dies doctorandorum
BOOK OF ABSTRACTS
2021

MF

UNIVERSITY OF OSIJEK, FACULTY OF MEDICINE OSIJEK
POSTGRADUATE DOCTORAL STUDY
OF BIOMEDICINE AND HEALTH

DIES DOCTORANDORUM 2021.

BOOK OF ABSTRACTS

Publisher:
JOSIP JURAJ STROSSMAYER UNIVERSITY OF OSIJEK, FACULTY OF MEDICINE OSIJEK

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Prepress and printed by:
Studio HS internet d.o.o. Osijek

Circulation:
50 copies

ISBN: 978-953-7736-57-6

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Foreword

Dies doctorandorum is an annual event which we are pleased to organize again, now in 2020. This is an open and public event inviting people from all spheres of life to participate, but the focus is mainly on our doctoral students whose work is opened to critique and debate.

In previous years this event provided doctoral students a platform to present their research to a wider audience. Hopefully they will use the time given this year in the same confident manner as in previous years. This event is also an opportunity for peers to immerse themselves into the research of other people and benefit from the scientific spirit provided here. I have no doubt that everyone will greatly benefit from plenty of fruitful discussions, debates and the general atmosphere which these kinds of events allow us to cultivate. Aside from the motivating discussions, I'm sure there will be other tacit knowledge offered such as the ability to understand how the scientific community works.

At the end of the day, Dies doctorandorum is a social event, offering the possibility and opportunity to socialize and connect with your peers, mentors and others. I'm sure there are a lot of you who share similar scientific interests and this event is offering the opportunity to connect with each other. It is possible that maybe some of you will further collaborate in the nearby future.

Despite the informal nature of Dies doctorandorum we will still analyze and observe the progress our PhD students. With this in mind, I would like to point out that the best poster presentations will be chosen by the Members of the Committee for Doctoral Studies and awarded the Dean's award.

As for the public, please keep in mind that some of our PhD candidates will get the opportunity to practice presenting their own ideas, concepts and research results for the first time. Therefore, reassuring and inspiring atmosphere is encouraged so our young candidates would feel satisfaction and pride related to their difficult and valiant efforts in scientific development.

Professor Jure Mirat, 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

PhD candidate: Ivan Abičić, M.D., ENT Clinic, Clinical Hospital Centre Osijek, Croatia

Mentor: Prof. Andrijana Včeva, M.D., Ph.D., ENT Clinic, University Hospital Centre Osijek; Faculty of Medicine Osijek, University of Osijek, Croatia

Mentor 2: Tihana Mendeš, M.D., Ph.D., ENT Clinic, Clinical Hospital Centre Osijek, Croatia

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

Hypothesis: 1. 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 study entry, 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: Influence of general and specific stress factors on the working capacity of healthcare workers in outpatient emergency medicine during the COVID-19 pandemic

PhD candidate: Antun Bajan, Faculty of Dental Medicine and Health Osijek, Osijek, Creoatia

Mentor: Assoc. Prof. Goran Krstačić, M.D. Ph.D., Polyclinic for prevention of cardiovascular diseases and rehabilitation Zagreb, Faculty of Medicine Osijek, Osijek, Croatia

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

Introduction: During the COVID-19 pandemic, stress among emergency workers increased significantly due to the new factors that did not exist before. Factors that can be considered predictors of occupational stress include fear of infection, reduced levels of safety and protection during emergency medical procedures, and marginalization of patients not suffering from COVID-19. The inability to cope with stress can also result in impaired quality of patient care, reduced productivity, absenteeism, high staff turnover, and high economic costs to the health system. Preservation of working ability in the field of emergency medicine is of great importance both for health professionals and for the wider community because of the importance and sensitivity of the work they perform. In the domain of quality of life, the data found in the scientific and professional literature indicate the need for intervention in terms of work environment and work organization related to the prevention and management of stress in the workplace.

Hypothesis: General and specific work stressors negatively affect the working ability of health workers in the intervention teams of the Institute of Emergency Medicine at the level of the Republic of Croatia during the COVID-19 pandemic.

Aims:
1. Examine the intensity of general and specific stressors in the workplace of doctors and nurses in the field of outpatient emergency medicine during the COVID-19 pandemic

2. Examine the working ability of health workers in outpatient emergency medicine, through the values of the index of work ability (indicators) of work ability (WAI) during the COVID-19 pandemic
3.Examine the influence of sociodemographic characteristics of respondents (gender, age, level of education) on the experience of stress and work ability during the COVID-19 pandemic
4. Identify predictors of stress and work ability among health care workers in the emergency medicine industry during the COVID-19 pandemic
5. To examine the relationship between the level of stress and the value of the working capacity index among health professionals in the field of emergency medicine during the COVID-19 pandemic
6. Compare the level of stress between health workers working in intervention teams and health workers from the control group

Materials/Participants and Methods: Respondents are doctors, nurses/technicians employed in outpatient emergency departments, regardless of educational level or workplace. According to the work tasks, according to the place and working conditions, the respondents will be divided into two groups:a group of cases and a control. The group of cases will consist of health professionals working in intervention teams- Team I, Team II and the Emergency Air Medical Transport Team. The exclusive criteria for this group are all employees who worked in the reporting unit. The second, control group, will consist of health professionals who work in the Team of the reporting unit, ie who do not work in intervention teams and are not exposed to the virus, and in all other characteristics will be harmonized with the participants of the case group. A number of at least 330 subjects was determined for the sample size. The basis for this number derives from the conditions for the use of statistical analyzes. The testing instrument consists of open-ended questions and two questionnaires: the Questionnaire on Stressors in the Workplace of Hospital Healthcare Professionals and the Work Ability Index Questionnaire (WAI Questionnaire).

Research plan: The case-control study will be conducted in twenty-one county institutes for outpatient emergency medicine at the level of the Republic of Croatia after obtaining the consent of the Ethics Committee of each institute, ie the Faculty of Medicine Osijek during the period from January to June 2021. The approach to solving the questionnaire will be anonymous and voluntary, which the respondents will confirm with their signature, and you can terminate your participation at any time without any obligations on the part of the respondents. Before completing the questionnaire, each respondent will receive an explanation of the survey and the purpose and goal of the survey.

Significance/Expected scientific contribution: The results of the research could contribute to the recognition, resolution and prevention of stress and safety in the work environment of the Institute of Emergency Medicine as part of the overall preservation of health in the workplace of health professionals. In addition to a comprehensive review of the risk and protective factors of work capacity in the outpatient emergency department during the Covid-19 pandemic, the results could be used as a starting point for stressor research and for developing prevention programs and treatment algorithms for potential new pandemics in the future.

MeSH/Keywords: COVID-19, Emergency Medicine, Work Capacity Evaluation, Occupational Stress, Surveys and Questionnaires, Health Personnel.

