used in this study.

The important findings of the present *in vitro* study were that both the ProTaper and ProTaper combined with ultrasound preparation techniques had harmful

effects on root dentin, as both techniques led to crack development in roots previously treated with resorcinol-formaldehyde paste. Most of the cracks were observed in the coronal rather than in the apical location of roots. Seemingly the ProTaper combined with the ultrasound preparation technique is more damaging to roots as compared to the ProTaper alone. Namely, the use of combination of instruments induced complete fractures and intradental cracks in root dentin. To identify cracks in such roots, two types of aids were considered; the use of different (x10 and x16) magnifications and the use of dye. Seemingly, there was no benefit in using higher level of magnification to identify the cracks. But, the use of methylene blue dye to assist crack identification was more effective in the group where the ProTaper alone was used for root canal preparation of teeth previously treated with RF paste. It is likely that methylene blue can be effectively used for a small craze line detection.

Shemesh et al. also found that the retreatment groups developed more defects than the primary treated groups and stated that retreatment procedures require more mechanical manipulations in the root canal; consequently, more dentin tissue is removed from root canal walls in retreatment cases (7).

It is still considered that teeth after endodontic treatments become weaker and are more prone to fracture than vital teeth (28). The strength of endodontically treated teeth has been analysed in several studies and has been associated with proprioreceptors (29), dehydration (30), lost of tooth tissue (29), mechanical preparation (31), irrigation (26) and intracanal medicaments (32), and extent of remaining tissues (33). Consequently, it is important to note that the strength of a tooth may be compromised due to mechanical root preparations and it can be concluded that the remaining volume of the dentine after root canal preparation is most relevant to tooth strength (34).

The present experiment evaluated only vertical loading, even though occlusal forces in oral environment are complex and diverse (35). We studied resistance to fracture of teeth previously treated with RF paste under different study conditions. Comparisons of resistance to fracture were made not only among the three experimental groups but also with pulp-vital teeth. The control group of teeth with vital pulp demonstrated the highest resistance to fracture, and the least resistance to fracture was found in the group which contained retreated roots with micro- cracks.

It is important to note that the present study *in vitro* clearly demonstated a cumulative detrimental effect to the risks of tooth strength, namely the use of the RF material, the retreatment of teeth treated with the RF paste, and the impact of the micro-cracks present in such retreated teeth. It is known that there is a possibility of undiagnosed cracks during endodontic retreatment. Our experiment demonstrated that the presence of cracks after retreatment poses an additional risk factor to the resistance to fracture of root dentin. These are considerable risks that may be encoun-

tered in clinical endodontic practices. However, any broad generalizations from the present findings should be drawn with caution, mainly due to the fact that we used an *in vitro* design to test the resistance to fracture.

In order to better investigate the root canal surface of teeth previously treated with RF paste, observation with a scanning electron microscope (SEM) was done. SEM is a popular tool for the visualization of surfaces of the root canal wall in order to observe dentinal tubules, the smear layer, various root canal filling materials and their contact with root dentin (36). In multi-rooted teeth treated with RF paste different discoloration of roots is often observed. Subsequently, it could be assumed that a possible effect of RF paste on dentin might be different. Therefore extensively discolored roots and roots without discoloration were used in this study in order to identify possible differences of the effect of RF paste on root dentin.

The root canal surfaces of scanned discolored root specimens had a usual profile, which could be observed by SEM. The smear layer was not detected as root canals were not cleaned and shaped. It should be mentioned that often clinicians did not have a goal to obturate root canals with RF paste to the entire length due to a strong believe that underlying pulp tissue could be fixated and this would ensure good treatment results (1). The most surprising finding of this study was the profile of root canal dentin of non-discolored root specimens. The morphology of dentin was quite unusual and similar to sclerotic dentin as only a few open dentin tubules were detected in the coronal part. It is quite difficult to explain our findings. It could be that sclerotic dentin is related to tubular sclerosis – a well known physiological phenomenon that starts in the apical root part and adverse coronally (37). Another factor influencing obliteration of tubules could be the toxicity of components of RF paste. It could be supposed that underlying pulp-vital tissues could provoke obliteration of dentinal tubules, because in the non-discolored roots endodontic treatment procedures were not performed as after amputation of the pulp the mixed RF paste was placed on the floor of pulp chamber.

In the present study multiple dentin defects were detected in all the scanned specimens despite the colour of roots. It should be mentioned that the defects were localized on the inner region of root canal walls. It might be assumed that the reason for the observed dentin defects could be the breakdown of the protein structure caused by the components of RF paste. Obviously more dentin defects were observed in the discolored roots where the canals were partially obturated with RF paste.

The results of this study showed that RF paste possesses good adhesion and deep penetration into dentinal tubules. The penetration depth could be directly related to flow ability. However, RF paste is not a premixed material and due to the lack of strict mixing recommendations the consistency could vary from thin to thick and it directly relies on liquid/power ratio.

Even though teeth that were treated with RF paste started getting a historical aspect, dentists still face a number of challenges in this field. Our study evaluates the impact of retreatment procedures on teeth previously treated with RF paste. The study investigates the following issues: the impact of retreatment procedures on frequency of tooth root cracks, the resistance of the root to fracture as well as the effect of RF paste on root dentin. All these factors could be important from the clinical perspective as they may be the reason of non-healing periapical destruction. The outcome of endodontic treatment depends on a multitude of mutually related factors that cannot be accounted for as a whole in one study. Thus the results obtained from both in *vitro* and *in vivo* studies could help a dentist to get a more accurate view towards evaluating the influence these factors may have on the outcome of the treatment.

4. Conclusions

1. The use of NiTi instruments alone as well as the combination of NiTi instruments and ultrasound devices during endodontic retreatment results in a damaging effect on root dentin. The usage of both methods cause more dentin cracks in coronal third of the root. The usage of the instruments combination method causes a higher number of cracks in the root, – complete fractures as well as intradental fractures of dentin being more frequent among them.
2. The identification of dentin cracks in teeth previously treated with RF paste was more effective when a combination of dye and microscope inspection was used.
3. The roots of teeth previously treated with RF paste are less resistant to fracture than teeth with vital-pulp.
4. The endodontic retreatment of teeth previously treated with RF paste reduces the resistance to fracture of root dentin and increases the risk of vertical fracture. Resistance to fracture significantly decreases by the presence of micro-cracks during retreatment.
5. RF paste not only causes different colour changes of the tooth tissue but also has a different impact on dentinal tubules. The structure of root dentin of discoloured teeth is typical, while the dentinal tubules of teeth that have no changes in colour are totally sclerotic. It is thought that the chemical changes in dentine that were caused by this material have an impact on defects in the dentin of both discoloured teeth and teeth with normal colour. RF paste has features of homogeniety as well as penetration into dentinal tubules.

6. Practical recommendations

1. During endodontic retreatment procedures of teeth that were previously treated with RF paste, dentists are recommended to avoid any long term usage of ultrasound instruments as well as rotary machine-driven instruments that have a large taper. These systems can be used after assesing the clinical need and reducing the time of usage to minimum. During the treatment with ultrasound instruments a continuous water cooling is necessary. It is also recommended to use instrument systems of smaller taper for root canal preparation.
2. In instances when any type of instrument is used it is recommended to carry out an early diagnostics on possible cracks after every additional endodontic retreatment procedure. The best way to perform diagnostics is by using dye – methylene blue – and magnification. This should be done keeping in mind the fact that the possibility of undiagnosed cracks still remains.
3. To evaluate the prognosis of a tooth previously treated with RF paste after the performed endodontic retreatment as well as risks and its suitability for abutment. It is recommended to avoid a large fixed dental prosthesis on such teeth.

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SANTRAUKA

Įvadas

Ilgą laiką Kinijoje, Indijoje, Prancūzijoje ir visose Rytų Europos šalyse šaknų kanalų gydymui buvo naudojama rezorcino formalino (RF) pasta (1, 2). Lietuvoje šis metodas buvo plačiai taikomas beveik iki 1994 metų. Todėl gydytojai odontologai iki šiol klinikinėje praktikoje sprendžia su šios medžiagos naudojimu susijusias endodontinio pergydymo problemas. Klinikinė patirtis leidžia teigti, kad rezorcino formalino pasta gydytų dantų pergydymas siejamas su sunkiai nuspėjama gydymo baigtimi. Iki šiol nebuvo atlikta tyrimų, nagrinėjančių gydymo procedūrų įtaką tokių dantų dentino paviršiui, jo mechaninėms savybėms. Tyrimų rezultatai rodo, kad endodontiškai gydytų dantų polinkis skilti yra didesnis (3).

Endodontinis (per)gydymas apima nemažai procedūrų, kurioms naudojamos skirtingos priemonės, pasižyminčios skirtingu poveikiu danties šaknies kanalo sienos paviršiui. Atliekant mechaninį šaknies kanalo ruošimą pirminio endodontinio gydymo metu, dentino paviršius pirmą kartą yra veikiamas tiesioginių mechaninių jėgų (vertikalaus instrumento spaudimo, trinties, sukuriamos tarp instrumento ir šaknies sienelių, ir kt.), o pergydymo atveju dantis patiria jau pakartotinį mechaninių jėgų, dažniausiai ir ultragarsinės vibracijos poveikį.

Per pastaruosius du dešimtmečius požiūris į šaknies kanalo mechaninio ruošimo principus pasikeitė iš esmės. Tai siejama su naujų lydinių, pavyzdžiui, nikelio ir titano (NiTi), įdiegimu į endodontinių instrumentų gamybą bei vis dažniau šaknų kanalų ruošimui naudojamais mašininiais instrumentais, kurie labai sutrumpino gydymo procedūros atlikimo trukmę, sumažino dažniausiai pasitaikančių komplikacijų skaičių (4, 5, 6). Technologijų pažanga gydytojams odontologams suteikia galimybę gydymui naudoti skirtingo tipo instrumentų derinius, o endodontiniam pergydymui dažniausiai naudojamas ultragarsinių ir mašininių instrumentų derinys. Kaip ir kiekviena nauja technologija, be minėtų pranašumų, ji yra siejama ir su tam tikrais trūkumais. Vienas iš jų yra šaknies dentino paviršiaus defektų didesnė tikimybė (4, 7, 8, 9). Dentino skilimų tikimybė atliekant endodontinio pergydymo procedūras yra dar vienas labai svarbus veiksnys, galintis turėti įtakos tokio tipo gydymo prognozei. Mikroskilimai, kanalo sienos paviršiaus netolygumai, turi įtakos danties atsparumo lūžiui mažėjimui (4, 10), o dėl funkcinio krūvio gali plisti ir sukelti vertikalų šaknies lūžį (11, 12, 13, 14).

Skilimai, be poveikio danties mechaniniam atsparumui, turi dar vieną svarbią reikšmę endodontinio gydymo baigčiai. Jie tampa bakterijų kolonijų sankaupų niša, apsaugota nuo gydomųjų veiksmų poveikio, o tai gali tapti tiesiogine gydymo nesėkmės priežastimi (11).

Rezorcino formalino pastos fizikinės savybės, jos poveikis danties audiniams ir dentino ultrastruktūrai nebuvo tirti ir todėl visiškai nežinomas gydymo naudojant šią medžiagą poveikis danties audiniams. Tik remiantis individualia klinikine gydytojų patirtimi yra teigiama, kad tokie dantys pasižymi didesniu trapumu. Šiuo tyrimu *in vitro* buvo siekiama ištirti atliekamų endodontinio pergydymo procedūrų įtaką dantims, gydytiems rezorcino formalino pasta.

Darbo tikslas – ištirti dantų, gydytų rezorcino formalino pasta, dentino paviršiaus ultrastruktūros ypatumus, naudojamų endodontijoje diagnostikos metodų patikimumą, endodontinio pergydymo procedūrų poveikį dentinui ir jo atsparumą skilimui *in vitro*.

Darbo uždaviniai

1. Įvertinti dentino skilimų skaičių, tipus, jų lokalizaciją po atliktų endodontinio pergydymo procedūrų.
2. Ištirti dažo medžiagos ir skirtingų mikroskopo didinimų efektyvumą vertinant rezorcino formalino pasta gydytų dantų skilimus.
3. Ištirti dantų, gydytų rezorcino formalino pasta ir turinčių gyvą pulpą, šaknies dentino atsparumą skilimui.
4. Įvertinti dantų gydytų rezorcino formalino pasta atsparumą skilimui po atlikto endodontinio pergydymo procedūrų, esant dentine mikropažaidų ir jų nesant.
5. Ištirti rezorcino formalino pasta gydytų dantų, turinčių skirtingą audinių spalvą, šaknų kanalų paviršių ir dentino ultrastruktūrą.

Medžiaga ir metodai

Tyrimui atlikti gautas VšĮ VUL Žalgirio klinikos etikos komiteto leidimas Nr. EK-2.

Endodontinio pergydymo procedūrų poveikio šaknies dentino paviršiui tyrimui buvo įtraukta rezorcino formalino pasta gydytų 80 pašalintų pirmųjų apatinio žandikaulio krūminių dantų susiformavusiomis šaknų viršūnėmis, kurių paviršiuje nebuvo matoma jokių skilimo požymių. Suformuotos dvi grupės, remiantis kanalo užpildo medžiagos kietumu ir tyrėjo gebėjimu praeiti šaknies mėginio kanalo spindį. Vertinta ir lyginta šaknies dentino skilimų skaičius ir tipai šaknies vainikinio ir viršūninio skerspjūvio paviršiuose, dažymo metileno mėlynuoju ir naudojimo skirtingo didinimo mikroskopą apžiūrai nauda skilimų diagnostikai.

Šaknies dentino atsparumo skilimui tyrimui buvo atrinkta 120 išrautų apatinio žandikaulio pirmųjų krūminių dantų susiformavusiomis šaknų viršūnėmis, kurių šaknų kanalai anksčiau pildyti minkštos konsistencijos rezorcino formalino pasta ir 40 apatinio žandikaulio pirmųjų krūminių dantų, turinčių susiformavusias šaknų viršūnes ir gyvybingą pulpą. Buvo suformuotos keturios mėginių grupės. Vertinta RF pastos ir endododontinio pergydymo įtaka (nesant ir atsiradus mikroskilimams jo metu) įtaką šaknies dentino atsparumui skilimo ir lyginta tarp eksperimentinių grupių ir su kontroline grupe - gyvybingą pulpą turinčių dantų.

Šaknų kanalų paviršiaus tyrimui SEM naudota 20 išrautų apatinio žandikaulio pirmų krūminių susiformavusiomis šaknų viršūnėmis dantų, kurių šaknų kanalai buvo pildyti rezorcino formalino pasta. Buvo sudarytos dvi mėginių grupės, priklausomai nuo danties audinių spalvos (1 grupė - būdingi šiai šaknų kanalų pildymo metodikai audinių spalvos pokyčiai - nuo rausvos iki tamsiai rudai raudonos spalvos, 2 grupė – turinčių natūralaus dentino audinių spalvą). Mikronuotraukos buvo atliktos x200, x1000 ir x2000 didinimais, vainikiniame, viduriniame ir viršūniniame šaknų kanalų paviršių trečdaliuose ir palyginta tarp grupių.

Rezultatai

Palyginus bendra dentino skilimų skaičių po endodontinio pergydymo atlikto PtoTaper NiTi mašininiais bei ProTaper NiTi mašininių ir ultragarsinių instrumentų deriniu, naudojant 10 ir 16 kartų didinimą ir dažo medžiagą ar jos nenaudojant, abiejose grupėse statistiškai

reikšmingai daugiau skilimų buvo nustatyta vainikinio šaknų mėginių trečdalio skerspjūvių paviršiuose.

Identifikuotų skilimų skaičius buvo didesnis 1 mėginių grupėje naudojant dažo medžiagą, o 2 grupėje statistiškai reikšmingai daugiau skilimų buvo rasta nenaudojant dažo medžiagos.

Šaknų viršūninių trečdalių paviršiuose tiek naudojant dažo medžiagą tiek jos nenaudojant išliko tokia pati tendencija kaip ir vainikinių trečdalių paviršiuose – skilimų diagnozuota daugiau naudojant instrumentų derinį, bet skirtumas nebuvo statistiškai reikšmingas.

Atlikus skirtingų dentino skilimų tipų radimo dažnio palyginimą tarp 1 ir 2 mėginių grupių, naudojant 10 ir 16 kartų didinimą be dažo medžiagos ir su dažo medžiaga, statistiškai reikšmingai daugiau visiškų ir vidinių dentino skilimų rasta *ProTaper* ir ultragarsinių instrumentų derinio grupės mėginiuose. Statistiškai reikšmingai daugiau dalinių skilimų nuo išorinio šaknies paviršiaus rasta taip pat 2 mėginių grupėje, abiejose grupėse jų daugiau nustatyta naudojant dažo medžiagą, bet skirtumai nebuvo statistiškai reikšmingi.

Palyginus keturias tiriamąsias grupes, didžiausias šaknų mėginių dentino atsparumas skilimui buvo gyvybingą pulpą turinčių dantų (C1 grupėje, neigiama kontrolė), o mažiausias nustatytas grupėje, kurioje po pergydymo buvo rasta dentino skilimų (E3 grupėje) (12 lentelė). C1 grupėje atsparumo skilimui vidurkis buvo 227,5±29,6 N/mm^2, E1 grupėje – 174,7±41,5 N/mm^2, E2 grupėje – 169,7±31,5 4/mm^2 ir E3 grupėje – 150,5±11,4 N/mm^2.

Rudai raudonos spalvos šaknų skenuotų SEM mėginių kanalo paviršiuje dentino morfologija buvo įprasta ir buvo aiškiai matomi atviri dentino kanalėliai, o vertinant natūralaus dentino spalvos šaknų kanalų paviršius, dentino vaizdas buvo neįprastas. Visų mėginių vainikiniame šaknies kanalo trečdalyje buvo matomi pavieniai atviri dentino kanalėliai, o viduriniame ir viršūniniame kanalo trečdaliuose vaizdas buvo labai panašus į sklerozinio dentino

Visuose abiejų eksperimentinių grupių šaknų kanalų trečdaliuose buvo matomi daugybiniai šaknies dentino defektai, tačiau vizualiai gerokai daugiau defektų buvo matoma rudai raudonų šaknų mėginiuose negu natūralios dentino spalvos šaknų mėginiuose.

SEM vertinimas parodė rezorcino formalino pastos gerą sukibimą su šaknies kanalo dentinu. Visuose rudai raudonos spalvos šaknų mėginių kanalų trečdaliuose matomi daugybiniai vientisi ir gana polimerizuoti rezorcino formalino pastos „siūlai“ (angl. *tags*), giliai įsiskverbiantys į dentino kanalėlius.

