dies doctorandorum
BOOK OF ABSTRACTS
2022

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POSTGRADUATE DOCTORAL STUDY
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Foreword

Dies doctorandorum is an annual and traditional event we are hosting again in 2022 live after a two-year break due to COVID 19 pandemics. This is an open and public event that welcomes participants with a focus on Ph.D. students, presenting their research work available for review and debate.

This event is designed to discuss students' Ph. D. research projects, provide constructive guidelines and lead research in the right direction so that students can successfully complete their postdoctoral studies in a timely manner. All members of the scientific community are expected to participate and this event is intended for all those who love and live science in order to exchange their experiences with each other and provide guidance to young colleagues at the beginning of their scientific careers. Furthermore, our goal is to encourage social contacts, and improve cooperation between mentors and Ph.D. students thus encouraging the progress of the scientific community as well as the postdoctoral study' program itself.

Job of the scientific community is to do work that is important – work that has an impact and makes a difference in the world, work that breaks new ground and solves important problems. A message to our Ph.D. students is to do work that you find interesting and important. Working on problems that interest you will make the process enjoyable and sustain you during the long nights that it can take to bring a project to fruition.

Furthermore, being part of a community is an important part of a scientific career. Building your community of scholars is utmost importance. Interacting with these scholars will enable you to keep up with and contribute to your area. It is often said that it is the smaller conferences where one learns more and has more satisfying interactions such as our event. And it is senior people that you meet at this event who will most likely be asked to write letters for your promotion and tenure decision.

Despite the informal nature of Dies doctorandorum the progress of our Ph.D. students will be analyzed. Therefore, the best poster presentations will be chosen by the Members of the Committee for Doctoral Studies and awarded the Dean's award.

In closing, I would like to reiterate the importance of working on important questions, being very rigorous and finding "real" not alternative facts, both exploiting and exploring research, and being part of a scientific community.

Professor Ivica Mihaljević, M.D., Ph.D.
Dean, Faculty of Medicine Osijek

Abstracts of annual seminars

Dissertation Proposal Title: Influence of pepsin on AID and APOBEC3 expression and hypertrophy of lymphatic tissue of adenoids and palatine tonsils.

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Mentor: Prof. Andrijana Včeva, M.D., Ph.D., ENT Clinic, Clinical Hospital Centre Osijek, Osijek, Croatia

Co-mentor: Tihana Mendeš, M.D., Ph.D., ENT Clinic, Clinical Hospital Centre Osijek, Osijek, Croatia

Introduction: Hypertrophy of the palatine tonsils and adenoids in a certain number of people can cause symptoms such as otitis media, deafness, swallowing problems and obstructive sleep apnea. Pepsin is an enzyme proteinase produced by gastric cells mucous membranes and can be present in many other parts of human body. Activation-induced cytidine deaminase (AID) and apolipoprotein B mRNA mediated catalytic polypeptide (A3) are a family of cytidine deaminases and play a significant role in immune system. In our research, we will determine whether pepsin plays a role in the expression profiles of AIDs and A3s.

Hypothesis: Pepsin affects AID and APOBEC3 expression with consequent lymphatic tissue hypertrophy of adenoids and palatine tonsils.

Aims:
1. To investigate the influence of pepsin on expression profile of AID and APOBEC3 in the development of hypertrophy of adenoids and palatine tonsils.
2. To examine the pathophysiological mechanism of the influence of AID and APOBEC3 on hypertrophy of adenoids and palatine tonsils.

Materials/Participants and Methods: The study will include 100 patients under the age of 18 with indications for tonsilloadenoidectomy. The patients will be divided into two groups, depending on pepsin presence in the samples. Tympanometry will be used to determine middle ear function. PCR, Elisa and immunohistochemistry will be used. Prior to joining the study, all patients will be offered to sign an informed consent document for participation.

Research plan: Subjects are patients with indications for tonsilloadenoidectomy. Before the operative procedure, a sample of patient's saliva will be collected for further analysis. After the procedure, samples of palatine tonsils and adenoids will be collected and subjected to immunohistochemistry staining and ELISA. The planned duration of the study is 14 months / until the planned number of patients is collected.

Expected scientific contribution: The primary scientific contribution would be in detecting the effect of pepsin on the expression of AID and APOBEC3 in lymphatic tissue as a mechanism of hypertrophy of the lymphatic tissue of the tonsils and adenoids vegetation.

Keywords: Waldeyer's lymphatic ring; Apolipoprotein B Editing Complex type 3; Activation - induced cytidine deaminase; Pepsin

Dissertation Proposal Title: Impact of SGLT 2 inhibitors on endothelial dysfunction in patients with diabetes mellitus type II

PhD candidate: Jerko Arambašić, M.D., Department for Heart and Vascular Diseases, University Center Hospital Osijek, Osijek, Croatia. Faculty of Medicine, University of Osijek. Croatia.

Mentor: Hrvoje Roguljić, M.D., Ph.D., Department for Heart and Vascular Diseases, University Center Hospital Osijek, Faculty of Dental Medicine and Health Osijek, Osijek Croatia. Faculty of Medicine, University of Osijek, Croatia.

Introduction: SGLT2 inhibitors represent novel therapeutic option in the treatment of type II diabetes mellitus. This class of antidiabetic drugs has been associated with the reduction in the composite of cardiovascular mortality and heart failure hospitalizations among patients with heart failure in multiple cardiovascular outcome trials. The mechanism of their beneficial cardiovascular impact is not yet fully clarified. One of the proposed cardioprotective mechanisms is a positive effect of SGLT inhibitors treatment on the endothelial dysfunction in diabetic patients.

Hypothesis: SGLT2 inhibitors have a benefitial effect on the endothelial function in diabetic patients

Aims: this study aimed to assess the effect of SGLT2 inhibitors treatment on the markers of the endothelial function and oxidative stress in patients with type II diabetes.

Materials/Participants and Methods: the study is including 40 patients of both sexes in whom therapy with SGLT2 inhibitors was initiated in the Department of diabetes, endocrinology and metabolism disorders of Osijek University Hospital Centre in 2022. The markers of endothelial dysfunction and oxidative stress (CRP, oxLDL, TNFα, PAI-1, vWF, IL-6, E-selectin and VCAM-1) will be measured in serum before and three months after initiation of the therapy. Patients with known macrovascular disease will be excluded.

Research plan: this prospective observational study will be conducted at the Department of diabetes, endocrinology and metabolism disorders of Osijek University Hospital Centre. The research will take 12 months.

Expected scientific contribution: insight in the percise mechanism of action of SGLT2 inhibitors could help us determine subgroups of patients that could benefit the most by using these drugs.

Keywords: Diabetes mellitus typ II, Endothelial dysfunction, SGLT2 inhibitors.

Dissertation Proposal Title: Why rheumatoid arthritis represents cardiovascular risk?

PhD candidate: Dražen Bedeković, M.D. Ph.D. Clinical Hospital Center Osijek, Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Mentor: Prof. Srđan Novak, M.D. Ph.D., Clinical Hospital Center Rijeka, Rijeka, Croatia

Introduction: The vast majority of published studies and meta-analyzes of studies to date have shown that patients with rheumatoid arthritis (RA) have a significantly higher risk of developing cardiovascular disease compared to the general population; about 50-60%, as well as increased mortality from cardiovascular diseases, especially from acute myocardial infarction, acute cerebrovascular disease as well as chronic ischemic heart failure. Classic cardiovascular risk factors include variable factors: arterial hypertension, dyslipidemia, insulin resistance or diabetes, cigarette smoking, low physical activity; and irreversible factors: family heritage burden, race, age, and gender. This cardiovascular risk study in patients with RA and osteoarthritis (OA) was conducted on a total of 201 subjects; 124 RA and 77 OA, over an eight-year trial period.

Hypothesis: Statistically significant difference exists in the incidence and prevalence of cardiovascular risk factors: arterial hypertension, diabetes, hypercholesterolemia, and cigarette smoking between RA and OA study groups during the study period.

Aims:

1. To determine whether there is an association of cardiovascular risk factors and chronic inflammatory disease (RA, study group) in relation to patients with osteoarthritis (OA, control group) during the follow-up period.
2. To determine whether there is a statistically significant difference in the incidence of cardiovascular disease in patients with RA (study group) compared to patients with OA (control group) during the follow-up period and to compare the available incidence data in the general population.
3. Analyze the impact of chronic inflammation (RA) on cardiovascular risk factors with regard to the duration of the disease and the success of the inflammation control - subgroup analysis.

Materials/Participants and Methods: The study is structured as a prospective clinical cohort trial. The main difference between the study and control groups is the high inflammatory activity in RA, while in OA the inflammatory activity is low.

Inclusion criteria:

- diagnosed with rheumatoid arthritis or osteoarthritis by a rheumatologist according to the valid 1987 American College of Rheumatology (ACR) classification criteria for rheumatoid arthritis, or ACR criteria for osteoarthritis
- consent to participate in the study, which the patient must confirm by signing the informed consent.

Exclusion criteria:

- non-fulfillment of inclusion criteria.
- diagnosed with cardiovascular disease before the first visit.
- rejection of patients after the first study visit in further participation.
- unavailability of patients after the first visit.
- for the analysis of chronic inflammation control, the omission of three or more visits of subjects from the test group (RA).

For patients who ceased to be available the reason for unavailability is recorded, if the patient died, the time of death, the cause of death and the existence of cardiovascular diseases or risk factors until the time of death were recorded.

Research plan:

1. Screening of patients according to inclusion and exclusion criteria.
2. First visit - inclusion of subjects in the study and control group, collection of relevant data, conducting clinical examinations, tests and diagnostic procedures (EGK, blood sampling for laboratory analysis)
3. Follow-up of study group on annual visits: tests showing the success of inflammation control.
4. Final visit - study and control group: collection of relevant data, conducting clinical examination, tests and diagnostic procedures (EGK, blood sampling for laboratory analysis)
5. Statistical analysis.
6. Publication of results.

Preliminary results:

MAIN AIMS:

Statistically significant difference between the study and control groups:
Prevalence of hypertension – no
Incidence of hypertension – no
Prevalence of cigarette smoking - yes RA higher (general and current; general population)
Prevalence of diabetes - no (yes in relation to the general population)
Prevalence of hypercholesterolemia - yes RA higher

Prevalence of metabolic syndrome – no
Incidence of CVD - no; RA 31.7%, OA 30.9%
SECOUNDARY AIMS:
Deaths average year: RA 71.5; OA 77; CVD cause RA 70.7%, OA 58.8%
CVD causes of death for RA are statistically significantly higher than for the general population.
Subgroup analysis - inflammation control (RA):
Incidence of CVD - yes, there are fewer good ones.
Incidence of decompensation - yes, there are fewer good ones.
Prevalence of hypertension – no
Prevalence of hypercholesterolemia (LDL) - good control has higher.
HDL values - good control has higher.
Prevalence of diabetes - no, GUK and HbA1c is lower with good control.
Subgroup analysis - duration of disease (<15, 15-25,> 25 years) RA and OA:
Incidence of CVD – no
Incidence of decompensation – no
Prevalence of hypertension – no
Prevalence of hypercholesterolemia (LDL) – no
Prevalence of diabetes - no
Prevalence of cigarette smoking - yes for RA <15g.
BMI yes for RA higher <15g and 15-25g

Significance/Expected scientific contribution: The scientific contribution of research is in the possible demonstration of the influence of chronic systemic inflammation on cardiovascular risk factors and the incidence of cardiovascular diseases. If a statistically significant difference between the study and control groups exists considering the incidence of cardiovascular diseases, as well as the incidence and prevalence of risk factors for the development of cardiovascular diseases, direct chronic inflammation impact on cardiovascular risk factors (individual or all) will be demonstrated.

MeSH/Keywords: rheumatoid arthritis, osteoarthritis, arterial hypertension, diabetes mellitus, hypercholesterolemia, cigarette smoking, cardiovascular risk

Acknowledgement: I would like to express my very great appreciation to Višnja Prus and the staff of Department of clinical immunology, allergology and rheumatology University Hospital Centre Osijek.

Abstract Title: Tumor markers can differentiate COPD from other benign lung inflammations: A pilot study

Part of the Disertation Proposal: Diagnostic significance of Carbohydrate Sulfotransferase 7 in Lung Cancer

PhD candidate: Gramos Begolli, M.D., University Clinical Center of Kosovo, Kosovo

Mentor: Assist. Prof. Željko Debeljak, University Hospital Centre Osijek, Croatia; Faculty of Medicine Osijek, University of Osijek, Croatia

Introduction: Lung cancer and chronic obstructive pulmonary disease (COPD) are serious global health concerns. By increasing oxidative stress and the consequent DNA damage, chronic exposure to pro-inflammatory cytokines, inhibition of DNA repair systems, and enhanced cellular proliferation, COPD could be a driving factor in lung cancer. It is important to differentiate malignant diseases from COPD and other benign inflammations.

Aims: Aim of this pilot study is to determine and compare the following lung tumor markers in COPD and other benign pulmonary inflammtions: carcioemryonic antigen (CEA), neuron specific enolase (NSE), cytokeratin fragment 21-1 (CYFRA) and Progastrin-releasing peptide (ProGRP).

Materials/Participants and Methods: 92 patients were enrolled in this study: 39 patients diagnosed with COPD and 53 with other benign pulmonary inflammations. Blood samples were taken from subjects and serum was used for determination of CEA, CYFRA 21-1, ProGRP and NSE. Tumor markers were mesured using electrochemiluminescence immunoassay by Cobas 411, Roche diagnostics in Osijek University Hospital. For estimation of diagnostic accuracy Receiver Operating Characteristics (ROC) analysis implemented in MedCalc version 20.104 software was used.

Results: ROC analysis of NSE and CYFRA 21-1 data has shown statistically significant differentiation of COPD from other benign pulmonary inflammations. In comparison to the other pulmonary inflammation group, NSE was elevated in COPD ($AUC = 0.895$, $p < 0.001$) while CYFRA was elevated in other inflammations ($AUC = 0.746$, $P < 0.01$). Diagnostic accuracy of ProGRP and CEA was not statistically significant.

Conclusion: Preliminary results show promising diagnostic accuracies of NSE and CYFRA 21-1 in differentiation of COPD from other benign pulmonary inflammations. This analysis has to be performed on a larger set as a part of tumor marker accuracy evaluation in malignant and benign pulmonary diseases diagnostics.

MeSH/Keywords: Tumor Markers, COPD, Pulmonary Inflammations, NSE, CYFRA 21-1

Dissertation Proposal Title: Use of cardiac biomarkers in assessing the significance of atherosclerotic coronary heart disease

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Mentor: Assoc. Prof. Ines Bilić Ćurčić, M.D., Ph.D., Dtp. of Pharmacology Faculty of Medicine Osijek, University of Osijek, Clinical Institute of Internal Medicine, University Hospital Osijek, Croatia

Introduction: Cardiac biomarkers, troponin (Tn), N-terminal pro-brain natriuretic peptide (NTproBNP), C-reactive protein (CRP), and galectin-3 (GAL-3 are diagnostically and prognostically significant in the survival rate of acute myocardial infarction and heart failure. GAL-3 proved to be the best marker of inflammation and myocardial fibrosis, with no dynamics (unlike NT-proBNP); however, the role of galectin-3 in stable coronary disease remains to be explored.

Hypothesis: Cardiac biomarkers (Tn, NTproBNP, CRP, and GAL-3) have predictive value in evaluating stable coronary heart disease (CHD) severity and correlate with its extensiveness.

Aims:
1. Determine serum concentration of galectin-3 in patients with coronagraphically proved CHD and in the control group.
2. Determine a relationship between serum galectin-3 concentration and NT proBNP, Tn, and CRP level in patients with coronagraphically proved CHD and in the control group.
3. Determine a relationship between studied cardiac biomarkers with Syntax scores I and II in patients with coronagraphically proved CHD and in the control group.
4. Monitor all-cause mortality, cardiovascular mortality rate, major adverse cardiac events (MACE), target lesion revascularization (TLR), heart failure rate, and atrial fibrillation rate in patients with coronagraphically proved CHD and in the control group.

Materials/Participants and Methods: This research would be conducted as a case-control study including 150 eligible patients who underwent coronary angiography

due to suspected CHD. The study would include patients with a diagnosis of angina pectoris in whom non-invasive diagnostic processing confirmed the suspicion of a CHD. According to the percutaneous coronary intervention (PCI) finding subjects will be divided into 3 groups: 1. subjects with coronary heart disease resolved with PCI; 2. subjects with coronary heart disease in which coronary artery bypass graft surgery (CABG) procedure is indicated; 3. control group without angiographically proven CHD. Exclusion criteria are previous myocardial infarction, previous heart failure, malignant disease, chronic liver disease, previously known chronic renal failure, kidney disease, acute infection, autoimmune disease, and diabetes mellitus.
To confirm or reject hypotheses at the level of significance of 5%, and for the accuracy of the test to be higher than 80%, it is estimated that each group of patients should have 50 subjects (PCI group, CABG group, control group).
The serum level of galectin-3 would be determined by the ELISA technique and compared with TnI, hsCRP, and NTproBNP serum levels.

Research plan:

1. Screening of patients according to inclusion and exclusion criteria.
2. Collecting serum samples for GAL-3, NTproBNP, Tn, CRP, and laboratory analysis
3. Statistical analysis for paper publication
4. Follow up for all-cause mortality, cardiovascular mortality, MACE, TLR, HF, FA for 3-5 years
5. Statistical analysis
6. publication

Expected scientific contribution: Patients with stable coronary disease and elevated galectin-3 levels are expected to have a higher hospitalization rate due to heart failure, higher mortality and AIM rate; and need myocardial revascularization, thrombosis, and stent restenosis more often. Long-term follow-up is crucial to identify patients with a high risk of adverse clinical outcomes.

MeSH/Keywords: Biomarker, galectin-3, cardiovascular disease, heart, heart failure

Dissertation Proposal Title: The role of adenosine A1 and A2a receptors in changes in vascular reactivity after hyperbaric oxygenation

PhD candidate: Vedran Đambić, M.D.

Mentor: Assist. Prof. Aleksandar Kibel, M.D., Ph.D., Department of Heart and Vascular Diseases, University Hospital Centre Osijek; Faculty of Medicine Osijek, University of Osijek, Croatia

Co-mentor: Assist. Prof. Zrinka Mihaljević, Ph.D., Institute and Department of Physiology and Immunology, Faculty of Medicine Osijek, University of Osijek, Croatia

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Introduction: Hyperbaric oxygenation (HBO_2) has the ability to alter protein expression, modulates signaling pathways, and influences vascular structure and function leading to changes in vasomotor responses. Adenosine receptors (AR) play an important role in the mechanism of action of oxygen as a vasoactive substance. New data suggest important effects of HBO_2 on oxidative stress and alteration of production of vasoactive metabolites in blood vessels; however, the role of adenosine A1 and A2a receptors is still underinvestigated.

Aims: This study aimed to determine the functional role of adenosine A1 and A2a receptors in changes of vascular reactivity in isolated middle cerebral arteries (MCA) of Sprague-Dawley (SD) rats, after acute and intermittent HBO_2.

Participants and Methods: Male and female healthy SD rats aged 8-10 weeks were divided into 3 groups: CTRL (control non HBO_2), A- HBO_2 (animals exposed to a single

HBO_2 for 2 hours) and 4D- HBO_2 (animals exposed to HBO_2 at a pressure od 2 bars for 2 hours daily for 4 days, with vascular experiments conducted on the fifth day). The rats were anesthetized with ketamine-chloride (3 ml/kg) and midazolam (0,5 ml/kg) and decapitated. The MCA were isolated, cannulated and pressurized for 60'at 80 mmHg to assess basal diameter using pressure myograph Danish Myo Technology. A1 adenosin receptor selective agonist CCPA or A2a adenosin receptor agonist CGS in concentrations from 10^{-5} to 10^{-10} M were added to the vessel chamber, and blood vessel diameter was measured after incubation of the drug for 15 minutes. Samples of surface brain blood vessels were used for qRT-PCR. Relative gene expression of A1R and A2aR genes (mRNA) was measured with HPRT1 as the housekeeping gene. Statistical analyses were performed with Two-way ANOVA tests; $p<0.05$ was considered significant.

Results: The MCA response to adenosine A1 receptor administration was significantly reduced in rats exposed to A-HBO2 compared with CTRL and I-HBO2, while the response was similar between the CTRL and 4D-HBO2 groups. Rats exposed to A-HBO2 compared with CTRL and I-HBO2 at higher drug concentrations (10-6 to 10-5 M) showed a reduced MCA response to adenosine A2a agonist administration. Acute exposure to HBO_2 did not significantly alter A1R or A2aR gene expression. There was a significant increase in A1R i A2aR expression in the 4D-HBO_2 group compared to CTRL.

Conclusion: The results suggest that acute exposure of rats to HBO2 leads to a reduced MCA response to stimulation of adenosine A1 and A2a receptors. Intermittent exposure of rats to HBO2 resulted in a similar MCA response to adenosine A1 and A2a receptor stimulation as in the control group. Intermittent exposure to hyperbaric oxygen acts as pseudohypoxia, resulting in a protective compensatory increase in adenosine A1R and A2aR gene expression. This suggesting that HBO_2 alters sensitivity to or expression of adenosine receptors.

Key words: adenosine receptors, cerebral blood vessels, flow-induced dilation, gene expression, hyperbaric oxygenation, Sprague-Dawley rats, vascular reactivity

Acknowledgements: This study was supported by the Faculty of Medicine Osijek institutional projects IP-08-MEFOS-2020 (PI Aleksandar Kibel) and IP-05-MEFOS-2021 (PI Zrinka Mihaljević).