Dissertation Proposal Title: Global DNA methylation in patients with chronic obstructive pulmonary disease

PhD candidate: Eugenia Basioli Kasap, M.D., internal medicine specialist, pulmology subspecialist; Head of the Polyclinic for Internal Medicine General Hospital Zadar

Mentor: Assist. Prof. Sanda Škrinjarić Cincar, M.D. Ph.D.

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

Introduction: Chronic obstructive pulmonary disease (COPD) is the third most common cause of death in the world and its prevalence, incidence and mortality rates increase with age. Disease is complex interaction between genetic predispositions and the influence of environmental factors, combined with the growth, development and aging of the organism. Quality of life patients with COPD is significantly impaired and associated with the symptoms of the disease and with a poor prognosis of the disease.

Hypothesis: Among patients in Slavonia and Dalmatia, given the different environment, climate and lifestyle, there are clinically significant phenotypic differences that could be associated with epigenetic influences.

Aims: The main goal of the study is to determine whether there are differences in global methylation between patients with COPD in two Croatian regions, Slavonia and Dalmatia, and whether these differences can be related to the clinical characteristics of the examined patients. The secondary objectives is to determine whether there is a difference in the quality of life, among COPD patients between the two regions, Slavonia and Dalmatia.

Materials / Participants and Methods: According to the research plan, 64 patients with a diagnosis of COPD confirmed by standard diagnostics will be included in each center and a total of 64 control subjects.

Research plan: All patients included in the study, will undergo standard lung function treatment (FeNO test, spirometry with a bronchodilation test, and diffusion capacity),

radiological imaging (CT), total IgE and 4 questionnaires (SF-36 - Short Form 36, SGRQ - St George's Respitatory Questionnaire, CAT questionnaire - COPD Assessment test and questionnaire on living conditions and habits).The main part of research is the determination of DNA methylation patterns from a venous blood sample (3-5 ml) in the examined patients with COPD, as well as in the control group. Global DNA methylation will be analysed from peripheral blood lymphocytes, by quantification of LINE-1 methylation using the MethyLight method.

Significance / Expected scientific contribution: We expect that patients with COPD in Slavonia and Dalmatia could show differences due to the environmental factors and the influence of epigenetics. These effects could be associated with the development of COPD as well as the manifestation of certain phenotypic characteristics. By discovering the differences we could work on a proposal for lifestyle changes that would affect the improvement of quality of life, which has already been recognised and implemented in the guidelines as one of the goals of COPD treatment.

MeSH / Keywords: COPD, phenotyps, epigenetics, glogal DNA methylation, quality of life

Dissertation Proposal Title: Why rheumatoid arthritis represents cardiovascular risk?

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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 (ECG, 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 (ECG, blood sampling for laboratory analysis)
5. Statistical analysis.
6. Publication of results.

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 Clinical Hospital Center Osijek.

Dissertation Proposal Title: Pregnant women mental health and its association with perinatal and early neonatal outcomes in viable pregnancies

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

Co-mentor: Prof. Siniša Šijanović, M.D. PhD., University Hospital Centre Osijek; Faculty of Medicine Osijek, University of Osijek, Croatia

Introduction: It is estimated that anxiety affects approximately 15 – 23 % of pregnant women and is associated with increased risk of negative maternal and child outcomes. Anxiety during pregnancy can cause premature birth, maternal weight gain and metabolic gestational diseases, intrauterine fetal growth restriction, low birth weight, low Apgar scores and overall negative perinatal and early neonatal outcome.

Hypothesis: Pregnant women who have higher levels of stress and anxiety during pregnancy have poorer perinatal and early neonatal outcome compared to those who gave lower levels of stress and anxiety.

Aims:
To determine incidence and main causes of stress and anxiety during pregnancy
Investigate effects of psychosocial stress on its own and in combination with other biomedical parameters on perinatal and early neonatal outcomes
To determine difference between anxiety as a temporary condition and anxiety as a trait in pregnant women and difference in outcome between those two groups
To evaluate the need for a support program for pregnant women and prenatal anxiety screening in pregnant women

Materials/Participants and Methods: In this randomized clinical trial, after giving a consent to participate in study, patients (gestational age 26 weeks and above) will be divided in two groups of high risk and low risk pregnancies and then into two subgroups of nulliparous and multiparous women. After they fill out the required questionnaire which contains general sociodemographic data and modified stress

and anxiety questionnaire the remaining data (laboratory values, obstetric history, perinatal and early postnatal period data etc.) will be filled out by a researcher from the patient's hospital data.

Research plan: Prior to the main study a pilot study will be carried out to determine which clinical tools are the best to evaluate the stress and anxiety in pregnancy and those will be, if necessary, translated and validated. After the selection and adaptation of questionnaires, the main study will be carried out.
Significance/Expected scientific contribution: Determination of incidence and causes of stress and anxiety in pregnancy and its effect on perinatal and early neonatal outcome in viable pregnancies as well as adaptation of questionnaire for stress and anxiety in pregnancy.

MeSH/Keywords: Anxiety, Neonatal outcome, Pregnancy outcome, Stress, Mental health

Abstract Title: Hemoglobin concentrations in temporarily deferred voluntary blood donors in Regional transfusion center Osijek, Eastern Croatia

Part of the Disertation Proposal: Relationship of lifestyle and hemoglobin levels in the population of volunteer blood donors

PhD candidate: Vladimir Cipek, mag. med. lab. diag., Fresenius Kabi

Mentor: Assoc. Prof. Marina Samardžija, M.D., Ph.D., transfusion specialist, Head of the Clinical Department for Transfusion Medicine at the University Hospital Osijek, Osijek, Croatia

Introduction: A voluntary blood donor (VBD) is a person who donates blood, plasma or cellular parts of blood according to the principles of voluntariness, solidarity, anonymity and free of charge. The precondition for donating blood is the health of the person donating blood, but there are situations when the health of the donor is impaired, and donor must be temporarily or permanently deferred.

Aims: Investigate the reasons for the temporary deferral from blood donation within voluntary blood donor population in regional transfusion centre Osijek.

Materials/Participants and Methods: The study population consists of all voluntary blood donors, those who donated whole blood units, and those who did not donate blood due to temporary deferral in the period from 2014 to 2018 of the Clinical Institute for Transfusion Medicine Osijek (CITM). Data were obtained from the national IT transfusion program "e - Delphyn". The study was designed as a retrospective study.

Results: Males donated 119,920 (83.3 %) donations and females 24,121 (16.7 %) donations. Although females constituted one third of the total donor pool, they made only 1/6 of all blood donations. Most frequent reason for deferral was a low haemoglobin concentration (39 % of all deferred donors), followed by blood pressure (26 %) and respiratory problems (3 %), while all other individual reasons were less than 3 %. The total number of temporary deferred donors due to haemoglobin concentration was 5773, including 3102 (54 %) females and 2671 (46 %) males. Of all temporarily deferred female donors, 44.82 % (3102/6921) was due to the low haemoglobin levels. Among temporarily deferred males 34.55 % (2671/7731) was due to the low haemoglobin levels.