Išvados

1. Endodontiniam pergydymui naudojami mašininiai nikelio ir titano lydinio instrumentai bei jų ir ultragarsinių instrumentų derinys turi žalingą poveikį šaknies dentinui. Abu metodai sukelia daugiau skilimų šaknies vainikiniame trečdalyje. Naudojant instrumentų derinius, šaknyje susidaro daugiau skilimų, tarp kurių gerokai dažnesni yra visiški ir vidiniai dentino skilimai.
2. Rezorcino formalinu gydytų dantų dentino skilimai identifikuojami efektyviau, jei naudojama ir dažo medžiaga, ir apžiūra mikroskopu.
3. Rezorcino formalino pasta gydytų dantų šaknys mažiau atsparios skilimui nei gyvybingą pulpą turinčių dantų.

4. Rezorcino formalino pasta gydytų dantų endodontinis pergydymas sumažina atsparumą šaknies dentino skilimui, padidindamas vertikalaus skilimo riziką. Pergydant šaknis atsiradę dentino skilimai labai sumažina jo atsparumą skilimui.
5. Rezorcino formalino pasta ne tik sukelia skirtingus danties audinių spalvos pokyčius, bet ir turi skirtingą poveikį dentino kanalėliams. Pasikeitusios spalvos dantų šaknų dentino struktūra tipiška, o spalvos pokyčių neturinčių dantų šaknų dentino kanalėliai yra visiškai sklerozavę. Manoma, šios medžiagos sukelti cheminiai dentino pokyčiai turi įtakos ir pasikeitusios, ir nepakeitusios spalvos dantų šaknies dentino skilimams. Rezorcino formalino pasta pasižymi homogeniškumu ir skvarbumu į dentino kanalėlius.

Arūnas Rimkevičius

THE STUDY OF PATIENTS WITH ORAL LICHEN PLANUS: CLINICAL INDICES, RISK FACTORS AND QUALITY OF LIFE

SERGANČIŲJŲ BURNOS PLOKŠČIĄJA KERPLIGE KLINIKINIŲ RODIKLIŲ IR RIZIKOS VEIKSNIŲ BEI GYVENIMO KOKYBĖS TYRIMAS

Biomedicinos mokslai, Odontologija (07 B)
Biomedical Sciences, Odontology (07 B)

Abbreviations

HPV – human papilloma virus
HIV– human immunodeficiency virus
HCV – hepatitis C virus
HBV – hepatitis B virus
SLV – systemic lupus erythematosus
GVH – „*Graft versus host*“ disease
HSV – *herpes simplex* virus
FFA – fibrotic frontal alopecia
MDA – malondialdehyde
DNA- deoxyribonucleic acid
df – number of degrees of freedom
mln – million
n – absolute term
p – statistical significance
proc. – per cent
PI – Confidence interval
SD – Standard Deviation
χ^2 – chi-square test
Žalgiris Clinics – Žalgiris Clinics of Vilnius University Hospital

1. Introduction

1.1. Problem under investigation and its relevance

Lichen planus is a chronic inflammatory disease of the skin and mucous membranes and presents itself as specific rash, clinical and histological changes. Lichen planus may present itself not just on the skin and mucous membranes, but may also affect nails and the hairy part of the head. It is a disease with a number of clinical variations.

Different countries count from 0.5 to 2.6 per cent of diagnosed cases of the disease. It may occur just in the skin (up to 44 per cent), in the mouth and skin, just in the mouth (up to 70 per cent) [1, 2, 3]. Most often it is middle-aged and elderly people that suffer from it. According to some of the authors, it is more frequent among female than male, 2.2 and 1.6 per cent, respectively [4]; there are countries where it is more frequent among male [5]. Lichen planus is found in families and it is believed that genetic predisposition of the disease is possible [6], sometimes children have it [5, 7, 8, 9]. Lichen planus is a disease resulting in discomfort for patients, often causing painful lesions in the skin and mucous membrane, having a doubtful treatment prognosis and questionable potential for malignancy [10]. Malignancy of lichen planus lesions is specified irregularly [11]. It has been established that 1.0-3.0 per cent of all lichen planus lesions tend to turn malignant [12]. More frequent malignancy is of erosive – ulcerative rather than of the atrophic forms of the disease [13].

The occurrence of lichen planus is influenced by: chronic sicknesses, disorders of autoimmune disbalance, genetic predisposition towards the disease, reactions

caused by medicines, dental materials, traumas of tissues, specific nutrition, addictions and psycho-emotional conditions of the patient [3, 14, 15, 16, 17, 18, 19, 20, 21]. It is believed that conditions like depression, stress or just excessive anxiety may cause appearance of the disease. [22].

No doubt that endocrinal pathology: thyroid diseases [23], diabetes mellitus [24], as well as hepatitis [15], gastrointestinal diseases [25], cardiovascular diseases, hypertension [9] and other sicknesses influence the occurrence of the disease and its progress.

The following are the local etiological risk factors: periodontal pathology, teeth restoration and/or improper oral hygiene, dental materials [26-30]. Addictions, such as smoking and use of alcohol are also named as etiological factors [31, 32, 33].

Many of lichen planus risk factors may be unknown so far [34]; therefore the research of potential risks associated with the disease are of importance.

Lichen planus considerably aggravates the quality of life of the patients [35, 36-38]; therefore, when investigating changes in the quality of life, the ability for adaptation in lichen planus, regular observation of such patients is recommended and the treatment orientated to secure the quality of life should be ensured by the therapist.

No precise data on the lichen planus risk factors nor on the quality of life of the patients are available neither in Lithuania nor in the neighbouring countries; therefore, aiming to improve the quality of treatment and prevention, to assess the predisposition factors of lichen planus and their influence on the disease, malignancy of the affected tissues, the quality of life of the patients, further investigations of the disease are significant.

1.2. Originality of the study and its practical value

Patients suffering from lichen planus experience not only physical discomfort but psychological as well. It is difficult to assess their quality of life because understanding of the quality of life differs among individuals, and the expectations of the patients are different. Variety of the clinical forms of the disease determines its different progress, the nature and the impact on the quality of life. So far no investigations of the condition of the oral lichen planus patients and the influence of the disease on the quality of life have been performed in Lithuania.

The frequency of clinical forms and localisation of lesions of the Lithuanian lichen planus patients has been investigated and assessed for the first time, the prevalence of oral candidosis among the patients has been identified, the prevalence of malignancy of tissues affected by lichen planus has been assessed, the relationship with dermatosis, cardiovascular and gastrointestinal diseases, hepatitis, diabetes mellitus has been assessed. Oral health of the lichen planus patients has been inves-

tigated and assessed. A relationship between lichen planus and the factors of social and clinical risks has been established.

With the help of psychometric scale various aspects of lives, daily activities, social relationships of lichen planus patients have been assessed; therefore the results of the study allows making recommendations for the lichen planus patients regarding improvement of the quality of life.

Practical value of the study: the data collected and scientific evidence –based research which will be valuable in individual assessment of social and clinical factors determining the occurrence of the disease and its progress for each patient. The study will be useful in organisation of medical assistance for lichen planus patients, implementing modern standards of diagnostics and treatment, modelling the attitude of odontology and medicine students, residents, dental practitioners and specialists of odontology and skin diseases towards establishment, treatment, prevention of the disease and the importance of improvement of the quality of life of the patients.

1.3. Goal and objectives of the study

Goal of the study is to investigate clinical indices of the patients suffering from oral lichen planus, the correlation between the disease and the risk factors and the quality of life of the patients.

Objectives of the study:

1. To assess socio-demographic and clinical indices of the oral lichen planus patients.
2. To establish the relationships between the risk of occurrence of oral lichen planus and the socio-demographic, local, generic factors and chronic diseases and health conditions.
3. To establish and assess the quality of life of the oral lichen planus patients.
4. To identify the risk factors which influence the quality of life of the oral lichen planus patients.

2. Research material and methods

2.1. Design of the study

For settlement of the objectives of the scientific study the analytical observational case- control study was selected and a permit No 158200-02-148-056 LP11 of the Ethics Committee of the Biomedicine Research of Lithuania was obtained.

Persons participating in the research were verbally and in written form informed about the planned research and written consents of all patients regarding participation in the research were obtained.

Scope of the research. The research on oral lichen planus was performed during the years 2010-2014 at Žalgiris Clinics. The total number of patients was 312; of those 274 patients agreed to participate in the research and were investigated, 137 lichen planus patients and the same number of patients without it.

2.2. Test groups

2 groups of patients were chosen for the research. One group consisted of the lichen planus patients (case group). The patients of the control group - the patients without lichen planus - were of similar age, sex and social status.

The group of lichen planus patients (case group) included all patients from the period 2010 to 2014 that were referred to Žalgiris Clinics for consultancy and treatment, and who had histologically confirmed lichen planus diagnosis. Based on the clinical symptoms of the disease all cases of lichen planus were divided into the following groups: papular, reticular, erosive-ulcerous, bullous, atrophic and plaque-like forms. The plaque-like lichen planus form was diagnosed for none of the patients participating in the research so it will not be mentioned in the study hereinafter.

The patients in control group were selected from those arriving to Žalgiris Clinics for consultations on other than lichen planus sicknesses. The patients in the control group were of similar age, sex and social status, and were interviewed and investigated the same way as the lichen planus patients.

2.3. Investigation of patients

All participants in the scientific study were interviewed using "General Patient's Health Questionnaire" and "Quality of Life Questionnaire" which were responded by the participants of both groups. An additional "Clinical Investigation Questionnaire" was answered only by the lichen planus patients. The lichen planus patients were investigated clinically, histologically; they were also subjected to the investigation of cytological mucous membrane scrapings from the lichen planus lesions.

2.3.1. Patients' survey

Information about the demographic data, oral health and local and systemic risk factors of the participants was collected with the help of a structured questionnaire. Aiming to reduce the number of unanswered issues, the questionnaire was not explicitly filled in; further personal interviewing was performed. The persons of other than the Lithuanian origin, speaking poor Lithuanian, were also interviewed individually.

The questions in the questionnaires were presented with suggested answer alternatives. The participants of the study had to choose the most acceptable for them answer.

2.3.1.1. Questionnaire on the quality of life

All participants were asked to answer questions about the quality of life (Annex 8). Based on similar studies on the quality of life of sick persons performed internationally and in Lithuania, an adapted questionnaire WHOQOL–100 of the WHO was used [40]. The questionnaire was designed based on similar international and Lithuanian studies on the patients' quality of life. The participants were asked about the demographic data and about the key factors which might have influenced their quality of life. By the content three groups of the questions about the quality of life are distinguished: satisfaction with the condition of oral health, satisfaction with own life and satisfaction with the overall health.

2.3.1.2. Questionnaire for evaluation of the patient's overall health condition

All participants filled in the questionnaire adapted and translated according to Karolinska institute (Sweden, 2004) questionnaire for evaluation of overall condition of the patient's health. Demographic data (sex, age, place of residence and marital status) were collected as well as the information about the way of life (smoking, use of alcohol) and social- economic information (education, social status). Aiming to find out the aetiology all patients were asked about generic risk factors: experienced stress, infections, chronic illnesses, use of medications and genetic factors (lichen planus among the family members). The patients were also asked to self-assess their health condition.

2.3.1.3. Questionnaire of clinical inspection

The same researcher additionally performed a clinical investigation employing a clinical investigation questionnaire aimed to find out if the patient was consulting for the first time or repeatedly, what the progress of the disease was, if there were any exacerbations of the disease; the clinical form of the disease was also established, as well as localisation of lesions and the link between lichen planus and other diseases of the mucous (psoriasis, bullous, seborrhoea, lupus erythematosus and etc.)

2.3.2. Collection of data

Before the research, aiming to reduce the influence of informational errors, the credibility of the questionnaire on the quality of life was checked (under the same

questionnaire a group of 10 patients was interviewed, and the same group was interviewed again after two weeks using the same questionnaire). It was attempted to find out whether the questions were understood by the patients, were they adequate and if there had been any problems when answering the questions.

The credibility of the questionnaire was assessed by test-retest, which was established assessing the correlation (correlation coefficients) between the primary and the repeated interview using the same methods.

2.3.3. Clinical examination of the oral lichen planus patient

Oral clinical examination consisted of assessment of the forms of lichen planus and investigation of possible local and systemic risk factors associated with the oral environment. Locations of the lichen planus lesions were assessed.

Clinical investigation of the lichen planus patient was performed by examination of the patient's mouth. The form of the disease (papular, reticular, erosive-ulcerous, bullous, plaque-like (hyperkeratosis), atrophic), the location in the mouth (buccal mucosa, gums, tongue, lips, the vestibule of the mouth, the palate) and its prevalence (skin, nails, genitals) were established. Examination of the skin and of the genitals was performed by a doctor dermato-venereologist. For the purpose of assessment of the aetiology of lichen planus the following local risk factors were assessed: bimetallism, poor oral hygiene, sharp edges of the teeth, tooth roots, chronic endodontic infection, chronic periodontal pathology, tooth decay, dental amalgam, composite fillings and removable and nor removable dental prosthesis.

2.3.4. Histology test

Incisive biopsy was used for confirmation of the lichen planus diagnosis. Biopsy was performed to all patients that had the clinical diagnosis of lichen planus. Before the test all participants of the study were introduced to the methodology of biopsy sampling and the complications of the test: possible bleeding at the place of stick or incision injury, after-surgery pain. Prior to the planned biopsy the patients were warned to stop taking anticoagulant medicines. The biopsy samples were from 3–10 mm × 3-4 mm × 3-4 mm size.

Incisive mucous biopsy was performed under local surface infiltration anaesthesia with 0.5-1 ml 4 per cent *Ubistesini forte* anaesthetic, with prior disinfection of the mouth tissues with the solution of 0.12 per cent chlorhexidine for 1 min.

The piece of oral surface mucous epithelia was fixed in a buffer formalin solution, placed into a test-tube marked with the patient initials, and forwarded for further investigation to the State Pathology Centre, where the test –tubes were covered with paraffin, painted with haematoxylin and eosin, and the histology test was

performed assessing the infiltration of the oral mucous with lymphocytes (T lymphocyte infiltration), hyperplasia of oral mucosa and hyperparakeratosis, ulceration of squamous epithelia due to erosion of basal layer and necrosis of keratinocytes. The presence of hyperplasia was assessed as the minimum, the medium and the maximum. This way the lichen planus diagnosis was specified and the presence or absence of atypical cells in the lesions was verified.

All procedures were performed after assessment of the overall and the oral health of the patients. The histology test was performed at the Pathology Centre of VUH Santaros Klinikos.

2.3.5. Cytology test

Scrapings from the surfaces of lichen planus lesions in the mouth were taken with the help of a scoop and spread on a glass slide and further forwarded to the laboratory for investigation. Aiming to establish the presence of *Candida* and atypical cells exfoliative cytology of the surface cells was used. The cytology tests were performed at the laboratory of VUH Žalgiris Clinics.

2.4. Statistical analysis

The statistical analysis was performed by IBM SPSS Statistics® software package – *Statistical Package for Social Sciences 22 for Windows*. For assessment of interdependency of qualitative features (or homogeneity) the criterion of chi square *(χ^2)* was used, in cases of low samples – the precise χ^2 criterion. Pairwise comparisons were made applying z criterion.

Socio-demographic, clinical local (related to the oral environment) and general factors, such as chronic diseases, use of medications, allergies, factors of life style, such as smoking and use of alcohol, influencing lichen planus disease were assessed applying the method of logistical regression. The results of univariate and multivariate logistic regressive analysis are described presenting the SR – odds ratio, and 95 per cent PI – confidence interval of probability ratio.

In the analysis of the quality of life of the patients the dispersive analysis and the General Linear Model of multiplicative analysis SPSS.22 was used. In the final step the necessary assumptions for this analysis were checked. The compliance of the model was assessed by the determination coefficient R^2. Equality of variance, dependant variable, normality of its probability distribution and the null hypothesis of the coefficients of the linear model were verified.

The observed differences or links between the characteristics were considered statistically significant when the level of significance (p value) calculated during

verification of statistical hypothesis was under the selected level of significance $\alpha = 0.05$ (p – value < 0.05).

3. Results

137 lichen planus patients participated in the investigation: 24 male (17.5 per cent) and 113 female (82.5 per cent). The average age of the patients was 55.43±14.26. The eldest lichen planus patient was 80, the youngest – 17 years old. The majority of the patients were from 51 to 60 years old.

Analysing the distribution of lichen planus patients by education it was established that 38.0 per cent of the lichen planus patients had higher non-university education or uncompleted higher education, 32.1 per cent had university education, 22.6 per cent had secondary education, and 7.3 per cent had primary or uncompleted secondary education.

Assessment of the marital status of the lichen planus patients has established that the most of the patients (73.7 per cent) were married or cohabited. The majority of lichen (77.4 per cent) lived in a big city.

Analysing the distribution of lichen planus patients by the social status it has been established that 36.5 per cent were specialists or servants, 31.4 per cent were retired on pension, 9.5 per cent were workers or technical staff. The most of the female lichen planus patients (38.1 per cent) were specialists or servants, and the majority of male lichen planus patients were specialists or servants (29.2 per cent) and retired on pension (29.2 per cent).

It has been established that 11 (8.0 per cent) of the lichen planus patients had relatives suffering from the disease, and the rest 126 patients (92 per cent) had ambiguous aetiology (idiopathic): male – 23 (18.3 per cent), female – 103 (81.7 per cent).

During the assessment of the clinical indices of the lichen planus patients it has been established that the most patients (45.3 per cent) had the diagnosis of the reticular form of lichen planus, 34.3 per cent – erosive-ulcerous, 8.0 per cent – papular, 10.9 per cent – atrophic and 1.5 per cent – bullous form of lichen planus. The plaque-like lichen planus form has been diagnosed for none of the patients participating in the investigation (Table 1). The reticular clinical form of lichen planus was more frequent among male (66.6 per cent) than among female (40.7 per cent), the difference was statistically significant (χ^2=8.756, p=0.003). No other statistically significant differences between male and female lichen planus patients have been found.

TABLE 1. Distribution of lichen planus patients by the clinical form of the disease

Clinical form	Total (n=137) n (per cent)	Male (n=24) n (per cent)	Female (n=113) n (per cent)	p- value
Papular	11 (8.0)	1 (4.2)	10 (8.8)	0.689*
Reticular	62 (45.3)	16 (66.6)	46 (40.7)	0.003
Erosive-ulcerous	47 (34.3)	6 (25.0)	41 (36.3)	0.290
Bullous	2 (1.5)	0 (0.0)	2 (1.8)	1.00*
Atrophic	15 (10.9)	1 (4.2)	14 (12.4)	0.469*
Plaque-like	Not found	Not found	Not found	

*Fisher's exact test

Analysis of the localisation of the lichen planus lesions showed that the patients most frequently had lesions on buccal mucosa (87.6 per cent), on gums (46.7 per cent), on the side surfaces of the tongue (39.4 per cent) and on the dorsum of the tongue (29.9 per cent). Other areas of the oral mucous were affected less frequently. It has been established that 6 lichen planus patients (4.4 per cent) had affected nails and genitals.