Dissertation Proposal Title: Changes in glycome composition in prostate cancer patients treated with luteinizing hormone-releasing hormone agonists

PhD Candidate: Maja Drežnjak Madunić, M.D., Department of Oncology, Clinical Hospital Center Osijek and Faculty of Medicine, University of Osijek, Croatia

Mentor: Prof. Martina Smolić, M.D., Ph.D., Faculty of Dental Medicine and Health and Faculty of Medicine, University of Osijek, Croatia

Introduction: Glycosylation is the enzymatic process of covalent attachment of carbohydrates (monosaccharides) or carbohydrate chains (oligosaccharides or polysaccharides), called glycans, to proteins and lipids to form glycoconjugates. Glycans are important structural elements of glycoproteins that determine the physicochemical properties such as solubility, conformation, counting, and stability of the molecule of which they are a component. The occurrence of mutations in the DNA molecule sequence leading to impaired N-glycosylation of proteins causes the development of severe hereditary metabolic diseases (CDG), while the complete inability to form N-glycans is embryonically lethal. Up to 10% of the human genome is responsible for the formation of the enzymes and transporters required for glycan synthesis. The composition of glycans is influenced by the number, type, and sequence of monosaccharides, the nature of the bond between the monosaccharides, and the position of the atoms between which the bond is formed, and is the result of the environmental conditions of the cell at a given time. Therefore, we can say that the physiological and biochemical state of a cell directly affects the total population of glycan structures or glycones of that cell and the whole organism. Because of the great diversity of their structures and their flexibility to change, glycans have evolved as molecules that contribute to structural diversity and fine-tuning of protein function, allowing cells and organisms to adapt rapidly to new environmental conditions without requiring mutations in the DNA molecular sequence. Changes in N-glycosylation of IgG antibodies accompany the occurrence of many pathological conditions in the human body. Malignant diseases - cancer, various inflammatory conditions - infections and injuries, autoimmune diseases and unhealthy lifestyle habits - smoking and excessive food intake leading to obesity - cause changes in the composition of N-glycan IgG antibodies, which usually lead to increased levels of agalactosylated N-glycans - main components of the inflammatory state of the organism. Recently, it has been reported that N-glycosylation of the IgG molecule can

be modulated by factors such as sex hormones. Hormone therapy is the most effective form of treatment for advanced prostate cancer. The action of androgens is critical for the growth and function of the normal prostate. However, androgens are also an important factor in controlling the growth of prostate cancer as up to 80% of prostate cancer cells contain androgen receptors. Therefore, hormone therapy for prostate cancer is based on lowering androgen level in the blood or blocking their biological effects. Luteinizing hormone-releasing hormone (LHRH) agonists are synthetic compounds that are very similar in biochemical structure to natural LHRH. Their action leads to a transient increase in blood gonadotropin levels, especially LH, and thus to an increase in testosterone levels (flare phenomenon), followed by an almost complete cessation of LH secretion, resulting in a decrease in testosterone levels to castration levels, usually within 2-3 weeks. The aim of this study is to investigate how and to what extent N-glycosylation of the IgG molecule is modulated by the use of LHRH agonists in patients with prostate cancer.

Hypothesis : Use of a luteinizing hormone-releasing hormone agonist causes a change in glycome composition in patients with prostate cancer.

Objectives: Prospective observation and comparison of the glycan profiles of the IgG molecule of patients with prostate cancer before the introduction of pharmacotherapy with LHRH agonists and 3 months, 6 months and 9 months after the introduction of therapy. At the different stages of LHRH agonist administration, investigate whether there is a correlation between the concentrations of administered LHRH agonists and the concentration of external hormones with the level of glycosylation properties of the Ig G molecule.

Participants and Methods: Patients with prostate cancer treated at the Department of Oncology, Osijek College Hospital will participate in the study. This prospective cohort study will include patients with newly diagnosed intermediate or high risk prostate cancer in whom the use of LHRH agonists is indicated. Exclusion criteria: acute infectious disease and metastatic disease. All research in this study involving human subjects and human-derived materials will be conducted in accordance with the ethical principles of the World Medical Association Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects (introduced June 1964, last amended October 2000). All participants will sign an informed consent form prior to participation in the study. To eliminate experimental variation, glycan data will be normalized and serially corrected before statistical analysis. To observe the mean effect in the difference of numerical variables between measurements at four measurement points in a group of patients, with a significance level of 0.05 and a strength of 0.95, the minimum required sample size is 36 subjects. (G * Power version 3.1.2).

Research plan: Introduction of subjects with prostate cancer for whom LHRH agonists are to be introduced into pharmacotherapy but who do not meet exclusive research criteria. Determination of glycan profiles of IgG molecule, concentration of administered LHRH agonists and concentration of sex hormones in patients with prostate cancer before introduction of pharmacotherapy with LHRH agonists and 3 months after introduction of therapy. Determination of glycan profiles of IgG molecule, concentration of administered LHRH analogues and concentration of sex hormones in patients with prostate cancer 6 and 9 months after introduction of therapy. To perform analytical analysis and statistical processing of the collected data.

Expected scientific contribution: To the best of our knowledge, this is the first study to examine the effects of drug castration on glycosylation of IgG in patients with prostate cancer. This study investigates whether LHRH agonist therapy results in a significant change in the glycan composition of IgG.

Keywords: prostate cancer, N-glycosylation, IgG, testosterone, LHRH agonists

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Dissertation Proposal Title: Effect of hemodialysis on *N*-glycan profile of total plasma proteins and immunoglobuline G

PhD Candidate: Ivan Durlen, M.D., University Hospital Dubrava, Zagreb, Croatia

Mentor: Assoc. Prof. Irzal Hadžibegović, M.D., Ph.D., University Hospital Dubrava, Zagreb. Faculty of Dental Medicine and Health Care, Osijek, Croatia

Introduction: Glycosylation is an essential post-translational modification affecting the structure and function of multiple proteins. Glycans as integral parts of protein structure are versatile and responsive to environmental stimuli, and undergo significant changes during non-homeostatic states. Glycans have an important role in the immune system. Chronic inflammation in patients receiving hemodialysis (HD) is a complex syndrome with various pathogenic mechanisms -patient or procedure related (oxidative stress, carbonyl stress, depleted anti-oxidants, foreign body exposure – catheters, grafts, filter membranes, dialysate quality). It contributes to protein energy malnutrition and much higher cardiovascular morbidity and mortality. Between 30 and 50 % of hemodialysis patients have elevated serum levels of inflammatory markers such as C-reactive protein and IL-6. HD session by itself can trigger inflammation in a way that is not always identifiable with the conventional markers.

Hypothesis: Hemodialysis session changes *N*-glycan profile of total plasma protein and IgG of patients receiving chronic hemodialysis

Aims: Main: Assessing the changes in *N*-glycan profile of total plasma protein and IgG after a hemodialysis sessions in patients on chronic hemodialysis. Secondary: Comparing the changes in *N*-glycan profile with the changes of other inflammatory markers (CRP, IL-6). Comparison of the changes in *N*-glycan profile in patients with high/low interdialytic weight gain (and NT-proBNP value) and in patients with different grades of anemia.

Materials/Participants and Methods: 50 patients on HD will be enrolled after signing the informed consent form. Their demographic data, medical history data, HD data, comorbidities, medications, BMI, blood pressure, NT-proBNP, would be collected at baseline. *N*-glycane profile, CRP, IL-6 will be made before the dialysis session, directly after the dialysis and 24 hours after the dialysis.

Research Plan: Measurements will be done before the first weekly dialysis session (3 days after previous session),immediately after the session and 24 hours after the session. After collecting the data statistical analysis will be done.

Significance/Expected Scientific Contribution: This is the first study to examine changes in the *N*-glycan profile in patients receiving chronic hemodialysis and could show short term changes in *N*-glycan profile caused by hemodialysis that could impact systemic inflammation response and endanger cardiovascular continuum.

MeSH/Keywords: ESRD, hemodialysis, inflammation, *N*-Glycan profile, *N*-glycosilation

Dissertation Proposal Title: The influence of COVID-19 virus disease on mental health, lifestyle, and health-related quality of life

PhD candidate: Ivica Fotez, M.D., Faculty of Dental Medicine and Health Osijek, Osijek, Croatia

Mentor: Assist. Prof. Ivan Miškulin, Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Co-mentor: Prof. Aleksandar Včev, M.D., Ph.D., Faculty of Dental Medicine and Health Osijek, Osijek, Croatia

Introduction: The COVID-19 pandemic and the measures to prevent the spread of the virus have not only had a significant impact on health systems around the world but also had a strong impact on the lifestyle of the population, especially on eating habits and physical activity. The feeling of insecurity due to the uncertainty of the pandemic situation as well as the preventive measures negatively affects the mental health of the population and health-related quality of life.

Hypothesis: COVID-19 virus disease (CVD) negatively affects mental health, lifestyle, and health-related quality of life where people with more severe clinical manifestations show a higher frequency of adverse effects in all observed features compared to those with milder clinical presentation of the disease and those who were not ill at all.

Aims:
- to investigate mental health, lifestyle, and health-related quality of life in subjects who had CVD and those who were not ill;
- to evaluate factors that can influence the interrelation between CVD and its consequences in the observed population;
- to evaluate the possibilities of prevention of adverse effects of CVD on mental health, life habits, and health-related quality of life in the observed population.

Materials/Participants and Methods: The cross-sectional study will include an adult population from Virovitica-Podravska County during the pandemic period of one year. The expected sample size is 1200 subjects divided into three groups: subjects with severe clinical manifestations of CVD treated in hospitals, subjects with mild

clinical manifestations of CVD treated at the primary level of healthcare, and subjects who were not ill. The subject's mental health will be evaluated with PTSD Checklist for civilians, Beck Anxiety Inventory, Beck Depression Inventory, and Pittsburg Sleep Quality Index. Lifestyle will be evaluated with the International Physical Activity Questionnaire Short Form and Food Frequency Questionnaire for the Croatian population. The Croatian SF36 questionnaire will be used for the evaluation of the health-related quality of life. Sociodemographic, socioeconomic and anamnestic data of all subjects will be collected through a specially designed questionnaire.

Research plan: Participation in this study will be voluntary and subjects will be recruited from The General county hospital in Virovitica and Health center in Virovitica.

Significance/Expected scientific contribution: This study will provide direct insight into the psychosocial and health consequences of CVD in the Croatian population. The study will enlighten factors that influence the interrelation between CVD and its consequences in the observed population thus providing information on the specific situation in Croatia which will further enable healthcare professionals to design appropriate public health measures to mitigate the adverse effects of a pandemic on Croatian population.

MeSH/Keywords: COVID-19 virus disease, mental health, lifestyle, quality of life, Croatia

Dissertation Proposal Title: The role of the devices operating under the continous positive airway pressure in the recovery process of cochlear receptor cells in patienets with opstrucitve sleep apnea

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Introduction: The inner ear is very sensitive to hypoxia due to its high metabolic activity and low resistance to the changes in the oxygen partial pressure. According to the available research literature, it has been observed that due to delayed, recurrent hypoxia in opstructive sleep apnea syndrom (OSAS) patients, there may be a damage to the cochlear receptor cells. Since the cochlear cells are surrounded by the lowest partial pressure of oxygen in the body, any additional change or disturbance in balance can further reduce it and lead to the damage with a consequent drop in the threshold of auditory sensitivity, primarily at high frequencies, and a potential hearing loss. The exact mechanism by which the auditory nerve impulse is more slowly transmitted in patients with OSAS has not been fully explained. The existing literature does not give uniform results and leaves room for additional analyzes and confirmations.

Hypothesis: Patients with opstructive sleep apnea syndrom (OSAS) have cohlear receptor cells damage because of prolonged, reccurent hypoxia. The use of a CPAP device (devices operating under the continous positive airway pressure) leads to the recovery of cochlear receptor cells.

Aims:

1. To determinate whether there is cochlear receptor cells damage in OSAS patients depending on the degree of the disease

2. Investigate whether the use of ventilation devices with continuous positive pressure and constant oxygen pressure in the airways can lead to the recovery of the cochlear receptor cells

Participants and Methods: The investigation work will be designed as original scientific research- prospective cohort study at Department od Otorhinolaringology and Head and Neck Surgery, University Hospital Center Osijek.
The participants will be devided in two gorups: target (n45) and control (n36). The target group will consist of patients with OSAS who have been previously examined by a neurologist for sleep disorders and have undergone polysomnography and been diagnosed with severe obstructive sleep apnea according to the AHI index (>30). The control group will consist of subjects in whom specific questionnaires excluded the existence of obstructive sleep apnea.

Research plan: All participanting in this study will complete the following questionnaires: STOP-BANG and Ephort drowsiness scale. Subjects of the target group with severe obstructive sleep apnea will be referred to an otorhinolaryngologist audiologist for complete examination and processing after examination by a neurologist. These participants will be examined by an audiologist after 6-8 months of continuous and adequate use of the CPAP device for reevaluation. Participants of a control group will be patients examined or treated in Department of Otorhinolaryngology and Head and Neck surgery for other diseases in whom specific questionnaires excluded the existence of obstructive sleep apnea. Audiological diagnostics will be performed on all patients on the same devices of the Department of Audiology and Phoniatrics and it will include Pure tone audiometry, Timpanometry, Acoustic Stapedius Reflex, Auditory Brainstem Response and Otoacoustic Emission.

Expected scientific contribution: To prove the existence of receptor hearing impairment in the patients with obstructive sleep apnea; and then to prove that the use of a of continuous positive airway pressure with constant oxygen pressure in patients with OSAS using CPAP devices leads to the recovery of the cochlear receptor cells whose damage occurred as a result prolonged recurrent hypoxia. In addition, to determine the importance of a broader diagnostic processing of patients with obstructive sleep apnea. It is important to note that no study has been published so far on the possibility of a recovery of cochlear receptor cells in this way.A secondary contribution would indicate that continuous positive airway pressure can be used as a method of treating damaged cochlear receptor cells damaged with other agents (e.g., noise, presbycusis).

Keywords: OSAS, cohlear receptor cells, haering, CPAP device, hypoxia

Dissertation proposal title: Correlation between perfusion angiography and clinical outcome following endovascular treatment in patients with critical limb ischemia

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Mentor: Asst. Prof. Tajana Turk, M.D., Ph.D., Diagnostic and interventional radiology clinical department, Osijek University Hospital, Osijek, Croatia

Introduction: Lower extremity ischemia and its consequences are one of the biggest health problems of modern world and its consequences vary from simple skin lesions to severe tissue damage, which requires amputation. One of the most important ways to treat lower extremity ischemia is percutaneous transluminal angioplasty (PTA). Patient's condition may improve immediately after the angioplasty, but it may also remain unchanged, or even worsen, regardless of the primary technical result. This may be the result of inadequate foot perfusion improvement, since its healing primarily depends on the microcirculation status. The use of perfusion angiography allows the quantification of foot perfusion status before and after endovascular treatment and could thus be of use in preoperative assessment of whether the patient is suitable for PTA or not.

Hypothesis: There is a correlation between the foot microcirculation status, measured by perfusion angiography pre- and postoperatively, and condition improvement in patients with critical limb ischemia. Improving the foot perfusion depends not only on the extent of endovascular intervention, but also on the comorbidities and demographic characteristics of the patient.

Aims:
1. to determine the parameters of perfusion angiography in patients before and after the endovascular treatment of their crural arteries
2. to determine whether there is a correlation between the perfusion angiography parameters and the extent of endovascular intervention
3. to determine whether there is an correlation between the perfusion angiography parameters and atherosclerosis factors
4. to determine whether there is a correlation between perfusion angiography parameters and the clinical outcome of the treatment (amputation free survival)

Materials/participants and methods: The research will include 200 respondents. Patients with peripheral arterial disease without critical limb ischemia, who underwent endovascular intervention on the supragenicular arteries, would form a control group (100 patients), and subjects with peripheral arterial disease in the stage of critical limb ischemia, who underwent endovascular intervention on infragenicular arteries, would form an experimental group (100 patients).

Research plan: The data which would be gathered on all patients include basic data, comorbidities, habits, symptoms and basic laboratory results. Perfusion angiography of the foot will be performed on any patient undergoing endovascular treatment before and after endovascular intervention so its parameters could be analysed afterwards. Each patient will have a follow-up examination 3 months and 6 months after the intervention to assess the clinical status.

Expected scientific contribution: To corroborate the fact that the preprocedural analysis of multiple parameters, as well as individual and multidisciplinary approach to the patient, bring better quality in the future decisions on the need and cost-effectiveness of treatment of lower extremity ischemia with PTA and, thus, reduce the frequency of unjustifiably indicated and long-term unprofitable interventions.

Keywords: Perfusion angiography, Lower-limb ischemia, Percutaneous Transluminal Angioplasty, Endovascular treatment, Peripheral Arterial Disease

Dissertation Proposal Title: Quantitative pharmacodinamic analysis of prostate cancer in relation to benign changes by contrast study of multiparametric examination of the prostate with magnetic resonance imaging

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Mentor: Assoc. Prof. Tamer Salha, M.D., Ph.D., Radiologist, Department of Radiology, Health care centre Osijek-Baranja County, Osijek, Faculty of Medicine, University of Osijek, Osijek, Croatia

Introduction: Multiparametric magnet (mpMR) of the prostate includes several parameters such as T1 and T2 pulse sequence, diffusion (DWI-diffusion weighted imaging) and ADC (apparent diffusion coefficient) map, dynamic contrast study (DCE-dynamic contrast enhanced) and spectroscopy. The prostate mpMR finding is interpreted by standardized currently valid PIRADS v2.1. The advantage of prostate mpMR is its extremely high negative predictive value between 90-93%. The disadvantage is that, despite the active and constant development of PIRADS categorization, there is still undesirable overlap with benign changes, with false positive findings resulting in unjustified biopsies, and insufficient precision of all sequences for individual, non-multiparametric analysis that allows potential space for their development. Since neoangiogenesis is a key factor in cancer growth, our focus is on quantitative DCE study. DCE study is divided into qualitative, semiquantitative and quantitative method of analysis. The quantitative method of DCE study analysis is the most sophisticated. Here, the computer program takes into account the parameters that affect the signal intensity of the target lesion-parameters related to contrast (type, osmolarity, tissue arrival time, AIF-arterial input function), blood flow and imaging settings and calculates pharmacokinetic permeability parameters based on one of several possible pharmacokinetic models. The most widespread is Tofts' pharmacokinetic model, and the parameters obtained by this method are Ktrans (constant transfer of contrast medium from intravascular space to extravascular extracellular space), Kep (constant transfer of contrast agent from extravascular extracellular space to intravascular space), Ve (volume of extravascular extracellular space) and iAUC (initial are under the gadolinium concentration curve; area below the gadolinium concentration curve). With this method we gain insights into the movement of the contrast agent between the intra and extravascular space at the time of imaging, which tells us about tumor angiogenesis. Neoangiogenesis is

required for the growth of all tumors. Increased need for nutrients and oxygen leads to the proliferation of blood vessels that are chaotic in layout and number and more permeable as opposed to benign or inflammatory conditions that may have increased flow due to increased requirements without changing vascular permeability. Using a qualitative and semiquantitative method of DCE study analysis, it is not possible to distinguish whether the increased flow in the change we detected is due to increased flow within normal blood vessels of the change or due to increased proliferation of permeable vessels. Quantitative DCE studies have proven promising in the diagnosis of numerous malignant tumors such as gliomas, head and neck tumors, breast, ovarian, endometrial, rectal, bone tumors, and a number of other conditions. As for the prostate, several studies have shown differences in the values of quantitative DCE study parameters in cancer relative to healthy tissue or benign conditions, and some studies have proposed cut-off values for individual parameters. The obtained values were compared in relation to morphologically healthy prostate tissue or benign lesions, and the values of individual studies were different. In this study, the values obtained in the found lesion and morphologically healthy prostate tissue will be compared with the values of the same parameters in the internal obturator muscle as a constant and reference tissue with the origin of vascular supply to the branches of the internal iliac artery as a prostate tissue. Normalization of DCE study parameters in relation to muscle has already been shown to be useful in many malignant processes. Another focus of research is prostatic intraepithelial neoplasia (PIN) as one of the most common causes of false-positive findings of multiparametric magnetic resonance imaging of the prostate. In everyday clinical practice, according to the currently valid PIRADS v2.1 categorization, individual lesions classified as PIRADS 3 or less often 4, benign changes such as prostatitis, atrophy, benign prostatic hyperplasia or atypical proliferation of small acini (ASAP). High-grade PIN is a problem due to the possible progression to clinically significant cancer. Research to date has suggested that diffusion could help distinguish PIN from cancer and that progression of signal intensity reduction on the ADC map or diffusion image derivative could suggest progression of PIN to cancer, while studies of a DCE study method have not shown significant role. The potential role of the quantitative method of dynamic contrast enhanced study in PIN has been insufficiently investigated.

Hypothesis: The values of the quantitative dynamic contrast enhanced study obtained parameters are significantly different in prostate cancer compared to healthy tissue. The internal obturator muscle, less susceptible to heterogeneity of structure, in relation to prostate tissue, can serve as a reference tissue for normalization and comparison of obtained values. PIN, as a proliferative process different from healthy prostate tissue and cancer tissue, has different values of dynamic contrast enhanced study obtained parameters compared to cancer and / or healthy tissue.

Aims:

1. To explore whether there is a significant difference in the quantitative dynamic contrast enhanced study obtained parameters in relation to morphologically healthy prostate tissue and internal obturator muscle and also to determine a cut-off value for detecting prostate carcinoma with 100% sensitivity and the lowest possible percentage of false positive findings
2. To examine whether there is a possible role of quantitative analysis of the dynamic contrast study in PIN, and differences in the values of parameters in relation to cancer and morphologically healthy prostate tissue

Materials/Participants and Methods: This study included a total of 190 patients examined with a multiparametric prostate magnet at the Clinical Institute for Diagnostic and Interventional Radiology of the Clinical Hospital Center Osijek in the period from January 2016. to 9.mj.2021. The study included patients who underwent a targeted biopsy that demonstrated prostatic intraepithelial neoplasia (PIN) or Gleason prostate cancer of 3 + 3 = 6 or greater, which is unilateral without opposite dissemination or extraprostatic propagation. For the negative control group of healthy patients, the inclusion criteria were a pre-magnetic resonance biopsy finding negative for prostate cancer with a PIRADS 1 or 2 prostate magnet finding, a stable PSA value, and a non-cancerous digitorectal examination finding. The exclusive criteria were chronic renal failure with elevated urea and creatinine, which was contraindicated the use of intravenous contrast agent, patients with prostate cancer who had previously undergone therapy, patients with technically inadequate or incomplete mpMR examination, patients who have a multiparametric examination of the prostate by magnetic resonance imaging lesion of the category PIRADS 3, 4 or 5 without available biopsy findings or biopsy was not performed. The final number of patients with PIN was 39 with 48 foci, and with prostate cancer Gleason score 3 + 3 = 6 or more is 56 with 70 foci. The total number of patients in the control group was 54. Data are collected from medical documentation and available pictorial material. The research was approved by the Ethics Committee of the Clinical Hospital Center Osijek and the Ethics Committee of the Medical Faculty of the J.J. Strossmayer University in Osijek Prostate mpMR analysis was performed by two specialist radiologists, one with 18 years of radiological experience, the other with 6 years of radiological experience, according to the guidelines of PI-RADS categorization (Prostate Imaging-Reporting and Data System) that was valid at the time of examination. All patient examinations were performed on a Simens Magnet 3T MRI system (Simens AG, Healthcare Sector) using a standard imaging protocol in accordance with ACR-American College of Radiology guidelines. Endocretal coil was not used. For the dynamic contrast study, T1 VIBE transverse dynamic contrast sequence was used after intravenous administration with an automatic injector 0.2mL / kg gadoterate meglumine, flow rate 4mL / s after

which intravenously administered 20mL saline at the same flow rate. The computer program used to analyze the recorded data of multiparametric examination of the prostate by magnetic resonance imaging is Simens Syngo.via WebViewer v2018.1.5.0. The parameters obtained by quantitative pharmacokinetic analysis are Ktrans (constant of contrast medium transfer from intravascular space to extravascular extracellular space), Kep (constant transfer agent of contrast agent from extravascular extracellular space to intravascular space), Ve (volume of extravascular curve space below) and iAUC.
Lesions were found by morphological sequence analysis (T1 and T2 sequences) then characterized in more detail by functional sequence analysis (DWI and ADC maps and dynamic contrast study) on the basis of which PIRADS categorization was determined and locoregional stage of the disease was estimated. The T2 sequence was used as a reference sequence to localize the lesion in a dynamic contrast study.
These parameters (Ktrans, Kep, Ve, iAUC) were measured in a detected lesion that was biopsied after mpMR prostate labeled PIRADS 3,4 or 5 identified as Gleason carcinoma of 3 + 3 = 6 or more and PIN, then morphologically healthy prostate tissue of cancer patients and control groups and in the internal obturator muscle.
In the development of quantitative pharmacokinetic parameters, the computer program uses the Tofts model with standardized population-based arterial input function (AIF). Arterial Input Function.