Conclusion: Haemoglobin is the major reason of the donor deferral. The haemoglobin concentrations of deferred and accepted donors were gender and age dependent. Due to the low haemoglobin levels, female donors are more frequently deferred than male donors.

MeSH/Keywords: blood donor, gender differences, deferral, haemoglobin

Dissertation Proposal Title: Evaluation of gingival crevicular fluid levels of sclerostin and dickkopf in periodontitis patients on chronic statin therapy

PhD candidate: Kristina Duspara, MPharm, Public-Health Institution „Gradske apoteke" Tuzla, Bosnia and Herzegovina**,** University of Osijek, Faculty of Medicine, Osijek, Croatia

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

Introduction: Periodontitis is a disease that extends to deeper periodontal structures: alveolar bone and periodontal ligament[1]. Gingival Crevical Fluid (GCF) is a physiological fluid that develops from the gingival plexus of the blood vessels and extends to the gingival sulcus[2]. GCF is a biochemical and microbiological treasure where potential biological markers could be isolated. The loss of bone mass that leads to the weakening of the tooth component and at the end of the tooth loss is associated with a number of biochemical processes. The activated Wingless/ß-catenin canonical (WNT) signaling pathway decreases bone resorption. Sclerostin (SOST) and dickkopf (DKK1) are its antagonists that are blocking the WNT receptors/co-receptors, interacting with WNT ligands and preventing their maturation to active forms[3]. Inactivation of DKK1 promotes bone healing process, while inactivation of SOST increases bone mass and bone density in humans and animals[3]. SOST and DKK1 values are higher in patients with diagnosed chronic periodontitis that are consuming cigarettes and have diabetes mellitus type 2[4]. Ordinating statins to those patients may lead to an anti-inflammatory effect[5]. Increasing expression of the bone marrow morphogenic protein-2 (BMP-2), statins are achieving anabolic effect on differentiation and osteoblastic acitivity in the sense of increasing osteoblastic synthetic activity, basically by inducing apoptosis of osteoclast activity[6].

Hypothesis: Cirrculating levels of SOST and DKK1 in GCF correate with statin levels in chronic periodontitis patients on statin therapy

Aims: To determine the levels of WNT inhibitors in GCF from buccal swabs of periodontal patients with or without statin therapy and to investigate the correlation of results from buccal swabs and GCF indicators of bone remodeling and statin levels of the participants and to evaluate expression of genes involved in bone remodeling pathway in periodontal participants with or without statin therapy.

Participants and Methods: This will be a single center case-control study of 128 participants at least. Participants will be included successively during one-year period if they agree to participate in the study and if they do meet inclusion criteria and do not meet the exclusion criteria. Inclusion criteria: periodontal patients on statin therapy (longer than 6 months) and periodontal patients without a therapeutic indication for statin administration as a control group. Exclusion criteria: taking drugs affecting bone metabolism (medicines for osteoporosis, corticosteroids for more than 3 months), patients with primary hyperparathyroidism, untreated hyperthyroidism, chronic renal, gastrointestinal, or liver disease will also be excluded from the study. Ethical approval for this study will be obtained from the Health Center Tuzla Review Board and Faculty of Medicine Review Board. All research involving human subjects and material derived from human subjects in this study will be done in accordance with ethical principles outlined in the World Medical Association Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects (initiated in June 1964, last amendment in October 2000). All participants will sign an informed consent form before being included in the study.

Research plan: Following recruitment, participants will be involved in dental treatment to detect the presence of periodontitis. Both patients diagnosed with periodontitis who are on statin therapy and those who are not will be included in the study. Sampling of GCF will be carried out at the Specialist Periodontal Clinic in Tuzla. Dental examination will detect the area in which the gingival pockets are expressed, and these teeth will be isolated (water, abrasion, optradame or cofferdam) and the field of work dried. With the sterile forceps, a sterile paper point of appropriate size (20 or 25 in diameter) based on the depth of the periodontal pocket and to the minimum resistance limit will be applied and held for 30 seconds to soak with GCF. A sample of buccal mucosa (buccal swab) will be taken at the same Clinic. The patient will rinse the mucosa with water, then each side of the inner wall of oral cavity will be wiped 6 times with a serrated paper part of the collector (Whatman™ OmniSwab). Two samples will be taken for each patient, placed in a container and stored on cold until analysis. The determination of gingival (GCF) and levels of SOST and DKK1 in buccal mucosa will be performed in the Laboratory of Pharmacology at the Faculty of Medicine in Osijek using a commercially available ELISA kits (Biomedica, Vienna, Austria) according to the manufacturer's instructions. RNA will be isolated from buccal swabs using commercially available kit RNAeasy Micro Kit (QIAGEN, Hilden, Germany) according to manufacturer's protocol.

Significance/Expected scientific contribution: This study will master and optimize the technique of GCF sampling. To the best of our knowledge, this will be the first study examining circulating levels SOST and DKK1 as antagonists of the WNT signaling

pathway in GCF and their correlation to statin therapy in chronic periodontal patients. This study will evaluate whether statin therapy interacts with periodontal disease, and whether the difference is significant with respect to patients not administered with statins.

MeSH/Keywords: WNT, SOST, DKK1, statins, periodontitis.

Acknowledgement: The support of Croatian Ministry of Science and Education dedicated to multi-year institutional funding of scientific activity at the J. J. Strossmayer University of Osijek, Osijek, Croatia—grant number: IP-2019-FDMZ-7 (to M.S.) is gratefully acknowledged.

Literature:

Wolf HF , Rateitschak-Pluss EM, Rateitschak KH, „Parodontologija". Naklada Slap 2009.
Barros SP, Williams R, Offenbacher S, Morelli T.Gingival Crevicular as a Source of Biomarkers for Periodontitis. Periodontol 2000. 2016;70(1):53-64.
Glass D.A.2nd, Bialek P, Ahn JD, Starbuck M, Patel M.S.M V*Clevers H, Taketo MM, Long F, McMahon AP, Lang RA,Karsenty G.Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation.Developmental Cell 8. 2005;751-764.
Miranda TS, Napimoga MH, Feres M, Marins LM, da Cruz DF, da Silva HDP, Duarte PM. Antagonist of Wnt/ ß-catenin signaling in the periodontitis associated with type 2 diabetes and smoking. J Clin Periodontol. 2018;45(3):293-302.
Sakoda K, Yamamoto M, Negishi Y, Liao JK, Node K, Izumi Y. Simvastatin decreases IL-6 and IL-8 production in epithelial cells. J Dent Res. 2006;85(6):520-523.
Zhou H, Xie Y, Baloch Z, Shi Q, Huo Q, Ma T. The effect of atorvastatin, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor (HMG-CoA), on the prevention of osteoporosis in ovariectomized rabbits. J Bone Miner Metab. 2017;35(3):245-254.