Analysing localisation of the lichen planus lesions by sex, it has been established that the gums were more frequently affected in females (53.1 per cent) than for male (16.7 per cent); this difference was statistically significant (χ^2=10.555; p=0.001). It has also been established that the skin was more often affected in females (23.9 per cent) than in males (4.2 per cent); the difference was statistically significant (χ^2=4.738; p=0.028).

TABLE 2. Distribution of cytological indices by the forms of lichen planus

Clinical form	Microbe flora		Candida			Actinomicetes		Atypical cells		Total
	abundant	sparse	abundant	sparse	none	present	none	present	none	
Papular	5 (45.5)	6 (54.5)	0 (0.0)	7 (63.6)	4 (36.4)	0 (0.0)	11 (100)	0 (0.0)	11 (100)	11
Reticular	21 (33.9)	41 (66.1)	10 (16.1)	20 (32.3)	32 (57.6)	5 (8.1)	57 (91.9)	0 (0.0)	62 (100)	62
Erosive-ulcerous	28 (59.6)	19 (40.4)	7 (14.9)	18 (38.3)	22 (46.8)	2 (4.3)	45 (95.7)	3 (6.4)	44 (93.6)	47
Bullous	0 (0.0)	2 (100)	1 (50.0)	0 (0.0)	1 (50.0)	0 (0.0)	2 (100)	0 (0,0)	2 (100)	2
Atrophic	5 (33.3)	10 (66.7)	2 (13.3)	8 (53.3)	5 (33.3)	1 (6.7)	14 (93.3)	0 (0.0)	15 (100)	15
Plaque-like	Not found									

The cytological test showed (Table 2) that at different forms of lichen planus in the scrapings of from the lesions bacteria were found. Abundant microbe flora was found in 59.6 per cent of the patients suffering from erosive-ulcerous form of lichen planus, in 45.5 per cent of the patients with papular form of lichen planus, in 33.9 per cent of the patients with reticular form and in 33.3 per cent of the patients suffering from the atrophic form of lichen planus.

Abundantly various types of *Candida* fungus were found in the participants, of those (N=10) 16.1 per cent of reticular and (N=7) 14.9 per cent of erosive-ulcerous cases. Of all clinical forms of lichen planus only in 5.8 per cent cases *Actinomyces* fungus was found. Of all lichen planus patients atypical cells were found in 2.2 per cent cases. Assessing the cytology test it has been established that atypical cells were found only in the erosive-ulcerous form of lichen planus (3 cases).

After the histological investigation (Table 3) by which infiltration of oral mucous with lymphocytes (T lymphocyte infiltration), hyperplasia of oral mucous, hyperparakeratosis and ulceration of squamous cell epithelium were assessed, it has been established that the most obvious (3+) epithelia hyperplasia was found in 11.3 per cent of reticular, 10.6 per cent of erosive –ulcerous and 50 per cent of bullous forms of lichen planus. The most obvious leucocyte infiltration was in 55.3 per cent of erosive-ulcerous, 50 per cent of bullous, 30.6 per cent of reticular and in 20 per cent of atrophic form of lichen planus cases. Hyperparakeratosis was the most frequently (74.5 per cent) found in the cases of erosive-ulcerous form of lichen planus. Ulceration of epithelia has been found in 76.6 per cent of erosive –ulcerous and in 46.7 per cent of atrophic forms of lichen planus. 3 cases of squamous carcinoma

TABLE 3. Distribution of histological indices by the clinical form of lichen planus

Clinical form	Lymphocyte infiltration n (per cent)			Hyperparakeratosis n (per cent)		Hyperplasia n (per cent)			Ulceration of epithelia n (per cent)		Carcinoma n (per cent)		Total
	1+	2+	3+	Yes	No	1+	2+	3+	Yes	No	Yes	No	
Papular	2 (18.2)	9 (81.8)	0 (0.0)	4 (36.4)	7 (63.6)	7 (63.6)	4 (36.4)	0 (0.0)	0 (0.0)	11 (100)	0 (0.0)	11 (100)	11
Reticular	6 (9.7)	37 (59.7)	19 (30.6)	40 (64.5)	22 (35.5)	26 (41.9)	29 (46.8)	7 (11.3)	10 (16.1)	52 (83.9)	0 (0.0)	62 (100)	62
Erosive-ulcerous	2 (4.3)	19 (40.4)	26 (55.3)	35 (74.5)	12 (25.5)	11 (23.4)	31 (66.0)	5 (10.6)	36 (76.6)	11 (23.4)	3 (6.4)	44 (93.6)	47
Bullous	0 (0.0)	1 (50.0)	1 (50.0)	1 (50.0)	1 (50.0)	0 (0.0)	1 (50.0)	1 (50.0)	2 (100)	0 (0.0)	0 (0.0)	2 (100)	2
Atrophic	4 (26.7)	8 (53.3)	3 (20.0)	6 (40.0)	9 (60.0)	13 (86.7)	0 (0.0)	0 (0.0)	7 (46.7)	8 (53.3)	0 (0.0)	15 (100)	15
Plaque-like	Not found												

were found in the erosive-ulcerous lichen planus (Table 3). No cases of plaque –like of lichen planus has been found.

Analysing overall condition of health of the lichen planus patients it has been established that 35.0 per cent of the lichen planus patients had chronic gastritis, 32.9 per cent had arterial hypertension, 25.6 per cent had benign tumours and 24.1 per cent suffered from thyroid diseases.

The results of the research showed that 13.1 per cent of the lichen planus patients were allergic to antibiotics, 8.0 per cent – to food products, 5.1 per cent – to tooth filling materials, plastic, metals, 3.6 per cent – to iodine and 11.7 per cent of the patients were allergic to other allergens. relationship between the socio-demographic factors and lichen planus.

By comparison of potential local factors of the patients and of those not sick with lichen planus (Table 4) it has been established that in the investigated group there were more patients with different metals in the mouth, with poor oral hygiene, sharp teeth edges, having chronic periodontal pathology and fixed dental prostheses than in the control group.

TABLE 4. Distribution of the local factors among the patients of lichen planus and those that not have the disease.

Factor	Total n=274 (per cent)	Control group n=137 (per cent)	Case group n=137(per cent)	p value*
Different metals present in the mouth	37 (13.5)	5 (3.6)	32(23.4)	<0.001
Poor oral hygiene	85 (31.0)	19 (13.9)	66 (48.2)	<0.001
Sharp edges of teeth	45 (16.4)	18 (13.1)	27 (19.7)	0.096
Teeth roots	21 (7.7)	16 (11.7)	5 (3.6)	0.021
Chronic endodontic infection	21 (7.7)	14 (10.2)	7 (5.1)	0.171
Chronic periodontal pathology	144 (52.6)	54 (39.4)	90 (65.7)	<0.001
Decays	47 (17.2)	30 (22.1)	17 (12.4)	0.054
Amalgam fillings	51 (18.6)	23 (16.8)	28 (20.4)	0.535
Composite fillings	166 (60.6)	83 (60.6)	83 (60.6)	1.000
Fixed dental prostheses	156 (56.9)	66 (48.2)	90 (65.7)	0.005
Removable dental prostheses	39 (14.3)	24 (17.5)	15 (11.0)	0.166

*Chi square or Fisher's exact test

Statistically significant local factors related to lichen planus have been identified: different metals in the mouth, poor oral hygiene, teeth roots, chronic periodontal pathology, dental decay, fixed dental and removable dental prostheses.

It was searched for local factors which associate with lichen planus. All possible local risk factors were included into the multi-dimensional analysis and the influence of the age was also considered (Table 4). Stepwise backward elimination regression was chosen with the help of which variables were maintained in the model, based on which the model most specifically accounted the presence of lichen planus. 74.3 per cent of the respondents –lichen planus patients and 72.8 per cent of those that did not have lichen planus were correctly classified. Totally, 73.5 per cent of the participants of the study were correctly identified.

The risk to get sick with lichen planus for the patients with different metals in the mouth was statistically significantly higher (SR=5.86) (p=0.001) if compared with those without metals in the mouth. The patients with poor oral hygiene were at statistically significantly higher risk of lichen planus (SR=6.68) (p<0.001) if compared with the patients with good oral hygiene. The patients that had roots of teeth in the mouth were at statistically significantly higher risk of lichen planus (p=0.014) (SR=0.19) if compared with the patients without teeth roots in the mouth. The patients having chronic periodontal pathology were at statistically significantly higher risk of lichen planus (p=0.001) if compared with the patients that did not have it. The patients with dental decay were at statistically significantly higher risk of lichen planus (SR = 0.28) (p=0.005) if compared with the patients without dental decay. The patients that had removable prostheses the possibility to get sick with lichen planus was statistically significantly higher (SR = 0.36) (p=0.023) if compared with the patients that did not have removable prostheses in their mouth (Table 5).

TABLE 5. Local factors significant for lichen planus in the multi-dimensional logistic analysis

Factors	SR (95 per cent PI)	p value
Different metals in the mouth	5.86(2.03-16.91)	0.001
Poor oral hygiene	6.68(3.19-14.00)	<0.001
Teeth roots	0.19(0.05-0.71)	0.014
Chronic periodontal pathology	2.57(1.44-4.60)	0.001
Dental decay	0.28(0.11-0.68)	0.005
Removable dental prostheses	0.36(0.15-0.87)	0.023

SR – odds ratio, PI – confidence interval, p – significance level; Compliance of the model with the data – $\chi 2$= 87.47, df=6, p<0.001; Model compliance – determination coefficient R^2=0.37.

The results of the research showed that in the group of the lichen planus patients (Table 6) there were more patients that had experienced stress, taking diuretics, anti-hypertension medicines, neuroleptics and other tranquilizers, and having contacts with chemical substances than in the control group.

TABLE 6. Distribution of generic factors among the lichen planus patients and those that do not have it

Factor	Total n=247 (per cent)	Control gr. n=137 (per cent)	Research gr. n=137 (per cent)	p value
Stress	181 (66.1)	75 (54.7)	106 (77.4)	<0.001
Infections	36 (13.1)	26 (19.0)	10 (7.3)	0.007
MEDICATIONS:				
Anti-inflammatory non-steroidal drugs	48 (17.5)	27 (19.7)	21 (15.3)	0.427
Diuretics or antihypertensive agents	77 (28.1)	32 (23.4)	45 (32.8)	0.106
Anti- TB agents	2 (0.7)	1 (0.7)	1 (0.7)	1.000
Neuroleptics, other tranquilizers	57 (20.8)	18 (13.1)	39 (28.5)	0.003
Steroid hormones	17 (6.2)	9 (6.6)	8 (5.8)	0.999
Antiarrhythmic drugs	22 (8.0)	8 (5.8)	14 (10.2)	0.180
Chemical substances	28 (10.2)	3 (2.2)	25 (18.2)	<0.001

*Chi square or Fisher's exact test

Statistically significant generic factors associated with lichen planus have been identified; namely, stress, infections, use of neuroleptics and other tranquilizers, chemical substances.

It was searched for generic factors which had the strongest link with lichen planus. All generic factors were included into the multivariate analysis; the influence of age has been considered as well. Stepwise backward elimination regression was chosen, with the help of which variables were maintained in the model, based on which the model most specifically prognosticated lichen planus. 81 per cent of the participants –lichen planus patients and 51.1 per cent of those that did not have lichen planus were correctly classified. Totally, 66.1 per cent of the participants of the study were correctly identified.

Based on the developed model of multivariate logistic regression (Table 7), it is possible to state that the patients experiencing stress have were statistically significantly (SR=2.45) (p=0.002) higher the chances to get sick with lichen planus than those not experiencing stress. It has been established that for the patients suffering from chronic inflectional diseases the chances to get sick with lichen planus are statistically significantly (SR=0,3) (p=0,006) higher if compared with those that do not have it. The patients that used neuroleptics and/or other tranquilizers had statistically significantly higher (p=0.012) chances to get sick with lichen planus if compared with those that did not use those medications. The patients that have chemical substnaces in their work environment have statistically significantly high-

er (SR=10.63) (p<0,001) chances to get sick with lichen planus if compared with those that have no contacts with chemical substances.

TABLE 7. Generic factors significant for lichen planus, according to multivariate logistic analysis

Factors	SR (95 per cent PI)	p value
Stress	2.45(1.41-4.27)	0.002
Infections	0.30(0.13-4.59)	0.006
Neuroleptics, other tranquilisers	2.35(1.20-4.59)	0.012
Chemical substances	10.63(2.91-38.85)	<0.001

SR – odds ratio, PI – confidence interval, p – significance level; Compliance of the model with the data – $\chi 2$= 50.33 df=4. p<0.001; Model compliance – determination coefficient R^2=0.22; The model prognosticated with the accuracy of 66.1 per cent.

Comparing the prevalence of chronic diseases and conditions in the control and the case groups (Table 8) it has been established that bullosa simplex, psoriasis, gastro-intestinal disorders, seborrhoea dermatitis and diabetes mellitus were common in the case group. The most of the patients in the research group suffered from gastrointestinal disorders (31.1 per cent).

TABLE 8. Distribution of chronic diseases and conditions among the lichen planus patients and those that do not have it

Factors	Total n=274 (per cent)	Control gr. n=137 (per cent)	Research gr. n=137 (per cent)	p value*
Bullosa simplex	4 (2.9)	0 (0.0)	4 (2.9)	0.122
Psoriasis	8 (5.8)	0 (0.0)	8 (5.8)	0.007
Chronic gastritis, gastric ulcer, other gastro-intestinal diseases	44 (31.1)	0 (0.0)	44 (31.1)	<0.001
Seborrhoea dermatitis	4 (2.9)	0 (0.0)	4 (2.9)	0.122
Diabetes mellitus	8 (5.8)	0 (0.0)	8 (5.8)	0.003
Lupus erythematosus	0 (0.0)	0 (0.0)	0 (0.0)	1.000

*Chi square or Fisher's exact test

During the research experienced stress was assessed. The majority of the respondents (54.9 per cent) indicated stress at work (arduous work) (Table 9). In the research group more patients specified as stress a death of a close family member, of a friend and a personal sickness.

TABLE 9. Distribution of experienced stress cases in the groups of lichen planus patients and those that do not have it

Experienced stress	Total n=244 (per cent)	Control gr. n=128 (per cent)	Research gr. n=116 (per cent)	p value*
Death of a spouse	37 (15.2)	19 (14.8)	18 (15.5)	0.999
Divorce	35 (14.3)	22 (17.2)	13 (11.2)	0.147
Conflicts in the family	55 (22.5)	28 (21.9)	27 (23.3)	0.999
Death of a close family member, of a friend	68 (27.9)	33 (25.8)	35 (30.2)	0.889
Personal sickness or trauma	66 (27.0)	31 (24.2)	35 (30.2)	0.672
Marriage	14 (5.7)	7 (5.5)	7 (6.0)	1.000
Stress at work, arduous work	134 (54.9)	68 (53.1)	66 (56.9)	0.904
Resignation, retirement	38 (15.6)	23 (18.0)	15 (12.9)	0.221
Change of the place of residence	21 (8.6)	14 (10.9)	7 (6.0)	0.172
Change of nutrition habits	13 (5.3)	10 (7.8)	3 (2.6)	0.085
Son or daughter leaving home	23 (9.4)	12 (9.4)	11 (9.5)	0.999
Personal victory, promotion at work	3 (1.2)	3 (2.3)	0 (0.0)	0.247
Financial problems, debts, loans	41 (16.8)	27 (21.1)	14 (12.1)	0.041
Problems with the law and order	30 (12.3)	27 (21.1)	3 (2.6)	<0.001
Other	10 (4.1)	2 (1.6)	8 (6.9)	0.103

*Chi – square test or Fisher's exact test

Analysing the overall condition of health it has been established that in the case group there were more patients suffering from epilepsy or another neurological disease, having experienced episodes of convulsions or fainting spells, undergoing psychiatric treatment, had cardiovascular diseases, chronic gastritis, thyroid diseases, underwent blood transfusion, had oncologic diseases or were allergic to medications.

The chronic diseases and conditions which had statistically significant correlation with oral lichen planus have been identified, namely, chronic gastritis, thyroid diseases, blood transfusion and malignant tumours.

We searched for chronic diseases and conditions which had the greatest correlation with lichen planus. Multivariate logistic analysis assessed chronic diseases and the influence of the age. Stepwise backward elimination regression was chosen with the help of which variables were maintained in the model, based on which the model most specifically prognosticated lichen planus. 63.2 per cent of the respondents –lichen planus patients and 74.5 per cent of those that did not have lichen planus were correctly classified. Totally, 68.9 per cent of the participants of the study were correctly identified.

Based on the multivariate logistic regression model (Table 10) it is possible to state that the patients with chronic gastritis have statistically significantly higher (SR=3.09) (p<0,001) risk to get sick with lichen planus if compared with those that do not have lichen planus. It has been established that for the patients with thyroid diseases the chances to get sick with lichen planus are statistically significantly (SR= 6.32) (p<0,001) higher if compared with those that do not have it. The patients that had a blood transfusion have statistically significantly higher (SR=3.47) (p=0.028) chances to get sick with lichen planus than the patients who had not experienced a transfusion. The patients who have or had malignant tumours have statistically significantly higher (SR= 6.22) (p<0.001) chances to get sick with lichen planus if compared with the patients without malignant tumours.

TABLE 10. Assessment of the influence of chronic diseases and conditions on occurrence of lichen planus, multivariate logistical analysis

Factors	SR (95 per cent PI)	p value
Chronic gastritis	3.09(1.64-5.83)	<0.001
Thyroid diseases	6.32(2.44-16.39)	<0.001
Blood transfusion	3.47(1.15-10.49)	0.028
Malignant tumours	6.22(2.24-17.33)	<0.001

SR– odds ratio, PI – confidence interval , p – level of significance. Model compliance with the data – $\chi 2$= 60.79, df=4, p<0.001; Model compliance – determination coefficient R^2=0.27; The model prognosticated with accuracy 68.9 per cent.

Based on the data of the research, the lichen planus patients assessed the quality of life in lower points than the patients in the control group. It has been established that the mean life quality point of the lichen planus patients was 68.1 (stand. n. 8.3, med. 68,3), of those that do not have it – 75.9 (stand. N. 7.3, med. 76,9), the difference is statistically significant (p<0.001). Analysing the life quality differences between male and female it has been established that the female lichen planus patients assessed the quality of life in lower points (the average 67 points) than those that did not have oral lichen planus (the average 75.7 points), the difference is statistically significant (p<0.001).

Analysing the assessment of the quality of life with the consideration of the age it has been established almost all oral lichen planus patients assessed the quality of life statistically significantly in lower points than those that did not have it, with the exception of the participants of the study from 31 to 40 years of age, whose assessment of the quality of life did not differ statistically significantly, irrespectively of having or not oral lichen planus.

Analysing the assessment of the quality of life with the consideration of education it has been established that the lichen planus patients with the secondary, higher non-university and the university education assessed the quality of life in statistically significantly lower points than the patients without lichen planus with the same education. The patients with lichen planus and without it who had primary and uncompleted secondary education assessed the quality of life the same.