Research plan: The programming language R will be used - https://cran.r-project.org/.
A nonparametric Mann-Whitney U-test will be used to compare quantitative pharmacokinetic data and to examine the statistical significance of differences in prostate cancer localization (peripheral zone or transition zone). The significance level is set to 0.05. The statistical dependence between the variables will be examined using the Sperman rank correlation coefficient. Logistic regression will be used to analyze the possibility of using individual traits in the identification of diseased tissue and to evaluate the diagnostic performance of pharmacokinetic traits in distinguishing prostate cancer or PIN from morphologically healthy prostate tissue and to select the most predictive model based on quantitative pharmacokinetic traits.
To assess the quality of the model, common indicators such as AIC - "Akaike Information Criterion" and AUC - "Area Under the ROC Curve" will be used.
Also specificity and sensitivity will be calculated for each possible limit value and the optimal limit value will be investigated using the Youden index.
It is planned to use methods that do not assume the normality of data distribution.
The selected sample size (70 lesions per group, ie 140 in total) will ensure a Mann-Whiteny U test strength of at least 85% at a significance level of 0.05 and an effect size of 0.5 (calculated using the G * Power application). this amount of data is large

enough to apply logistic regression. To compare cancer and PIN, the selected sample size (48 PIN lesions and 70 cancer lesions) will provide a test strength of 80% with an effect size of 0.5 and a significance level of 0.05.

Significance/Expected scientific contribution: The new knowledge and insights that the proposed study brings is that the comparison of the quantitative pharmacokinetic parameters in prostate tissue in relation to the internal obturator muscle as a reference tissue less susceptible to heterogeneity, facilitates application and allows standardization of quantitative analysis of dynamic contrast studies and increasing the sensitivity and specificity for prostate cancer detection. Also new knowledge and insights that the proposed study brings is the study of the role of quantitative pharmacokinetic parameters of a dynamic contrast study in prostatic intraepithelial neoplasia.

MeSH/Keywords: Magnetic Resonance imaging; Muscle; Perfusion; Permeability; Prostate Cancer;

Abstract Title: Hip and Knee Osteoarthritis in Philadelphia Chromosome-Negative Myeloproliferative Neoplasms

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Mentor: Prof. Silva Zupančić-Šalek, M.D., Ph.D., "Holy Spirit" Clinical Hospital, Faculty of Medicine Osijek, University of Osijek, Osijek , Croatia

Introduction: Hip and knee osteoarthritis (OA) affect approximately 10-15% of persons older than 60 years. Considering that Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs) are typically diagnosed in older age and characterized by chronic inflammation, bone marrow remodeling and frequent osteoporosis, we hypothesized that OA may be highly prevalent in MPNs.

Aims: To investigate the prevalence of hip and knee OA in MPNs and its associated risk factors.

Materials/Participants and Methods: This is an ongoing prospective two-center study (dr. Josip Benčević General Hospital and General Hospital Šibenik). Consecutive patients with essential thrombocythemia (ET), polycythemia vera (PV) and myelofibrosis (MF) diagnosed according to 2016 World Health Organization criteria are being prospectively included. All participants with suspected hip and knee OA are being evaluated by an orthopedic surgeon and undergo X-ray imaging of the symptomatic joint(s) with OA being defined as grade ≥1 according to the radiological Kellgreen-Lawrence classification; grades 3-4 represent moderate and severe OA, respectively. Relevant clinical and laboratory data, as well as the MPN Symptom Assessment Form (MPN-SAF), are being recorded at the time of study entry. High-risk ET and PV patients are defined as those having age >60 years and/or history of thrombosis. The chi-square, the Mann-Whitney U, and the t-test were used. MedCalc Statistical Software® was used for all analyses.

Results: Fifty MPN patients (ET=18, PV=20, MF 12) were recruited; mean age was 61.5 years (±13.17), 26 (52%) were females, 44 (88%) had symptoms suggestive of OA and 35 (70%) had radiologically confirmed OA. The prevalence of hip OA was similar among all three disease entities (61.1% vs. 60% vs. 66.7%), whereas knee OA was more

prevalent in PV (65%) and MF (58.3%) than in ET (22.2%). Moderate to severe (grade 3-4) hip OA and knee OA were present in 13 (26%) and 9 (18%) MPN patients. The presence of OA in MPNs was generally associated with high-risk disease (p=0.020), older age (p=0.046), higher body mass index (p=0.015), presence of cardiovascular risk factors (p=0.030), hemoglobin ≤125g/L (p=0.045), hyperuricemia (p=0.076) and high (>8) MPN-SAF (p<0.001).

Conclusion: Out preliminary data suggest that OA may have strikingly high prevalence (60-80%) in MPNs, with a substantial proportion of MPN patients having moderate to severe OA (18-26%). Finally, the presence of OA in MPNs seems to be predominantly associated with older age, higher body mass index and cardiovascular risk factors,

MeSH/Keywords: Osteoarthritis, Hip; Osteoarthritis, Knee; Polycythemia Vera; Primary Myelofibrosis; Thrombocythemia, Essential

Acknowledgement: Božena Coha, Ivan Krečak, Marko Lucijanić

Dissertation Proposal Title: Influence of psyhotropic drugs on the occurrence of falls and/or fractures in institutionalized and uninstitutionalized elderly persons

PhD candidate: Anja Jakovčević, M.D., family medicine specialist, Health Center Županja, Croatia

Mentor: Assist. Prof. Mario Ćurković, Ph.D., family medicine specialist, Health Center of Osijek, Baranja County

Introduction: The number of people over the age of 65 has been increasing in recent decades, not only in developed but also in developing countries and makes up an increasing share of the general population. Falls and fractures are a significant problem for the elderly population. Every year, 28-35% of people over the age of 65 experience a decline, and the number rises to 32-42% for people over the age of 70. Taking more drugs is considered a risk factor for a decline due to the harmful effects of drug interactions or drugs and diseases. The main group of drugs associated with an increased risk of falls are psychotropic drugs (benzodiazepines, antidepressants and antipsychotics), and it has been observed that sedatives and hypnotics have also often been associated with falls in the elderly. Family physicians should recognize the impact of psychotropic drugs on the incidence of falls / fractures in the elderly, and better manage psychotropic drugs and optimize psychotropic therapy.

Hypothesis:

1. The use of psychotropic drugs is associated with an increased risk of falls and / or fractures in the elderly
2. Benzodiazepines increase the risk of falls and /or fractures in the elderly more than antidepressants

Aims:

Main goals:

1) Investigate whether the use of psychotropic drugs is associated with falls and / or fractures
2) Examine the impact of institutional accommodation and length of stay in the institution of the elderly on the incidence of falls and / or fractures

Secondary goals:

a) Influence of personal causes on the occurrence of falls and / or fractures (age, sex, chronic diseases, education, marital status, previous falls as the best predictor of falls)
b) the impact of the quality of life of the elderly on the occurrence of falls and / or fractures

Materials/Participants and Methods: 128 respondents will be included in the research. The estimated sample size is 60 respondents from institutions - Family Home for the Elderly and the Infirm "St. Anthony" Šag and the remaining 68 respondents will be recruited from the specialist family medicine office, Assistant Professor Mario Ćurković, PhD, Health Center of Osijek-Baranja County and family medical office Posavski Podgajci - Rajevo Selo, Health Center Županja, team leader Anja Jakovčević, MD, family medicine specialist.
All survey participants will be asked to complete a questionnaire containing a total of 40 questions. The survey consists of 8 questions on SOCIODEMOGFRAFIC data (gender, age, place of residence, marital status, education, medications used, previous falls, chronic illnesses-arterial hypertension, COPD, diabetes mellitus, osteoarthritis, obesity, Parkinson's disease), 26 questions from the standardized questionnaire of the World Health Organization on quality of life - WHOQOL-BREF, 6 questions from the standardized questionnaire for assessing the risk of falls in the elderly - MORSE FALL SCALE. In addition to filling out questionnaires, all subjects will be tested for blood levels of psychotropic drugs in the blood in the clinical biochemical laboratory of KBC Osijek.

Research plan: We started the research in two specialist family medicine surgeries. Respondents older than 65 who consume one or more psychotropic drugs (anxiolytics, antipsychotics, antidepressants, hypnotics) were given questionnaires with 40 questions and instructions were issued for determining the concentration of psychotropic drugs in the blood. Since it takes several weeks for the medical-biochemical laboratory to process the samples, we are still waiting for the findings. The findings will be sent to us via the CEZIH portal and we will have an accurate insight into the concentrations of the required drugs in the blood of the subjects. We will do the same with the respondents from the Home for the Elderly and the Infirm. We plan to conduct the research for 12 months because we believe that we need this time frame to collect relevant data.

Expected scientific contribution: It will be important in this research to realize that the use of individual psychotropic drugs or their combinations may be important risk factors, leading to an increased risk of falls and / or fractures in the elderly. Avoiding

these drugs and their combinations, better managing these drugs, using these drugs for a shorter period of time, and regularly reviewing prescribed medications by your family doctor could significantly reduce the incidence of falls and / or fractures in the elderly. Reducing falls and consequent injuries, especially fractures, can significantly affect the quality of life of the elderly, reduce mortality, reduce the cost of health care facilities, which allocate them to patients during their hospitalizations and rehabilitation.

Keywords: psyhotropic drugs, falls, fractures, elderly people

Dissertation Proposal Title: Dynamics of change in blood coagulation factors dependent on liver function before and after liver transplantation

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Mentor: Assist. Prof. Irena Jukić, M.D., Ph.D., , Croatian Institute of Transfusion Medicine, Medical Faculty Osijek, Osijek, Croatia

Introduction: Patients with terminal liver failure (complete absence of function) are candidates for liver transplantation. They have reduced or absent ability to produce blood clotting factors. After liver transplantation, we expect that the transplanted liver will begin functioning in the shortest possible time and thus the synthesis of blood clotting factors, which is not the case in all patients after liver transplantation.

Hypothesis: Synthesis of blood clotting factors does not have the same dynamics in all patients after liver transplantation. Dynamics of recovery of blood coagulation factors can be an indicator of liver function recovery and a predictor of transplant success or a predictor of graft rejection.

Aims: To examine blood coagulation factors in patients before and after liver transplantation. To find out which coagulation factors are synthesized slower in the absence or delay of liver function recovery after transplantation. To examine the influence of liver graft quality on the recovery of blood coagulation factors in recipients.

Materials/participants and Methods: The subjects in this study would be a group of at least 100 patients who will undergo a liver transplant at the University hospital Merkur, in Zagreb. Each of the examinees will be acquainted with the aim of the research and will sign the consent for conducting the research as a confirmation of their consent to participate in the study. For pre-transplant parameters, the findings usually required before liver transplantation will be used. No additional research intervention is required for all of the above.

Research plan: After liver transplantation, coagulation factors will be monitored at several time intervals (7 days, 1 month, 3 months, 6 months). Blood samples will be

taken and all results obtained will be analyzed. In addition, the results will be compared with regard to the initial diagnoses and the reasons that led to the necessity for liver transplantation.

Significance/Expected scientific contribution: This research will contribute to better understanding of the delay or absence of blood coagulation factors synthesis in liver transplant patients. This could lead to targeted diagnosis of individual clotting factors deficiency and, if necessary, application of targeted treatment. Research will show whether blood coagulation factors are a good indicator of liver function recovery, in correlation with other biochemical and clinical parameters, and whether they can be a predictor of liver transplant success. Ultimately, the results could contribute to even greater success of transplant medicine as well as reducing the consumption of valuable blood products.

MeSH/Keywords: liver transplantation, blood coagulation factors, transplants, liver function tests, delayed graft function

Dissertation Proposal Title: Analysis of two xenograft materials after socket preservation

PhD candidate: Matej Karl, M.D., Ordinacija dentalne medicine Matej Karl

Mentor: Assoc. Prof. Marko Matijević, M.D., Ph.D., Health Center Osijek

Introduction: Resorption is the inevitable physiological process that follows tooth extraction, that occurs because of quantitative and qualitative changes of the bone tissue. In the aftermath bone volume is reduced and the morphology of the ridge has changed. To prevent resorption and maintain alveolar ridge macro and micro architecture socket preservation technique is used. Empty alveola is augmented with substitute material to prevent excess resorption and to maintain sufficient bone volume to place a dental implant.

Hypothesis: Xenograft bone augmentation materials with different purification and preparation processes show similar tissue response and are viable options for bone augmentation.

Aims: The aim of this study is to evaluate histological and histomorphometrical findings of two different xenografts after socket preservation in patients with indications for delayed implant placement.

Materials/Participants and Methods: The study consists of two phases. In the first phase patients with indications for tooth extraction will for participation. Patients suffering from systemic diseases that could affect end result will be excluded..
At the begining of the clinical part of the study patients were divided randomly into two groups of equal size in which postextraction sites were augmented with two different xenogenic bovine materials in accordance with group affiliation. Standard surgical minimaly invasive techniques and materials will be used in procedures.

Research plan: During the first phase selected teeth were extracted. Site of extraction was exposed by raising mucoperiostal falp of full thickness. Empty alveola was curretaged to eliminate any form of debris from the wound. Sockets were then augmented. After the augmentation alveola was covered with resorbable collagen membrane and the wound was closed using single sutures.

After the six months of healing surgical site will be reopened to insert dental implant.. Prior to implant site preparation bone samples will be harvested using trephine burrs with various sizes in diameter, as the situation would allow. Samples will be fixated in 4% formaldehyde until analysis.
Samples will be stained using hematoxyolin-eosin technique and analysed.

Expected scientific contribution: We expect to confirm already established protocols and analyse bone tissue reactions on xeongraft materilas that are most widely used.

Keywords: xenograft, socket preservation, dental implants, bone augmentation, alveolar ridge

Dissertation Proposal Title: The Role of Global DNA Methylation as an Epigentic Marker in the Chronic Obstructive Pulmonary Disease

PhD candidate: Eugenija Basioli Kasap, M.D., General Hospital Zadar, Croatia

Mentor: Assist. Prof. Sanda Škrinjarić Cincar, M.D., Ph.D., Faculty of Medicine, University of Osijek, Osijek, Croatia

Co-mentator: Prof. Ljubica Glavaš Obrovac, Ph.D., Faculty of Medicine, University of Osijek, Osijek, Croatia

Introduction: Chronic obstructive pulmonary disease (COPD) is a progressive disease with a poor prognosis. Environmental and genetic factors are known to play a role in the development of COPD, but little is known about epigenetic pathways involved in disease development. One od the main epigenetic mechanisms is DNA methylation. Cites od DNA methylation can be inherited by DNA replication and catalyzed by DNA methyltransferases (DNMT).

Aims:1.To examine the values of global DNA methylation in patients with COPD in Croatia and differences between patients and control subjects. 2.To examine whether there are differences in the clinical characteristics of COPD and global DNA methylation between patients living and treated in Osijek and Zadar. 3.Examine the relationship of global DNA methylation findings with risk factors for COPD, clinical characteristics, associated diseases, lifestyle and quality of life of patients.

Participants and methods: The study will include 64 patients with COPD treated in Osijek and 64 patients treated in Zadar and a control group of 64 subjects. Detailed anamnestic data collection, complete pulmonary function tests (PFT), computed tomography (CT) of the chest, and ultrasound of the heart will be done for all patients. All patients will complete 4 validated questionnaires to assess symptoms and quality of life (SF-36-Short Form 36, SGRQ-St Georges Respitatory Questionnaire, CAT (COPD assessment test) and mMRC (Modified Medical Research Council) as well as life habits form that include a modified validated MDSS (Mediterranean Diet Serving Score) questionnaire. A blood sample will be stored at -20 ° C until DNA is isolated from leukocytes, and after that, absolute quantification of global DNA methylation by colorimetric method will be performed.

Results: According to the preliminary results, the population in both centers coincides in age, sex, smoking history, disease severity and symptoms, but differs in some characteristics that could be interpreted by different environmental conditions. By the cluster method, the subjects were grouped into two clusters, the first larger with lower methylation and the second smaller with higher methylation, and they differ from each other in terms of smoking, coughing and expectoration.

Conculsion: The expected contribution is to determine the possible association of global DNA methylation with lung function, clinical characteristics, disease severity and quality of life of COPD patients and the possibility of clinical applicability of determining global DNA methylation as a biomarker.

MeSH / Keywords: Epigenetics,Global DNA methylation,COPD,Quality of life,Lifestyle

Dissertation Proposal Title: Thermographic analysis of the metabolic activity of the breast in healthy women

Part of the Dissertation Proposal: Significance of thermographic analysis of the metabolic activity of the breast

PhD candidate: Dejan Kečkeš, M.D., Clinic for Tumours, Faculty of Medicine Osijek, Osijek, Croatia

Mentor: Prof. Sven Kurbel, M.D., Ph.D., UHC Osijek, Faculty of Medicine Osijek, Policlinic "Aviva", Zagreb, Croatia

Introduction: The risk of breast cancer (BC) laterality emergence can be associated with breast size, vascularity and metabolic activity.
Thermography is an indicator of physiological function, unlike MMG, magnetic resonance imaging (MRI) and ultrasound, which reveal anatomical characteristics.
The thermography measures breasts' thermal print based on the metabolic activity of cells.
Digital infrared thermal imaging (DITI) can register temperature distribution patterns to a sensitivity of 0.08 °C or less, and deviations from the symmetry pattern can be identified.
The thermal symmetry of the healthy human body has a definition to be a maximum value of 0.570 °C for two bilateral regions of interest.
Paired organs in a human body may have anatomical and physiological asymmetry, which could influence the laterality of cancer occurrence.
The analysis focuses on characterising women's basal steady temperature profiles in specifically defined regions of interest and determining the thermal symmetry values for a healthy woman and standard patterns of temperature distribution in the whole breast.
The more significant the temperature difference in a smaller area of breast interest is, the more suspicious area will be.
The reason of that are pathophysiological and morphological changes of the breast.
Considering that the tumour is making the whole breast warmer, its presence in a specific quadrant will cause even higher temperature release in the same quadrant.
These thermal variations can be measured using thermography.
Thermography provides a significant advantage as an early indicator of breast disorder, owing to the first emergence of thermal signs indicating errant function.
Infrared thermography is a method, which detects infrared energy emitted from objects, converts it into temperatures and can display an image of temperature distribution.

Breasts have an average temperature difference of less than 0.5 °.
The real challenge in natural science is to understand, recognise and classified these temperature changes as a higher metabolic activity due to hormonal changes and consequently a higher demand for the blood inflow, inflammation or pre-cancerogenesis stage.
The perception that breast cancer appears more commonly in the left than the right side breast has been of interest to the scientific community for many years.
However, the reason for the increased appearance of breast cancer in the left breast is still unclear.
The presence of an abnormal, asymmetric infrared heat pattern of the breast increases a women's risk of getting breast cancer at least 10-fold (Gautherie and Gros).

Aims: Establishment of the laterality of breast temperature changes in healthy women and its association with breast's size, vascularity, and metabolic activity.
Calculating of the degree of thermal asymmetry and vascular characteristics for the risk of developing breast cancer.
Making the National Thermographic Protocol (NTP) for women with higher temperature asymmetry who should have frequent follow-ups and monitoring because standardised protocol will enable us to observe temperature dynamics, record them and later analyse and compare them for a better assessment and evaluation of the cancer risk prediction.

Materials and Methods: 340 healthy women between 19-66 years of age underwent non-contact thermographic imaging during preventive screening.
In a thermally controlled room without air circulation, where the room temperature was maintained between 20-21 °C with a relative humidity of 50-65%.
During the thermographic imaging, the participants were asked to stand and keep their hands on their neck one meter away from the camera.
The recording was taken at the moment of the maximal inhalation with the arms above the head.
An infrared (IR) thermographic camera (FLIR T335; FLIR Systems Pty Ltd, Australia) with a detection range of -20 ºC to +650 ºC, with thermal sensitivity less than 0.05 ºC, IR resolution of 320 x 240 focal plane array detector (76,800 pixels) and with the image frequency of 9Hz was used
Analysis of the data was performed by using the FLIR Tools software (FLIR Systems, Inc., North Billerica, MA, USA).
Method of analysis (thermogram): Ville Marie Breast Thermography Grading Scale methodology as adopted by the Thermography Service of Integrative Life Solutions (Clemmons, NC).

Thermograms were classified into 1 of 5 thermobiological (TH) groups based on vascularity and breast temperature, where TH-1 represents (Normal) absence of any vascular pattern to mild vascular symmetry and TH-2 (Normal Vascular) represents significant but asymmetrical vascular pattern to moderate vascular asymmetry, if stable. The last three groups of this classification are TH-3 (Equivocal), one abnormal sign, TH-4 (Abnormal), two abnormal signs and TH-5 (Severely Abnormal), three abnormal signs.
A unilateral pathology or any other condition that created an abnormal circulation were recorded as an asymmetrical thermographic pattern if the temperature difference was above 0.570 ^{o}C (Fig.2).
Unfortunately, though, there is no screening tool currently available that provides 100% predictability of a cancerous tumour's presence.
The only definitive diagnostic tool is a biopsy.
A unilateral pathology or any other condition that created an abnormal circulation were recorded as an asymmetrical thermographic pattern if the temperature difference was above 0.570 ^{o}C.
Breast glandular tissue density was classified based on the MMG (BIRADS) classification system.
Vascularity was determined after visualising the thermograms and inspecting the pattern of blood vessel distribution using the grey-scale visualisation method (Fig.1) on the FLIR operative system.
Descriptive statistical analysis was used to evaluate data.
The Student's dependent paired t-test was used to test the statistical significance of the collected data between the mean values obtained for the left and right breast.