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

PhD candidate: Mirjana Grebenar Čerkez, M.D., Department of Otorhinolaryngology and Head and Neck Surgery, University Hospital Centre Osijek; Faculty of Medicine Osijek, University of Osijek, Croatia

Mentor: Assist. Prof. Darija Birtić, M.D., Ph.D, Department of Otorhinolaryngology and Head and Neck Surgery, University Hospital Centre Osijek; Faculty of Medicine Osijek, University of Osijek, Croatia

Co-mentor: Assist. prof. Željko Zubčić, M.D:, Ph.D., Department of Otorhinolaryngology and Head and Neck Surgery, University Hospital Centre Osijek; Faculty of Medicine Osijek, University of Osijek, Croatia

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 an original scientific research, a prospective cohort study at the Department of Otorhinolaringology and Head and Neck Surgery, University Hospital Center Osijek. The participants will be devided in two groups: target and control. 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 have been diagnosed with moderate or severe obstructive sleep apnea according to the AHI index. The control group will consist of subjects in whom specific questionnaires excluded the existence of obstructive sleep apnea.

Research plan: All participants of this study will complete the following questionnaires: STOP-BANG and Ephort drowsiness scale. Subjects of the target group with moderate or 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 in the 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: Hip and Knee Osteoarthritis in Patients with Chronic Philadelphia Chromosome-Negative Myeloproliferative Neoplasms

PhD candidate: Hrvoje Holik, M.D., General Hospital „Dr. Josip Benčević", Slavonski Brod, Croatia

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

Introduction: Osteoarthritis (OA) is a chronic inflammatory disease of various joints that affects the middle-aged and elderly population. Chronic Philadelphia chromosome negative myeloproliferative neoplasms (MPN) are rare malignancies of indolent course. In addition to myeloproliferation, this disease is also characterized by chronic inflammation, while the malignant clone is the main driver of the inflammatory reaction. It is hypothesized that chronic inflammation present in MPN may also be the cause of OA in these patients and that consequently more patients with MPN have OA than they would have if they did not have MPN.

Hypothesis: The prevalence of symptomatic and radiological OA of the hip and knee in MPN patients is higher than in the general population.

Aims:
to determine the prevalence of hip and knee OA in patients with MPN
to compare the prevalence of hip and knee OA between MPN subtypes
to examine the relationship between the occurrence and severity of the hip and knee OA with the characteristics of MPN patients
to compare the burden of symptoms measured by the MPN SAF questionnaire between patients with hip and knee OA and those without OA

Materials/Participants and Methods: The study will include minimally 100 MPN patients who are treated in the General Hospital „Dr. Josip Benčević", Slavonski Brod and the General Hospital Šibenik. The MPN diagnosis in patients was made according to the criteria of the World Health Organization from 2016.

Research plan: Subjects data such as age, sex, body weight and height, body mass index, performance status, time of diagnosis, duration of disease, treatment modality,

family history, cardiovascular disease would be taken from existing medical records or during medical exam. The subjects will undergo laboratory and radiologically diagnostics. X-rays of the hip and knee joints findings will be read according to the Kellgren-Lawrence classification and the subjects will be examined by an orthopedist who will diagnose OA. The diagnosis of symptomatic OA will be made based on the criteria of the American Rheumatological Society. The diagnosis of radiological OA will be made on the basis of radiological treatment according to the Kellgren-Lawrence classification. Subjects will complete the MPN SAF and OAKHQOL questionnaire that measures the quality of life of patients.

Significance/Expected scientific contribution: If hypothesis is confirmed, possible subgroups of MPN patients would be defined and in such patients, early diagnosis of hip and knee OA and timely treatment can reduce complications and could lead to a better quality of life and possible reductions in the cost of OA treatment.

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

Dissertation Proposal Title: The association between circulating miRNA 151-5p levels and plasma cholinesterase activity with postoperative cognitive dysfunction after cardiac surgery

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

Mentor: Prof. Slavica Kvolik, M.D., Ph.D., University Hospital Centre Osijek; Faculty of Medicine Osijek, University of Osijek, Croatia

Introduction: Postoperative cognitive dysfunction (POCD) after cardiac surgery is the most common postoperative complication. Recent findings suggest that the systemic inflammatory response to surgery, anaesthesia and patient-related factors may be associated with neuroinflammation and POCD, but the pathophysiology is still not sufficiently understood. Depression of plasma cholinesterase (ChE) activity may lead to an amplified neuroinflammatory response, and miRNAs may be important mediators of the profound molecular and cellular changes in neuroinflammation. Nevertheless, the role of miRNAs and ChE in the occurrence of POCD remains unclear, and biomarkers that could lead to the detection, prevention and treatment of POCD are still being investigated.

Hypothesis: Higher perioperative circulating miRNA-151-5p levels and lower plasma ChE activity are associated with the development of POCD after cardiac surgery. High postoperative miRNA-151-5p and low preoperative ChE value are predictors of POCD after coronary artery bypass (CABG) surgery.

Aims: To examine the perioperative circulating MiRNA-151-5p concentration and plasma ChE activity in patients undergoing CABG surgery, and their association with POCD.

Materials/Participants and Methods: The study will include 50 patients undergoing elective CABG surgery under standard general anaesthesia and cardiopulmonary bypass protocol. Haemodynamic parameters and brain tissue oxygenation will be monitored by the Hemosphere platform. MiRNA-151-5p, ChE, C-reactive protein (CRP), procalcitonin (PCT), and haemoglobin will be measured before surgery, immediately after surgery and 24 h after surgery. CRP, PCT, haemoglobin, urea and creatinine will

be further measured on a daily basis. POCD screening will be assessed preoperatively, 24 h and 72 h after surgery, and 6th postoperative day using a validated screening tool (Mini Mental State Exam and Montreal Cognitive Assessment).

Research plan: This prospective observational study will be conducted at the Department of Anaesthesiology, Reanimation and Intensive Care and at the Department of Cardiac Surgery of Clinical Hospital Osijek. The research will begin after the approval of the institutional ethics committee. It will take 12 months.

Significance/Expected scientific contribution: Examining of inflammatory response pathways will contribute to better understanding of the POCD and the finding of biomarkers that enable prevention, timely diagnosis and treatment of POCD in cardiac surgery patients.

Keywords: postoperative cognitive complications, cardiac surgical procedures, cholinesterase, miRNA, neurophysiological monitoring.

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

PhD candidate: Damir Kirner M.D., University Hospital Centre Osijek, Croatia

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. Liver damage due to drugs may cause variety of histological forms from steatosis to cirrhosis. Amiodarone is a worldwide used antiarrhytmic drug with the possibility to induce hepatotoxicity and mitochondrial dysfunction. Mechanisms leading to non-alcoholic fatty liver disease (NAFLD) have not yet been clearly clarified.

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

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, amiodarone, drug induced liver injury, lipogenesis, mitochondrial dysfunction

Abstract Title: Does the endothelium of competitive athletes benefit from the consumption of n-3 PUFAs, selenium, lutein, and vitamin E enriched hen eggs?