Analysing the assessment of the quality of life of the patients with lichen planus and without it with the consideration of marital status it has been established that married and cohabiting, single and widows and widowers assessed the quality of life in lower points than the patients without lichen planus; the difference is statistically insignificant.

Analysing the assessment of the quality of life of the patients with lichen planus and without it with the consideration of the place of residence it has been established that both lichen planus patients living a big city and in countryside assessed the quality of life in lower points than the patients without the disease; the difference is statistically significant.

Investigating the assessment of the quality of life of the patients with lichen planus and without it with the consideration of the social status it has been established that workers, technical staff, specialists, servants, retired persons, house-wives with lichen planus assessed the quality of life in lower points than those without lichen planus; the differences are statistically significant. It has been established that businessmen, top managers, students, school children and the unemployed both lichen planus patients ant patients without it assessed the quality of life the same.

Table 11 presents the data illustrating the link between the quality of life and the socio-demographic factors. It has been established that in the case group the quality of life is the most related to the sex (F=10.5, p=0.001), a little bit less with the place of residence (F=9.5, p=0.003). Meanwhile, it the control group the strongest link is between the quality of life and the education (F=4.14, p=0.008). Other socio-demographic factors did not have any statistically significant link with the quality of life.

Having assessed by the method of regressive analysis the intensity of the links (value of β coefficient, positive – increases, negative – decreases) between the quality of life and the socio-demographic factors (the age, the sex, the marital status, the place of residence) it has been established that in the case group the quality of life was better of the male (β=6.20; PI 2.42-9.98) comparing with the female, of those living in a big city (β=5.19; PI 1.85-8.53) comparing with the ones living in other cities and the countryside. In the control group the quality of life was better only of the participants with university education (β=7.34, PI 2.11-12.56) comparing with the respondents with primary and incomplete secondary education. Other socio-demographic factors did not have any statistically significant difference for the quality of

TABLE 11. Relationship between the quality of life and the socio-demographic factors

Factor	Case group[a]					Control group[b]				
	Sum of mean squares	df	Mean square	F	p	Sum of mean squares	df	Mean square	F	p
Model compliance	1901.8	11	172.9	2.9	0.002	1172.6	11	106.6	2.2	0.019
Free member	214356.8	1	214356.8	3609.0	<0.001	262139.9	1	262139.9	5376.5	<0.001
Age	208.8	5	41.8	0.7	0.622	401.5	5,	80.3	1.65	0.152
Sex	626.2	1	626.2	10.5	0.001	27.0	1	27.03	0.55	0.460
Education	38.7	3	12.9	0.2	0.884	605.2	3	201.7	4.14	**0.008**
Marital status	132.2	1	132.2	2.2	0.138	0.4	1	0.408	0.01	0.927
Place of residence	561.4	1	561.4	9.5	0.003	3.4	1	3.4	0.069	0.793
Random error	7424.3	125	59.4			6045.8	124	48.8		

a – $R^2 = 0.20$ (case); b – $R^2 = 0.16$ (control)

TABLE 12. Correlation between the quality of life and the local risk factors

Factor	Case group[a]					Control group[b]				
	Sum of mean squares	df	Mean square	F	p	Sum of mean squares	df	Mean square	F	p
Model compliance,	641.1	11	58.3	0.8	0.608	1313.9	11	119.5	2.5	0.007
Free member	45707.8	1	45707.8	**652.6**	<0.001	53774.2	1	53774.2	1129.1	<0.001
Different metals in the mouth	31.8	1	31.8	0.45	0.502	101.5	1	101.5	2.1	0.147
Poor oral hygiene	238.6	1	238.6	3.4	,067	40.1	1	40.1	0.8	0.360
Sharp teeth edges	117.8	1	117.8	**1.682**	,197	8.4	1	8.4	**0.2**	**0.675**
Teeth roots	2.952	1	2.952	**,042**	,838	105.2	1	105.2	2.2	0.140
Chronic endodontic infection	2.0	1	2.0	**,029**	,865	56.6	1	56.6	1.2	0.278
Chronic periodontal pathology	60.4	1	60.4	**,862**	,355	553.8	1	553.8	11.6	0.001
Dental decay	,1	1	,1	**,001**	,978	73.9	1	73.9	1.6	0.215
Dental amalgam	23.6	1	23.6	**,337**	,562	11.2	1	11.2	0.2	0.628
Composite fillings	193.0	1	193.0	**2.755**	,099	71.0	1	71.0	1.5	0.225
Fixed dental prostheses	4.3	1	4,.	**,061**	,805	116.8	1	116.8	2.5	0.120
Removable dental prostheses	1.1	1	1.1	**,016**	,899	4.1		4.1	0.1	0.771
Random error	8684.5	124	70.0			5905.8	124	47.6		

a – $R^2 = 0.07$ (case); b – $R^2 = 0.18$ (control)

life. Considering the above, the above-mentioned characteristics were not included into the stages of risk factors' investigation.

The data given in Table 12 show the correlation between the quality of life and the local risk factors. It has been established that in the research group the quality of life does not statistically significantly correlate with the local risk factors. Meanwhile, in the control group the most intensive correlation between the quality of life and the chronic periodontal pathology has been found (F=11.6, p=0.001). The other factors did not have any statistically significant correlation with the quality of life.

Having assessed by the method of regressive analysis the intensity of the correlation between the quality of life and the local factors (different metals in the mouth, poor oral hygiene, sharp teeth edges, teeth roots, chronic endodontic infection, chronic periodontal pathology, dental decay, dental amalgam, composite fillings, fixed dental prostheses, removable dental prostheses) it has been established that only in the control group the quality of life is better of the respondents without chronic periodontal pathology (β=4.91, PI 2.06-7.76) if compared with the participants of the study that do have chronic periodontal pathology. The other local factors did not have statistically significant difference on the quality of life. Therefore the above-mentioned indications were not included into the steps of the study of risks factors.

TABLE 13. Correlation between the quality of life and the generic risk factors

Factor	Case group grupė[a]					Control group[b]				
	Sum of mean squares	df	Mean square	F	p	Sum of mean squares	df	Mean square	F	p
Model compliance	1240.5	9	137.8	2.2	,029	467.9	9	**52.0**	1.0	,462
Free member	12627.8	1	12627.8	198.3	<0.001	14421.4	1	14421.4	271.2	<0.001
Stress	120.7	1	120.7	1.9	,171	3.8	1	3.8	,1	,789
Chronic infections	2,1	1	2.1	,0	,858	25.6	1	25.6	,5	,489
Anti-inflammatory	462.5	1	462.5	**7.3**	,008	75.6	1	75.6	1.4	,235
Diuretics	17.8	1	17.8	,3	,598	37.5	1	37.5	,7	,403
Anti-TB drugs	1.2	1	1.2	,0	,892	,0	1	,0	,0	,988
Neuroleptics,	186.6	1	186.6	2.9	,089	128.7	1	128.7	2.4	,122
Steroid hormones	93.9	1	93.9	1.5	,227	19.7	1	19.7	,4	,544
Antiarrhythmic medications	105.6	1	105.6	1.7	,200	10.0	1	10.0	,2	,666
Chemical substances	3.4	1	3.4	,1	,817	,0	1	,0	,0	,978
Random error	8085.7	127	63.7			6753.741	127	53.2		

a – R^2 = 0.13 (case); b – R^2 =0.07 (control)

Table 13 presents data showing correlation between the quality of life and the generic factors. It has been established that only in the case group the quality of life the most intensively correlates with the use of anti-inflammatory medications (F=7.3, p=0.008). The other generic factors had no statistically significant link with the quality of life.

Having assessed by the method of regressive analysis the intensity of the correlation between the quality of life and the general factors (stress, chronic infections, anti-inflammatory medications, diuretics, anti-TB drugs, neuroleptics, steroid hormones, antiarrhythmic medications, chemical substances) it has been established that only in the case group the quality of life was better of the patients that did not use anti-inflammatory non-steroid medications (β=5.35, PI 1.42-9.29) comparing them with the ones that took them. The other generic factors had no statistically significant link with the quality of life.

Table 14 presents data showing the link between the quality of life and the experienced stress. It has been established that in the research group the quality of life the most correlates with a personal sickness or trauma (F=5.5, p=0.020). In the control group the quality of life has the strongest link also with a personal sickness or a trauma (F=6.1, p=0.015), slightly less with the death of the spouse (F=5.8, p=0.018). No any other statistically significant links between the stress and the quality of life has been found.

Having assessed by the method of regressive analysis the intensity of the link between the quality of life and the experienced stress (death of the spouse, personal sickness or trauma, stress at work) it has been established that in the case group the quality of life was better of the patients that had no personal sicknesses or (β=3.79,

TABLE 14. Correlation between the quality of life and the episodes of experienced stress

Factor	Case group[a]					Control group[b]				
	Sum of mean squares	df	Mean square	F	p	Sum of mean squares	df	Mean square	F	p
Model compliance	472.6	3	157.5	2.4	,074	712.5	3	237.5	4.9	,003
Free member	252846.0	1	252846.0	3798.3	<0.001	315436.4	1	315436.4	6445.3	<0.001
Death of the spouse	8.7	1	8.7	,1	,719	281.7	1	281.7	5.8	,018
Personal sickness or trauma	367.5	1	367.5	5.5	,020	298.9	1	298.9	6.1	,015
Stress at work	155.5	1	155.5	2.3	,129	2.7	1	2.7	,1	,813
Random error	8853.6	133	66.6			6509.1	133	48.9		

a – R^2 = 0.051 (case); b – R^2 =0.10 (control)

PI 0.60-6.99), comparing them with those that had it. In the control group the quality of life was better of the participants in the study who did not have a personal sickness or trauma (β=3.59, PI 0.72-6.47), if compared with the ones that had it; and the same was with the patients that did not experience the death of a spouse (β=4.26, PI 0.75-7.78) comparing with those that did. Stress at work had no statistically significant influence of the quality of life. Considering that the above-mentioned factor has not been included into the risk factors' study steps.

In Table 15 the data showing correlation between the quality of life and the analysed risk factors are presented. It has been established that in the case group the quality of life the most correlates with the sex (F=11.7, p=0.001), the place of residence (F=7.7, p=0.006) and with poor oral hygiene (F=4.7, p=0.032). In the control group the quality of life the most correlates with chronic periodontal pathology (F=11.4, p=0.001), fixed dental prostheses (F=8.2, p=0.005), use of neuroleptics (F=4.4, p=0.039) and education (F=3.4, p=0.021).

Having assessed by the method of regressive analysis the intensity of correlation between the quality of life and the risk factors it has been established that in the case group the quality of life is better of the male patients (β=6.35, PI 2.68-10.02) if compared with female patients, of those living in a big city (β=4.55, PI 1.31-7.80) and of those with good oral hygiene (β=3.04, PI 0.27-5.81). In the control group the quality of life is better of the participants of the study with university educa-

TABLE 15. Correlation between the quality of life and the risk factors

Factor	Case group[a]					Control group[b]				
	Sum of mean squares	df	Mean square	F	p	Sum of mean squares	df	Mean square	F	p
Model compliance	2101.2	10	210.1	3.7	<0.01	1753.2	10	175.3	4.0	<0.01
Free member	59510.0	1	59510.0	1037.8	<0.01	72546.1	1	72546.1	1671.6	<0.01
Sex	671.1	1	671.1	11.7	,001	35.1	1	35.1	,8	,370
Place of residence	442.4	1	442.4	7.7	,006	24.3	1	24.3	,6	,456
Education	43.0	3	14.3	,2	,861	438.3	3	146.1	3.4	,021
Poor oral hygiene	270.2	1	270.2	4.7	,032	27.1	1	27.1	,6	,431
Chronic periodontal pathology	77.3	1	77.3	1.3	,248	492.6	1	492.6	11.4	,001
Fixed dental prostheses	35.2	1	35.2	,6	,435	356.9	1	356.9	8.2	,005
Neuroleptics	95.9	1	95.9	1.7	,198	189.5	1	189.5	4.4	,039
Age	55.4	1	55.4	1.0	,328	83.7	1	83.7	1.9	,167
Random error	7224.9	126	57.3			5468.4	126	43.4		

a – R^2 = 0.23 (case); b – R^2 =0.24 (control)

tion (β=7.01, PI 2.09-11.93), university education and incomplete higher education (β=5.16, PI 0.07-10.26), of those having no chronic periodontal pathology (β=4.51, PI 1.86-7.16) and of the ones not taking neuroleptics (β=3.67, PI 0.19-7.15).

4. Discussion

The study investigated the correlations between the clinical indices of the lichen planus patients and the risk factors and self-assessment of the quality of life of the patients.

Among the scientific literature a number of scientific studies showing that the disease is more common in female than in male can be found [4, 12, 41, 42, 43, 44]. In our research the majority (82.5 per cent) of the lichen planus patients were women.

Lichen planus is a disease of the middle-aged people, but scientific literature describes individual cases when it is found in the mouths of children [7, 13, 34]. The results of our study showed that the majority of the lichen planus patients were from 51 to 60 years old and the data corresponded to the data presented by the scientists from Brazil, Sweden and Italy [41, 43, 45].

According to the data of the study the majority (45.3 per cent) of the patients had reticular form of lichen planus, and none of the patients participating in the research had the diagnosed form plaque-like lichen planus. If compared with the data of the researches from other countries, our data corresponded to the data of the scientists from Sweden, Croatia, Pakistan, Bangladesh and Iran, who had established that reticular form of lichen planus prevailed the investigated residents, but in Thailand the obtained data were different: atrophic and erosive-ulcerous forms of lichen planus prevailed [42, 43, 46, 47, 48].

Of all oral lichen planus patients in 2.2 per cent of clinical cases in the places of lesions atypical cells were found. Investigation of Iran residents showed that the disease turned malignant in 0.07 per cent patients. In Holland the malignant form developed in 0.5 per cent of cases, in Island – 1.0 per cent, and in Israel – 5.8 per cent [46, 49, 50]. Swedish scientists note the malignancy trend in oral lichen planus as slow but increasing [51].

The results of our study showed that in 87.6 per cent of the patients lesions were on buccal mucosa and the data obtained corresponded to the data of scientists from Brazil, Pakistan, Japan and Sweden [41, 45, 47, 52]. Different data were obtained in Spain where investigation of the lichen planus patients established that the tongue is the most frequently affected place [4, 44].

According to the data of our research, the probability to get sick with lichen planus increases if oral hygiene is insufficient, when patients have different metals or

tooth roots in the mouth, sick with chronic periodontal pathology or dental decay, with increased dental plaque, wearing removable dental prosthesis and experiencing stress. This is confirmed by the studies of other scientists. The study performed by *S.* Pourshahidi et al. (2011) proved that the patients of lichen planus had experienced statistically significantly more stress than the control group of healthy participants [53]. It is important to note that stress, anxiety, strain and depression might induce exacerbation of lichen planus and formation of erosions [48, 54, 55]. Medications, especially antibiotics, diuretics, anti-TB medications, neuroleptics of phenothiazine group [56, 57], dental materials [26 - 29], toothpaste, trauma [30], alcohol, spicy food, and smoking may provoke most frequently single-sided and unevenly spread lichenoid lesions in the mouth [31]. Increased amount of dental plaque as local irritant induces occurrence of lichen planus [29]. According to the data of our research, the patients taking neuroleptics and/or other tranquilisers have more chances to get sick with lichen planus than those that do not take it. Scientific research has proved that viruses and bacteria, among which *Helicobacter pylori* persisting in gastrointestinal tract, are significant for occurrence of lichen planus [58, 59]. The results of our research showed that the patients with chronic gastritis had 3.09 times higher risk of lichen planus, if compared with those that did not have the disease. Higher probability to get lichen planus has been established for the patients with malignant tumours, thyroid diseases, those who had experienced blood transfusion or for the patients that had chemical substances in their work environment.

It has been proved that of all human health parameters the oral health has the highest influence on the quality of life. The results of our study showed that in the case group the male had better quality of life than the female, the same with those living in the big city if compared with the countryside residents and the patients with good oral hygiene.

5. Conclusions

- The majority of the lichen planus patients were over 50 years old. 82.5 per cent of them were female. 87.6 per cent of the patients had lesions on buccal mucous. 45.3 per cent had the diagnosis of reticular form of lichen planus. Atypical cells were found only in the cases of erosive-ulcerous form of lichen planus (2.2 per cent of the cases).
- Statistically significant local lichen planus factors were different metals in the mouth, insufficient oral hygiene, tooth root, chronic periodontal pathology, dental decay and fixed and removable dental prostheses. Stress, chronic inflectional diseases, use of neuroleptics and tranquilisers, as well as work

with chemical substances were the main generic risk factors for oral lichen planus.

- The probability to have oral lichen planus was higher for the persons with chronic gastritis, thyroid diseases, malign tumours and blood transfusion. Bullous simplex, psoriasis, disorders of the gastrointestinal tract, seborrhoea dermatitis and diabetes mellitus are more frequent with oral lichen planus than in its absence.
- The quality of life of the oral lichen planus patients was statistically significantly worse than of the persons not having the disease. The worse quality of life of the oral lichen planus patients was conditioned by: female sex, life in small settlements and insufficient oral hygiene.

6. Practical recommendations

1. We suggest preparing an informational factsheet for dental practitioners, specialists in odontology and the patients suffering from the disease about the risk factors to be avoided and how to improve the quality of life in cases of oral lichen planus, and to periodically update it.

2. We recommend for the dental practitioners to actively participate in scientific conferences, seminars and practical workshops of topics of lichen planus and other premalignant sicknesses.

3. For the purpose of improvement of timely diagnostics of oral lichen planus dental practitioners and oral care professionals should carefully examine oral mucosa and, in case of discovered variations, to forward the patient for consultancy of professional doctors – specialists.

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SANTRAUKA

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Plokščioji kerpligė yra lėtinė uždegiminė odos ir gleivinių liga, pasireiškianti būdingu bėrimu, klinikiniais ir histologiniais pokyčiais. Plokščioji kerpligė gali pasireikšti ne tik odoje ir gleivinėse, bet ir pažeisti nagus bei plaukuotąją galvos dalį. Tai liga, turinti keletą klinikinių variantų.

Skirtingose šalyse diagnozuojama nuo 0,5 iki 2,6 proc. susirgimo atvejų. Liga gali vystytis tik odoje (iki 44 proc.), burnoje ir odoje, tik burnoje (iki 70 proc.). Dažniausiai serga vidutinio amžiaus ir pagyvenę žmonės. Kai kurių autorių duomenimis, moterys serga dažniau nei vyrai, atitinkamai 2,2 proc. ir 1,6 proc., yra šalių, kur vyrai serga dažniau nei moterys.