Results: There were no significant size differences between the left and the right breast ($p=0.209$). Dependent on the differences in the sizes of the left and the right breast, three groups were determined (the left and the right breast were of equal size; the left breast was larger than the right; the right breast was larger than the left one (Fig.3) Maximum, minimum and average temperatures of the left and the right breast are shown in Table 2.
There was a significant difference for the maximum temperature between the left and the right breast ($t= 3.196$, $p<0.02$), as well as for the average temperature ($t= 3.558$, $p<0.01$).
There was no significant difference between the left and the right breast considering the minimum temperature ($t= 1.534$, $p>0.05$).
Data analysis has been done per age group, and there was no temperature difference dependent on the age group.
Vascularity features of the breast were observed as well and its influence on heat production

Avascular breasts were found in 27% (Fig.5) and equal vascularity in 11% (Fig.6) of participants; the left breast with more vessels was found in 38%, and the right breast with more blood vessels was found in 24% of subjects.

Conclusions: Thermography is currently the only method that can detect physiological- pathophysiological changes of metabolic activity in the breast, specifically in high-risk regions for cancer occurrence.
Left breasts were found to have significantly higher maximum and average temperature than right breast.
The left breast was larger in 39% and more vascularised in 38% of cases.
Heart heat signature did not influence the heat distribution pattern as much as it was expected.
It can also raise suspicions of pre-cancerous stages based on temperature distribution differences, so radiotherapy could be avoided.
The standardised National Thermographic Protocol (NTP) will enable us a better assessment and evaluation of the cancer risk prediction.
When combined with other examinations, thermography may contribute to the best possible evaluation of breast health or pathology.

MeSH/Keywords: Thermal imaging, Thermal breast asymmetry, Breast thermography, Left breast

Acknowledgement: The guidance of Prim. Zvonimir Zore, MD, Ph.D. and his many hours spent on recording thermographic images is gratefully acknowledged.
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Title of dissertation proposal: Association of lipogenesis and mitochondrial dysfunction's gene expression and biomarker levels with interpatient variability in the frequency and severity of amiodarone-induced liver injury

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Mentor: Assoc. Prof. Robert Smolić, M.D., Ph. D., University of Osijek, Faculty of Medicine Osijek, Osijek and Faculty of Dental Medicine and Health Osijek, Osijek, Croatia

Introduction: Drug-induced liver injury (DILI) is one of the more frequent causes of liver damage. The real incidence in clinical practice is inknown. Amiodarone is a frequently used antiarrhytmic drug known to induce acute and chronic liver injury. The mechanisms leading to amiodarone-mediated liver injury have not yet been fully elucidated.

Hypothesis: Gene expression and serum levels of proteins involving in process of lipogenesis and mitochondrial dysfunction are the markers of amiodarone-mediated liver injury and steatohepatitis.

Aim: The aim of this study is to investigate frequency and severity of amiodarone-induced liver injury at baseline, 6 and 12 months after the involvement in the study.

Participants and Methods: 85 patients with amiodarone introduced therapy will be included in this prospective cohort study. A self-reported questionnarie will be used to investigate association of age, sex, anthropometrical parameters, dietary and lifestyle factors with severity of amiodarone-induced liver injury assesed by sheer elastography at baseline, 6 and 12 months after treatment. Standard biomarkers of liver damage, serum levels and gene expression of SREBP-1, PPAR gamma, PPAR alpha, MTTP, IL-8, TNF-alpha and TNF-beta involved in process of lipogenesis and mitochondrial dysfunction will be determined by ELISA and RT-PCR, respectively at baseline, 6 and 12 months after the involvement in the study.

Research plan: Following recruitment, participants will be assessed for liver injury by sheer elastography and mitochondrial dysfunction's gene expression and lipogenesis biomarkers and these findings will be correlated to dietary and lifestyle habits at baseline, 6 and 12 months after the involvement in the study.

Expected scientific contribution: Determination of frequency and severity of amiodarone-induced liver injury. Assessment of genes and proteins involved in process of lipogenesis and mitochondrial dysfunction as potential quantitative markers of amiodarone-induced liver injury. Ultimately, to establish methods for prevention of amiodarone-induced liver steatosis occurence.

Keywords: non-alcoholic fatty liver disease, steatohepatitis, amiodarone, drug induced liver injury, mitochondrial dysfunction

Abstract Title: Reducing oxidative stress in athletes with consumption of n-3 PUFAs, selenium, lutein, and vitamin E enriched hen eggs, is it sufficient?

Part of the Disertation Proposal: The effect of intake of nutrient enriched functional food on microvascular endothelial function in athlete - randomized controlled study

PhD candidate: Luka Kolar, M.D., National Memorial Hospital Vukovar**,** Vukovar, Croatia

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Co-Mentor: Marko Stupin, M.D., Ph.D., Osijek University Hospital, Osijek, Croatia

Introduction: Strenuous training that competitive athletes practice often generates injuries, immune suppression, oxidative stress, and dietary disbalance. Analyses of dietary habits in various athletes' groups found that a substantial proportion of the studied populations did not reach the dietary goals for many macro- and micronutrients, including those with antioxidant properties. Functional food rich in antioxidant nutrients, including n-3 polyunsaturated fatty acids (n-3 PUFAs), selenium, vitamin E and lutein, could prove to be an excellent solution to the poor nutritional habits of the athletes.

Aims: The aim of the present study was to determine the effect of three-week consumption of n-3 PUFAs, selenium, lutein, and vitamin E enriched hen eggs on biomarkers of oxidative stress and antioxidant defense in competitive athletes.

Materials/Participants and Methods: 31 competitive athletes were divided to Control group (N=14) who ate three regular hens' eggs/daily, and Nutri4 group (N=17) who ate three n-3 PUFAs, selenium, lutein, and vitamin E enriched hen eggs/daily for 3 weeks. Before and after respective dietary protocol body mass index (BMI), body composition and serum lipid profile were measured. As a markers of oxidative stress, serum protein concentration of 8-iso prostaglandin F2α (8-iso-PGF2α) and hydrogen peroxide and peroxynitrite (DCF-DA) formation in peripheral blood mononuclear cells (PBMCs) were measured, while serum enzyme activity of antioxidant enzymes catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) were measured as markers of antioxidant defense.

Results: Following dietary protocol, BMI, body composition and serum lipid profile did not change in both Nutri4 nor Control group. Serum protein concentration of 8-iso-PGF2α and DCF-DA level in PBMCs significantly decreased in Nutri4, but not in Controls following the respective dietary protocol. Serum activity of CAT, GPx and SOD did not significantly change in both Nutri4 and Controls following diet protocol.

Conclusion: Three-week consumption of n-3 PUFAs, selenium, lutein, and vitamin E enriched hen eggs significantly reduced oxidative stress level in competitive athletes potentially by affecting non-enzymatic, or other enzymatic (beside measured) antioxidant mechanisms.

MeSH/Keywords: oxidative stress, n-3 PUFAs, selenium, vitamin E, lutein, functional food, athletes.

Acknowledgements: The study was funded by European Structural and Investment Funds to Science Centre of Excellence for Personalised Health Care, the Josip Juraj Strossmayer University of Osijek, Scientific Unit for Research, Production and Medical Testing of Functional Food, # KK.01.1.1.01.0010.

Abstract Title: Fetal dose estimation in radiotherapy of the breast carcinoma during the pregnancy using newly developed anthropomorphic phantom of a pregnant woman in the second trimester

Part of the Dissertation Proposal: Development of anthropomorphic phantom of a pregnant woman in the second trimester and fetal dose estimation in radiotherapy of breast carcinoma during pregnancy

PhD candidate: Vjekoslav Kopačin, M.D., University Hospital Osijek, Faculty of Medicine Osijek, Osijek, Croatia

Mentor: Assist. Prof. Hrvoje Brkić, Faculty of Medicine Osijek, Osijek, Croatia

Co-Mentor: Assist. Prof.Tajana Turk, University Hospital Osijek, Faculty of Medicine Osijek, Osijek, Croatia

Introduction: Breast carcinoma incidence during pregnancy is 1:3000 – 10000 and there are mixed clinical opinions if radiotherapy in such cases should be performed. It depends on fetal dose during radiotherapy and it has been shown that, for fetal doses above 0.05 Gy, and especially for doses above 0.1 Gy, the radiation risk for conceptus increases. There is a number of dosimetry approaches (experimental and computational) for fetal dose estimation, but uncertainties are often large or not known.

Aims: To develop computational and physical anthropomorphic phantom of the pregnant patient in the 2nd trimester of pregnancy. It will be used for fetal dose estimation in breast cancer radiotherapy of the pregnant patient, but also for analyses of the radiation sources in order to further optimize the radiation treatment plan. The results will be used to adjust the guidelines for radiotherapy treatment of breast cancer in pregnancy.

Materials/Participants and Methods: The phantoms of the pregnant woman are based on a 37 y.o. patient. The gestational age of the fetus at the time of the MRI scan was 17 weeks. The voxelized phantom is developed and has a total of 38 organs and structures with voxel dimensions 1.86 x 1.86 x 1.86 mm. The physical phantom was developed using materials that are determined according to their physical and

radiological properties. The radiotherapy plan for breast irradiation was developed using Varian Eclipse 15.6 planning software. The prescribed dose is 50 Gy in 25 fractions. The mean dose to the breast is 50.3 Gy and the maximum dose is 53.5 Gy. To calculate the fetal dose Monte Carlo simulations were performed (using MCNP 6.2). Experimental radiotherapy on the physical phantom was performed according to plan with Siemens Oncor Expression linear accelerator.

Results: Preliminary data acquired using Monte Carlo simulation in computational phantom shows that the fetus will receive 0.117% of the dose delivered to the target volume, i.e. of 50.3 Gy prescribed to the patient, the fetus receives 0.059 Gy, which is a little above the 0.05 Gy threshold. Only 8.5% of the photons reaching the fetus are caused by the leakage of the accelerator head, while 91.5% of the dose comes through the mother's body as scattered radiation. According to preliminary dosimetry measurements with RPL dosimeters with the physical phantom, a dose to the fetal head is 0.83 Gy, and to the thorax is 0.76 Gy of the 50.3 Gy prescribed to the patient in the 25 fractions.

Conclusion: During the radiotherapy of breast carcinoma in the 2nd trimester of a pregnant females, the fetal dose will be comparable to the threshold of radiation risk for conceptus. It shows that it could be possible to achieve fetal doses below 0.05 Gy with proper optimization of the radiotherapy treatment plan. Preliminary dosimetry measurements with the physical phantom shows that there is a negligible discrepancy between MCNP simulation and experimental radiotherapy on the physical model. With the further radiotherapy plan optimization, doses to the fetus could be reduced to acceptable ranges.

MeSH/Keywords: Breast Carcinoma; Dosimetry; Phantom, Physical and Computational; Pregnant Woman; Radiotherapy

Dissertation Proposal Title: Epigenetic indicators as a prognostic factors in oral cavity and oropharyngeal cancers

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Mentor: Assist. Prof. Željko Zubčić, M.D., Ph.D., Clinical Hospital Center Osijek, Osijek, Croatia

Introduction: Head and neck cancer (HNC) is the sixth most common malignancy. The basic principle of treatment of such patients is still mutilating surgery with possible postoperative radiotherapy.
We will try to define whether there are any epigenetic biomarkers that could help us identify patients with oral / oropharyngeal cancer (OOPC) who have a better / worse prognosis depending on the presence of individual biomarkers. In this way, we could also adjust the treatment and influence the prognosis of these patients.

Hypothesis: Specific epigenetic markers affect the prognosis and survival of patients with OOPC.

Aims:

Define which epigenetic markers are elevated in patients with OOPC
To determine if there is a difference between the expression of epignetic markers in patients with OOPC
To examine whether increased expression of certain epigenetic markers affects the locoregional spread of the tumor itself
Associate increased expression of a particular epigenetic marker with patient survival and prognosis

Materials and methods: The study would include at least 50 patients with OOPC who were primarily surgically treated at the Clinic for OMS CHD, Zagreb or at the Clinic for ENT and OMS UCH Mostar in the period from the 1/1/2017. to the 1/1/2020. Epigenetic markers will be isolated from tumor samples taken during surgery.
The clinical status of the patient after surgical treatment will be monitored for at least one year after surgery, which includes the presence or absence of primary tumor, time of recurrence, time of regional metastasis, time of distant metastasis, survival

in months. Also, epigenetic markers will be determined from tumor samples by DNA methylation via IM EPIC BeadChip array. After the obtained analysis of DNA methylation of patient samples, these results will be correlated with clinical monitoring of patients.

Research plan: The study would be conducted in part retrospectively and prospectively. It would include at least 50 patients who were primarily surgically treated for primary OOPC. Various epigenetic markers were isolated from tumor samples of these patients at the Institut Ruđer Bošković. These same patients will be monitored clinically for at least one year after surgical treatment and their clinical parameters will be monitored and correlated with the findings of epigenetic analysis.

Expected scientific contribution: This research could have a scientific contribution to the detection of epigenetic markers through DNA methylation or expression of mRNA that could identify patients with better or worse prognosis and survival. Namely, if it is proven that a particular epigenetic marker has an impact on the prognosis and outcome of treatment, then already during tumor biopsy, ie diagnosis, we could identify these patients and adjust the treatment modality itself.

MeSH / Keywords: Epigenesis, Mouth neoplasms, Oropharyngeal neoplasms, Survival, Prognosis

Abstract Title: Estimation of dietary salt intake and 24-h urine parameters in children with essential arterial hypertension

Part of the Disertation Proposal: Relationship between juvenile essential arterial hypertension and vascular reactivity in systemic and cerebral circulation

PhD candidate: Martina Kos, M.D.

Mentor: Assist. Prof. Ivana Jukić, M.D., Ph.D., Faculty of Medicine Osijek, University of Osijek, Croatia

Co-mentor: Assoc. Prof. Silvija Pušeljić, M.D., Ph.D., University Hospital Centre Osijek; Faculty of Medicine Osijek, University of Osijek, Croatia

Affiliation of candidate (and Co-mentor): Clinic of Pediatrics, University Hospital Centre Osijek, J. Huttlera 4, 31000 Osijek, Croatia; Department of Pediatrics, Faculty of Medicine Osijek, University Josip Juraj Strossmayer Osijek, J. Huttlera 4, 31000 Osijek, Croatia.
Affiliation of mentor: Institute and Department of Physiology and Immunology, Faculty of Medicine Osijek, University Josip Juraj Strossmayer Osijek, J. Huttlera 4, 31000 Osijek, Croatia.

Introduction: Elevated blood pressure (BP) in childhood may be associated with considerable organ damage and increased risk of cardiovascular disease in adulthood. The development of arterial hypertension is influenced by genetic and environmental factors, with high salt intake being a known environmental contributor. In children, dietary salt intake is also related to blood pressure and the WHO strongly recommends a low salt intake, as well as a high, energy-based potassium intake to prevent adult hypertension.

Aims: This study aimed to estimate daily salt intake in children with essential arterial hypertension and in normotensive children, and to assess the relationship between their blood pressure values and 24-h urinary sodium excretion.

Participants and Methods: Total of 26 children participated in this study; 12 children with essential arterial hypertension (HT) and 14 normotensive children (NT) (both

sexes, age ranged 12-17). Systolic, diastolic and mean blood pressure and heart rate were measured. 24-h urine samples were analysed for excreted sodium concentration whereas daily salt intake based on 24-h urinary sodium excretion was calculated using appropriate formula. Statistical analysis was conducted by t-test (between groups), and Pearson's or Spearman's correlation test was used to determine the correlations between blood pressure (SBP, DBP and MAP) and 24-h urinary sodium excretion ($p< 0.05$ was considered statistically significant).
Study was approved by Ethical Committees of Faculty of Medicine Osijek and University Hospital Centre Osijek, and all participant`s parent/guardian gave written informed consent.

Results. Body mass index (BMI) was similar between groups. Calculated salt intake was significantly higher in children with essential hypertension compared with normotensive children. Systolic (SBP), diastolic (DBP) and mean (MAP) arterial blood pressure were significantly higher in children with essential arterial hypertension compared with normotensive children, while there were no difference in heart rate between HT and NT groups. 24-h urinary sodium excretion was significantly higher in the HT compared with the NT group. 24-h urinary sodium excretion was positively associated with SBP ($r=0.673$), DBP ($r=0.513$) and MAP ($r=0.651$).

Conclusions. Even though BMI was similar, 24-h urinary sodium excretion was significantly higher in hypertensive children indicating their higher daily salt intake, which is associated with higher blood pressure in these children.

MeSH/Key words: child, blood pressure, hypertension, salt intake, sodium

Dissertation Proposal Title: Clinical Significance of N1-Methyladenosin (m^1A) Modification of RNA Molecules in Development of Oral Cancer.

PhD candidate: Ana Kvolik Pavić, M.D., University Hospital Centre Osijek, Osijek, Croatia

Mentor: Assist. Prof. Dinko Leović, M.D., Ph.D., University Hospital Center Zagreb, Croatia

Introduction: Oral squamous cell cancer is a common malignancy arising predominantly under carcinogenic influence of tobacco and alcohol consumption. Despite improvements in diagnosis, surgical and oncological treatment, the survival of patients with oral cancer hasn't improved in the last 50 years. More studies are needed to elucidate tumorigenesis and find possible treatment for this type of cancer. Diversity of genetic code is expressed with various modifications of DNA and RNA molecules that can silence or enhance transcription. The modifications of RNA molecule are reversible, with sets of enzymes that introduce in ("writers"), read it ("readers") and eliminated it ("erasers") from the RNA. One such modification is m1A modification of RNA molecule that has been studied in gastrointestinal, ovary, breast, prostate, pancreas, kidney, and non-small cell lung cancer, but not in oral cancer yet.

Hypothesis: m^1A modification of RNA is implicated in development and progression of oral squamous cell cancer.

Aims: The aim of this study is to determine levels of enzymes involved with m1A modification from publicly available transcriptomic databases and select those with statistically significant differential expression in Head and Neck Cancer tissue samples. These finding with then be correlated with oral cancer samples collected from patients, and their clinical data.

Materials/Participants and Methods: The study will be conducted on oral cancer samples collected in University Hospital Center Zagreb Department of Otolaryngology, Head and Neck Surgery and Osijek University Hospital, Department of Maxillofacial and Oral surgery. Gene expression will be studied using quantitative polymerase chain reaction (qPCR) and levels of m^1A RNA modification by immunohistochemistry using anti-m^1A antibody.

Research plan: Public transcriptomic databases like TCGA, GEO and GTex will be surveyed using on-line bioinformatic tools to discover which of so far known enzymes involved in m^1A processing are differentially expressed in HNC. Tissue samples and medical data will be collected from Croatian Oral Cancer patients. Gene expression levels of the selected m^1A processing enzymes will be determined by qPCR and m^1A levels by IHC. Experimental results will be correlated with clinical data, with emphasis on prognostic and predictive significance of m^1A RNA modification and its processing enzymes for oral cancer patients.

Significance/Expected scientific contribution: Oral cancer is a relatively common disease with high morbidity and mortality. The m^1A modification of RNA or its processing enzymes could possibly be used as predictors of outcome or potential new therapeutic targets.

MeSH/Keywords: Oral Cancer, Carcinogenesis, tRNA Methyltransferases, Gene Expression, Survival Analysis

Dissertation Proposal Title: The correlation of calpain 1 serum activity and concentrations of interleukin 33 with the development of acute respiratory distress syndrome in patients with SARS-Cov-2 virus-induced pneumonia

PhD candidate: Domagoj Loinjak, M.D., University Hospital Centre Osijek; Faculty of Medicine Osijek, University of Osijek, Croatia

Mentor: Assoc. Prof. Robert Smolić, M.D. Ph.D., Faculti of Medicine Osijek, Faculty of Dental Medicine and Health Osijek, University of Osijek, Croatia

Co-mentator: Assist. Prof. Lana Maričić, M.D., Ph.D., University Hospital Centre Osijek; Faculty of Medicine Osijek, University of Osijek, Croatia

Introduction: Acute respiratory distress syndrome (ARDS) is one of the most severe complications of pneumonia caused by the SARS-CoV-2 virus. The development of ARDS is associated with increased cytokine production, while research on animal models indicates that interleukin 33 (IL-33) is one of the more significant among them. It represents a nuclear factor that is released from cells in response to damage and acts on inflammatory cells by amplifying the inflammatory response. Calpain 1, a protease whose activity is associated with fibrotic changes that develop within ARDS, also plays a role in the development of ARDS. The relationship between IL-33 and calpain 1 has been insufficiently investigated, especially regarding the role of calpain 1 as an IL-33 activator.

Hypothesis: Increased calpain 1 serum activity and elevated IL-33 serum concentrations contribute to the development of ARDS in patients with SARS-CoV-2 virus-induced pneumonia.

Aims: The aims of this study were to examine calpain 1 activity and IL-33 concentrations in serum in patients with SARS-CoV-2 virus-induced pneumonia who developed ARDS, determine their relationship and dependence on the method of respiratory support (oxygen therapy, mechanical ventilation) and to compare their values with acute inflammatory response marker values.

Participants and Methods: The study included 80 adult patients with a diagnosis of SARS-CoV-2 virus-induced pneumonia based on a positive PCR test and chest

X-ray findings. Patients were divided into two groups: examined group (patients who developed ARDS) and control group (patients who didn't develop ARDS). The study will exclude patients over the age 80, patients with malignant and neurodegenerative diseases, as well as patients with acute coronary or cerebrovascular events. Peripheral venous blood was sampled from patients to measure calpain-1 activity (fluorometry), IL-33 concentration (ELISA), and acute inflammatory response markers (CRP, fibrinogen, IL-6). A hospital information system will be used for gathering of medical history information, as well as data on the course, methods and the outcome of treatment.

Research plan: This study is conducted at the University Hospital Osijek and Faculty of Medicine Osijek. Sample collection was performed over a period of 6 months at the Respiratory Centre and the Department of Infectology. The samples were initially processed and stored at the Faculty of Medicine Osijek, where further measurements will also be performed. Blood sampling was performed between the 10^{th} and 20^{th} day of the disease's course (the first day being the day the symptoms first appeared).