Part of the Disertation Proposal: The effect of regular exercise and intake of n-3 polyunsaturated fatty acids, vitamin E, selenium and lutein enriched functional food on microvascular endothelial function in athletes.

PhD candidate: Luka Kolar, M.D., National Memorial Hospital Vukovar, 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: Marko Stupin, M.D., Ph.D., Osijek University Hospital

Introduction: It is well accepted that regular exercise, but also micronutrients as n-3 polyunsaturated fatty acids (PUFAs), selenium, vitamin E and lutein, have the potential to preserve cardiovascular health and improve endothelial function affecting rather similar mechanisms. However, researches have shown that the dietary goals for various nutrients are not reached in substantial proportion of competitive athletes. Moreover, there is a paucity of data on the interactive effect of these two divergent interventions on endothelial function in competitive athletes.

Aims: The aim of the present study was to determine the effect of n-3 PUFAs, selenium, lutein, and vitamin E enriched hen eggs consumption on endothelium-dependent and endothelium-independent vasodilation of forearm skin microcirculation, and to test whether such functional food consumption will modify microvascular adaptation to a stress challenge 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. Body mass index (BMI), body composition and serum lipid profile were measured before and after respective dietary protocol. Endothelium-dependent (post-occlusive reactive hyperemia, PORH; and iontophoresis of acetylcholine, AChID) and endothelium-independent responses (iontophoresis of sodium nitroprusside, SNPID) of skin microvascular blood flow was assessed by laser Doppler flowmetry

before and after protocol, and in pre- and post- acute exhausting exercise (AEE) sessions at both study visits.

Results: BMI, body composition and serum lipid profile did not change in both Nutri4 and Control group following dietary protocol. PORH and AChID at rest significantly increased, and SNPID at rest remained unchanged in Nutri4 group, while none were significantly changed in Control group. ΔPORH responsiveness range to AEE significantly increased in Nutri4, but not in Control group, while ΔAChID and ΔSNPID responsiveness to AEE were not significantly changed compared to baseline measurement in both study groups.

Conclusion: N-3 PUFA, selenium, lutein and vitamin E enriched hens' eggs consumption contributes to enhanced microvascular endothelial function at rest, and also beneficially modifies peripheral microvascular adaptation to the acute exercise stress challenge in competitive athletes.

MeSH/Keywords: exercise, n-3 PUFAs, selenium, vitamin E, lutein, functional food, endothelium

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, Clinical Hospital Center Osijek, Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Mentor: Assist. Prof. Hrvoje Brkić, Faculty of Medicine Osijek, University of 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 will be 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).
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.

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. It is yet to be validated could the physical phantom be used in dosimetry measurements.

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

Abstract Title: Relationship between microvascular reactivity and anthropometric parameters in juvenile 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., University Hospital Centre Osijek; Faculty of Medicine Osijek, University of Osijek, Croatia

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

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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: Primary hypertension in children is emerging as a significant public health problem, as its prevalence increases in parallel with the current obesity epidemic. Elevated blood pressure (BP) in childhood may be associated with considerable organ damage and increased risk of cardiovascular disease in adulthood. Endothelial dysfunction is considered an important factor in the pathogenesis of atherosclerosis and hypertension, and endothelium-dependent vasomotor impairment is an early indicator of functional atherosclerotic changes that precede morphological changes.

Aims: This study aimes to assess the relationship between the forearm skin microvascular reactivity to vascular occlusion (post occlusive reactive hyperemia; PORH) and anthropometric parameters in children with essential arterial hypertension and in normotensive children.

Participants and Methods: Total of 25 children participated in this study; 12 children with essential arterial hypertension (HT) and 13 normotensive children (NT) (both sexes, age ranged 9-18). Post-occlusive reactive hyperemia (PORH) in skin microcirculation following 1 minute of vascular occlusion period was assessed using laser Doppler flowmetry. Anthropometric parameters, blood pressure and heart rate were measured. Statistical analysis was conducted by t-test (between groups), and Pearson's or Spearman's correlation test was used to determine the correlations between microvascular endothelial function (PORH) and corresponding variables (BMI, SBP, MAP). $p< 0.05$ 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. Sistolic (SBP), diastolic (DBP) and mean (MAP) arterial blood pressure were significantly higher in children with essential arterial hypertension compared to normotensive children. Children from HT group had higher body mass index (BMI) and waist-to-hip patio (WHR), while there were no differences in heart rate between HT and NT groups.
PORH was significantly lower in HT group compared to NT individuals.
Statistically significant positive correlation was found between BMI and SBP ($r = 0.522$) and between BMI and MAP ($r = 0.575$). PORH was negatively associated with BMI ($r = -0,531$), SBP ($r = -0.410$) and MAP ($r = -0.461$).

Conclusions. High blood pressure attenuated microvascular function in children. Arterial blood pressure is related to obesity (BMI).

MeSH/Key words: child, hypertension, endothelium, microcirculation, postocclusive hyperemic response

Abstract: Changes in the proteome of extracellular vesicles shed by rat liver after subtoxic exposure to acetaminophen

Part of Disertation Proposal: Quantitative analysis of proteoms of liver and hepatocellular carcinomas with the focus on secreted extracellular vesicles

PhD candidate: Anamarija Kovač Peić, M.D.; General hospital „Dr. Josip Benčević", Slavonski brod, Crotia

Mentor: Prof. Marija Heffer, M.D., Ph.D., Faculty of Medicine Osijek, University of Osijek, Croatia

Introduction: Liver damage after abuse of several agents, like alcohol, energy drinks, different bacterial and fungal toxins, as well as pre- and post-market drug complications are frequent causes of damage and as a final consequence also failure of this vital organ. Several drugs like painkillers are responsible for so-called drug-induced liver injury (DILI). DILI represents a severe clinical and economical challenge. Acetaminophen (APAP) is the painkiller and antipyretic drug, when used in therapeutic doses, APAP overdose is a leading case of DILI, especially in the presence of alcohol consume.
Most frequently used models od liver injury are poisoning by galactosamine and lipopolysaccharides from cell envelopes of different bacteria, like *Escherichia coli*. These and similar toxic agents promote acute liver inflammation following by damage of this organ. DILI, alcohol abuse, over consume of alcohol and painkillers and their combination, as well as bacterial toxins and mycotoxins, can cause severe liver disease and finally failure of this vital organ. There are several models for both DILI and alcoholic liver disease, mostly by use of rat or mice as experimental animals.
Analysis of body fluids, especially plasma, serum and urine has a long history and change of concentration of proteins that are synthesized in the liver, as well as changes in their posttranslational modifications is an important diagnostic and prognostic tool. Omics investigations of body fluid samples can provide additional information on the way of diagnosis and treatment. In diagnostics of liver diseases liquid biopsy can supplement or even completely replace the invasive and painful liver biopsy.
Extracellular vesicles (EVs) are released by liver, and they can be also detected in body fluids. Changes in MVs composition after liver injury and under other pathological conditions is became an important indication on the way for diagnosis of liver