Plokščioji kerpligė aptinkama šeimose, todėl manoma, kad galima šios ligos genetinė predispozicija, kai kada serga vaikai. Plokščioji kerpligė – tai liga, sukelianti ligoniams diskomfortą, neretai lemianti skausmingus odos ir gleivinių pakitimus, turinti abejotiną gydymo prognozę ir diskutuotiną potencialą supiktybėti. Įvardijama nevienoda plokščiosios kerpligės pažeistų audinių malignizacija. Nustatyta, kad 1,0–3,0 proc. visų sergančiųjų plokščiąja kerplige pažeidimų linkę piktybėti. Dažniau piktybėja erozinė-opinė, rečiau – atrofinė ligos formos.

Plokščiosios kerpligės išsivystymui turi reikšmės lėtinės ligos, autoimuninio disbalanso sutrikimas, genetinis polinkis sirgti šia liga, vaistų, odontologinių medžiagų sukeltos reakcijos, audinių trauma, tam tikra mityba, žalingi įpročiai bei paciento psichoemocinė būklė. Manoma, kad tokios būklės kaip depresija, stresas ar tiesiog perdėtas nerimas gali lemti ligos atsiradimą.

Neabejotinai endokrininė patologija: skydliaukės ligos, cukrinis diabetas; taip pat hepatitai, skrandžio ir žarnyno ligos, širdies ir kraujagyslių ligos, hipertenzija bei kitos ligos turi įtakos plokščiosios kerpligės atsiradimui ir ligos eigai.

Vietinės etiologinės rizikos veiksniai: periodonto patologija, dantų restauracijos ar / ir netinkama burnos higiena, odontologinės medžiagos. Žalingi įpročiai, pavyzdžiui, rūkymas ir alkoholio vartojimas, taip pat įvardijami kaip etiologiniai veiksniai.

Daugybė plokščiosios kerpligės rizikos veiksnių gali būti ir kol kas nežinomų, dėl to svarbūs tyrimai, nagrinėjantys galimas, susijusias su šia liga rizikas.

Plokščioji kerpligė itin blogina ligonių gyvenimo kokybę, todėl, tiriant gyvenimo kokybės pokyčius, žmonių sugebėjimą adaptuotis susirgus burnos plokščiąja kerplige, rekomenduojamas nuolatinis šių pacientų stebėjimas, būtinybė gydytojui taikyti į paciento gyvenimo kokybės užtikrinimą orientuotą gydymą.

Apie burnos plokščiosios kerpligės rizikos veiksnius ir pacientų gyvenimo kokybę Lietuvoje ir kaimyninėse šalyse tikslių duomenų nėra, todėl, siekiant pagerinti mūsų šalyje gyvenančių pacientų gydymo kokybę, profilaktiką, įvertinti plokščiosios kerpligės vystymąsi predisponuojančius veiksnius, jų įtaką ligai, pažeistų audinių malignizaciją, sergančiųjų gyvenimo kokybę, svarbūs tolesni ligos tyrimai.

Darbo tikslas – ištirti pacientų, sergančių burnos plokščiąja kerplige, klinikinius rodiklius, ligos sąryšį su rizikos veiksniais ir pacientų gyvenimo kokybe.

Darbo uždaviniai:

1. Įvertinti sergančiųjų burnos plokščiąja kerplige sociodemografinius ir klinikinius rodiklius.
2. Nustatyti rizikos susirgti burnos plokščiąja kerplige sąsajas su sociodemografiniais, vietiniais, bendriniais veiksniais bei lėtinėmis ligomis ir sveikatos būklėmis.
3. Nustatyti ir įvertinti sergančiųjų burnos plokščiąja kerplige gyvenimo kokybę.
4. Nustatyti rizikos veiksnius, turinčius įtakos sergančiųjų burnos plokščiąja kerplige gyvenimo kokybei.

Medžiaga ir metodai

Šios mokslinės studijos uždaviniams išspręsti buvo pasirinktas analitinis stebimasis atvejo ir kontrolės tyrimas, kuriam buvo gautas Lietuvos biomedicininių tyrimų etikos komiteto leidimas Nr. 158200-02-148-056 LP11 (5 priedas).

Asmenys, dalyvaujantys tyrime, buvo žodine ir rašytine forma informuoti apie numatomą vykdyti tyrimą (6 priedas) ir buvo gauti raštiškai visų pacientų sutikimai dalyvauti tyrime (7 priedas).

Tyrimo apimtis. Burnos plokščiosios kerpligės tyrimas atliktas 2010–2014 metais Žalgirio klinikoje. Iš viso kreipėsi 312 pacientų, iš kurių sutiko dalyvauti tyrime ir buvo ištirti 274 pacientai, t. y. 137 pacientai, sergantys plokščiąja kerplige, ir tiek pat asmenų, nesergančių šia liga.

Rezultatai

Tyrime dalyvavo 137 asmenys, sergantys plokščiąja kerplige: 24 vyrai (17,5 proc.) ir 113 moterų (82,5 proc.). Sergančiųjų amžiaus vidurkis buvo 55,43 ± 14,26 metų. Vyriausiam pacientui, sergančiam plokščiąja kerplige, buvo 80 metų, o jauniausiam – 17 metų. Didžioji dalis sergančiųjų plokščiąja kerplige buvo 51–60 m. amžiaus.

Vertinant sergančiųjų plokščiąja kerplige klinikinius rodiklius, nustatyta, kad didžiajai daliai (45,3 proc.) pacientų buvo diagnozuota tinklinė plokščiosios kerpligės forma, 34,3 proc. – erozinė-opinė, 8,0 proc. – papulinė, 10,9 proc. – atrofinė ir 1,5 proc. – pūslinė plokščiosios kerpligės forma. Apnašinė plokščiosios kerpligės forma nebuvo diagnozuota nė vienam iš tyrime dalyvavusių pacientų.

Analizuojant sergančiųjų plokščiąja kerplige pažeidimų lokalizaciją, pastebėta, kad pacientams buvo dažniausiai pažeista žandų gleivinė (87,6 proc.), dantenos (46,7 proc.), liežuvio šoniniai paviršiai (39,4 proc.) ir liežuvio nugarėlė (29,9 proc.).

Citologinis tyrimas parodė, kad, esant skirtingoms klinikinėms plokščiosios kerpligės formoms, pažeidimų nuograndose aptikta bakterijų. Aptikta gausi mikrobinė flora 59,6 proc. sergančiųjų erozine-opine plokščiosios kerpligės forma, 45,5 proc. sergančiųjų papuline, 33,9 proc. sergančiųjų tinkline ir 33,3 proc. sergančiųjų atrofine plokščiosios kerpligės formomis.

Gausiai įvairių *Candida* grybelio rūšių buvo aptikta pas tiriamuosius, iš jų (N = 10) 16,1 proc. tinklinės ir (N = 7) 14,9 proc. erozinės-opinės plokščiosios kerpligės atvejų. Iš visų klinikinių plokščiosios kerpligės formų atvejų tik 5,8 proc. atvejų aptikta *Actinomyces* grybų. Iš visų sergančiųjų burnos plokščiąja kerplige 2,2 proc. klinikinių atvejų pažeidimų vietose aptikta atipinių ląstelių. Vertinant citologinį tyrimą, nustatyta, kad atipinės ląstelės buvo aptiktos sergant tik erozine-opine plokščiosios kerpligės forma (3 atvejai).

Atlikus histologinį tyrimą, kuriuo buvo įvertinta burnos gleivinės infiltracija limfocitais (T limfocitų infiltracija), burnos gleivinės hiperplazija, hiperparakeratozė ir plokščiojo epitelio opėjimas, nustatyta, kad ryškiausia (3+) epitelio hiperplazija yra 11,3 proc. tinklinės, 10,6 proc. erozinės-opinės ir 50 proc. pūslinės plokščiosios kerpligės formos atvejų. Ryškiausia leukocitų infiltracija buvo 55,3 proc. erozinės-opinės, 50 proc. pūslinės, 30,6 proc. tinklinės ir 20 proc. atrofinės plokščiosios kerpligės formų atvejų. Dažniausia (74,5 proc.) hiperparakeratozė buvo stebima erozinės-opinės plokščiosios kerpligės formos atvejais. Epitelio išopėjimas nustatytas 76,6 proc. erozinės-opinės ir 46,7 proc. atrofinės plokščiosios kerpligės formų atvejų. Tyrimo metu nustatyti 3 plokščialąstelinės karcinomos atvejai sergantiesiems erozine-opine plokščiąja kerplige. Apnašinės plokščiosios kerpligės formos ligos atvejų nenustatyta.

Analizuojant sergančiųjų plokščiąja kerplige bendrą sveikatos būklę, nustatyta, kad 35,0 proc. šių pacientų taip pat buvo diagnozuotas lėtinis gastritas, 32,9 proc. – arterinė hipertenzija, 25,6 proc. – nepiktybiniai navikai, 23,4 proc. – piktybiniai navikai ir 24,1 proc. – skydliaukės ligos.

Tyrimo rezultatai parodė, kad 13,1 proc. sergančiųjų plokščiąja kerplige buvo alergiški antibiotikams, 8,0 proc. – maisto produktams, 5,1 proc. – plombavimo medžiagoms, plastmasėms, metalams, 3,6 proc. – jodui ir 11,7 proc. – kitiems alergenams.

Pacientams, turėjusiems skirtingų metalų burnoje, galimybė susirgti plokščiąja kerplige buvo statistiškai reikšmingai (SR = 5,86) (p = 0,001) didesnė lyginant su pacientais, neturėjusiais skirtingų metalų burnoje. Pacientams, kurių burnos higiena buvo bloga, galimybė susirgti plokščiąja kerplige buvo statistiškai reikšmingai (SR = 6,68) (p < 0,001) didesnė lyginant su pacientais, kurių burnos higiena buvo gera. Pacientams, kurių burnoje buvo rasta dantų šaknų, tikimybė susirgti plokščiąja kerplige buvo statistiškai reikšmingai (p = 0,014) didesnė (SR = 0,19) lyginant su pacientais, kurių burnoje nebuvo dantų šaknų. Pacientams, kuriems būdinga lėtinė periodonto patologija, galimybė susirgti plokščiąja kerplige buvo statistiškai reikšmingai (p = 0,001) didesnė lyginant su pacientais, kuriems nebūdinga lėtinė periodonto patologija. Pacientams, turintiems dantų ėduonį, galimybė susirgti plokščiąja kerplige buvo statistiškai reikšmingai (SR = 0,28) (p = 0,005) didesnė lyginant su pacientais, kurie neturėjo dantų ėduonies. Pacientams, kurie turėjo išimamus protezus, galimybė susirgti plokščiąja kerplige buvo statistiškai reikšmingai (SR = 0,36) (p = 0,023) didesnė lyginant su pacientais, kurie neturėjo išimamų dantų protezų burnoje.

Remiantis sudarytu daugiamatės logistinės regresijos modeliu galima teigti, kad pacientams, patiriantiems stresą, galimybė susirgti burnos plokščiąja kerplige buvo statistiškai reikšmingai (SR = 2,45) (p = 0,002) didesnė lyginant su pacientais, nepatiriančiais streso. Nustatyta, kad pacientams, sergantiems lėtinėmis infekcinėmis ligomis, galimybė susirgti burnos plokščiąja kerplige statistiškai reikšmingai (SR = 0,3) (p = 0,006) didesnė lyginant su pacientais, nesergančiais lėtinėmis infekcinėmis ligomis. Pacientams, vartojantiems neuroleptikus ir / ar kitus raminamuosius vaistus, galimybė susirgti burnos plokščiąja kerplige statistiškai reikšmingai (p = 0,012) didesnė nei nevartojantiems šių vaistų asmenims. Pacientams, kurių darbo aplinkoje yra cheminių medžiagų, galimybė susirgti burnos plokščiąja kerplige buvo statistiškai reikšmingai (SR = 10,63) (p < 0,001) didesnė lyginant su pacientais, neturinčiais sąlyčio su cheminėmis medžiagomis.

Remiantis daugiamatės logistinės regresijos modeliu, galima teigti, kad pacientams, sergantiems lėtiniu gastritu, galimybė susirgti burnos plokščiąja kerplige buvo statistiškai reikšmingai didesnė (SR = 3,09) (p < 0,001) lyginant su asmenimis, nesergančiais lėtiniu gastritu. Nustatyta, kad pacientams, sergantiems skydliaukės ligomis, galimybė susirgti burnos plokščiąja kerplige statistiškai reikšmingai (SR = 6,32) (p < 0,001) didesnė lyginant su asmenimis, nesergančiais skydliaukės ligomis. Pacientams, kuriems buvo atliktas kraujo perpylimas, galimybė susirgti burnos plokščiąja kerplige statistiškai reikšmingai didesnė (SR = 3,47) (p = 0,028) nei pacientams, kuriems nebuvo perpiltas kraujas. Pacientams, kurie turi ar turėjo piktybinių auglių, galimybė susirgti plokščiąja kerplige statistiškai reikšmingai (SR = 6,22) didesnė (p < 0,001) lyginant su pacientais, kurie neturėjo piktybinių auglių.

Remiantis atlikto tyrimo duomenimis, sergantieji burnos plokščiąja kerplige gyvenimo kokybę vertino žemesniais balais nei kontrolinės grupės pacientai. Nustatyta, kad sergančiųjų burnos plokščiąja kerplige gyvenimo kokybės balo vidurkis buvo 68,1 (stand. n. 8,3, med. 68,3), nesergančiųjų – 75,9 (stand. n. 7,3, med. 76,9), skirtumas statistiškai reikšmingas ($p < 0,001$). Analizuojant gyvenimo kokybės skirtumus tarp vyrų ir moterų, nustatyta, kad sergančios plokščiąja kerplige moterys gyvenimo kokybę vertino žemesniais balais (vidurkis 67 balai) nei nesergančios burnos plokščiąja kerplige (vidurkis 75,7 balo), skirtumas statistiškai reikšmingas ($p < 0,001$).

Regresinės analizės metodu įvertinus ryšių stiprumą tarp gyvenimo kokybės ir rizikos veiksnių nustatyta, kad tiriamojoje grupėje gyvenimo kokybė geresnė pacientų vyrų ($\beta = 6,35$, PI 2,68–10,02) lyginant su moterimis, taip pat studijos dalyvių, gyvenančių dideliame mieste ($\beta = 4,55$, PI 1,31–7,80) ir turinčių gerą burnos higieną ($\beta = 3,04$, PI 0,27–5,81). Kontrolinėje grupėje gyvenimo kokybė geresnė studijos dalyvių, turinčių aukštąjį universitetinį išsilavinimą ($\beta = 7,01$, PI 2,09–11,93), aukštąjį universitetinį ir nebaigtą aukštąjį išsilavinimą ($\beta = 5,16$, PI 0,07–10,26), neturinčių lėtinės periodonto patologijos ($\beta = 4,51$, PI 1,86–7,16) ir nevartojančių neuroleptikų ($\beta = 3,67$, PI 0,19–7,15).

Išvados

1. Didžioji dalis sergančiųjų burnos plokščiąja kerplige buvo vyresni negu 50 m. amžiaus asmenys, 82,5 proc. jų – moterys. 87,6 proc. pacientų buvo pažeista žandų gleivinė. 45,3 proc. pacientų buvo diagnozuota tinklinė plokščiosios kerpligės forma. Atipinių ląstelių buvo aptikta sergant tik erozine-opine plokščiosios kerpligės forma (2,2 proc. atvejų).
2. Statistiškai patikimai reikšmingi vietiniai burnos plokščiosios kerpligės veiksniai buvo skirtingi metalai burnoje, nepakankama burnos higiena, dantų šaknys, lėtinė periodonto patologija, dantų ėduonis bei fiksuoti ir išimami dantų protezai. Stresas, lėtinės infekcinės ligos, neuroleptikų ir raminamųjų vaistų vartojimas bei darbas su cheminėmis medžiagomis buvo pagrindiniai bendriniai burnos plokščiosios kerpligės rizikos veiksniai.
3. Galimybė susirgti burnos plokščiąja kerplige didesnė asmenims, sergantiems lėtiniu gastritu, skydliaukės ligomis, turintiems piktybinių navikų ir po kraujo perpylimo. Paprastoji pūslinė, žvynelinė, virškinamojo trakto sutrikimai, seborėjinis dermatitas ir cukrinis diabetas dažniau nustatomi sergant burnos plokščiąja kerplige negu nesergant šia liga.
4. Sergančiųjų burnos plokščiąja kerplige gyvenimo kokybė buvo statistiškai reikšmingai blogesnė negu šia liga nesergančių asmenų. Sergančiųjų burnos plokščiąja kerplige blogesnę gyvenimo kokybę lėmė moteriškoji lytis, gyvenimas nedideliame mieste ir nepakankama burnos higiena.

Eglė Vindašiūtė-Narbutė

CEMENTO PERTEKLIAUS ŠALINIMO NUO VAINIKĖLIŲ, CEMENTUOJAMŲ ANT DANTŲ IMPLANTŲ, VERTINIMAS

FACTORS ASSOCIATED WITH REMOVAL OF THE CEMENT EXCESS IN IMPLANT-SUPPORTED

Biomedicinos mokslai, odontologija (07B)
Biomedical Sciences, Odontology (07B)

Įvadas

Tyrimo aktualumas

Populiariausias vainikėlio ar tilto tvirtinimo prie implanto būdas yra cementavimas. Deja, tam, kad ši procedūra galėtų būti atliekama saugiai, gydytojams vis dar trūksta tam tikros informacijos ir nurodymų šiais klausimais:

a) po cementavimo gali likti nepastebėtas ir nepašalintas cemento perteklius. Sutariama, kad toks cemento likutis – vienas iš veiksnių, turinčių įtakos aplink implantą esančių audinių uždegimui (periimplantitui) atsirasti;

b) nėra jokių oficialių nurodymų, kurioje vietoje minkštųjų audinių atžvilgiu turėtų būti implanto atramos laiptelis cementuojant;

c) nėra įvertinti kiti klinikiniai veiksniai (implanto vieta, skersmuo), kurie gali būti svarbūs siekiant užtikrinti saugią cementavimo procedūrą;

d) nėra patvirtinta, kad rentgenologinis tyrimas yra patikimas metodas nustatyti ir įvertinti, ar buvo pašalintas visas cemento perteklius.

Tyrimo tikslas – nustatyti ir įvertinti veiksnius, kurie daro įtaką cemento šalinimui nuo vainikėlių, cementuojamų ant dantų implantų.

Tyrimo uždaviniai

1. Įvertinti, ar cementavimo ribos gylis turi įtakos cemento likučių šalinimo kokybei.
2. Palyginti du neaptikto cemento likučių įvertinimo metodus: svėrimą ir kompiuterizuotą planimetrinį.
3. Išsiaiškinti rentgenologinio tyrimo patikimumą vertinant cemento likutį pricementavus vainikėlį.
4. Įvertinti, kokią įtaką implanto vieta žandikaulyje turi neaptikto cemento kiekiui.
5. Nustatyti, ar implanto skersmuo turi įtakos cemento likučių šalinimo efektyvumui.
6. Išsiaiškinti, ar zonos aplink implanto kraštą dydis yra svarbus cemento likučių šalinimo veiksnys.
7. Įvertinti, kokiais atvejais galima visiškai pašalinti cemento likučius.