Expected scientific contribution: The contribution of this study is to establish the role of calpain-1 activity and IL-33 concentration in serum of patients with ARDS caused by SARS-CoV-2 pneumonia.

Keywords: Acute Respiratory Distress Syndrome, Calpain 1, COVID 19, Interleukin-33, SARS-CoV-2 virus

Dissertation Proposal Title: The effects of consumption of functionally enriched hen eggs on endothelial activation and inflammation in patients with acute coronary syndrome

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Mentor: Aleksandar Kibel, M.D., Ph.D., Department of Heart and Vascular Diseases, University Hospital Center Osijek, Faculty of Medicine, University of Osijek, Osijek, Croatia

Introduction: Acute coronary syndrome (ACS) is an acute atherothrombotic event in the coronary arteries. High levels of low-density lipoprotein (LDL) cholesterol increase oxidative stress and inflammation, ultimately leading to decreased nitric oxide bioavailability. Increased levels of oxidative stress and production of high levels of proinflammatory cytokines (IL-1, IL-6, TNF-α) lead to the development of endothelial dysfunction.

Hypothesis: A diet that includes consumption of functionally enriched hen eggs (n-3 polyunsaturated fatty acids, vitamin E, lutein, selenium) will have a positive effect on reducing oxidative stress and inflammatory responses in patients with ACS.

Aims: This aim of this study was to investigate the effects of consumption of functionally enriched hen eggs on pro- and anti-inflammatory cytokines, and on oxidative stress in patients with ACS.

Materials/Participants and Methods: The study includes at least 28 participants, male and female, with ACS over the age of 18 years. Pro-inflammatory and anti-inflammatory markers will be determined using the Invitrogen ProcartaPlex Multiplex Immunoassay System on the Luminex 200 Analyzer. The activity of antioxidant enzymes will be determined using molecular methods. Oxidative stress and the antioxidant capacity measurements will be performed spectrophotometrically using methods for TBARS (Thiobarbituric acid reactive substances) and FRAP (Ferric reducing ability of plasma). Selenium concentrations will be determined by spectrometry method.

Research plan: The study was prospective, interventional, and randomized. Twenty-eight patients with existing ACS were divided into two groups, the experimental group (N=15), who ate three enriched hen eggs/daily, and the control group (N=13), who ate three regular hen eggs/daily for 3 weeks. Pro-inflammatory (IL-1β, IL-6, TNF-α) and anti-inflammatory markers (IL-10, IL-17) will be determined before and after the dietary protocol. The activity of antioxidant enzymes (glutathione peroxidase; GPx, superoxide dismutase; SOD, catalase; CAT) and oxidative stress (TBARS) and antioxidant capacity (FRAP) will be measured before and after the diet protocol. Selenium concentration will be measured before and after the dietary protocol.

Significance/Expected scientific contribution: Whether the progression of the development of the atherosclerotic process in cardiovascular disease can be prevented by influencing oxidative stress without detrimental effect on health. In addition, it will be important to elucidate the mechanisms by which such functional foods affect the microcirculation and thus the possible positive effects in the context of patients who have had acute coronary syndrome.

MeSH/Keywords: acute coronary syndrome; oxidative stress; endothelial dysfunction; functional food; anti-inflammatory potential.

Abstract Title: Immunohistochemical Expression of Wnt-4 Protein in Clear Cell Renal Carcinoma

Part of the Disertation Proposal: Immunohistochemical expression of Wnt-4 as an indicator of the biological behavior of renal cell carcinoma

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Mentor: Assist. Prof. Tvrtko Hudolin, M.D., Ph. D., Osijek University hospital, Osijek, Croatia

Co-Mentor: Assist. Prof. Ivan Miškulin, Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Introduction: Kidney cancer accounts for about 2–3% of cancers worldwide and is one of the most common urological malignancies, with more than 330,000 newly diagnosed cases per year, mostly in Europe, North America, Australia and Japan. It is estimated that there are about 85,000 new cases of kidney cancer in the European Union each year, while about 35,000 patients die from the disease. Due to the widespread use of imaging modalities such as ultrasound, computed tomography (CT) or magnetic resonance imaging, most patients with kidney cancer are now diagnosed at an earlier stage of the disease, with better treatment outcomes. Renal cancer is a complex disease consisting of several subtypes, of which clear cell carcinoma (ccRCC) is the most common form that causes significant morbidity and mortality, so a better understanding of its biology is very important.
Normal kidney development is a complex process involving many molecules, and one of the most important is the family of wingless binding integration site proteins (Wnt) required for grouping cells for complete epithelial differentiation, i.e., nephron formation. However, changes in Wnt proteins have also been shown in various types of tumors, such as hepatocellular carcinoma, thyroid cancer and head and neck squamous cell carcinoma, but also in adrenal gland tumors and kidney cancer. In this study, we investigated the immunohistochemical expression of one member of the Wnt family, Wnt-4, which has been attributed the most important role not only in kidney development, but also in different kidney diseases. The aim of this study was to determine Wnt-4 presence and its potential role as an indicator of ccRCC biological activity

Aim: Investigate immunohistochemical expression of Wnt-4 protein in healthy and tumorous tissue after surgery.

Materials/Participants and Methods: ***Patients***
This retrospective cohort study included a group of 185 patients with ccRCC who underwent surgery at the Department of Urology, University Hospital Centre Zagreb, from September 2015 to April 2019. Patients did not have any prior therapy or known renal disease that could affect treatment outcome or Wnt-4 expression. Demographic and patient data including information about age, sex, CT findings, clinical stage, tumor location, type of surgery (partial vs. radical nephrectomy), as well as pathohistological results were collected, analyzed and correlated with Wnt-4 expression. Patients were followed up for at least one year after the surgery in accordance with European urological association (EUA) guidelines. We used the 2017 TNM classification and Fuhrman's grading system. The study was approved by the Institutional Ethical Review Board.

Immunohistochemistr: The tissue was fixed in 10% buffered formalin, dehydrated in ascending order of alcohols, embedded in paraffin blocks, and cut to a thickness of 3–4 microns. Antigen unmasking was performed in TP-Link High Buffer pH 9.0 3-in-1. After unmasking, the tissue was incubated with the primary anti-WNT-4 (B-6) antibody Santa Cruz Biotechnology, Inc. (Santa Cruz Biotechnology, Inc., Dallas, Texas, U.S.A.) diluted 1:50, for 30 min at room temperature. After the incubation with the primary antibody, samples were incubated for 10 min with a buffer-washed peroxidase blocking reagent, and the tissue was incubated with the EnVision FLEX/HRP secondary antibody (Agilent, Santa Clara, California, U.S.A.) for 30 min. The entire staining procedure was done in Autostainer Link 48 (Agilent, Santa Clara, California, U.S.A.). Immunostaining was semi-quantitatively evaluated for intensity (0 = negative; 1(+) = weak; 2(++) = moderate; and 3(+++) = strong staining). For detecting Wnt-4 expression in healthy tissue we used macroscopically and histologically healthy kidney tissue.
STATISTICAL ANALYSIS: Statistical processing and analysis of the data were performed using the program STATISTICA 6.1 (StatSoft Inc., Tulsa, Oklahoma, USA). Patient demographic data were described by descriptive statistics (numerical data) and frequency tables (descriptive data). Comparison of Wnt-4 expression in macroscopically and microscopically healthy tissue and in tumor tissue was performed with a t-test for independent samples. Comparison of Wnt-4 and tumor grade and comparison of Wnt-4 and tumor stages were performed using analysis of variance (ANOVA) and Fisher LSD test. Comparison of Wnt-4 and TNM groups, subgroups, and suspected metastases was performed using a t-test for independent samples. The relationship between Wnt-4 and the biggest diameter of the tumor size was

analyzed by correlation. Multivariate Cox regression and Kaplan–Meier curve were used in survival analysis. The statistical differences between several different groups of patients were tested by the chi-square test.
RESULTS: Wnt-4 expression in healthy tissue was high, on average 2.8 (0–3), and in tumor tissue on average 1.2 (1–3) (p <0.001, statistical significance at a significance level of 99% ($\alpha = 0.01$)). Most of our patients had grade 2 or 3 tumors, and a proportional correlation between Wnt-4 positivity and increasing grade was found ($p = 0.403$), but without statistically significant difference. Approximately equal Wnt-4 expression (1.18 vs. 1.17) was observed when comparing pathological stages T1 and T3 (since there were only eight T2 and two T4 patients, they were excluded from the analysis) ($p = 0.929$). The same results were reported for T1a vs. T1b and T3a vs. T3b subgroups. When we compared the tumor size in centimeters for all patients, there was a poor negative correlation between tumor size and Wnt-4 expression, i.e., larger tumors had less Wnt-4 expression ($r = -0.1240$, $p = 0.093$, statistical significance at a significance level of 90% ($\alpha = 0.1$)). Patients with suspected metastatic disease had higher mean Wnt-4 expression (1.5 vs. 1.1) compared with patients without metastases, but without significant differences between them ($p = 0.104$)
The average follow-up time was 33 months (12–60). There was no difference in survival rates between Wnt-4 negative and Wnt-4 positive groups ($p = 0.578$)

Conclusion: The role of Wnt-4 protein in the kidney tissue has not been studied in detail so far, and this research will provide additional data on its expression in normal or tumor tissue of renal cell carcinoma.

Keywords: clear cell renal carcinoma; Wnt signaling pathway; Wnt-4 protein; immunohistochemistry

Dissertation Proposal Title: Presence of human papilloma virus in pathohistological samples of foreskin after circumcision

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Mentor: Assist. Prof. Ksenija Marjanović, M.D., Clinical Department of Pathology and Forensic Medicine at Clinical Hospital Centre Osijek, Croatia

Introduction: Genital infections with human papilloma virus (HPV) are almost ubiquitous in the sexually active population. HPV causes significant morbidity in male, where they manifest as genital warts, and in female population, where certain types of virus can lead to neoplastic changes in the genitals, primarily the cervix. Epidemiological data for the Republic of Croatia shows that in 2019 the prevalence of high-risk types of HPV, in the population of women with cervical cancer, was 83%. In addition to cervical cancer, HPV has been linked to other anogenital neoplasms, including vulvar and penile cancers. According to the vaccination calendar in Croatia, HPV vaccination is not mandatory, and persons of both sexes can be vaccinated at the age of 14, before the age of expected sexual intercourse. Men are usually asymptomatic and act as HPV carriers. There is some evidence of the presence of HPV infection in sexually inactive populations, i.e. in children. Given that there is no screening for HPV infection in children and sexually active men and that our institution does not routinely perform pathohistological examination of the foreskin after circumcision, we will investigate the prevalence of HPV in pathohistological samples of the foreskin.

Hypothesis: In sexually active men, the prevalence of high-risk types of HPV in pathohistological samples of the foreskin is higher compared to sexually inactive men. Pathohistological diagnosis is more accurate in detecting high-risk types of HPV compared to foreskin swabs.

Aims:
1. Investigate the prevalence of HPV in the prepuce preparations of adult men and children
2. Investigate the most common types of HPV in the above samples
3. Prove the presence of HPV in sexually inactive men (children)
4. Compare diagnostic methods for detecting HPV DNA by detection from foreskin swabs and pathohistological samples of the foreskin

Materials/Participants and Methods: Men in whom circumcision is indicated for medical reasons will participate in the study. The expected total number of respondents is 120. All the patients will be informed about the study and they will sign an *Informed consent term* prior to their inclusion.

Research plan: Once the research hypothesis has been defined, a further research work plan will consist of forming an *Informed consent* for patients who will be invited to participate in the research work. Research work together with the informed consent will be presented to the Ethics Committee of Clinical Hospital Centre and Faculty of Medicine, University of Osijek. This is followed by the next phase, which includes sample collection of previously mentioned patients. When sufficient samples are collected (120), a pilot research study will be formed to represent the doctoral dissertation. Swabs of foreskin will be taken with an appropriate sampling set, which includes a transport medium and a cytological brush. Then it will be analysed at the Department of Microbiology Osijek. Also, genomic DNA will be isolated from pathohistological samples and the amount of HPV DNA will be measured by spectrophotometry. Obtained concentrations of HPV DNA will be analysed by the Cobas Z device on the Departmant of Pathology Osijek.

Significance/Expected scientific contribution: To contribute to current knowledge about the very high prevalence of HPV not only in the sexually active population but also in children. Comparing two methods in detecting of high-risk HPV , the foreskin swab (the gold standard for the diagnosis of HPV) and pathohistological finding. Future research could examine what impact mandatory HPV vaccination in children (sexually inactive) would have on the prevalence of HPV and HPV types in men and development of cervical cancer in women.

MeSH/Keywords: HPV, Foreskin, Prevalence, Circumcisio

Abstract Title: Anterior cervical discectomy and fusion with osseous allograft: a comparison of clinical outcomes and complication rates between single and multiple level procedures

Part of the Disertation Proposal: This study aims to show multilevel cervical discectomy and fusion as equaly safe and successful surgical method od treatment of the degenerative diseases of the cervical spine as single level surgery, with comparable complication rate. Our hypothesis is that the number of fused levels does not play a decisive role in surgical outcome, when surgery is perform by single surgeon with the required surgical skills, single operative protocol and unique protocol of postoperative care.

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Introduction: Anterior cervical discectomy and fusion (ACDF) is considered the gold standard for many degenerative diseases of the cervical spine. Anterior nerve root decompression via anterior cervical discectomy (ACD) with or without fusion for radiculopathy is associated with rapid relief (3-4 months) of arm/neck pain, weakness, and/or sensory loss compared with physical therapy (PT) or cervical collar immobilization. Anterior cervical discectomy and ACD with fusion (ACDF) are associated with longer term (12 months) improvement in certain motor functions compared to PT. Other rapid gains observed after anterior decompression (diminished pain, improved sensation, and improved strength in certain muscle groups) are also maintained over the course of 12 months. However, comparable clinical improvements with PT or cervical immobilization therapy are also present in these clinical modalities (Class I). Conflicting evidence exists as to the efficacy of anterior cervical foraminotomy with reported success rates of 52-99% but recurrent symptoms as high as 30%
Single level herniated cervical disc causes radiculopathy of the nerve which surpasses the neuroforamen which leads to a pain with specific dermatome and specific

neurological deficits with motor weakness in the muscle which is innervated by the nerve. Very often, there are several herniated discs and the symptoms which cannot be localized to one level only. In this case there is an indication for multiple level surgery.

In multiple studies, overall morbidity rates for ACDF varied from 13.2% to 19.3%; with dysphagia (1.7%-9.5%), postoperative hematoma (0.4%-5.6%); epidural hematoma 0.9%, exacerbation of myelopathy (0.2%-3.3%), symptomatic recurrent laryngeal nerve palsy (0.9%-3.1%), cerebrospinal fluid (CSF) leak (0.5%-1.7%), wound infection (0.1-0.9%-1.6 Horner's syndrome (0.06%-1.1%), respiratory insufficiency (1.1%), esophageal perforation (0.3%-0.9%, with a mortality rate of 0.1%), and instrument failure (0.1%-0.9%)(10). The most common cause of readmission of ACDF is systemic infection and sepsis, followed by pulmonary complications after ACDF (11). Recent prospective cohort study on 159 patients who underwent ACDF with more then 10 years follow up has shown that patient surgery outcomes were not related to age, gender, number of levels treated, and minimally to preexisting degeneration at the adjacent level, that the use of narcotic pain medication decreased substantially and neurological deficits almost all resolved. Patient self-reported success ranged from 85% to 95%. Over the long term, additional surgery for pseudarthrosis (10%) occurred in the early follow-up period, and for adjacent segment degeneration (21%), which occurred linearly during the >10-year follow-up period

It has been postulated that the number of complications in the cervical spine surgery rises with number of operated segments(13). Recent retrospective study suggested that 4-level ACDF is not necessarily associated with a greater number of or more severe complications than 3-level ACDF(14).

Prolonged operative time is associated with increased odds of healthcare utilization and transfusion after single-level ACDF, with operative times greater than 91 minutes which may carry higher odds of postoperative complications (16). Recent meta-analysis demonstrated a higher rate of dysphagia with multiple-level ACDF than with single-level ACDF at a period of 12-24 months (3). Recurrent laryngeal nerve (RLN) palsy is a common and potentially debilitating complication of anterior cervical discectomy and fusion (ACDF), however recent meta analysis could not find differences between single-and multiple level ACDF (17). Long-segment anterior cervical fusions shows their fusion rates exceeding most of the reported fusion rates for similar procedures in the literature, with rates similar to those reported for short-segment ACDFs and that three-level and 4-level ACDF procedures are viable options for cervical spine pathology.

Aims: Aims of this study are to interrogate which of the following factors show statisticaly significant impact on the surgical outcome of anterior cervical discectomy and fusion using osseous allograft: age, gender, body mass index (BMI), smoking,

diabetes, neurological deficits prior and following surgery, VAS (visual analogue scale) pain assessment prior and following surgery, LOS (length of stay), complication rate and the fusion rate. Further aim is to determine which of these factors play a significant role in treatment of single level and of multiple level surgery and which is the final impact of number of fused levels in the successful outcome.

Materials/Participants and Methods: This is a retrospective study based on analysis of patients history, operative reports and radiological findings in 1123 patients. After approval of the study by the appropriate hospital institutional review board (Semmes-Murphey Clinic/Baptist Memorial Hospital, Memphis, USA), we evaluated the records from all patients undergoing first-time ACDF for cervical radiculopathy and/or myelopathy due to degenerative disc disease and/or cervical spinal canal stenosis. Surgery was done over a 13-year period (June 1, 2003 to January 31, 2016) by Kenan Arnautovic, MD, PhD, who used the standard anterior approach from the left side. Fusion was achieved with cadaver allografts (Medtronic; Fridley, Minnesota) with the Elite Vision titanium plate and variable screws (Medtronic). Operative reports, hospital and outpatient clinic charts, and radiographic studies were reviewed by two individuals independently. Operative reports, inpatient and outpatient history and radiological studies (postoperative X-ray of the cervical spine) were retrospectively analyzed with regard to following parameters: age, gender, BMI, fused level, neurological deficits, pain assessment, presence of risk factors (smoking, diabetes), complication rate and presence of radiological fusion on control X-ray of the cervical spine following surgery. The data has been distributed in two groups – group of patient with single level and group of patients with multilevel surgery. Favorable surgical outcome has been defined as postoperative pain reduction, absence of postoperative complications and radiological signs of fusion in the postoperative course of treatment. Surgical outcome has been compared between the single level and multiple level surgery**.**

Results: Overall, 1123 patients were operated on, of which 485 (43%) were men and 638 (57%) were women. The mean age of patients is 50 years. Overall 40.5% of patients underwent one-level surgery, 34.4 % two-level, 21.9 % three-level, and 3.2% four-level surgery. The fusion rate was 99.56%. Three patients underwent additional surgery because of non-union, one patient had a ventral revision, and two underwent additional posterior fusion. A total of 560 patients (49.87%) were discharged home on the same-day. Of the remaining 563 patients (50.13%), 510 patients (90.58%) who stayed overnight left the hospital the next day and 96.27% within 3 days. Table 2. shows the overview of the patient characteristics according to length of stay (Same Day vs. Overnight patients). Pain was the most common symptom which rapidly recovered following surgery. Only 21 patients stayed in the hospital longer 3 nights or

more, with patients with multi-level surgery having more often longer hospital stay than the single level surgery.
The most common complication was infection in 11 patients followed by hematoma in 9, Horner syndrome in 5, non-union in 5, lower plate screw breakage in 2 and screw pullout in 1 patient (0.08%). Complications occurred most frequently in patients 50 to 59 years of age. Overall, complications occurred in 1.75% of patients who underwent one-level surgery, 2.84% who had two-level, 5.7% who had three-level, and 19.4% with four-level surgery. Significantly more complications were in patients who had three- or four-level surgery. Patient who stayed overnight were significantly older and had more frequent diabetes. Smoking and BMI were equaly distributed between the two groups. Preoperative pain level was significantly lower in overnight cohort, and this cohort had significantly higher percentage of patients with multilevel surgery. Postoperative pain reduction did not show differences between the two groups. Neurological outcome was not different between patients with single- and multilevel surgery. Non-union occured more frequently in patients with multilevel surgery.

Conclusion: To our knowledge, this study is unique since it is the first single-surgeon study which compares single level and multiple level surgery using osseous allograft in 1123 patients, which minimizes the risk of bias which could emerge due to differences in experience of the surgeon, operative technique and operative protocol. This study has shown two-level cervical discectomy and fusion to be as successful and safe procedure as single level anterior cervical discectomy and fusion, with comparable complication rate. Three- and four level anterior cervical discectomy and fusion have shown higher complication rate.

Keywords: cervical spine, anterior cervical discectomy and fusion, osseous allograft, single-level ACDF, multiple level ACDF, complication rate

Dissertation Proposal Title: Quality of life of patients with laryngopharyngeal reflux as a basis for an innovative approach to treatment

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Mentor: Prof. Andrijana Včeva, M.D., Ph.D., Department of otorhinolaryngology, Head and Neck Surgery, University Hospital Centre Osijek, Osijek, Croatia

Introduction: Laryngopharyngeal reflux (LPR) is a newer clinical entity. The quality of life of patients with laryngopharyngeal reflux is significantly impaired. The diagnosis is made on the basis of a detailed anamnesis, clinical examination of the larynx and determination of the reflux index score (RSI) and reflux finding score (RFS) according to Belafsky. We still do not have an adequate questionnaire on the quality of life of patients with laryngopharyngeal reflux with good psychometric properties, to assess the quality of life and evaluate the success and impact of therapeutic methods and eating habits on the quality of life of patients with laryngopharyngeal reflux.

Hypothesis: Laryngopharyngeal Reflux Health-Related Quality of Life questionnaire (LPR-HRQL) has good psychometric properties in the Croatian cultural environment in patients with laryngopharyngeal reflux and is associated with the severity of laryngopharyngeal reflux before and after treatment.

Aims:

1. Validate and translate the LPR-HRQL questionnaire from English into Croatian, determine its psychometric properties (reliability and validity) and evaluate the comprehensibility of the translated questions.
2. Examine whether the LPR-HRQL questionnaire can be used as a simple method of assessing the quality of life of patients with laryngopharyngeal reflux treated with different therapeutic methods.
3. Examine whether there is a difference in the quality of life of patients with laryngopharyngeal reflux in relation to the severity of laryngopharyngeal reflux before and after treatment.
4. Examine the impact of diet on the onset and course of the disease and quality of life in patients with laryngopharyngeal reflux.