diseases. These nanoparticles are a heterogeneous population with a size between 30 and 1000 nm (and up to 2000 nm in the case of apoptotic bodies). They are selectively enriched by proteins, lipids, and ribonucleic acids and released from EVs producing cells. The smallest ones, the exosomes, play a crucial role in both physiological and various pathological processes. They also have an emerging role in biomarker discovery, and recently also in therapeutic applications for treatment of diseases.
Liver-derived EVs can be also secreted into the bloodstream or excreted by urine. They are carriers for specific mRNAs and micro RNAs, as well as intracellular proteins and proteins coming from different cellular membranes. Microvesicular RNAs are early indicators of organ injury, and/or other diseases like viral hepatitis or malignancy. EVs contain several proteins, and some of them are specifically enriched in these membrane-enveloped nanoparticles. Specific changes of proteome of MVs are consequence of organ injury, malignant modification, or other pathological conditions, e.g. viral infection or inflammation. Consequently, EVs are important source of disease biomarkers. Their use as important tools for disease treatment, especially for delivery of anti-cancer drugs is recently topic of intensive studies .
Composition of liver EVs depends of particular cell state. The presence of some liver-specific proteins indirectly supported the assumption for presence of liver-derived EVs in the bloodstream and other body fluids, and few years later it was also experimentally confirmed by detecting some liver-derived proteins in exosome-like vesicles purified from mouse and/or rat urine or serum samples . Human liver biopsy is a very invasive and painful process, and the identification of biomarkers for detection of pathological changes in this organ, preferably in body fluids, should be a method of choice. For identification of possible biomarker candidates, the investigation of EVs isolated from livers or shed into body fluids of model animals was performed. In EVs released by cultured primary hepatocytes, several members of liver-specific cytochromes P450, uridinediphosphate-glucuranosyl-transferase (UGT) and glutathione S-transferase (GST) protein families were identified . Rodríguez-Suárez et al. detected some heat-shock proteins [HSP90 and HSP70] as potential biomarker candidates for experimental galactosamine induced hepatitis in rats. Higher concentration of these two proteins was also detected in EVs released by primary hepatocytes as well as in serum of treated rats.

Aims: In order to determine the proteome changes in exosomes during DILI we treated rats with an overdose of acetaminophen. Instead of the use the supernatant of culture of primary hepatocytes [27-29], EVs were collected by perfusion of an isolated liver from control rats and rats treated acetaminophen, and striking differences were observed in their size and distribution. To determine if there were also differences in their proteome, EVs collected from normal and acetaminophen treated rat livers were solubilized, digested with trypsin and analyzed by LC-ESI-MS/MS.

Materials and methods: Studies were approved by the Institutional Committee for the Animal Care and Use at the Rhode Island Hospital. Six-to-eight week old male Fischer-344 rats (Jackson Laboratories, Farmington, CT, USA) were used. Euthanasia was performed using CO_2 inhalation followed by cervical dislocation.

To obtain MV shed under conditions close to those in situ, the portal vein of livers from untreated controls and from animals treated with 500mg/kg acetaminophen was canulated and the cannula attached to a peristaltic pump. Following perfusion at 37°C with Hank's balanced salt solution (HBSS) containing heparin to remove blood cells, the liver will be removed and submerged in 80 ml of Hepatozyme serum free medium (Thermo Fischer Scientific, Waltham, MA, USA]. The isolated liver was be perfused at 37 C at a flow rate of 3ml/min/g of liver. This high rate is necessary to assure adequate oxygenation in the absence of red blood cells. The normal blood flow for the rat liver is 1.25ml/min/g of liver .

Microvesicles were harvested from culture medium conditioned by minced liver or liver slice cultures or by perfusion through the isolated liver. Culture was centrifuged at low speed to remove cells and passed through a 1.2 μm filter (Sartorius, Bohemia, NY, USA) to remove debris. The filtrate containing exosomes (30-100nM) and microvesicles (100-1000 nm) will be concentrated by centrifugation at 100K x g onto a 27%/68% sucrose cushion as described by Hong et al. Microvesicles collected at the interface will be resuspended in PBS, and submitted to fractionation by size exclusion chromatography as described by Rood et al [36]. Fractions corresponding to the excluded volume will be concentrated on a sucrose cushion. MVs yields will be quantitated by determining total protein recovered in the MV containing fractions. Vesicle content was determined as previously described .

The ultracentrifuge pellets of liver MVs were fixed with 3% (v/v) glutaraldehyde in 0.15M sodium cacodylate buffer, then post-fixed with 1% (w/v) osmium tetroxide (Electron Microscopy Sciences, Hartfield, PS, USA). Further sample preparation was performed according to Ref. [16]. Ultra-thin sections were examined by transmission electron microscopy (TEM) using a Morgagni 268-transmission electron microscope (Philips, Rogers, AR, USA) and images were collected with an AMT Advantage 542 CCD camera system (Woburn, MA, USA).

Proteins from MVs isolated from control rat livers and livers from acetaminophen-treated rats were separated by SDS-PAGE under reducing conditions. The amount of protein loaded in each well was 10 μg. After separation proteins were transferred to a PVDF membrane (Serva, Heidelberg, Germany) and further treated as described previously . Afterwards, membranes were treated with rabbit anti-annexin antibody (Sigma-Aldrich/Merck, St. Louis, MO, USA) in 0.5% (w/v) nonfat milk/TTBS overnight at 4°C. The detection was performed according to previously described procedure .

For the incorporation of MVs proteins into polyacrylamide gel and "tube gel" proteolytic digestion, the method developed by Lu and Zhu was modified. Microvesicle

fractions containing 100µg proteins were solubilized with 2% (w/v) SDS, 6M urea, 25 mM NH_4HCO_3, pH 8.0 and further incubated at 37°C for 30 min. The sample was than reduced with 50 mM dithiothreitol at 56°C for 1 h and alkylated with 40 mM iodoacetamide at room temperature in the dark for 45 min. The reduced and alkylated proteins were then incorporated into a polyacrylamide gel as described previously . After polymerization, the gel was cut into small pieces, washed, dehydrated, and completely dried in vacuum centrifuge. Proteolytic digestion was performed with trypsin (Sigma) in 40 mM NH_4HCO_3, 10% (v/v) acetonytrile (CAN) overnight at 37°C. Peptides were extracted from the gel using sequential extraction with 200 µL of 25 mM NH_4HCO_3, 200 µL of 0.1% (v/v) trifluoroacetic acid (TFA) in water, 200 µL of 0.1% TFA in ACN, and 200 µl of 100% ACN. The solutions were then combined and concentrated in a SpeedVac.