Ginamieji teiginiai

1. Cementavimo ribos gylis turi įtakos cemento likučių šalinimo kokybei.
2. Tiek svėrimo, tiek kompiuterizuotas planimetrinis metodai yra vienodai tikslūs nustatant cemento likučių kiekį.
3. Atliekant rentgenologinį tyrimą galima nustatyti cemento likučių kiekį po valymo.
4. Implanto vieta yra svarbus veiksnys, nuo kurio priklauso cemento likučių pašalinimas.

5. Implanto skersmuo turi įtakos cemento likučių šalinimo efektyvumui.
6. Zonos aplink implanto kraštą dydis turi įtakos cemento likučių kiekiui po valymo.

Tyrimo reikšmė

Šio tyrimo rezultatai bus svarbus mokslinis įrodymas formuluojant tam tikras rekomendacijas:

1. Odontologams protezuotojams – kaip užtikrinti, kad po cementavimo ant implantų b ūtų visiškai pašalintas nereikalingas cementas.
2. Keičiant dabartinius gydymo nurodymus ir siūlant į cementavimo protokolą įtraukti tam tikras gydymo naujoves.

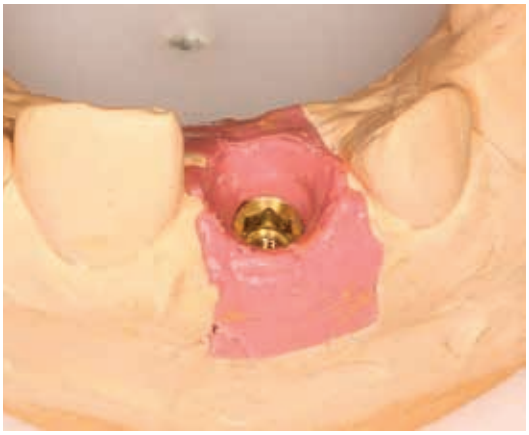
Tyrimo medžiaga ir metodai

Atliekant šį darbą vykdyti du tyrimai: bandomasis *in vitro* tyrimas ir perspektyvusis klinikinis tyrimas.

Implanto atramos laiptelio padėties įtaka cemento likučių kiekiui po protezų ant implantų cementavimo. *In vitro* tyrimas

Modelių ruošimas ir laboratorinės procedūros

Atliekant tyrimą naudoti dvidešimt penki modeliai su priekinių dantų srityje įtvirtintais 3,5 mm skersmens implantų analogais („Internal“, gam. „BioHorizons“, Birmingamas, Alabama, JAV). Padarytas implanto, esančio paciento burnoje maždaug 5 mm po dantenomis, atspaudas. Dantų technologas iš ketvirtosios klasės gipso pagamino modelius („Heraeus“, gam. „Kulzer GmbH“, Hanau, Vokietija). Minkštiesiems audiniams imituoti naudotas A tipo silikonas „GumQuick Plus“ (gam. „Dreve Dentamid GmbH“, Una, Vokietija) (žr. 1 pav.).



1 PAV. Tyrimo modelis su įtvirtintu implantu ir minkštųjų audinių imitacija

Tas pats dantų technologas išliejo dvidešimt penkias individualias implantų atramas ir tiek pat metalinių vainikėlių, naudodamas lydinį „Starbond CoS“ (gam. „S&S Scheftner GmbH“, Maincas, Vokietija; sudėtis: 59 proc. Co, 25 proc. Cr, 9,5 proc. W ir 3,5 proc. Mo). Atramos suskirstytos į penkias grupes pagal implanto atramos laiptelio poziciją (žr. 2 pav.):

1 grupė (kontrolinė) – 1 mm virš dantenų;
2 grupė – ties dantenomis;
3 grupė – 1 mm po dantenomis;
4 grupė – 2 mm po dantenomis;
5 grupė – 3 mm po dantenomis.

2 PAV. Individualios lietos atramos su skirtingais laipteliais

Kiekviename vainikėlyje iš gomurio pusės padaryta anga, kad po cementavimo jį būtų galima atsukti nuo implanto (žr. 3 pav.).

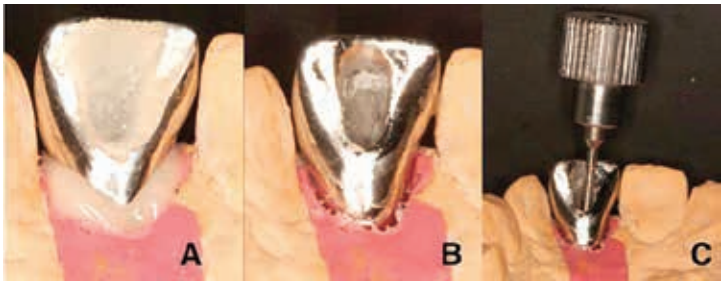
Šios gomurinės angos reikalingos tam, kad po cementavimo būtų galima lengvai atsukti atramos varžtą ir nuo implanto nuimti atramą bei prie jos pricementuotą metalinį vainikėlį. Vainikėliai ir atramos dalys, kurios liečiasi prie minkštųjų

3 PAV. Atramos ir vainikėliai su gomurinėmis angomis po cementavimo

audinių, buvo nupoliruoti guminiais 3 mm storio ir 22 mm skersmens poliravimo ratukais „Polysoft“ (gam. „Renfert“, „Hilzinger“, Vokietija).

Cementavimas ir cemento likučių šalinimas

Naudotas derva modifikuotas stiklo jonomerinis cementas „Fuji Plus“ (gam. „GC“, Tokijas, Japonija). Prieš cementavimą kiekvieno protezo atrama padengta ortodontiniu vašku „Wax Pak“ (gam. „3M UNITEK“, Monrovija, Kalifornija, JAV), kad cementuojant ant prisukimo varžto nepatektų cemento. Taip pat užplombuotos („Gradia Anterior“, gam. „GC“) ir gomurinės angos, kad neištekėtų cemento perteklius ir kad būtų galima tiksliau imituoti klinikinę situaciją, kai vainikėlis – be gomurinės angos. Cementas paruoštas pagal gamintojo instrukciją, plonas jo sluoksnis užteptas ant visų vidinių vainikėlio paviršių ir vainikėlis lengvai pirštais spaudžiant uždėtas ant atramos (žr. 4 pav., A). Sukietėjus cementui plieniniu zondu



4 PAV. Procedūros eiliškumas: A – ant atramos uždėtas vainikėlis; B – nuvalytas cementas; C – atsuktas atramos prisukimo varžtas ir nuo implanto nuimta atrama su pricementuotu vainikėliu, kad būtų galima įvertinti

5 PAV. Cemento likučiai ant vainikėlių, cementuotų skirtingame gylyje

(„Dentsply International Inc.“, Milfordas, Delaveras, JAV) ir dantų siūlu (gam. „Curaprox“, Krynsas, Šveicarija) cementas valytas, kol įsitikinta, kad pašalintas visas jo perteklius (žr. 4 pav., B). Po šios procedūros pašalinta plomba ir vaškas, atsuktas atramos prisukimo varžtas ir nuo implanto nuimta atrama su pricementuotu vainikėliu, kad jį būtų galima įvertinti (4 pav., C). Cemento likutis, priklausomai nuo cementavimo ribos pozicijos, matomas 5 paveiksle.

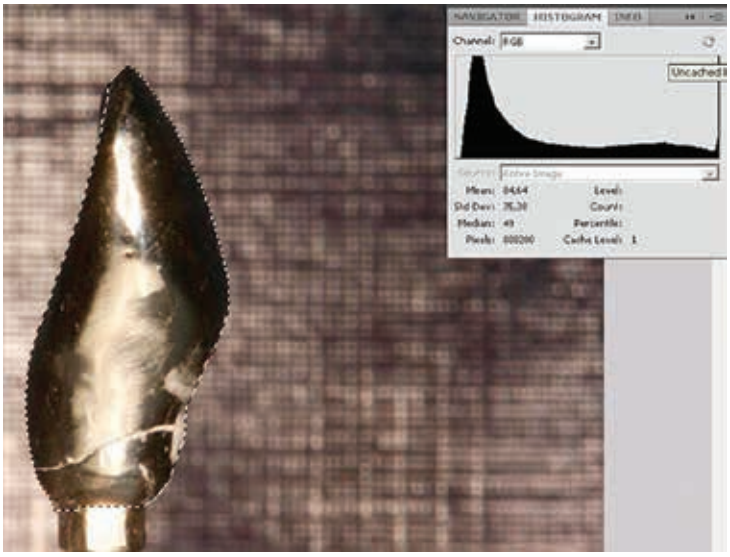
Neaptiktų cemento likučių vertinimas

Neaptikti ir nepašalinti cemento likučiai vertinti dviem būdais – sveriant ir kompiuterizuotu planimetriniu metodu.

Planimetrinis metodas ypač naudingas, kai reikia įvertinti viso mėginio paviršiaus ir cemento likučių dengiamo ploto santykį. Taikant šį metodą pirmiausia specialiai sukurtu įrenginiu nufotografuoti visi mėginio kvadrantai (mezialinis, distalinis, lūpinis ir liežuvinis) taip, kad būtų išlaikytas vienodas atstumas tarp mėginio ir fotoaparato (gam. „Canon“, Niujorkas, JAV) (žr. 6 pav.).

6 PAV. Vienodu atstumu fotografuojami visi mėginio kvadrantai

Nuotraukos analizuotos naudojant programą „Adobe Photoshop“ (gam. „Adobe Systems Ltd Europa“, Uksbridžas, Jungtinė Karalystė). Visose nuotraukose kiekvieno mėginio paviršiaus plotas apibrėžtas rankiniu žymekliu. Cemento likučiams apskaičiuoti naudoti kontūro formavimo (angl. *pen tool*) ir žymėjimo (angl. *make path*) įrankiai. Visas paviršius buvo pažymėtas, pikseliai apskaičiuoti naudojant histogramos funkciją (7 pav.), taip pat pažymėtos ir įvertintos nuotraukos vietos, kuriose buvo matyti cemento likučių. Tada apskaičiuotas cemento likučių dengiamo ploto ir viso mėginio paviršiaus ploto santykis. Vienas mėginio paviršius laikytas



7 PAV. Pasirinkto ploto pikselių skaičiavimas

statistiniu vienetu, todėl kiekvienas mėginys fotografuotas ir matuotas keturis kartus (distalinis, mezialinis, lūpinis ir liežuvinis paviršiai atskirai) – taip gauta po dvidešimt kiekvienos grupės mėginių nuotraukų ir jų vertinimų.

Vertinant antruoju būdu, buvo sveriami cemento likučiai. Nuo kiekvieno mėginio pašalinti cemento likučiai atskirai sverti naudojant analitines skaitmenines svarstykles „Vibra“ (gam. „Shinko Denshi“, Tokijas, Japonija) su0,0001 g paklaida (žr. 8 pav.). Statistiniu vienetu laikytas mėginys, tad kiekvienoje grupėje buvo po 5 mėginius.

8 PAV. Analitinės skaitmeninės svarstyklės

Statistinė analizė

Statistinė analizė atlikta naudojant programą „SPSS“, veikiančią operacinėje sistemoje „Windows v. 17“. Pirmiausia apskaičiuotos vidutinės vertės su standartiniu nuokrypiu. Dėl mažos imties pasirinktas nepriklausomas K (Kruskall-Wallis) testas, skirtas neparametriniams

duomenims analizuoti. Atliekant šį testą siekta nustatyti, ar implanto atramos laiptelio padėties gylis turi įtakos tam, kiek lieka neaptikto ir nepašalinto cemento. Statistiškai reikšmingi lyginamieji grupių duomenys gauti naudojant Mann-Whitney testą. Pagal paprasto išskaidymo grafinį pavaizdavimą ir Spearmano koreliacijos koeficientą nustatytas dviejų metodikų santykis. Statistiškai patikimas rezultatas gautas tada, kai p = 0,05.

Klinikiniai veiksniai, turintys įtakos cemento pašalinimo nuo vainikėlių, cementuojamų ant dantų implantų, kokybei. Prospektyvusis klinikinis tyrimas

Pacientai

Tyrimo dalyviai – Vilniaus implantologijos centro klinikos pacientai. Į tyrimą įtraukti pacientai, atitinkantys šiuos kriterijus: 1) ne jaunesni nei 18 metų; 2) kuriems trūko vieno danties bet kurioje burnos vietoje ir šalia kurio buvo gretimi dantys; 3) kuriems buvo galima atlikti chirurginę operaciją; 4) kurie pasirašė informuoto asmens sutikimo formą ir leidimą duomenis naudoti tyrimo tikslais. Į tyrimą neįtraukti šių kriterijų neatitikę pacientai ir tie, kurių burnos higiena buvo nepakankama, kurie atsisakė bendradarbiauti, rūkė, sirgo alkoholizmu ir diabetu.

Tyrime dalyvavo 65 pacientai (33 vyrai ir 35 moterys) nuo 20 iki 75 metų amžiaus (vidurkis – 37,4 ± 1,2 m.). Tyrimo protokolą patvirtino Vilniaus regioninis biomedicininių tyrimų etikos komitetas (protokolas Nr. 158200-02-457-132).

Tyrimo planas

Atliekant tyrimą įsriegti 65 šešiakampiai implantai („Internal Tapered“, gam. „BioHorizons“, Birmingamas, Alabama, JAV) – 35 viršutiniame ir 30 apatiniame žandikauliuose: 4 priekinėje (kandžių ir iltinių dantų) (6,2 proc.), 22 kaplių (33,8 proc.) dantų ir 39 krūminių dantų srityse (60 proc.). Implantų skersmenys buvo tokie: 21 implantas – 3,5 mm (32,3 proc.), 34 implantai – 4 mm (52,3 proc.) ir 10 implantų – 5 mm (15,4 proc.) skersmens.

Implantų įsriegimas

Implantai su vienodo skersmens implanto atramos jungtimis („Internal Tapered“, gam. „BioHorizons“, Alabama, JAV) įsriegti atlikus rentgenologinį įvertinimą. Pacientams profilaktiškai skirta 2 g amoksicilino („Ospamox“, gam. „Biochemie“, Austrija) valandą prieš operaciją. Vietinė nejautra sukelta 4 proc. artikaino

hidrochoridu („Ubistesin“, gam. „3M ESPE“, Vokietija), pjūvis darytas taip, kad liktų bent 2 mm minkštųjų audinių bukalinėje pusėje. Implantai sriegti pagal gamintojų rekomendacijas – 1 mm virš kraštinio kaulo. Sriegimo vieta pamatuota, kad būtų sudarytas bent 1,5 mm tarpas iki gretimų dantų ir 1 mm tarpas tarp alveolės keteros (iš lūpinės ir liežuvinės pusių) ir implanto. Priklausomai nuo klinikinės situacijos pasirinkti skirtingo skersmens implantai (3,5, 4,0 ir 5,0 mm), kurie įsriegti vienos operacijos metu. Tyrimui atlikti naudoti implantai, pagaminti iš Ti-6Al-Nb 49 metalų lydinio; implantų paviršius pašiurkštintas naudojant tirpią implanto dengimo medžiagą (angl. *resorbing blasting media*, RBM). Implanto kaklelio viršus turėjo poliruotą 0,5 mm dalį – jungtį su atrama. Įsriegus implantą, prisuktos gijimo galvutės, audiniai užsiūti 5/0 siūlu („Assucryl“, gam. „PGA“, Šveicarija) be tempimo.

Cementavimas

Nustačius, kad implantai įsriegti sėkmingai, protezavimo procedūros apatiniame žandikaulyje pradėtos po 2 mėnesių, o viršutiniame – po 4 mėnesių gijimo. Implantai laikyti sėkmingai prigijusiais ir protezuojama, jei:

1) rentgeno nuotraukoje nematyti pašviesėjimo prie implanto; 2) nėra kliniškai pastebimo implanto paslankumo; 3) nėra pūliavimo, skausmo ar kitų patologinių reiškinių.

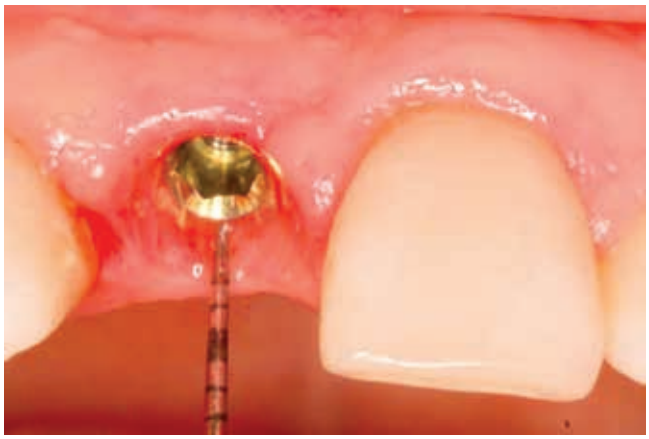
Atspaudai nuimti naudojant atviro šaukšto metodiką. Vienmomenčiam atspaudui buvo naudojama A-polivinilsiloksano bazė („Variotime“, gam. „Heraeus Kulzer“, Hanau, Vokietija).

Naudoti cementuojami-prisukami vainikėliai, nes tokiu atveju po cementavimo galima nuimti vainikėlį (žr. 9 pav.).

9 PAV. Standartinė atrama ir vainikėlis prieš cementavimą

Cementavimo ribos gylis

Implanto įsriegimo gylis vertintas iš mezialinės, distalinės, liežuvinės ir lūpinės pusės, atsukus gijimo galvutę. Matuota ženklintu periodontologiniu zondu su 1 mm padalomis (gam. „Hu-Friedy“, Čikaga, JAV). Vertinant galutinius duomenis, cementavimo ribos gylis buvo skaičiuojamas atėmus 1,5 mm, nes laiptelio pozicija ant standartinės implanto atramos yra 1,5 mm virš implanto ir atramos susijungimo taško. Virš implanto esanti implanto atramos vieta matuota keturis kartus: iš mezialinės, distalinės, lūpinės ir liežuvinės pusės (žr. 10 pav.).