Materials/Participants and Methods: The study will include 200 patients. Prior to joining the study, all patients will be offered to sign an informed consent document for participation. To determine LPR, RSI and RFS, questionnaires will be used. The eating habits of patients will be examined with a Food Frequency Questionnaire (FFQ). Quality of life of patients will be examined with LPR-HRQL and SF-36 questionnaires. Patients with LPR will be randomly divided into two subgroups. The first patient subgroup will be treated with 20 mg Pantoprazole twice a day and hygienic-dietary measures for 60 days. The second patient subgroup will be treated with 20 mg Esomeprazole twice a day and hygienic-dietary measures for 60 days.

Research plan: At the first visit, patients will be diagnosed with LPR based on otorhinolaryngological examination and results of RSI and RFS questionnaires. Also, all patients (control and research group) will fill out FFQ, LPR-HRQL and SF-36 questionnaires. Patients with LPR will be treated with hygienic-dietary measures and 20 mg Pantoprazole or 20 mg Esomeprazole (depending on random classification into subgroups) twice a day for 60 days. 30 days and 60 days after initial examination, patients will fill out FFQ, LPR-HRQL, SF-36, RSI and RFS questionnaires. The planned duration of the study is 14 months / until the planned number of patients is collected.

Expected scientific contribution: By validating and translating a questionnaire on the quality of life of patients with laryngopharyngeal reflux with good psychometric properties into Croatian, we will be able to assess the quality of life of patients with laryngopharyngeal reflux and evaluate the success and impact of therapeutic methods on the quality of life of patients with laryngopharyngeal reflux. The clinical contribution of this study is to standardise access to patients with laryngopharyngeal reflux, to avoid unnecessary variations in the choice of therapeutic method, to monitor the improvement of quality of life, and thus improve and accelerate patient recovery as well as increase quality of life.

Keywords: Laryngopharyngeal reflux; quality of life; Pantoprazole; Esomeprazole; eating habits.

Abstract Title: Verification of the automated ELISA method for hepcidin-25 in serum

Part of the Disertation Proposal: Verification of the ELISA method for hepcidin-25 in serum

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Mentor: Assist., Prof. Sanja Mandić, Department of Chemistry, Biochemistry and Clinical Chemistry, Faculty of Medicine, University of Osijek, Croatia, Institute of Clinical Laboratory Diagnostics, Osijek University Hospital, Croatia

Co-mentor: Prof, Ines Banjari, Department of Food and Nutrition Research, Faculty of Food Technology Osijek, Croatia

Introduction: Hepcidin-25, a protein generated in the liver is the master protein in regulating iron homeostasis. Its main mechanism is binding to the ferroportin resulting in its internalization and degradation, following increased intracellular iron storage while plasma iron concentration and dietary iron absorption are decreased. So far, hepcidin-25 concentration in serum, was measured by different methods, resulting in incomparable outcomes and complicated results interpretation. Most commonly, techniques such us mass spectrometry (MS) and solid phase enzyme-linked immunosorbent assay (ELISA) are used.

Aims: The aim was to verify the fully automated ELISA method standardized against the MS for hepcidin-25 in serum, using the DRG Hybrid XL analyzer (DRG Instruments GmbH, Marburg, Germany).

Materials/Participants and Methods: Commercially available control samples in two levels were analyzed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines EP15-A2 on Method Verification. Procedure included the assessment of the accuracy (repeatability, intra-assay precision (CVi)) and day-to-day accuracy (intermediate precision), inter-assay precision (CVg)), measurement uncertainty (bias %) and verification of the reference interval (RI) of healthy subjects (20 male blood donors > 18 years of age, following guidelines on venipuncture and hemoglobin

concentration > 135 g/L). Hepcidin-25 was measured by the automated ELISA. All data were calculated using the Excel program and verification of the RI was calculated using the MedCalc program (version 12.4.0.0. MedCalc Software, Marakerke, Belgium).

Results: Intra-assay coefficients of variations (CV) for C1 and C2 were 9.06 % and 4.48 %, respectively. Inter-assay CVs were 8.88 % (C1) and 5.55 % (C2). Calculated bias was 32.84 % (C1) and 20.17 % (C2). Optimal minimum CVs for precision (calculated as CVi x 0.25) was 2.27 % (C1) and 1.2 % (C2). RI declared by the manufacturer were verified and confirmed (mean 10.18 µg/L (SD 9.93)).

Conclusion: Verification of the fully automated ELISA method for hepcidin-25 in serum performed on the DRG Hybrid XL analyzer met the analytical acceptance criteria with desirable analytical reproducibility and is suitable for implementation in the routine work.

MeSH/Keywords: hepcidins, enzyme-linked immunosorbent assay, mass spectrometry, iron, homeostasis

Dissertation Proposal Title: Use of lipidomics and changes in lipidomicstatus in patients treated with psycho-pharmacotherapy

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Mentor: Prof. Dunja Degmečić, M.D., Ph.D., Psychiatry Clinic, Clinical Hospital Center Osijek; Faculty of Medicine, University of Osijek, Osijek, Croatia

Introduction: Many diseases reveal a significant change in lipids during their profiling from body fluids, tissues, or cells compared to the lipid of healthy people. Lipidomics is widely involved in research in almost all diseases. The study of lipid biochemistry will not only provide insight into specific roles of molecular lipid species in health and disease but will also support the identification of potential biomarkers for the establishment of preventive or therapeutic approaches for human health. All available information raises the question of whether mutations in sterols biosynthesis enzymes lead to devastating developmental disturbances and changes in concentrations of available sterols that can lead to general psychological and functioning disturbances.

Hypothesis: Psychopharmacs significantly alter the lipid profile in the selected population of patients treated at the Psychiatric Clinic.

Aims:
- To determine the impact of individual groups of psychopharmaceuticals on lipidomic status
- Establish the correlation between sociodemographic characteristics and changes in the lipid status of the selected population
- To connect comorbidity diagnoses and their interfering with lipid status

Materials/Participants and Methods: The study will include patients who are treated on an outpatient and inpatient basis at the Clinic of Psychiatry. It is planned to conduct a detailed clinical and psychiatric examination of patients and check diagnostic criteria according to the ICD 10 classification for individual diagnostic categories, and an extended questionnaire on sociodemographic data will be developed. Blood samples will be collected in serum tubes. In all serum samples, drug types and concentrations will be determined by serum extraction followed by spectrometric analysis.

Research plan: Collecting samples and conducting research. Analysis of samples. Analysis of results. Publication of the pilot study based on the results obtained. Publication of final results of work.

Significance: The use of lipidomics in clinical studies would provide new insights into the profiling of lipid and pathophysiological mechanisms underlying mental disorders. The main aim of this research is to establish a detailed lipid status of patients suffering from psychological disorders who are in treatment with different groups of psychopharmacs. The aim is also to gain insight into the concentration of psychopharmacs in the patient's blood, whether as monotherapy or polytherapy and to correlate the concentration of psychopharmacs in the blood with different segments of lipid status.

MeSH/Keywords: lipidomics, cholesterol, psychopharmacotherapy, psychological disorders, ICD-10

Abstract: Diagnostic biomarkers of early-onset sepsis (EOS) in preterm infants

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Mentor: Prof. Silvija Pušeljić, M.D., Ph.D., Clinic for Pediatrics, UHC Osijek Faculty of Medicine, University of Osijek, Osijek, Croatia

Introduction: Neonatal sepsis is a clinical syndrome that results from a systemic inflammatory response to an invasive bacterial infection. It's a severe form of disease with very high mortality. EOS occurs within the first 3-5 days of birth, the causative agents are mostly ante/intraparal transmitted. It has numerous risk factors, sudden onset, and a fulminant course. Current diagnostic criteria are often very nonspecific. Early diagnosis and timely treatment are crucial. Presepsin (soluble CD14 subtype) is a newer biomarker that shows good potential in the diagnosis of EOS, grows very quickly after the onset of infection, has very high sensitivity, and is almost 100% specific.

Hypothesis: Presepsin is a new suitable biomarker whose elevated umbilical cord blood values can be a good predictor for the development of EOS in newborns within the first 72 hours of life.

The primary aim of the study is to determine the diagnostic value of serum concentration of presepsin in EOS, in preterm infants, with a birth weight of 2499 grams and less, and gestational age of 36+6/7 weeks and less.

Secondary aims:
To determine the levels and interrelationships of presepsin, procalcitonin, and high sensitivity CRP from umbilical cord blood in the examined group of patients, and to determine their time-determined dynamics in EOS.
To determine the incidens of EOS in preterm infants with very low birth weight.
To determine the cut-off values of presepsin in EOS and the influence of placental pathology on the levels of previously mentioned biomarkers in EOS.

Materials/Participants and Methods: Prospective cohort research will be conducted at the Department of Neonatology of the Pediatrics Clinic and the Maternity of the UHC Osijek. The study will be conducted between 2022 and 2023 and it is planned to include 166 preterm infants of both sexes. Inclusive criteria: birth weight of 2499 grams and less, and gestational age of 36+6/7 weeks and less.

Research plan: Blood samples taken from the umbilical cord immediately after birth will be stored and analyzed in the Central laboratory of UHC Osijek. The infants will be observed and treated by neonatologists from the Clinic for Pediatrics of UHC Osijek. Diagnostics data and complete history of diseases of premature infants and their mothers will be analyzed.

Significance: Through this study, we want to point out the possibility of using presepsin as a new biomarker in neonatology and control of biochemical parameters directly from umbilical cord blood, which represents a valuable source of diagnostic data, in a non-invasive manner. We want to emphasize the importance of very early detection of bacterial infections in newborns and the prevention of life-threatening consequences and complications of EOS.

Keywords: Early-onset neonatal sepsis, Preterm infants, Presepsin, Procalcitonin, Placental pathology.

Abstract title: Do patients with stable coronary heart disease and an absolute indication for surgical revascularization (significant LMCA or LAD), in whom surgery is not possible for pathoanatomical reasons, have significantly higher mortality than patients with stable coronary heart disease treated surgically?

Part of the Dissertation Proposal: Comparison of overall mortality in group of patients with absolute indication for surgical revascularization in whom surgery is not possible due to pato anatomical reasons with similar patients who were surgically treated.

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Introduction: Patients with disease of LMCA and proximal LAD (significant or higher stenosis) have absolute indication for revascularization, either percutaneous (PCI procedure) or surgical (CABG procedure). In this group of patients there is small population of patients in which there is too high risk for PCI and due to mostly pato - anatomical reasons and/or heavy calcification there is also no possibility for surgical treatment. For this group only option is conservative treatment. It is our goal to reevaluate this group of patients and see if they are adequately treated comparing to similar group of patients who were treated surgicalley.

Aims: Comparison of three-year mortality of patients from the observed group versus the control group.

Materials/Participants and Methods: This is a retrospective study involving patients treated at our clinic. Stable patients with coronary heart disease, without significant valvular disease, were included who were presented at our cardio-surgical consilium between 2010 and 2015 with absolute indication for surgical revascularization, in which surgical treatment is not possible due to pathological and anatomical reasons. 70 patients are included in this group. Regarding the control group, included were patients with stable coronary heart disease, without significant valvular disease, who were surgically treated on at our hospital between 2010 and 2012. The control group included 279 patients. A 3-year telephone follow-up was performed in both groups of patients.

Results: Base line characteristics were in observed group were similar with control group (male 71%vs79%, p0.193, DM 40.8%vs39.5%, p0.837, HLP 43.7%vs48.6%, p0.563, peripheral artery disease 26.8%vs 18.5%, p0.122, prior myocardial infarction 43.7%vs41.3%, p0.713, prior PCI 21.1%vs23.4%, p0.680). Our primary end point was overall mortality and there was no significant difference in observed group vs control group (15,5%vs9.8%, p0.168).

Conclusion: There is no significant difference in mortality in these two groups of patients witch means that conservative therapy and incomplete revascularization is as good treatment option as is surgical revascularization for this select group of patients.

MeSH/Keywords: coronary artery disease, surgical treatment for coronary artery disease, conservative treatment for coronary artery disease, incomplete revascularization, coronary artery disease mortality.

Dissertation Proposal Title: The influence of health education on vaccination, attitudes and knowledge of the school population related to vaccination and HPV infection

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Introduction: HPV causes a wide range of diseases, from genital warts to cancers of the oropharyngeal region, cervix, penis and anus. More than 90 % of cervical cancer cases are due to infection with oncogenic types of HPV. Several randomized studies have unequivocally shown that HPV vaccination prevents precancerous lesions. Therefore, HPV vaccination has become standard in many countries. There are still no studies to show the influence of health education on vaccination, attitudes and knowledge of the school population related to vaccination and HPV infection.

Hypothesis:
1. In Osijek-Baranja County, there is a low level of vaccination against HPV in the school population, which is related to the lack of knowledge of students about vaccination and HPV infection itself and their negative attitudes towards vaccination.
2. Health education will increase the proportion of vaccinated people, the level of knowledge and alleviate the negative attitudes of students related to HPV vaccination and HPV infection.

Aims:
1. Investigate the proportion of vaccination, knowledge and attitudes of students towards vaccination against HPV infection;
2. Investigate whether there is a difference in HPV vaccination and knowledge and attitudes of school children about HPV vaccination and HPV infection depending on gender, place of residence (city and village), education of parents (NK, SSS, VŠ, VSS, mr, dr) and the occupation of parents (medical and non-medical occupations);
3. Investigate the impact of health education on students' knowledge and attitudes related to HPV infection and HPV vaccination;

4. Investigate whether attitudes and knowledge about HPV vaccination and HPV infection differ between students who have opted for vaccination and students who have not opted for vaccination;
5. Investigate whether attitudes and knowledge about HPV vaccination and HPV infection differ between students who were involved in health education about HPV infection and vaccination and students who were not.

Materials/Participants and Methods: The research will include eighth grade students from selected primary schools. Prior to joining the study, all parents will be offered to sign an informed consent document for their children's participation. Also, all parents will be asked to complete a short questionnaire that will include sociodemographic data and The Carolina HPV Immunization Attitudes and Beliefs Scale – CHIAS (McRee, Brewer, Reiter, Gottlieb i Smith, 2010.; Delač, Korajlija, 2019.). In addition to sociodemographic data, the student questionnaire will contain several scales that will assess knowledge about HPV and the vaccine, as well as an assessment of one's own knowledge (Caskey, 2009.; Delač, Korajlija, 2019.), and a scale of health beliefs and attitudes about HPV (MacArthur, 2017.; Delač, Korajlija, 2019.). Students, who will participate in the research, will be divided into 2 groups: a group of respondents who will have health education, which is why they will solve the questionnaire 4 times, and a control group that will fill out the questionnaire 2 times - at the beginning and end of the research.

Research plan: During first visit to the primary school, all students involved in the research will solve a questionnaire. Then, a group of respondents will have a short lecture on health education, after which they will again solve the survey questionnaire, as well as 4 weeks and 8 weeks after the health education. The control group of students will also re-solve the survey questionnaire after 8 weeks. The planned duration of the research is one school-academic year.

Expected scientific contribution: The proposed research will more clearly define the factors that affect students' knowledge and attitudes about HPV vaccination and HPV infection in general. In addition, the impact of health education on students' knowledge and attitudes about HPV vaccination and HPV infection in general will be determined, as well as the impact of this public health intervention on vaccination. This approach will create the preconditions for designing a comprehensive program for the prevention of HPV infection in the studied population based on evidence. Finally, the great professional contribution of this research will be reflected in the improvement and advancement of preventive health care for students.

Keywords: Attitudes; Health education; Human papillomavirus (HPV); Knowledge; Vaccination

Disertation Proposal Title: Effect of atorvastatin and rosuvastatin on metabolic, endothelial and cognitive function in postmenopausal women

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Introduction: Postmenopause is defined as a period in a woman's life that begins after the last menstrual period, and is characterized primarily by hormonal changes that occur due to reduced number and / or reduced function of ovarian follicles. It is divided into early, up to 70 years of age, and late, which occurs after 70 years of age. The state of postmenopause can be defined retrogradely only 1 year after the cessation of menstrual bleeding in women, and after other organic and hormonal causes of amenorrhea are excluded. The average age at which menopause occurs is around the age of 50. Thus, women spend almost a third of their lives in postmenopause. Both in the World and in the Croatia as many as 18% of the total population are postmenopausal women due to the increase in human life expectancy and the aging of the population. The first 5 years before menopause (the last menstrual period in a woman's life) and the first 5 years after menopause are considered to be very specific period in which the greatest hormonal and metabolic changes occur. This period is called perimenopause.
In granulosa ovarian cells during perimenopause there is a decrease in the production of inhibins A and B, which results in an increase in follicle-stimulating hormone (FSH), and an increase in its negative feedback is reduced by the reduced production of estradiol in the ovaries. The level of luteinizing hormone (LH) remains unchanged for several years, and later there is an increase in the concentration of luteinizing hormone. Elevated levels of follicle-stimulating hormone stimulate follicles to grow, but follicle maturation and ovulation are less common, leading to a lack of corpus luteum progesterone production, which is one of the reasons for elevated perimenopausal estradiol levels. Elevated estrogen levels and decreased progesterone levels eventually lead to anovulation, shortened cycles, dysmenorrhea, endometrial hyperplasia, and polyps and fibroids also become common. Immediately before menopause, follicles are increasingly resistant to the influence of follicle-stimulating hormone, so the concentrations of inhibin, progesterone and estradiol fall further

down. The ovarian stroma produces more testosterone and androstenedione under the influence of follicle-stimulating and luteinizing hormone, and through circulation androstenedione reaches adipose tissue where it is converted into testosterone and estrone. Over time, levels of sex hormone-binding globulin (SHBG) and growth hormone levels decrease, which reduces the production of androgens in the adrenal glands.

Menopause changes not only the function of the ovaries, as an organ of the reproductive system, but also the function of the adrenal gland, hormonally active adipose tissue and the entire central nervous and cardiovascular system, which ultimately affects the body as a whole. It is important to emphasize that the values of individual hormones also depend on the manner in which menopause occurs, so we distinguish between natural and iatrogenic menopause.

In addition to affecting ovarian function and consequently the menstrual cycle and the sexual and urinary system of women, changes in postmenopausal hormone levels lead to vasomotor symptoms, and various changes in the neurological system such as sleep disorders, cognitive impairment, depressive disorders and many others. It is necessary to mention a very important effect on the cardiovascular system. Estrogens have a beneficial effect due to the following: they stimulate vasodilation by acting on the endothelium of blood vessels through prostacyclin, thromboxane and nitric oxide, have a beneficial effect on cholesterol and lipoproteins in terms of raising HDL-cholesterol levels, lowering LDL-cholesterol levels and total cholesterol. Estrogens also have a positive effect on coagulation and fibrinolysis by reducing the concentration of factor VII, fibrinogen and PAI-1, as well as reducing the amount of abdominal adipose tissue and improving the metabolism of glucose. Consequently, estrogen deficiency may be associated with increased cardiovascular risk (more common coronary heart disease and cerebrovascular stroke), and these diseases are becoming the leading cause of death in women of this age.

After the age of 65, cardiovascular diseases, including complications such as acute myocardial infarction and cerebrovascular stroke, are more common in women than in men with more common fatalities. Due to the specifics of hormonal status and changes that occur at the cardiovascular, neurological and endocrinological levels in postmenopausal women, it is necessary to adjust both preventive and therapeutic approaches especially at the level of primary health care.

Inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (statins) are a group of drugs used to treat hypercholesterolemia and have been shown to successfully prevent further progression of atherosclerosis and to reverse it. Many studies have shown, in relation to these effects, a reduction in the prevalence of cardiovascular diseases and mortality from cardiovascular diseases in patients receiving statin therapy. This therapy acts at the level of the liver, in hepatocytes, by competitive inhibition of the enzyme HMG-CoA reductase, whose function is to

produce mevalonic acid, a precursor of cholesterol. Reductase inhibitors also cause an increase in the number of low-density lipoprotein (LDL) receptors which leads to an increase in the rate of LDL catabolism and hepatic extraction of very low-density lipoprotein (VLDL) residues from the blood, which ultimately leads to a decrease in LDL cholesterol levels. In addition to the mentioned effect, the effect of lowering the concentration of triglycerides in the plasma is achieved, as well as a small increase in the concentration of HDL cholesterol. Cardiovascular risk and mortality caused by cardiovascular incidents are reduced, so we can say that statin therapy has a very important role in both primary and secondary prevention of cardiovascular diseases. In Croatia, the most commonly used statin molecules are atorvastatin and rosuvastatin.
The causal role of LDL cholesterol, and other apo-B-containing lipoproteins, in the development of atherosclerotic cardiovascular disease (ASCVD) is demonstrated beyond any doubt by genetic, observational, and interventional studies. Prolonged lower LDL cholesterol is associated with lower risk of ASCVD and the absolute benefit of lowering LDL cholesterol depends on the absolute risk of ASCVD and the absolute reduction in LDL cholesterol, so even a small absolute reduction in LDL cholesterol may be beneficial in a high- or very-high-risk patient.
The 2019 ESC / EAS Guidelines presented recommendations for the primary and secondary prevention of cardiovascular disease and the use of statin therapy in the treatment of cardiovascular risk-dependent dyslipidemias in patients. Special emphasis in the current guidelines is placed on reducing the concentration of LDL cholesterol in order to prevent cardiovascular incidents. Recommended LDL cholesterol values for patients with very high cardiovascular risk are less than 1.4 mmol / L with a reduction in baseline of 50% or more, for patients with high cardiovascular risk are less than 1.8 mmol / L with a reduction in baseline of 50 % or more, for patients with moderate cardiovascular risk less than 2.6 mmol / L, and for patients with low cardiovascular risk less than 3.0 mmol / L.
New 2021 ESC / EAS Guidelines presented new recommendations. Systematic or opportunistic CV risk assessment in the general population in men >40 years of age and in women >50 years of age or postmenopausal with no known ASCVD risk factors may be considered.

Hypothesis: The use of statin therapy (atorvastatin and rosuvastatin) in postmenopausal women contributes to better regulated parameters of metabolic function: total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, insulin resistance, plasma glucose and urate and better endothelial function, measured by serum endocan and endoglin levels, but also contributes to the improvement of the speed of reactions and cognitive functions contrast to postmenopausal women without statin therapy.

Aims:

1. To determine the effect of statin therapy (atorvastatin / rosuvastatin) on metabolic functions in postmenopausal women measured by the effect on total serum cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, insulin-like growth factor 1 (IGF-1), plasma glucose and urate levels.
2. To determine the effect of statin therapy (atorvastatin / rosuvastatin) on endothelial function in postmenopausal women measured by serum endocan and endoglin levels.
3. To determine the effect of statin therapy (atorvastatin / rosuvastatin) on cognitive function in postmenopausal women, including the effect on response rate.
4. To determine the existence of differences in the effect of statin therapy on metabolic, endothelial and cognitive function depending on the type of statin therapy (atorvastatin / rosuvastatin).