In some samples, in-gel deglycosylation was performed to facilitate the tryptic digestion of highly glycosylated proteins as previously described. Shortly: washed and dried gels were rehydrated with digestion buffer containing 25 mU PNGase F (ProZyme, Inc., San Leandro, CA, USA.) in 25 mM NH_4HCO_3. Deglycosylation was performed at 37°C overnight. Gels were washed and sonicated, and then completely dried in a speed vacuum. Tryotic digestion was then performed following the protocol described above.

A 75 µm x 12 cm column containing 3 µm Monitor C18 resin (Orochem Technologies, Inc., Lombard, IL, USA) and having an integrated 10 µm ESI emitter tip ("Self-Pack" PicoFrit column; New Objective, Woburn, MA, USA) was used for separation of tryptic peptides in the front of nano-LC-MS/MS. Solvent A was 0.1 M acetic acid in water and solvent B was 0.1 M acetic acid in ACN. Peptides were eluted with a linear gradient (0-70% solvent B over 60 min), operated at 200 nL/min. using an Agilent 1200 HPLC (Agilent Technologies, Santa Clara, CA). The nano LC was hyphened with a LTQ Velos Orbitrap Velos mass spectrometer (Thermo Scientific, San Jose, CA) with a 1.8 kV ESI voltage. The nano-LC-MS/MS analysis was performed as previously described [39]. Shortly: Full MS scans in the *m/z* range of 300-1700 at a nominal resolution of 60,000 were collected, followed with the acquisition of MS/MS spectra for the ten most abundant ions in the LTQ ion trap. Only ions having a charge state ≥ 2 were considered for collision-induced dissociation.

MS/MS spectra were searched against the Uniprot rat protein using the Mascot algorithm v.2.3.2 provided by Matrix Science. The exact procedures were previously given [39]. Shortly: Mascot searches were performed with the following parameters: trypsin enzyme specificity, 2 possible missed cleavages, 20ppm mass tolerance. Search parameters specified a differential modification of oxidation on methionine and a static modification of carbamidomethylation (+57.0215 Da) on cysteine. Protein quantification was performed using ProteoIQ software v. 2.3.05 (BioInquire, Bogart, GA, USA) with spectra count data. To provide high confidence on peptide sequence

assignment and protein identification, data were filtered with following stringent criteria: Mowse score > 28 for all charge states, at least 2 peptides per protein, 1% peptide false discovery rate (FDR) and 1% protein FDR.

Results: Isolated perfused liver offers a means to collect MV shed under conditions that approach those *in situ*. This approach provides large quantities of highly enriched MVs, making further fractionation based on size or expression of cell-type specific markers a feasible undertaking. Based on TEM analysis MVs shed by normal liver have different size, starting with a diameter larger 250 nm, 100-250 nm, and smaller than 100 nm. Acetaminophen intoxication enhances shedding of a population of very small MVs, suggesting injury increases shedding of MV from a particular cell type e.g. endothelial cells or causes a change in the process by which MV are formed or released . Major differences (arrows) were apparent in the protein composition shown by SDS-PAGE (see arrows) that will be further analyzed by LC-MS/MS.
The problem of detection of "real-vesicular proteins" was recently addressed by Choi et al.. We early recognized this problem when working with plasma membrane proteins, and different sample preparation strategies, in-gel tryptic digestion, as well glycoprotein deglycosylation before trypsin digestion were applied . This strategy yielded in detection of hydrophobic proteins with one or more membrane-spanning domains in both preparations of liver MVs. Cytosolic intravesicular proteins actin as well as tubulin that according to these Choi et al. were "protected from the action of trypsin" were also detected in both MVs fractions after use of presented strategies for sample preparation and proteolytic digestion.
Out of over 200 proteins, only 46 (26%) of them are detected in both MV preparations . The protein pattern in MVs fraction of non-treated liver differs significantly from this one in the fraction of MVs isolated from the liver of acetaminophen treated rats, where more different proteins were detected. This result was validated by multiple MS analyses of both fractions as well as by presented analysis by SDS-PAGE that also shows large difference in protein patterns between two samples . To exclude difference in protein compositions that is caused by liver damage during perfusion, presence of some proteins that are markers for liver cells' injury was controlled. Aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) are markers for hepatocellular injury. In both preparations AST was not detected at all, and concentrate of LDH in MVs is at the threshold of detection limit. Purine nucleoside phosphorylase, an enzyme that is localized in non-parenchymal cells such as vascular endothelium and Kupffer cells was detected only in acetaminophen-treated cells . However, this enzyme is also marker of oxidative injury that can also be consequence of toxicity of applied high drug concentration.
Cytochromes P450 (CDP450) are a superfamily of liver enzymes that oxidize steroids and fatty acids. These enzymes are also responsible for oxidation and clearance of

xenobiotics like. Together with uridinediphoshate-glucuronosyl-transferase (UGT), glutathione S-transferase (GST) and carbamoyl phosphate synthetase these proteins are discussed as biomarkers for hepatocyte-derived biomarkers. Above listed proteins were detected only in MVs isolated from normal liver, and not in the MVs from treated liver. At the other hand, proteins from the annexin family, namely annexin A1, annexin A3, and annexin A5 were detected only in MVs shed by liver after injury . Annexin A6 was detected in both MV fractions. However, the concentration of this plasma membrane associated protein was much higher in MVs of acetaminophen-treated liver. Stepwise disappearance of these proteins, especially of the members of the CD P450 protein superfamily and of alpha-1-macroglobulin in liver derived MVs, as well as the appearance or increasing concentration of "typical MVs proteins" like members of the annexin family can be additional tool for early detection of liver injury .

Conclusions: Healthy liver cells shad MVs into blood stream and other body fluid. Similar process occurs in liver cells after both the injury caused by different toxic agents and after malignant modification, that also secrete MVs by use of identical or similar mechanisms. However, already by electron microscopy, differences in size and form of these MVs can be observed . Liver MVs are identified as important source of potential disease biomarkers. They can be detected by use of so-called liquid biopsy and can be used for simple and fast diagnosis of liver injuries and/or malignant changes of this organ.
Change in the proteome liver MVs was mostly studied on the analysis of microvesicles secreted by primary hepatocytes that were grown in cell culture, and important biomarker candidates for liver injury were identified. The liver is a complex organ, and hepatocytes constitute about 80% of the cell population of the liver. Residual 20% are occupied by other cell populations like Kupffer cells, hepatic stellate cells, endothelial cells and mesothelial cells that also play very important role in liver function. These cells also shed MVs, and they can be detected in body fluids. The liver perfusion animal model presented here can be applied as useful addition to already used methods for identification of biomarker candidates for both liver injury and malignancy. It also offers a useful alternative for very aggressive and stressful liver biopsy. The absence or low concentration of biomarkers for liver injury caused by the perfusion demonstrates that the artifacts in MVs proteome caused by the perfusion procedure are minimal, and that this protocol can be used for their isolation and further analysis.