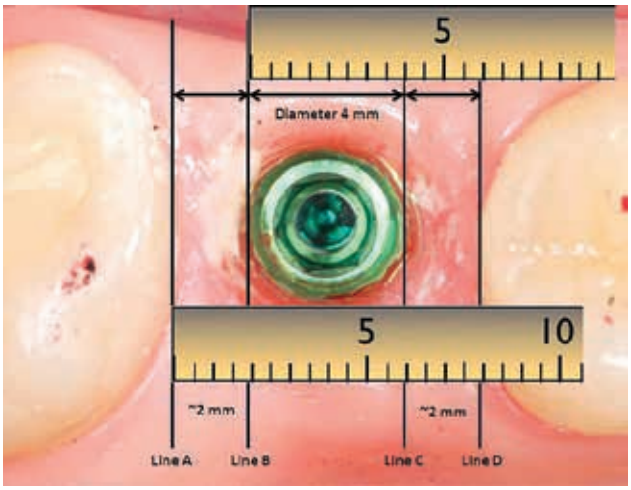
10 PAV. Implanto įsriegimo gylio matavimas iš liežuvinės pusės

Zona aplink implanto kraštą

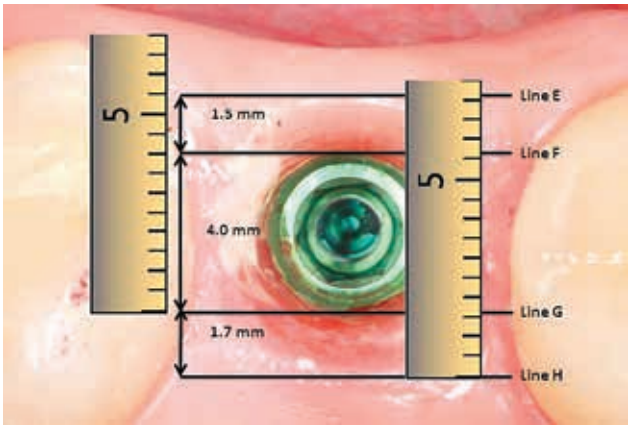
Paprastai protezavimo srityje zona aplink implanto kraštą (angl. *undercut*) yra apibrėžiama kaip tam tikra vieta, suformuota iš bet kurio paviršiaus, esančio žemiau danties viršutinio kontūro matomos linijos, ir pasirinktos protezavimo vietos.

Šiame tyrime zona aplink implanto kraštą suprantama kaip atstumas nuo išorinio implanto kaklelio taško (linija B, C, F, G, žr. 11 pav. ir 12 pav.) iki aplink vainikėlį esančių dantenų krašto arba iki gretimo danties (žr. 11 pav. ir 12 pav. linija A, D, E, H) horizontaliai. Dar galimas ir Tomo Linkevičiaus pasiūlytas apibrėžimas – atstumas tarp cementavimo linijos ir vainikėlio profilio linijos.

Zona aplink implanto kraštą matuota keturiose vietose – atstumas nuo paties kraštinio implanto kaklelio taško iki gretimo danties iš mezialinės ir distalinės pusės (atstumas tarp linijų: nuo A iki B ir nuo C iki D, žr. 11 pav.) bei atstumas nuo paties kraštinio implanto kaklelio taško iki išorinio minkštųjų audinių krašto iš lūpinės ir liežuvinės pusės (atstumas nuo E iki F ir nuo G iki H, žr. 12 pav.). Implantas fotografuotas taip, kad nuotraukoje būtų aiškiai matyti visi šeši šešia-



11 PAV. Zonos aplink implanto kraštą matavimas iš mezialinės ir distalinės pusės



12 PAV. Zonos aplink implanto kraštą matavimas iš lūpinės ir liežuvinės pusės

kampės implanto veržlės kampai. Pagal implanto skersmenį nuotrauka sukalibruota naudojant skaitmeninę liniuotę. Vertinta naudojant operacinės sistemos „Windows 2010“ programinę įrangą „Microsoft PowerPoint“. Pridėjus liniuotę, atstumas nuo implanto krašto iki gretimo danties buvo matuojamas iš mezialinės ir distalinės pusės (žr. 11 pav.). Liežuvinis-lūpinis matavimas matomas 12 paveiksle.

Tiksliai pamatavus keliose vietose iš skirtingų perspektyvų (lūpinės, liežuvinės, mezialinės ir distalinės) nustatytos tokios zonos aplink implanto kraštą: a) iki 1 mm – 118 atvejų; b) 1–2 mm – 96 atvejai; c) 3 mm ir daugiau – 46 atvejai.

Cemento šalinimas

Cementavimas ir cemento valymo procedūros vykdytos labai panašiai, kaip ir atliekant *in vitro* tyrimą. Prieš cementavimą standartinė atrama buvo prisukta prie implanto, o prisukimo vieta izoliuota ortodontiniu vašku („Wax Pak“, gam. „3M Unitek“; Monrovija, Kalifornija, JAV) (žr. 13 pav.).

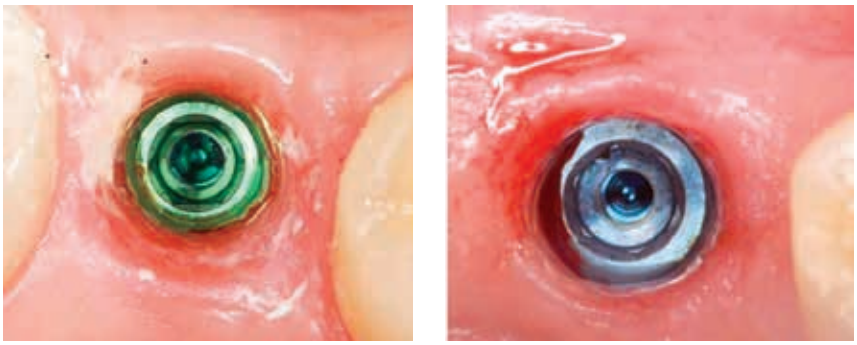
Okliuzinės vainikėlių angos užplombuotos („Gradia Anterior“, gam. „GC“, Tokijas, Japonija), kad cementuojant neištekėtų cemento perteklius ir būtų galima tiksliau imituoti klinikinę situaciją, kai vainikėlis yra be okliuzinės angos (žr. 14 pav.).

13 PAV. Prisukta standartinė atrama

14 PAV. Užplombuota vainikėlio anga

Cementas („GC Fuji Plus“) pagal gamintojų nurodymus sumaišytas atskirai kiekvienam vainikėliui. Plonu sluoksniu padengtas visas vidinis vainikėlio plotas ir švelniai spaudžiant pirštu uždėtas ant atramos. Cementui sukietėjus iki reikiamos konsistencijos, plieniniu zondu („Dentsply International Inc.“, Milfordas, Delaveras, JAV) ir dantų siūlais („Vitis“, gam. „Dentaid“, Barselona, Ispanija; gam.

15 PAV. Vainikėlis, kurį galima vertinti



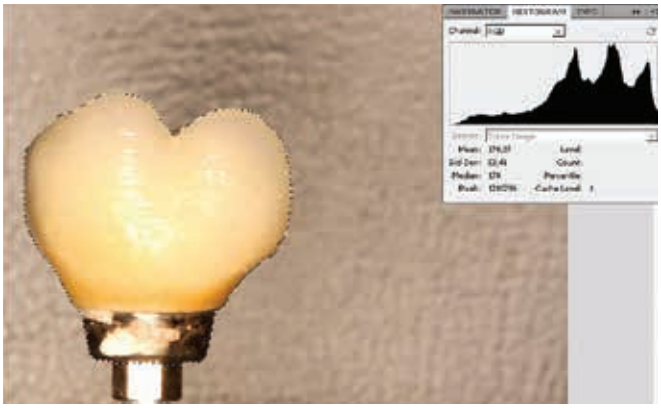
16 PAV. Vaizdas prieš vertinimą

„Curaprox“, Krynsas, Šveicarija) cementas valytas, kol įsitikinta, kad pašalintas visas jo perteklius. Cementą visais atvejais šalino tas pats gydytojas.

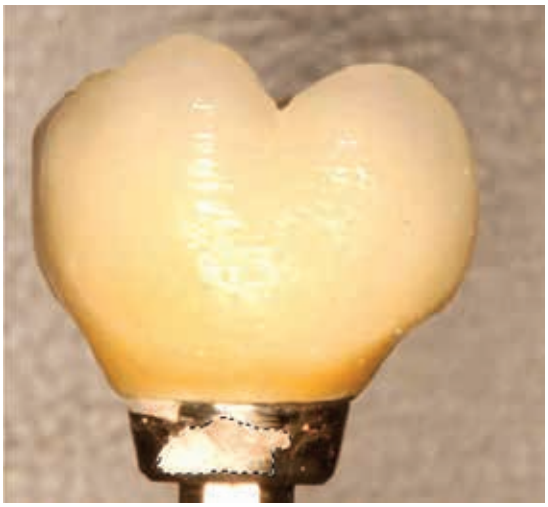
Rentgenologiniam vertinimui naudota „RVG Windows Trophy 5.0“ programa ir lygiagreti rentgeno nuotraukų atlikimo technika („Rinn“ tipo, gam. „Dentsply“, JAV). Jei po šio vertinimo vis dar buvo cemento, valymas kartotas, kol rentgeno nuotraukoje nesimatė jokių cemento likučių. Po šios procedūros pašalinta plomba ir vaškas, atsuktas atramos prisukimo varžtas ir nuo implanto nuimta atrama su pricementuotu vainikėliu, kad jį būtų galima įvertinti (žr. 15 pav.). Taip pat padarytos implanto nuotraukos (žr. 16 pav.).

Neaptikto cemento kiekio įvertinimas

Nuėmus vainikėlį, padaryta implanto ir aplink jį esančių audinių nuotrauka, kad būtų galima tinkamai įvertinti cemento likučius. Fotografuota specialiai sumontuotu įrenginiu, kuris leido išlaikyti standartizuotą 16 cm atstumą nuo foto-



17 PAV. Visas vainikėlio paviršius, apskaičiuotas pikseliais



18 PAV. Cementu dengtas paviršius, skaičiuotas pikseliais

aparato iki vainikėlio. Nuotraukos analizuotos naudojant programą „Adobe Photoshop“ (gam. „Adove Systems Ltd Europa“, Uksbridžas, Jungtinė Karalystė) – taip nustatytas cementu padengto ir viso vainikėlio paviršiaus santykis.

Toliau atliekant tyrimą vertintas okliuzinis implanto ir aplinkinių audinių vaizdas nuotraukose. Pažymėti keturi taškai, skaidantys implantą į lygias keturias dalis, tada nubrėžtos linijos, susikertančios per implanto centrą ir padalijančios jį į keturias lygias dalis. Kiekvienos dalies paviršius (neįtraukiant paties implanto ploto) ir cemento dengiamos vietos pažymėtos, kad būtų galima apskaičiuoti santykį.

Po šio vertinimo vainikėliai nusiųsti į laboratoriją, kad būtų pašalintas cementas ir atliktas poliravimas. Minkštieji audiniai nuvalyti nuo cemento likučių, o implantas ir aplink jį esantys audiniai praplauti skysčiu („Perio-Aid“, 0,12 proc., gam. „Dentaid“). Nupoliravus tie patys vainikėliai dezinfekuoti ir prisukti prie implantų,

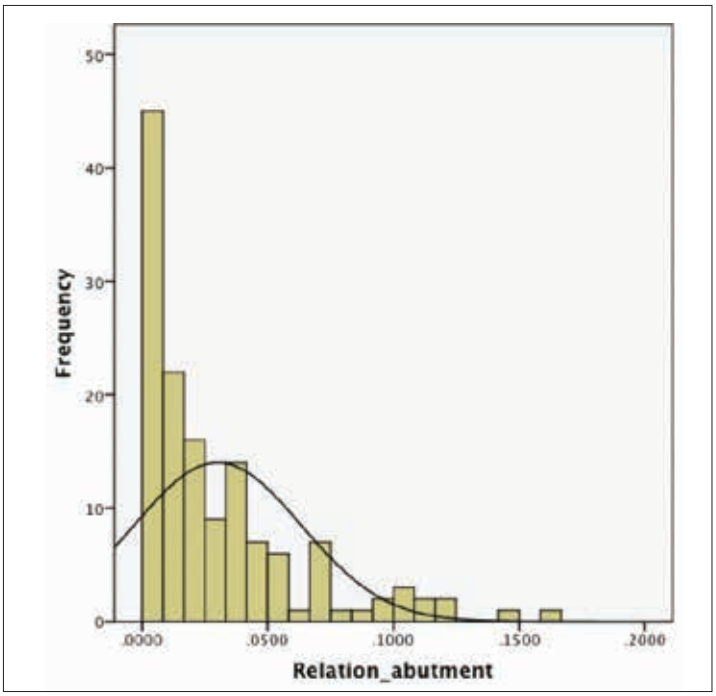
19 PAV. Visas kvadranto plotas (pikseliais) ir cemento likučių dengiamas plotas (pikseliais), kad būtų galima apskaičiuoti jų santykį

o prisukimo angos uždarytos politetrafluoretileno juostele (kaip pasiūlė Moraguez ir Belser 2010 m.) ir užplombuotos („Gradia Anterior“, gam. „GC“).

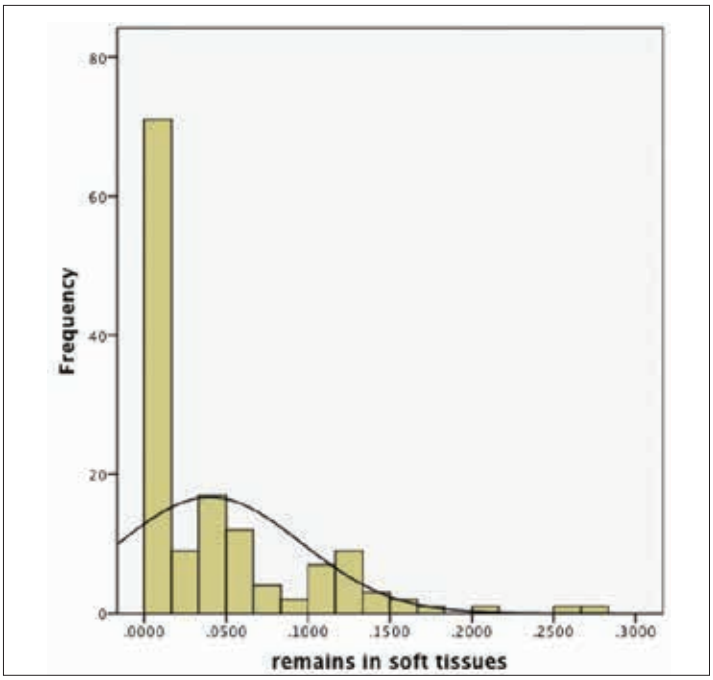
Statistinė analizė

Statistinė analizė atlikta naudojant tokią pat programą, kaip ir atliekant *in vitro* tyrimą. Statistiniu vienetu laikytas implanto kvadrantas, nes to paties implanto skirtingose vietose zonos aplink implanto kraštą ir cementavimo ribų gyliai buvo skirtingi.

Statistiškai analizuoti kiekvienos grupės duomenys, vidurkiai ir standartinės klaidos, taip pat sudaryta diagrama, iš kurios galima spręsti, ar duomenys tinkamai pasiskirstę (žr. 1 ir 2 diagramas). Duomenys buvo neparametriniai, tad atliktas K (Kruskall-Wallis) testas. Be to, reikėjo išsiaiškinti, ar daugiau cemento lieka, jei implanto atramos laiptelis yra kuo žemiau dantenų, kai zona aplink implanto kraštą didėja, o esant teigiamai koreliacijai (Kruskall-Wallis testas rodė statistinį reikšmingumą) lyginamiesiems grupių duomenims pateikti naudotas Mann-Whitney U testas. Apskaičiuoti vidurkiai buvo statistiškai reikšmingi, tinkamu intervalu laikytas 95 proc., $P \leq 0,05$.



1 DIAGRAMA. Cemento likučiai ant atramos, apskaičiuoti pikseliais



2 DIAGRAMA. Cemento likučiai ant minkštųjų audinių, apskaičiuoti pikseliais

Implanto skersmuo, zona aplink implanto kraštą ir cementavimo ribos gylis laikyti ranginiais (angl. *ordinal*) kintamaisiais, o vietos kintamasis – nominaliuoju (angl. *nominal*) kintamuoju, todėl atliktas tik Mann-Whitney testas, kad būtų galima palyginti skirtingų vietų ir cemento likučių santykį.

Rezultatai

In vitro tyrimas

Skirtingas cemento likučių kiekis buvo rastas ant visų mėginių. Visų grupių rezultatus sudarė šie duomenys: 1) cemento likučių svoris gramais; 2) cemento likučių dengiamo ir viso mėginio kvadranto paviršiaus santykis (žr. 1 lentelę).

K (Kruskall-Wallis) testas parodė reikšmingą neaptikto cemento likučių kiekio padidėjimą, kai laiptelis buvo kuo žemiau minkštųjų audinių. Atliekant šį testą, sverta (P = 0,00) ir skaičiuotas santykis (P = 0,00).

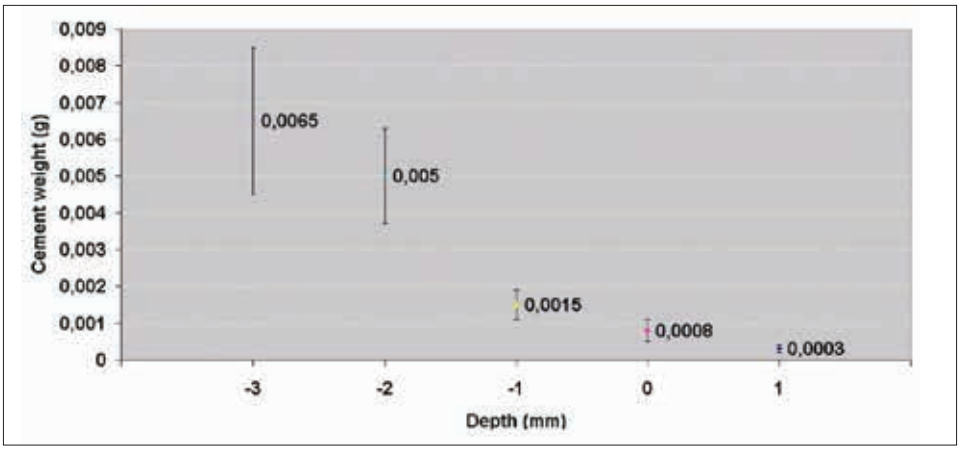
1 LENTELĖ. Cemento likučių apskaičiavimas skirtingose grupėse

Grupė	Cemento likučių svoris ± SP (g)	Santykis ± SP
1 grupė (virš dantenų)	0,0003 ± 0,0001	0,0111 ± 0,0212
2 grupė (ties dantenomis)	0,0008 ± 0,0003	0,0165 ± 0,0192
3 grupė (1 mm po dantenomis)	0,0013 ± 0,0005	0,0572 ± 0,0288
4 grupė (2 mm po dantenomis)	0,0051 ± 0,0013	0,1158 ± 0,0547
5 grupė (3 mm po dantenomis)	0,0063 ± 0,0021	0,1171 ± 0,0594

2 LENTELĖ. Neaptikto cemento likučių kiekio padidėjimas (svorio (P = 0,00) ir santykio atžvilgiu (P = 0,00)) laipteliui esant kuo žemiau minkštųjų audinių

	Gylis	N	Vidurkis
Santykis	–3 mm	20	74,30
	–2 mm	20	76,60
	–1 mm	20	53,98
	0	20	27,53
	1 mm	20	21,10
	Iš viso	100	
Svoris	–3 mm	5	21,40
	–2 mm	5	19,60
	–1 mm	5	12,60
	0	5	8,40
	1 mm	5	3,00
	Iš viso	25	
Statistika		Santykis	Svoris
χ^2		64,476	21,825
Df		4	4
Reikšminga (P)		0,00	0,00

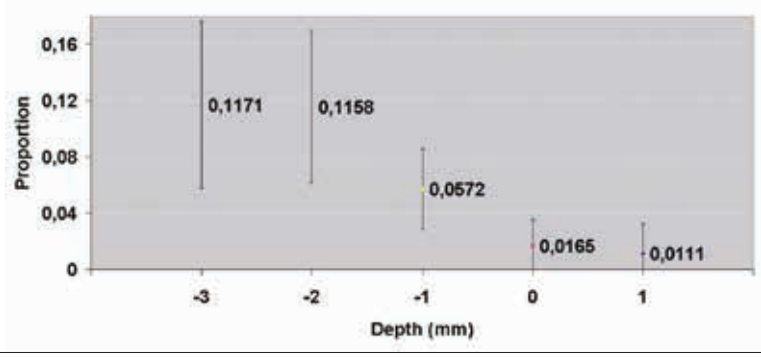
Mann-Whitney testas vertinant cemento likučių kiekį parodė statistiškai reikšmingų skirtumų tarp grupių (P ≤ 0,05), išskyrus ketvirtąją ir penktąją grupes (P > 0,05) (žr. 3 diagramą ir 3 lentelę).