Participants and Methods: The research will include women from the Osijek-Baranja County, who are patients in family medicine offices in the Osijek-Baranja County Health Center. The estimated number of respondents is 400. The research will be conducted after the approval of the Ethics Committee of the Faculty of Medicine in Osijek and the Ethics Committee of the Osijek-Baranja County Health Center. Assessment of metabolic function will be performed by laboratory measurement of triglyceride levels, total cholesterol, LDL cholesterol, HDL cholesterol, plasma glucose and urate serum levels. Before blood sampling the subjects must be fasting for at least 10 hours. At each sampling, one sample of venous blood will be taken without the addition of anticoagulants for analysis. Samples will be centrifuged at 3500 rpm for 10 minutes before analysis. The biochemical methods to be used are: UV photometry with hexokinase, photometry with cholesterol oxidase (CHOD-PAP), photometry with glycerophosphate oxidase, UV photometry with uricase and homogeneous enzyme-linked immunosorbent assay. Assessment of endothelial function / dysfunction is planned to be performed by measuring serum levels of endocan and endoglin. Endocan and endoglin ELISA commercial kit will be used to measure the concentration of these molecules according to the manufacturer's instructions, and measurements will be performed at the beginning of the study (1st measurement) and at the end of the study after 24 months or achieving LDL cholesterol targets according to current guidelines). Cognitive functions in patients will be examined by MoCA (Montreal Cognitive Assessment) test, which is an important tool for detecting cognitive disorders, including mild ones, as well as the initial stages of other neurological diseases (Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis, sleep disorders). Assessment of cognitive function will be complemented by the use of the MMSE (Mini Mental State Exame) test, which for many years has been the gold standard in detecting the initial stages of cognitive

impairment. The assessment of cognitive functions will also include a controlled rate assessment test, which will be performed after each of the planned blood sampling with the Human benchmark, a free guide available online, and validated for a rough computer-based rate assessment. The test will consist of three specific measurements (test performance). An average of three measurements expressed in milliseconds will be entered in the research collection table. Descriptive statistical methods will be used to describe the frequency distribution of the investigated variables. All variables will be tested for normality of distribution by the Kolmogorov-Smirnov test, and depending on the result, parametric or non-parametric methods will be applied for their further processing. Categorical data will be presented in absolute and relative frequencies. Mean values of continuous variables will be expressed by arithmetic mean and standard deviation for normally distributed variables and median and interquartile range in cases where variables are not normally distributed. Differences in categorical variables will be tested by $\chi 2$ test and Fisher's exact test. Differences in numerical variables will be tested by t-test as parametric or Mann-Whitney U test as nonparametric test, and for more than two samples by ANOVA test or Kurskal Wallis as nonparametric test. The correlation between the variables will be expressed by the Pearson correlation coefficient in cases where the variables follow the normal distribution or by the Spearman correlation coefficient in the cases when the variables do not follow the normal distribution. All P values are two-sided. The significance level will be set at $p < 0.05$. MedCalc Statistical Software version 18.11.3 will be used for statistical analysis.

Research plan: The research will include women from the Osijek-Baranja County, who are patients in family medicine offices in the Osijek-Baranja County Health Center. The estimated number of respondents is 400. Inclusive criteria will be: age 45 to 65 years, postmenopause (absence of menstruation in the past 1 year or longer), the presence of indications for the use of statin therapy based on lipidogram values according to current guidelines for the treatment of hyperlipoproteinemia. Exclusive criteria will be: age less than 45 and older than 65, iatrogenic menopause, malignant disease, hormone replacement therapy, alcoholism, type 1 and 2 diabetes, unregulated hypertension in which there is a need for intensive correction during follow-up (2 years), antidepressant and beta-blocker therapy, active liver disease with persistently elevated transaminases, unregulated chronic kidney disease and need for dialysis. Each respondent will be informed in an understandable way about the research method, research objectives and will sign a written informed consent to participate in the research. Respondents must participate in the survey voluntarily and will be guaranteed anonymity. The follow-up time of the subjects will be until the target values of LDL cholesterol are reached (individual recommendation based on the cardiovascular risk of the subjects) for a maximum of 24 months. It is planned

to perform measurements in several stages. The first measurement at the beginning of the test will include measurement of total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, IGF-1, urate, fasting plasma glucose, endocan and endoglin levels. Further measurements will be performed every 8 weeks, until the target values of LDL cholesterol are reached for maximum 24 months if the subjects do not achieve the target values of LDL cholesterol recommended in the guidelines. Every 8 weeks, the dose of statin therapy will be increased up to the maximum recommended doses if the target LDL cholesterol values are not reached. Endocan, endoglin, and IGF-1 levels will be measured at the beginning and end of the study (reaching the LDL cholesterol target or the end of follow-up after 24 months). The study will be organized as a prospective study with two main groups of subjects: subjects using atorvastatin and subjects using rosuvastatin (each group will contain an equal number of subjects). Treatment in both groups will be started with the lowest recommended dose of statin therapy with an increase in the dose of statin therapy every 8 weeks in case of non-achievement of target values, until the target LDL cholesterol targets are reached or until the end of the study. Atorvastatin therapy will be administered in doses of 10 - 20 - 40 - 80 mg, and rosuvastatin therapy in doses of 5 - 10 - 20 - 40 mg. The impact on the metabolic parameters will be compared depending on use of statin therapy, the type of statin therapy and the dose of statin therapy used. The impact on endothelial function will also be monitored. Response rate measurements and cognitive function tests will be performed at the beginning of the study, then every 8 weeks, until the target LDL cholesterol targets are reached or the research is completed.

Expected scientific contribution: Statin therapy has an important place in both primary and secondary prevention of cardiovascular diseases and is widely available in the health care system of Croatia. In postmenopausal women the risk of developing cardiovascular diseases is greatly increased due to metabolic changes so the impact of lowering LDL cholesterol on insulin resistance, endothelial function measured by serum levels of endocan and endoglin, but also on other metabolic parameters (levels of glucose, urate, triglycerides, serum HDL cholesterol) as well as cognitive function, with special reference to the speed of reactions, should be assessed thoroughly. Research on the impact of statin therapy in postmenopausal women will complement and expand knowledge about prevention, which is easily available in primary health care, and can potentially create framework guidelines for the use of therapy, but also monitoring its effects.

Keywords: hydroxymethylglutaryl-CoA reductase inhibitors, postmenopause, metabolism, endothelial function, reaction time, lipids, cardiovascular system

Abstract Title: Predicting future development of COPD (MARKO study)

Part of the Disertation Proposal: Assessment of the predictive value of the MARKO questionnaire for the development of chronic obstructive pulmonary disease in smokers during a five-year follow-up

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Introduction: Chronic obstructive pulmonary disease (COPD) is a global leading cause of morbidity and mortality, responsible for approximately 5 million deaths per year, with an expected significant increase by 2025. The main risk factor for developing COPD is tobacco smoke, but only <1/3 of smokers develop COPD during their lifetime. Early diagnosis should allow an early intervention and thus possible prevention of COPD progression. Symptoms and health-related loss of quality of life (HRQoL) often precede diagnostically significant loss of lung function. As existing HRQoL questionnaires are too complicated (Chronic Respiratory Questionnaire - CRQ, St. George Respiratory Questionnaire - SGRQ), and/or were not developed for the purpose of early detection of COPD (COPD Assessment Test - CAT), we constructed, developed, validated and we plan to assess the predictability of this simple self-applicable questionnaire to identify early HRQoL changes related to the future incident cases of COPD (MARKO questionnaire).

Aims: To determine the predictability of MARKO questionnaire and/or its domains, individually or in combination with other markers and characteristics (gender, smoking history, lung function, 6-minute walk test (6MWT), exhaled breath temperature (EBT), hsCRP) for the incident COPD in subjects at risk over 2 years monitoring period.

Participants and Methods: Patients aged 40-65 years of both sexes were recruited and analyzed, and followed for 2 years according to inclusion and exclusion criteria.
Inclusion criteria: active smokers aged 40 to 65 with a smoking history of at least 20 pack-years, who at the time of inclusion were not diagnosed with COPD.

Exclusive criteria: (a) being treated for any clinically relevant chronic disease that significantly affects quality of life; (b) on immunosuppressive therapy; (c) significant acute respiratory illness 4 weeks before screening; (d) hospitalized for a period of 3 months prior to screening; (e) myocardial infarction, stroke or transient ischemic attack during the 6 months prior to screening; (f) diagnosis of asthma; (g) unable to perform the diagnostic protocol.
During the initial visit to the pulmonologist, and after signing the informed consent and inclusion by the GP, a detailed diagnostic workout was done: patients completed 3 self-assessment questionnaires - MARKO, SGRQ and CAT, ECRHS II, medical history and physical, laboratory (CBC, hsCRP), lung function tests and EBT.
At the follow-up after 2 years, the following workout was performed: 3 self-assessment questionnaires - MARKO, SGRQ and CAT, history and physical, lung function tests and EBT.
Statistical analysis: The analysis of the MARKO questionnaire is focused on the validity of the construct by factor analysis and the predictability for the incident COPD. Outcome-related variables in univariate analysis at the $p < 0.20$ level were analyzed using multivariate regression analysis. Statistical analysis was performed using the statistical software package Statistica version 12 (StatSoft, Inc. Tulsa, OK). The results with the significance level $p < 0.05$ were considered significant.

Results: A sample of 232 subjects (58.6% male), mean (SD) age 52.1 (7.1) years with 36.6 (17.3) pack-years of smoking was reassessed after 2 years. Exploratory factor analysis of MARKO questionnaire isolated three distinct domains. We found out that a change in EBT after a cigarette (ΔEBT) and the second domain of MARKO questionnaire were significantly predictive for future COPD development (AUC 0.86, $p<0.001$) and for advanced disease (AUC 0.83, $p<0.001$). The second domain of MARKO questionnaire comprised questions about severe cold with cough and bronchitis and the use of antibiotics for it during previous year.

Conclusion: Our preliminary data shows that the MARKO questionnaire (second domain) combined with EBT (change after a cigarette smoke) could potentially serve as early markers of future COPD in smokers.

MeSH/Keywords: Health related quality of life (HRQOL), Questionnaire, Chronic obstructive pulmonary disease, Smoking habit, Predictive markers, Validity

Title of abstract: Effects of preoperative anxiety, depression and pain on quality of postoperative recovery after radical prostatectomy

Dissertation proposal title: Effects of preoperative anxiety, depression and pain on quality of postoperative recovery and patient satisfaction after radical prostatectomy

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Introduction: Prostate cancer is the second most frequently diagnosed cancer in men with 1.4 million new cases annually worldwide. Radical prostatectomy is one of the main treatment methods. Patients with prostate cancer are often in psychological stress and pain preoperatively. Facing diagnosis of cancer with its health, social and financial implications and making decisions about optimal treatment modality is very stressful for majority of patients. Radical prostatectomy is effective treatment for prostate cancer but often leads to urinary and sexual dysfunction. Sources of preoperative anxiety are concerns about the outcomes of surgery and anesthesia, pain, nausea and vomiting, fatigue, loss of dignity and cognitive impairment. Symptoms of anxiety and depression can be induced, or existing ones aggravated.
Anxiety and depression are associated with increased acute pain scores and opioid consumption. Correlation of anxiety and depression with worse oncologic outcomes and lower quality of life is well established but data on their effects on quality of postoperative recovery are scarce, especially for patients after radical prostatectomy.
Patients with preoperative pain can have higher levels of acute postoperative pain. They are also at increased risk of developing chronic pain postoperatively. Since different types of surgery are assocciated with different patterns and mechanisms of acute and chronic pain studies that examine pain outcomes should be procedure specific. Results of studies that examined severity of acute postoperative pain after radical prostatectomy are inconsistent.

Aims: The aims of this study are to examine the effects of preoperative depression, anxiety and pain on quality of postoperative recovery and acute postoperative pain after radical prostatectomy.
Methods: This prospective observational study was conducted at the Department of Urology, General Hospital Zadar, Croatia. Eligible patients were those with prostate cancer scheduled for laparoscopic or open radical prostatectomy. Excluded patients were those with severe comorbidities or cognitive impairment precluding use of questionnaires and objective assessment of postoperative recovery and pain, alcohol or drug abuse and language barrier.

Study protocol: Patents were approched day before surgery by one of two researchers and explained study protocol. After written informed consent was obtained patiens completed psychological tests. Researchers were available for additional clarification or assisstance. Anxiety was assessed with State-Trait Anxiety Inventory (STAI-S and STAI-T) and depression with Center for Epidemiological Studies Depression Scale (CES-D). Patients who had pain at any site in the previous week completed Brief Pain Inventory (BPI). Quality of recovery-40 (QoR-40) questionnaire was administered as a baseline measure.
Demographic and clinical data included age, educational level, BMI, ASA physical status, comorbidities, PSA level, Gleason score, type of surgery, duration of anesthesia and surgery, intra and postoperative complications, estimated blood loss and duration of hospitalization.
Anesthesia protocol was standardized. General anesthesia was induced with fentanyl 1.5-2.5 mcg/kg, propofol 1.5-2 mg/kg and rocuronium 1 mg/kg. For maintenanace of anesthesia sevoflurane 1 MAC in air/O2 mixture was used. Boluses of fentanyl 50-100 mcg and rocuronium 10-20 mg were added as needed. Patiens were ventilated in pressure control mode with tidal volumes 6-8 ml/kg IBW and PEEP 5 ml H2O. Respiratory rate was adjusted to maintain normocapnia. Ketoprofen 100 mg was administered approximately 30 minutes before the end of surgery. For postoperative analgesia tramadol, paracetamol and ketoprofen were used.
Postoperative recovery was assessed on postoperative days 1-3 using the Quality of recovery-40 score (QoR-40). Numeric rating scale 0-11 was used to assess the intensity of postoperative pain at rest (NRS-R) and movement (NRS-M) at 1, 6 and 24 h post-surgery.

Measures: Stait-Trait Anxiety Inventory (STAI)
State-Trait Anxiety Inventory is self-reported measure of anxiety composed of two questionnaires with 20 item each. Questionnaires measure actual level of anxiety (STAI-S) and general level of anxiety (STAI-T). Items are scored on four-point Likert scale. Scores range from 20 to 80 with higher scores indicating higher levels of anxiety.

Center for Epidemiological Studies Depression (CES-D)
The CES-D scale is a short self-reported scale that measures symptoms associated with depression experienced in the past week. It consists of 20 items scored on four-point Likert scale (0-3). Scores range from 0 to 60. Score ≥ 16 identifies individuals at risk for clinical depression.
Brief Pain Inventory (BPI)
Brief Pain Inventory includes four items that report sensory dimension of pain or pain severity and seven items that report reactive dimension of pain or inteference with daily function. Items are rated on 0-10 scale. To measure pain severity, arithmetic mean of four severity scores (BPI-S) and "worst pain" (BPI-W) were used. To measure of interference with daily function arithmetic mean of seven interference items was used (BPI-I). Brief pain inventory was validated by Croatian Pain Society.
Quality of Recovery-40 (QoR-40)
Quality of Recovery-40 is a patient rated questionnaire for evaluation of postoperative recovery included in patient-reported outcome measures (PROMS). It consists of 40 questions that cover five key dimensions comprising postoperative recovery; physical comfort (12 items), emotional state (9 items), psychological support (7 items), physical independence (5 items) and pain (7 items). The items are scored on a five-point Likert scale. Total score ranges from 40 (worst possible recovery) to 200 (best possible recovery). Psychometric validation of Croatian version of QoR-40 was performed in the group od 162 mixed surgery patients. Internal consistency (Cronbach's α) of the global QoR-40 was high (0.93) and of the QoR-40 dimensions was moderate to high (≥ 0.714). QoR-40 was found to be valid and reliable questionnaire for measuring quality of postoperative recovery in Croatian population with psychometric properties similar to the original version (Sulen N et al, article accepted for publication).

Results: One hundrend and sevety five patients were screend for participation. Three patient were ineligible due to comorbidities and two patients due to language barrier. Five patients refused participation and three patients returned their base-line questionnaires uncompleted and withdrew consent. After 162 patients completed base-line questionnaires another two patients were excluded due to postoperative bleeding that necessitated reoperation. Finaly 160 patients were included in the study. Demographic and clinical data are presented in Table 1.

Table 1. Demographic and clinical data

Preoperative variables	
Age (years) mean IQR	63.7 (59.5-68)
BMI mean IQR	27.6 (25.2-29.3)
Education level I -primary school n(%)	15 (9.4)

-secondary school n (%)	90 (56.3)
-college (3 years) n (%)	19 (11.9)
-college (≥4 years) n (%)	36 (22.5)
ASA status -II n (%)	127 (79.4)
-III n (%)	33 (20.6)
PSA (ng/ml) median IQR	7.7 (5.7-13)
Gleason score ≤ 6 n (%)	34 (21.3)
3+4 n (%)	85 (53.1)
4+3 n (%)	24 (15.0)
≥8 n (%)	17 (10.6)
Preoperative pain present n (%)	33 (20.6)
Intraoperative variables	
Type of surgery - laparoscopic n (%)	88 (55.0)
-open n (%)	72 (45.0)
Duration of anesthesia (min) median IQR	185 (160-210)
Duration of surgery (min) median IQR	155 (135-185)
Lymphadenectomy -not performed n (%)	78 (48.8)
-negative lymph nodes n (%)	64 (40.0)
-positive lymph nodes n (%)	18 (11.3)
Intraoperative blood loss (ml) median IQR	275 (200-400)
Crystalloid infusion (ml) median IQR	2000 (1500-2100)
Anesthesia complications n (%)	5 (3.1%)
Intraoperative surgical complications n (%)	8 (5.0)
Postoperative variables	
Postoperative complications n (%)	26 (16.3)
Patients receiving blood transfusion (intra and postoperative) n (%)	9 (5.6)
Duration of hospitalization (days) mean IQR	8.74 (8-9)

Preoperatively, mean (SD) STAI-S, STAI-T and CES-D scores were 36.4(10.2), 34.5 (9.1) and 10.3 (9.2), respectively. QoR-40 scores on days 1-3 were 164.0 (15.9), 176.6 (14.5) and 183.5 (12.3) respectively.

There are significant negative correlations of STAI-S, STAI-T and CES-D scores with QoR-40 scores on 1st to 3rd postop. day (r -0.30 to -0.47; $P<0.05$) (Table 2).

Table 2. Pearson correlation coefficients between QoR-40 on the first postoperative day and STAI-T, STAI-S , CES-D and NRS scores

	QoR-40 1. day
NRS-R 6h	-0.267 (< 0.001)
NRS-R 24h	-0.317 (< 0.001)
NRS-M 6h	-0.329 (< 0.001)
NRS-M 24h	-0.34 (< 0.001)
STAI-T	-0.385 (< 0.001)
NRS-R 1h	-0.397 (< 0.001)
NRS-M 1h	-0.406 (< 0.001)
CES-D total	-0.461 (< 0.001)
STAI-S total	-0.464 (< 0.001)

Thirty-three patients (20.6%) reported presence of pain in preoperative period. Pain was mainly of musculoskeletal origin with only one patient reporting pain at surgery site. Mean (SD) BPI-W, BPI-S and BPI-I scores were 3.4 (1.5), 2.3 (1.1) and 2.0 (1.2) respectively.
NRS-R scores at 1,6, 24 h were 2.8 (2.1), 2.8 (1.6) and 2.4 (1.8) and NRS-M scores at 1,6,24 h were 4.2 (2.1), 4.3 (1.7) and 4.2 (1.7) respectively.
BPI-W and BPI-S had significant positive correlations with NRS-R and NRS-M at 6h (r 0.42 to 0.53; P<0.05). BPI-S and BPI-I scores had negative correlation with preoperative QoR-40 score (r -0.37, -0.47 respectively, P<0.05) but BPI scores did not correlate with postoperative QoR-40 scores.

Conclusion: Preoperative anxiety and depression had negative correlation with postoperative quality of recovery after radical prostatectomy. Preoperative pain was mild on average and mainly of musculoskeletal origin with only one patient reporting pain at surgery site. Preoperative pain correlated with postoperative pain at 6h but did not affect quality of postoperative recovery assessed with QoR-40. Screening for anxiety and depression in preoperative period with therapeutic intervention could improve quality of postoperative recovery after radical prostatectomy.

Key words: depression, anxiety, pain, postoperative recovery, prostatectomy

Acknowledgement: Associate Professor Milan Milošević, MD, PhD, Assistant Professor Miroslav Župčić, MD, PhD and Professor Boris Mraović, MD contributed to analysis and interpretation of data and to preparation of manuscript.

Abstract Title: Consumption of hen eggs enriched with n-3 PUFAs, selenium, vitamin E and lutein incites anti-inflammatory conditions in healthy participants

Part of the Dissertation Proposal: "Influence of consumption of eggs enriched with functional compounds on endothelial function and oxidative stress levels in healthy young subjects - a randomized controlled study"

PhD candidate: Petar Šušnjara, mag. med. lab. diagn., Institute and Department of Physiology and Immunology, Faculty of Medicine, Josip Juraj Strossmayer University of Osijek, Croatia

Mentor: Prof. Ines Drenjančević, M.D., Ph.D., Institute and Department of Physiology and Immunology, Faculty of Medicine Osijek, University of Osijek, Croatia

Co-Mentor: Assist. prof. Zrinka Mihaljević, Ph.D., Institute and Department of Physiology and Immunology, Faculty of Medicine Osijek, University of Osijek**,** Croatia

Introduction: Inflammation and/or oxidative stress induce endothelial activation and contribute to its functional impairment, ultimately increasing the risk of developing early atherosclerotic lesions. Overwhelming levels of endogenous and exogenous reactive oxygen species (ROS) in antioxidant-lacking environment lead to oxidative stress-related DNA damage and immune system activation. On one hand, ROS initiate pro-inflammatory cytokines' gene expression through activation of specific pathways resulting in lymphocyte and macrophage recruitment. On the other hand, inflammatory processes also lead to exaggerated oxidative stress through ROS overproduction and reduced antioxidant activity. Such cascade of damaging events results in protein denaturation, lipid peroxidation and apoptosis which can lead to low-grade inflammation of vascular wall. Thus, increasing the antioxidative defence mechanisms may be beneficial in preventing or attenuating pro-inflammatory conditions that lead to endothelial dysfunction and, ultimately to cardiometabolic diseases, even in healthy persons, ahead of clinical manifestation of diseases.

Aims: Present study aimed to evaluate effects of consumption of enriched eggs on oxidative status and inflammatory conditions in healthy young volunteers.

Participants and Methods: This was a randomized, double-blind, placebo-controlled study (part of ID: NCT04564690, the study was registered on the Clinical trial under the title: Effect of Enriched QUARTET® Hen Eggs on Cardiovascular Function in Cardiovascular Patients and Healthy Individuals). Subjects were divided in Control group (N = 14; W/M = 6/8) who consumed 3 regular hen eggs per day (n-3 PUFAs content ~438 mg/per day; selenium content ~0.05 mg/per day; lutein content ~0.33 mg/per day; vitamin E content ~1.79 mg/per day), while Nutri4 group (N = 20; W/M = 9/11) consumed 3 enriched hen eggs per day (n-3 PUFAs content ~1026 mg/per day; selenium content ~0.06 mg/per day; lutein content ~1.85 mg/per day; vitamin E content ~3.29 mg/per day) for three weeks. Samples were taken before and after the respective dietary protocols. Serum concentrations of lipid mediators and cytokines were measured with ELISA assays and antibody-based, magnetic bead reagent kits on Luminex platform, respectively. Serum oxidative stress and antioxidant capacity were measured using standardized methods, while gene expression in peripheral blood mononuclear cells (PBMCs) was measured via real time PCR. The study protocol and procedures conformed with the standards set by the latest revision of the Declaration of Helsinki and were approved by the Ethical Committee of the Scientific Center of Excellence for Personalized Health Care, Josip Juraj Strossmayer University of Osijek (Cl: 602-04/14-08/06; No: 2158-610714-114) and Ethics Committee of the Medical Faculty Osijek CLASS: 602-04 / 20-08 / 07 REGISTRATION NUMBER: 2158-61-07-20147.

Results: Serum levels of pro-inflammatory interleukin 17A (IL-17A) were decreased and nNOS expression was increased in Nutri4 group, while serum concentrations of pro-inflammatory LTB4, PGE2, PGE3 produced via cyclooxygenase (COX) pathways were significantly increased in Control group. Antioxidative enzyme activity was measured for superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) and there were no significant differences detected in Control or Nutri4 group after respective dietary protocols. There were also no significant changes in serum TBARS or FRAP in either the control or the Nutri4 group compared to the corresponding baseline values.

Conclusion: This study population consisted of young, healthy adults without underlying comorbidities; thus no oxidative stress or major anti-inflammatory effects were detected. Decreased serum levels of pro-inflammatory IL-17A and an increased nNOS expression in Nutri4 group, together with alteration of metabolites produced via COX pathways in Control group, suggest a shift towards anti-inflammatory conditions in participants who consumed enriched hen eggs, while such changes were not observed in Control group. Present results suggest that combined action of n-3 PUFAs and antioxidants play an important role in nNOS activation pathway and this cooperation may have protective role in resting, non-inflammatory conditions. The change in nNOS expression was surprising and grants further investigation.

MeSH / Keywords: functional food, inflammation, n-3 PUFAs, nNOS, IL-17

Acknowledgements: The study was funded by European Structural and Investment Funds to Science Centre of Excellence for Personalized Health Care, the Josip Juraj Strossmayer University of Osijek, Scientific Unit for Research, Production and Medical Testing of Functional Food, # KK.01.1.1.01.0010.

Abstract Title: Hyperuricemia in kidney transplant recipients

PhD candidate: Mila Vasilj Mihaljević, The Health Centre Vukovar, Croatia

Mentor: Assist. Prof. Tihana Šimundić, Clinical Hospital Centre Osijek, Croatia

Introduction: In modern society, elevated uric acid levels seem unfavorable. Ever since Garrod discovered that hyperuricemia was the cause of gout in the 19th century, the harmful effects of uric acid on cardiovascular and kidney disease have been increasingly proven and documented.
Hyperuricemia can occur due to decreased urate elimination, or it can occur due to increased production, or it can be due to combinations of these conditions. Hyperuricemia is quite common after kidney transplantation. The prevalence of hyperuricaemia in renal transplant recipients ranges from 19% to 55% in patients who did not receive cyclosporine A (CsA) as immunosuppressive and from 30% to 84% in patients treated with CsA. According to various studies, risk factors for hyperuricaemia after kidney transplantation include decreased glomerular filtration rate (GFR), diuretic use, existing history of hyperuricaemia, treatment with calcineurin inhibitors, especially CsA, male gender, diabetes, hypercalcemia, and weight gain.

Aims: The aim of the study was to show the relationship between serum urate levels and renal graft function, and to show the difference in blod urate levels between genders, BMI, blood pressure, and therapy in the patient wih kidney graft.

Materials/Participantsand Methods: The study included all adult transplant patients who are monitored at the Department of Nephrology of the Clinical Hospital Center in Osijek. Data (age, sex, previous illness, therapy, height, weight, blood pressure values, BMI, glucose blood levels, cholesterol, and urate) was collected from the Department of Nephrology for each patient by accessing the hospital from medical records.

Results: The study was conducted on 177 transplant patients (54.2% men and 45.8% women). Creatinine and urate values are significantly lower in women compared to men, while there are no significant differences in eGFR, GUK, and CRP values relative to gender. Malnourished participants have significantly lower urate values, and obese participants have the highest urate values. Also with higher urate values are higher values of body mass index, systolic pressure and creatinine, and lower eGFR values.

Due to the use of immunosuppressants, eGFR values were significantly lower in participants on cyclosporine compared to patients on tacrolimus. Given urate values, 67.2% of them have hyperuricemia.

Conclusion: Elevated serum urate levels are associated with poorer graft renal function. There is a significant difference of serum urate levels concerning gender, BMI, and elevated systolic blood pressure. There is no significant difference in urate value relative to the type of immunosuppressive therapy.

Keywords: Hyperuricemia;kidney graft; kidney transplantation; uric aci

Abstract Title: Characteristics of plateletpheresis donors with aggregates in apheresis-derived platelet concentrates

Part of the Disertation Proposal: Participants and Methods – allocation of examinees in the studied (aggregates) and control group

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Mentor: Assist. Prof. Irena Jukić, M.D., Ph.D., Croatian Institute of Transfusion Medicine, Zagreb; Medical Faculty Osijek, Croatia

Introduction: Plateletpheresis is a method of platelet concentrate (PC) preparation from a single donor. Apheresis-derived platelet concentrates (APC) account for about 17 % of total PC production at Croatian Institute of Transfusion Medicine and are mainly used as transfusion support for children and immunized patients. Phenomenon of aggregates (AGR) is well known and appears more often in APC than in buffy-coat-derived PC. The reason of AGR appearance is still a research topic. Apart from difference in preparation process, which may cause the appearance of AGR in APC, we also noticed that AGR appear more often in APC from certain donors.

Aims: To examine characteristics of donors, apheresis procedures and APC with AGR and compare them with control group (CG).

Materials/Participants and Methods: In the observed period (2018 - 2020), 720 APC with AGR from 296 donors were recorded. A CG consisted of 216 APC without AGR from 211 donors without recorded history of AGR in the observed period. Statistical analysis was performed in MedCalc Software version 20.009. As the data did not follow normal distribution, non-parametric tests were used with level of significance $p < 0.05$.

Results: A total of 144 donors had ≥ 2 APC with AGR (568 donations, 78.9 %) and 31 donors had ≥ 5 APC with AGR (276 donations, 38.3 %). There were 53 (7.4 %) donations from women in AGR group and 15 (7 %) in CG. Donors of APC with AGR were significantly older ($P = 0.034$), had significantly more donations ($P < 0.001$), less white blood cells ($P = 0.003$) and platelets in circulation ($P < 0.001$). Apheresis procedures lasted significantly shorter with larger blood volume processed, but with

lower volume of anticoagulant infused in APC with AGR ($P \leq 0.001$). Blood type B was more frequent in AGR group ($P = 0.022$), but there was no significant difference in RhD phenotype. There were significantly more nonconforming APC in AGR group (27.2 %) than in CG (3.3 %) with $P < 0.001$.

Conclusion: This study clearly shows differences in characteristics of donors of APC with AGR compared to CG, which implies donor-related factor. Since formation of AGR is a process which theoretically involves initial phase of coagulation, there might be coagulation-related donor factor influencing AGR appearance, that has not been investigated to date. Based on identified risk factors for AGR appearance from this and future studies, pathophysiological background for AGR formation in APC could be clarified. This knowledge could be implemented in everyday practice for a new approach in apheresis donor selection, in order to avoid wastage of blood components, needless donor exhaustion and unnecessary financial expenses.

MeSH/Keywords: blood platelets, plateletpheresis, platelet aggregation, blood donors, donor selection

Dissertation Proposal Title: Differences in psychological – social - demographic characteristics, symptoms, and risk factors of the persons who come for a medical treatment for the first time in gastroenterologist clinic due to dyspeptic difficulties considering indications for endoscopy.

PhD candidate: Mile Volarić, M.D., University Clinical Hospital Mostar

Mentor: Assoc. Prof. Ljiljana Trtica Majnarić, M.D., Ph.D., Faculty of Medicine Osijek-Department of Family Medicine, Osijek, Croatia

Introduction: Chronical undiagnosed dyspepsia is a term for operating dyspepsia diagnosis which constantly lasts for a longer period (at least 3 months) and which started at least 6 months before defining a final diagnosis of upper gastrointestinal tract disorder by endoscopy. The latest researches show that upper gastrointestinal tract disorders are a heterogeneous group of disorders having the background in different biological- psychological- social factors and pathological- physiological mechanisms. The connection between psychological factors and development of upper gastrointestinal tract diseases has been well known. It is established that chronical psychological stress and the sensitivity of the person to a stress represent important risk factors for this disease as well as taking aspirin, consuming alcohol or smoking cigarettes.

Hypothesis: The persons with chronical dyspepsia who distinguish from each other by setting indications for endoscopy examination are different due to psychological - social- demographic characteristics, symptoms and risk factors.

Aims: To examine the following differences in persons with undiagnosed dyspepsia: psychological-social- demographic characteristics, symptoms related to gastrointestinal tract and risk factors. Next, considering the indication for endoscopy examination: to research the connection between certain psychological - social-demographic characteristics and risk factors with setting an indication for endoscopy examination and the examination result.

Materials/Participants and Methods: The persons coming for a medical treatment for the first time due to chronical dyspepsia problems - data is collected for > 100 persons.

Research plan: For the needs of this research, the persons who will be considered to be those with chronical undiagnosed dyspepsia represent all the patients who were sent to gastroenterologist due to a longer period of disorder, as well as those who didn't set the indication for endoscopy and those who did set the same indication. The data will be taken from anamnesis, lab results and standardized questionnaire related to reaction to stress.

Significance/Expected scientific contribution: Creation of a protocol according to which persons who come to the gastroenterologist clinic for the first time due to chronical dyspepsia problem should be categorized and sent to a further medical treatment. A support to doctors of Family Medicine to make a decision about who of the patients with dyspepsia should be sent to gastroenterologist clinic.

MeSH/Keywords: Dyspepsia; Endoscopy; Stress, Physiological; Helicobacter pylori; Risk factors

Abstract Title: Relation of proangiogenic and antiangiogenic VEGF cytokines in STEMI patients

PhD candidate: Marin Vučković, Clinical hospital center Osijek, Osijek, Croatia

Mentor: Assist. Prof. Sandra Makarović, Clinical hospital center Osijek, Osijek, Croatia

Introduction: Mortality and morbidity of patients with ST elevation myocardial inarction(STEMI) si not only because of mechanical obstruction of coronary artery but also result in biochemical disturbances. Vascular endothelial growth factor(VEGF-A) 165 is important factor of angiogenesis through inducing migration and proliferation of endothelial cells, increased vascular permeability, and modulating trombogenesis. In alternative ways of genetic cutting it could be changed in isoform - VEGF-A165b, that has the same affinity for combining with VEGF receptor, but it makes protein phosphorylation unsuccessful, resulting in antiangiogenesis.

Hypothesis: VEGF-A 165/VEGF-A 165b ratio is important for sucessfull myocardial recovery after STEMI.

Aims: Compare plasma levels of VEGF-A 165 and VEGF-A165b before, 24 and 96 hours after, and one month after primary percutaneus coronary intervention(pPCI) in STEMI patients with angiological marker of successful revascularisation TIMI flow (Thrombolysis in myocardial infarction), and clinical signs of successful revascularisation
Evaluate global and regional longitudinal myocardial deformation by echocardiography in acute and chronic state after STEMI and compare result with plasma levels of VEGF-A 165 and VEGF-A165b
Evaluate mayor adverse cardiovascular events 6 months after STEMI(worsening of heart failure(HF), hospitalization because of HF, reinfarction, need for coronary revascularisation, death from HF, death from cardiovascular cause, all cause death) with levels of VEGF-A 165 and VEGF-A165b

Materials/Participants and Methods: In this prospective cohort study 45 consecutive patients will be enrolled with first STEMI without exclusion factors.(known HF, malignancy, chronic liver and kidney disease, acute inflamatory disease). Plasma levels of VEGF-A 165 and 165b will be detected with ELISA kits. Echocardiography exam will evaluate global and regional longitudinal myocardial deformation.

Research plan: In all patients blood samples will be taken before, 24 and 96 hours after, and month after PCI. Echocardiography exam will be done before hospital discharge and one month after STEMI. Patients will be followed by invastigators predischarge, one month and 6 months after discharge ambulatory or by phone.

Significance/Expected scientific contribution: We are expecting to show imbalans of proangigenic and antiangiogenic factors of VEGF family in STEMI patients despite optimal PCI. Finaly, we are expecting to prove that reduced heart function after STEMI is a result of imbalanced corellation of VEGF-A165/VEGF-A165b, and it could be messured through clinical adverse events. The inhibition of antiangiogenic factor could ultimatly reduce mortality and morbidity burden in STEMI patients.

MeSH/Keywords: ST elevation myocardial infarction, VEGF-A 165, VEGF-A 165b, PCI, antiangiogenic

Abstract Title: Maternal Salt Intake and Microvascular Endothelial Function During the Third Trimester of Healthy Pregnancy

Part of the Disertation Proposal: Association between the amount of salt intake during pregnancy with systemic and uteroplacental vascular function of the mother, and the pregnancy outcome

PhD candidate: Martina Vulin, M.D., University Hospital Center Osijek, Osijek, Croatia

Mentor: Assist. Prof. Andrijana Muller, M.D., Ph.D., University Hospital Center Osijek, Osijek, Croatia

Co-Mentor: Assoc. Prof. Ana Stupin, M.D., Ph.D., Faculty of Medicine Osijek, Osijek, Croatia

Introduction: Even in healthy individuals, high-salt (HS) loading leads to endothelial dysfunction, a hallmark of cardiometabolic diseases, independently of the changes in blood pressure (BP). Still, the effect of the amount of daily salt intake during healthy pregnancy on maternal endothelium-dependent vascular reactivity has not yet been investigated.

Aims: This study aimed to assess the effect of the amount of daily salt intake during the third trimester of pregnancy on maternal microvascular reactivity to stimuli in healthy pregnant women.

Materials/Participants and Methods: The present study was designed as a cross-sectional study in which all the data for each pregnant woman were obtained at a one-time point between 37 and 40 weeks of gestation. Daily salt intake was estimated based on 24-h urinary sodium excretion using appropriate formula. Microvascular endothelium-dependent vasodilation in response to vascular occlusion (PORH), iontophoresis of acetylcholine (AChID) and local heating (HEAT), as well as endothelium-independent vasodilation in response to iontophoresis of sodium nitroprusside (SNP) was assessed by Laser Doppler flowmetry (LDF).

Results: Preliminary results of the present study involve data obtained from 27 healthy pregnant women. According to the amount of daily salt intake, pregnant

women were divided in low-salt (LS, <5 g of salt/day, N=4), normal-to-high salt (NHS, 5.0-7.5 g of salt/day, N=12) and high-salt (HS, >7.5 g of salt/day, N=11) group. All pregnant women were normotensive, and BP values did not differ between groups. HEAT was significantly lower in HS compared to LS and NHS group, while PORH and AChID tended to be lower in HS compared to LS and NHS group, but without statistical significance. SNPID did not significantly differ between the groups. There was weak to moderate negative correlation between daily salt intake and functional markers of microvascular endothelium-dependent vasodilation (PORH, AChID and HEAT).

Conclusion: The amount of daily salt intake significantly affects maternal endothelium-dependent microvascular reactivity during the third trimester of healthy pregnancy, in particular by reducing responses mediated by nitric-oxide (NO).

MeSH/Keywords: high-salt intake; endothelium; microcirculation; pregnancy; laser Doppler flowmetry

Acknowledgement: This study was supported by the Faculty of Medicine Osijek Institutional Research Projects IP-10-MEFOS-2021 (PI Ana Stupin) and IP-02-MEFOS-2022 (PI Ana Stupin).

Abstract Title: Biomechanical analysis of the m. gracilis and the superficial third of the m. quardiceps femoris tendons concerning the biomechanics of the medial patellofemoral ligament

Part of the Dissertation Proposal: Biomechanical analysis of the m. gracilis and the superficial third of the m. quardiceps femoris tendons concerning the biomechanics of the medial patellofemoral ligament

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Mentor: Assist. Prof. Zoran Zelić, M.D., Ph.D., University Hospital Centre Osijek; Faculty of Medicine Osijek, University of Osijek, Croatia

Introduction: The medial patellofemoral complex, which consists of the medial patellofemoral ligament [MPFL] and the medial patellotibial ligament, is the major passive stabilizer of the patellofemoral knee joint. It has been shown that the rupture of MPFL is a major pathological consequence of patellar dislocation and that MPFL is a major passive stabilizer in patellofemoral instability and lateral patellar displacement. Reconstruction of MPFL is generally accepted method of treatment for these conditions. Many techniques have been developed to reconstruct MPFL and their goal is to achieve the anatomical reconstruction of MPFL.

Aims: To define morphometrical and biomechanical caracteristics of quadriceps femoris tendon and distal gracilis tendon, to compare given results of quadriceps femoris tendon and distal gracilis tendon and finally to compare them to biomechanical and morphological caracteristics of MPFL

Materials/Participants and Methods: The research was conducted on 16 samples of the human tendon, of which there are 8 gracilis tendon and 8 quadriceps tendon from the archival material of the Department of Anatomy from the Faculty of Medicine, University J. J. Strossmayer Osijek. On a specifically constructed module made by the Mechanical Engineering Faculty in Slavonski Brod tendon properties were examined and data analyzed. After a standard cyclic load, the measured data like the maximum elongation force, tensile strength, extensibility, stiffness and the module of elasticity were compared with previously conducted studies

Results: Tensile strength is significantly higher in gracilis tendon (26 MPa - 92 MPa) than in quadriceps tendon (30 MPa - 44 MPa). The extensibility is significantly higher in the quadriceps tendon (10% - 15%) than in the gracilis tendon (13% - 17%). Regarding stiffness (N/mm) there are no significant differences between the groups of gracilis and quadriceps tendons. The module of elasticity is significantly higher in gracilis tendon (235 MPa - 855 MPa) in comparison to quadriceps tendon (239 MPa - 361 MPa).

Conclusion: Considering this study on a sample of 8 quadriceps tendons and 8 m. Gracilis tendons, the biomechanical properties of quadriceps tendons showed better biomechanical properties and closer values to the original mediopatellar ligament, which could have an impact when selecting transplants for its reconstruction.

Keywords: medial patellofemoral ligament, biomechanics, tendon of the quadriceps femoris, tendon m. gracilis

Abstract Title: GANT61 and Lithium Chloride Inhibit the Growth of Head and Neck Cancer Cell Lines Through the Regulation of GLI3 Processing by GSK3β

Part of the Disertation Proposal: Role and Regulation of the „ GLI CODE" in head and neck tumours

PhD candidate: Vedran Zubčić M.D., University Hospital Osijek, Osijek, Croatia

Mentor: Assist. Prof. Dinko Leović, M.D., Ph.D., University Hospital Center Zagreb, Zagreb, Croatia

Introduction: Head and neck squamous cell carcinoma (HNSCC) are tumours of various sites of the head and neck region. In HNSCC, cancer stem cells (CSC) are responsible for tumor initiation, progression, and metastasis, but also for drug resistance and recurrence. Signaling pathways often activated in CSC include the Hedgehog-Gli (HH-GLI). GLI proteins regulate the transcription of many genes involved in proliferation, differentiation, cell cycle regulation, stemness, angiogenesis, invasiveness, and pathway autoregulation through PTCH1 and GLI1

Aims: Almost all studies dealing with HH-GLI pathway inhibition in HNSCC tested the upstream pathway inhibitors. In most of them, only GLI1 was stained, and its nuclear localization was associated with metastasis, poor survival, tumor size, and recurrence .It is recently demonstrated that GLI3 is important in the CSC population of HNSCC and is involved in cell proliferation, invasion, and stemness of these cells. GLI proteins can be also activated by non-canonical signaling and can bypass this upstream inhibition. That is the reason we decided to investigate downstream inhibitors on several HNSCC cell lines. We focused our research on a direct GLI inhibitor GANT-61, and lithium chloride (LiCl), a GSK3β inhibitor.

Materials/Participants and Methods: The study was conducted on a five HNSSC commercial cell lines . For gene/protein extraction, cells were treated with GANT61, or LiCl and were collected and then used for either RNA or protein extraction

Quantitative Real-Time Polymerase Chain Reaction. RNA was extracted from cell pellets. Expression of *PTCH1*, *GLI1*, *GLI2* and *GLI3* genes were measured. **Western blotting** and **Immunoprecipitation and Coomassie Staining** were used as methods also.

Results: HH-GLI signaling pathway genes PTCH1, GLI1, GLI2, and GLI3 are expressed in all analyzed HNSCC cell lines. GLI3 shows the strongest expression in all analyzed cell lines . The same expression pattern is visible at the protein level. The full-length GLI3 protein shows the strongest expression of all GLI proteins . The PTCH1 protein was detected in all cell lines.
Conclusion: GANT61 and LiCl, downstream HH-GLI pathway inhibitors, inhibit the proliferation and colony forming capability of HNSCC cells. This suggests that the downstream components of HH-GLI signaling are activated at least partly non-canonically in HNSCC. The main effector of HH-GLI signaling in HNSCC is the GLI3 protein and is responsive to GANT61 and LiCl inhibition. Therefore, downstream inhibition of HH-GLI signaling in HNSCC may be a promising therapeutic strategy.

MeSH/Keywords: Hedgehog signaling, HNSCC, GLI, GANT61, LiCl

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