3 DIAGRAMA. Neaptikto cemento likučių kiekis (svoris gramais), priklausomai nuo laiptelio vietos

3 LENTELĖ. Skirtumas tarp grupių

Grupė	Cemento svoris	Santykis
1 ir 2	P = 0,008	P = 0,054
2 ir 3	P = 0,025	P = 0,000
3 ir 4	P = 0,009	P = 0,000
4 ir 5	P = 0,344	P = 0,910
Pabrauktos vertės rodo statistinį reikšmingumą		

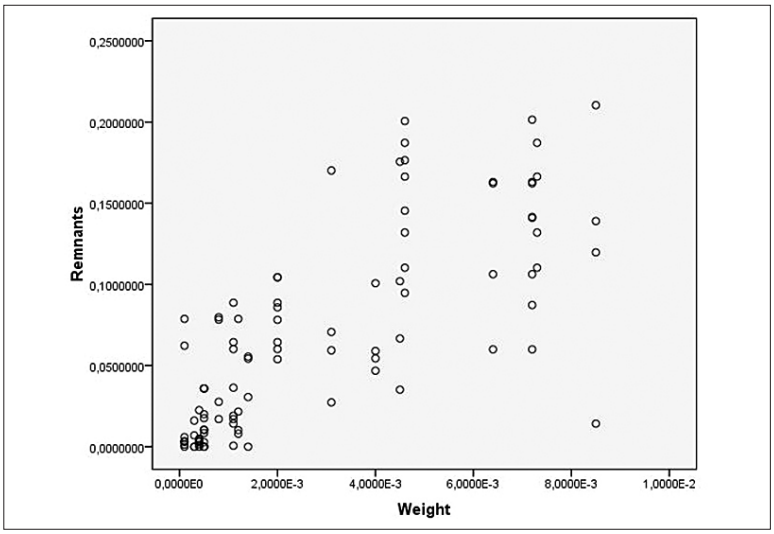


4 DIAGRAMA. Neaptikto cemento likučių kiekis, priklausomai nuo laiptelio vietos

Santykio vertinimas parodė statistiškai reikšmingą skirtumą tarp grupių ($P \leq 0,05$), išskyrus pirmąją ir antrąją bei ketvirtąją ir penktąją grupes ($P > 0,05$) (žr. 4 diagramą ir 4 lentelę).

Didžiausias neaptikto cemento kiekis buvo rastas implanto atramos laipteliui esant 2 mm ir 3 mm po dantenomis, mažiausias – 1 mm virš dantenų.

Paprasto išskaidymo grafinis vaizdas parodė teigiamą matavimų pasiskirstymą (žr. 5 diagramą), o Spearmano koreliacijos koeficientas – reikšmingą santykį tarp abiejų matavimo technikų ($r = 0,889$; $P = 0,00$).



5 DIAGRAMA. Matavimų išskaidymas

Klinikinis tyrimas

Tyrimo apimtys padidėjo nuo 65 iki 260 implantų, nes kiekvienas implantas matuotas keturis kartus. Pagal implanto atramos laiptelio gylį sudarytos keturios grupės (duomenys pateikti 5 lentelėje):

1 grupė – ties minkštaisiais audiniais (16 atvejų);

2 grupė – 1 mm po dantenomis (58 atvejai);

3 grupė – 2 mm po dantenomis (90 atvejų);

4 grupė – 3 mm po dantenomis (96 atvejai).

4 LENTELĖ. Duomenys

Laiptelio gylis	Zona aplink implanto kraštą	Vieta	Skersmuo
0 mm – 16 atvejų	1 mm – 118 atvejų	4 priekiniai (6,2 %)	3,5 mm – 21 (32,3 %)
–1 mm – 58 atvejai	2 mm – 96 atvejai	22 kapliai (33,8 %)	4,0 mm – 34 (52,3 %)
–2 mm – 90 atvejų	≥3 mm – 46 atvejai	39 krūminiai (60 %)	5,0 mm – 10 (15,4 %)
–3 mm – 96 atvejai	–	–	–
260 atvejų	260 atvejų	65 implantai	65 implantai

Cementavimo ribos gylis

5 LENTELĖ. Lyginamieji grupių duomenys

Grupė	Cemento ir vainikėlių santykis pikseliais ± SP	Cemento ir minkštųjų audinių santykis pikseliais ± SP
Gylis 0 mm	0,002 ± 0,001	0,014 ± 0,006
Gylis –1 mm	0,024 ± 0,005	0,052 ± 0,011
Gylis –2 mm	0,036 ± 0,004	0,057 ± 0,009
Gylis –3 mm	0,055 ± 0,007	0,071 ± 0,012

Statistiškai reikšmingas cemento likučio padidėjimas pastebėtas, kai laiptelis buvo kuo žemiau dantenų (P = 0,000). Reikšmės turėjo ir vieta (P = 0,0045) (žr. 6 lentelę).

6 LENTELĖ. Neaptikto cemento likučių padidėjimas, laipteliui esant kuo žemiau, ir jo vietos įtaka rezultatams

Cementas ir vainikėlis	N	Mediana
1 grupė	16	0,002
2 grupė	58	0,009
3 grupė	90	0,027
4 grupė	96	0,043
Iš viso	260	
P = 0,000		

Cementas ir vainikėlis	N	Mediana
Cementas ir minkštieji audiniai		
1 grupė	16	0,000
2 grupė	58	0,023
3 grupė	90	0,035
4 grupė	96	0,035
Iš viso	260	
P = 0,045		

Statistiškai reikšmingas skirtumas tarp visų grupių (P ≤ 0,05) nustatytas, kai buvo vertintas cemento perteklius ant atramos ir vainikėlio, taip pat tarp 1 ir 2 grupių (P ≤ 0,05), kai vertintas cemento kiekis ant minkštųjų audinių (žr. 7 lentelę).

7 LENTELĖ. Lyginamieji grupių duomenys

Grupė	Cemento likučių kiekio padidėjimas	
	Ant vainikėlio	Ant minkštųjų audinių
1 ir 2	P = 0,000	P = 0,005
2 ir 3	P = 0,014	P = 0,439
3 ir 4	P = 0,003	P = 0,491

Implanto vieta

8 LENTELĖ. Rezultatai pikseliais, priklausomai nuo implanto vietos

Grupė	Cemento ir vainikėlio santykis pikseliais ± SP	Cemento ir minkštųjų audinių santykis pikseliais ± SP
Priekiniai	0,030 ± 0,008	0,034 ± 0,012
Kapliai	0,038 ± 0,004	0,073 ± 0,011
Krūminiai	0,040 ± 0,004	0,070 ± 0,009

Statistiškai reikšmingo skirtumo tarp cemento likučių ant vainikėlio ir minkštųjų audinių, priklausomai nuo implanto vietos (visos p vertės buvo didesnės nei 0,05), nepastebėta (žr. 9 lentelę).

9 LENTELĖ. Cemento likučių ant vainikėlio ir minkštųjų audinių skirtumas tarp grupių

Grupė	Cemento likutis (santykis pikseliais)	
	Ant vainikėlio	Ant minkštųjų audinių
Priekiniai ir kapliai	P = 0,497	P = 0,061
Kapliai ir krūminiai	P = 0,798	P = 0,754
Priekiniai ir krūminiai	P = 0,425	P = 0,065

Implanto skersmuo

10 LENTELĖ. Rezultatas, apskaičiuotas pagal implanto skersmenį

Grupė	Cemento ir vainikėlio santykis pikseliais ± SP	Cemento ir minkštųjų audinių santykis pikseliais ± SE SP
3,5 mm skersmuo	0,033 ± 0,004	0,074 ± 0,013
4,0 mm skersmuo	0,077 ± 0,004	0,077 ± 0,009
5,0 mm skersmuo	0,039 ± 0,008	0,021 ± 0,007

Statistiškai reikšmingas cemento likučių sumažėjimas pastebėtas, kai implantai buvo platesni (p = 0,026), tačiau nebuvo reikšmingos koreliacijos tarp cemento likučių ant atramos ir didesnio implanto skersmens (p = 0,600).

Lyginamieji grupių duomenys pateikti 11 lentelėje.

11 LENTELĖ. Lyginamieji grupių duomenys pagal implanto skersmenį ir cemento likučių kiekį

Grupė	Cemento likučiai (santykis pikseliais)	
	Ant vainikėlio	Ant minkštųjų audinių
3,5 mm ir 4,0 mm	P = 0,011	P = 0,754
4,0 mm ir 5,0 mm	P = 0,012	P = 0,009
3,5 mm ir 5,0 mm	P = 0,050	P = 0,012

Zonos aplink implanto kraštą dydis

12 LENTELĖ. Lyginamieji grupių duomenys pagal zoną aplink implanto kraštą

Grupė	Cemento ir vainikėlio santykis pikseliais ± SP	Cemento ir minkštųjų audinių santykis pikseliais ± SP
Zonos aplink implanto kraštą dydis – 1 mm	0,035 ± 0,004	0,054 ± 0,009
Zonos aplink implanto kraštą dydis – 2 mm	0,040 ± 0,004	0,081 ± 0,010
Zonos aplink implanto kraštą dydis – 3 mm	0,048 ± 0,012	0,084 ± 0,022

Stiprus ryšys tarp zonos aplink implanto kraštą ir cemento likučių kiekio matyti ne tik ant minkštųjų audinių (p = 0,004), bet ir ant vainikėlio ir atramos (p = 0,046).

13 LENTELĖ. Lyginamieji grupių duomenys pagal zoną aplink implanto kraštą

Grupė	Cemento likučiai (santykis pikseliais)	
	Ant vainikėlių	Ant minkštųjų audinių
1 mm ir 2 mm	P = 0,005	P = 0,002
2 mm ir 3 mm	P = 0,049	P = 0,039
1 mm ir 3 mm	P = 0,003	P = 0,002

Visų cemento likučių pašalinimas

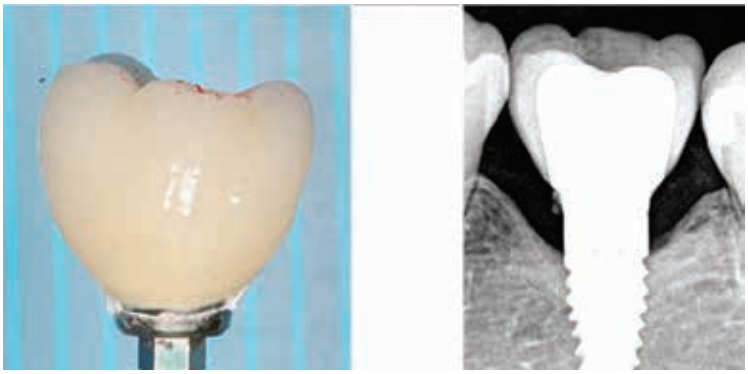
Skirtingas kiekis cemento likučių rastas ant beveik visų mėginių ir minkštųjų audinių. Duomenys apie cemento nebuvimą ant atramos ir minkštųjų audinių pateikiami 14 lentelėje. Pažymėtina, kad nors daugeliu atveju cemento nebuvo, tačiau cemento likučius ant atramos ir minkštųjų audinių skaičiuojant kartu, jo kiekis procentais smarkiai didėjo. Atlikti tyrimai rodo, kad cementas greičiausiai stipriai prikimba prie atramos ir vainikėlio arba prie minkštųjų audinių.

14 LENTELĖ. Visų cemento likučių pašalinimas pagal šiame tyrime analizuotus veiksnius

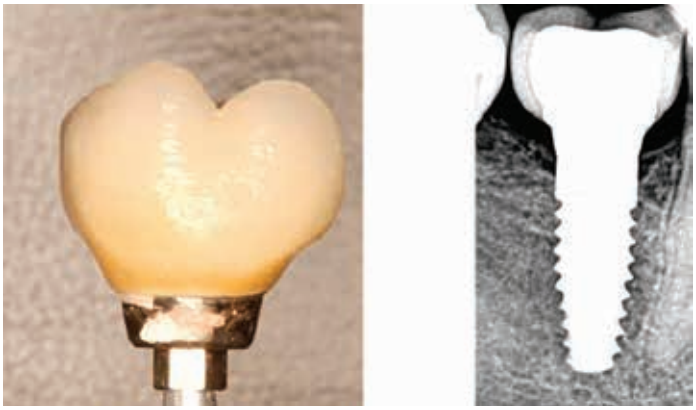
Grupės	Visų cemento likučių pašalinimas nuo vainikėlio	Visų cemento likučių pašalinimas nuo minkštųjų audinių	Visų cemento likučių pašalinimas nuo atramos vainikėlio ir minkštųjų audinių
Gylis 0 mm (N = 16)	5 (31,2 %)	15 (93,8 %)	4 (25 %)
Gylis –1 mm (N = 58)	3 (5,2 %)	22 (37,9 %)	3 (5,2 %)
Gylis –2 mm (N = 90)	6 (6,7 %)	28 (31 %)	3 (3,3%)
Gylis –3 (N = 96)	1 (1,0 %)	35 (36 %)	0 (0 %)
Zonos aplink implanto kraštą dydis – 1 mm (N = 118)	13 (11,0 %)	65 (55,1 %)	9 (7,6 %)
Zonos aplink implanto kraštą dydis – 2mm (N = 96)	6 (6,3 %)	34 (35,4 %)	2 (2,1 %)
Zonos aplink implanto kraštą dydis – 3 mm (N = 46)	1 (2,2 %)	12 (26,1 %)	1 (2,2 %)
Priekiniai (N = 16)	0 (0,0 %)	9 (56,3 %)	0 (0,0 %)
Kapliai (N = 88)	8 (9,1 %)	34 (38,6 %)	4 (4,5 %)
Krūminiai (N = 156)	12 (7,7 %)	68 (43,6 %)	8 (5,1 %)
Skersmuo – 3,5 mm (N = 84)	7 (8,3 %)	33 (39,3 %)	3 (3,6 %)
Skersmuo – 4,0 mm (N = 136)	11 (8,1 %)	59 (43,4 %)	8 (5,8 %)
Skersmuo – 5,0 mm (N = 40)	2 (5,0 %)	19 (47,5 %)	1 (2,5 %)

Rentgenologinis įvertinimas

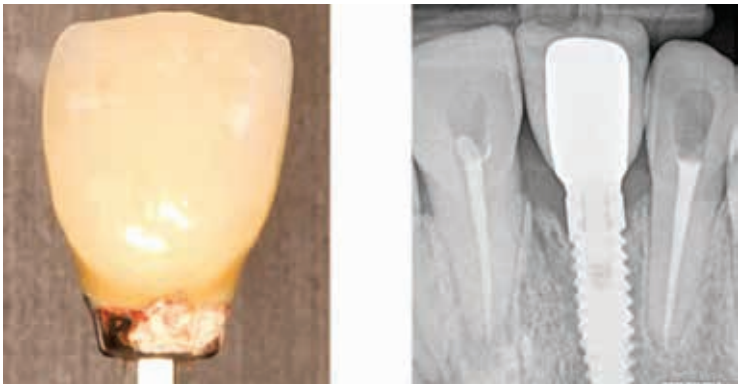
Atlikus pirmąjį radiologinį vertinimą, cemento likučiai mezialinėje pusėje buvo matomi 5 atvejais iš 65 (7,7 proc.) ir distalinėje pusėje 7 atvejais iš 65 (10,7 proc.). Toliau pateiktose keliose tyrimo nuotraukose matyti skirtumas tarp rentgenologinį ir vainikėlio nuotraukų.



20 PAV. Tik iš mezialinės pusės darytose rentgeno nuotraukose matomi cemento likučiai



21 PAV. Jei cemento likučiai yra lūpinėje pusėje, jų rentgeno nuotraukoje nematyti



22 PAV. Jei cemento likučiai yra lūpinėje pusėje, jų rentgeno nuotraukoje nematyti

Išvados

1. Kuo giliau yra implanto atramos laiptelis, tuo daugiau lieka neaptikto cemento, prikibusio prie atramos, vainikėlio ir minkštųjų audinių apie implantus.
2. Tiek svėrimo, tiek kompiuterizuotas planimetrinis metodas yra vienodai tikslūs nustatant cemento likučio kiekį.
3. Daugeliu atveju radiologinis tyrimas nėra tinkamas būdas cemento likučiams aptikti.
4. Implanto vieta neturi įtakos cemento nuvalymo kokybei.
5. Esant didesniam implanto skersmeniui, minkštuosiuose audiniuose lieka mažiau cemento, tačiau tai neturi įtakos cemento kiekiui, likusiam ant atramos ir vainikėlio.
6. Esant didesnei zonai aplink implanto kraštą, lieka daugiau neaptikto cemento.
7. Atliekant šį tyrimą visiškai pašalinti cemento nebuvo įmanoma dėl daugelio veiksnių.

Praktinės rekomendacijos

1. Cementuojant vainikėlį, cementavimo linija turėtų būti matoma, kad būtų galima tinkamai pašalinti cemento likučius. Tam naudotinos individualios atramos.

2. Rentgenologinis cemento likučių vertinimas neturėtų būti laikomas patikimu metodu, jei cementavimo linija yra žemiau minkštųjų audinių.

3. Jei tik įmanoma, reikėtų siekti, kad nesusidarytų zona aplink implanto kraštą, t. y. kad sutaptų cementavimo krašto ir išorinė vainikėlio linijos.

4. Jei tik įmanoma, patariama naudoti prisukamus vainikėlius.

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