Key words: Liver injury, Microvesicles, Perfusion, Proteome, Biomarker Candidates

Dissertation Proposal Title: Effect of high salt diet on vascular production of nitric oxide (NO) and reactive oxygen species (ROS) in *Tff3*$^{-/-}$/C57BL/6N knockout mice

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

Co-mentor: Prof. Ines Drenjančević, M.D., Ph.D., Faculty of Medicine Osijek, University of Osijek, Croatia

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Introduction: *Tff3* gene knockout mice (*Tff3*$^{-/-}$/C57BL/6N) have changes in lipid metabolism which may affect vascular function. This study aimed to asses the effect of high salt (HS) diet on vascular production of NO and ROS in carotid arteries of *Tff3*$^{-/-}$ knockout mice and their wild type controls (WT, C57BL/6N).

Methods: Male, ten-weeks-old transgenic *Tff3*$^{-/-}$/C57BL/6N and WT/C57BL/6N (parental strain) healthy mice were divided in LS (0.4% NaCl) and HS (4% NaCl in rodent chow fed for 1 week) groups, water *ad libidum*. After anaesthesia (ketamine-chloride and midazolam), mice were decapitated and carotid arteries were isolated and cannulated on pressure myograph with (Δ100 mmHg) or without flow (Δ0 mmHg), in the absence/presence of the nitric oxide synthase (NOS) inhibitor L-NAME (10^{-4}M) and superoxide dismutase mimetic TEMPOL (100 μmol l^{-1}). NO production was determined by 4,5-diaminofluorescein (DAF-2DA, 5 μM) while ROS production was determined by dihydroethidine (DHE, 20 μM) fluorescence assay.
Statistical analyses were performed with One-way ANOVA test; $p<0.05$ was considered significant. All experimental procedures conformed to the European Guidelines for the Care and Use of Laboratory Animals (directive 86/609) and were approved by local and national Ethical Committee (No.2158/61-02-139/2-06; No.2158/61-07-14-119).

Results: Basal (no-flow) production of NO was significantly decreased in WT HS mice compared to WT LS mice. Other groups exhibited similar levels of NO production

under no-flow conditions. L-NAME significantly decreased NO production in all groups, except in *Tff3*$^{-/-}$ HS mice. Under flow conditions, NO production in WT HS mice was significantly lower than in WT LS mice. NO production in *Tff3*$^{-/-}$ LS knockout mice was significantly lower than in WT LS mice. NO production in *Tff3*$^{-/-}$ HS mice was similar to *Tff3*$^{-/-}$ LS mice. L-NAME significantly decreased NO production in all groups of mice, except in *Tff3*$^{-/-}$ HS mice.
Basal (no-flow) ROS production was significantly increased in WT HS compared to the WT LS group and in *Tff3*$^{-/-}$ HS compared to *Tff3*$^{-/-}$ LS group. Tempol significantly decreased ROS production in WT HS and *Tff3*$^{-/-}$ HS groups. Under flow conditions, ROS production was significantly increased in WT HS compared to WT LS group and in *Tff3*$^{-/-}$ HS compared to *Tff3*$^{-/-}$ LS group. Tempol significantly decreased ROS production in all groups.

Conclusion: HS diet significantly decreased the flow-induced NO production in WT mice and increases generation of ROS in both strains.

Key words: *Tff3* gene, flow-induced dilation, oxidative stress, high salt diet

Acknowledgements: This study was supported by the Croatian Science Foundation under the project IP-2014-09-6380 (V-ELI Athero), VIF-2018-MEFOS-09-1509 grant and Faculty of Medicine Osijek Institutional grant #IP-1-MEFOS2019 and #IP-2-MEFOS2020 (PI Ines Drenjančević).

Dissertation Proposal Title: Effects of low salt diet on Th17-mediated inflammation and vascular reactivity in patients with psoriasis vulgaris

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Introduction: Vulgar psoriasis (PV) is a chronic, recurrent, immune-mediated inflammatory skin disease. Recent research has shown that increased NaCl intake promotes the expansion of proinflammatory Th17 cells and regulatory T cells. Recent studies in patients with autoimmune diseases have shown that a short-term low-salt diet alters the balance of Th17 / Treg lymphocytes in favor of anti-inflammatory regulatory T lymphocytes that secrete IL-10. Since PV is a prototype of Th17-mediated inflammation, which is characterized by endothelial dysfunction, in the proposed study we will investigate the possibility of affecting the balance of Th17 / Treg cells and recovery of vascular reactivity in skin microcirculation in PV patients by regulating daily NaCl intake.

Hypothesis: Reduced salt intake leads to a change in the balance of Th17 and Treg lymphocytes, a reduction in inflammation and improvement in vascular reactivity in patients with psoriasis vulgaris.

Aims: 1. To determine the effect of low-salt diet on the balance of Th17 and Treg lymphocytes in patients with psoriasis vulgaris
2. To determine the effect of low-salt diet on vascular reactivity in skin microcirculation and the share of endothelium-dependent and endothelium-independent factors in vasodilation in patients with psoriasis.

Materials/Participants and Methods: The study will include 20 subjects with moderately severe PV, who will follow a diet with reduced salt intake (<3,75 g/d) for 2 weeks. Venous blood samples will be taken before enrollment and on day 14 after enrollment for blood count analysis and determination of concentrations of pro-inflammatory and anti-inflammatory cytokines. The proportion of Th17 lymphocytes

and regulatory T lymphocytes in the population of peripheral blood CD4 helper T cells will be determined by flow cytometry. Blood flow and vascular reactivity in skin microcirculation will be measured by the LDF method on two occasions, before inclusion in the study and two weeks after conducting a low-salt diet.

Research plan: In the first year, subjects will be included in the study, samples will be collected, and clinical parameters determined. In the following 6 months, samples will be processed on flow cytometry and Luminex. The following year is planned for statistical processing, writing and publishing a scientific papers and writing of the doctoral thesis.

Significance/Expected scientific contribution: Upon completion of the proposed study, it will be known whether changing dietary habits in terms of reduced salt intake can modulate Th17-mediated inflammation underlying psoriasis and thus the course of the disease, the development of cardiovascular comorbidities, and the quality of life of psoriasis vulgaris.

MeSH/Keywords: Psoriasis; Diet, Sodium-Restricted; TH-17 Cells; T-Lymphocytes, Regulatory; Microcirculation

Dissertation Proposal Title: Correlation of breast arterial calcification on mammography with peripheral arterial disease grading by color Doppler imaging in female patients on chronic hemodialysis in relation with mineral and bone disorder.

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Mentor: Prof. Lada Zibar, M.D., Ph.D., Internist - Nephrologist, Department of Nephrology, Clinical Hospital Merkur, Zagreb; Department for Pathophysiology, Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Introduction: Female patients on chronic hemodialysis (CHD) have frequent mineral and bone disorder (MBD). MBD is an additional risk for cardiovascular disease in these patients. Peripheral arterial disease (PAD) is a manifestation of vascular disease. Breast arterial calcification (BAC) is relatively negligible on mammography finding and often not described, although there is an evidence of correlation with other circulatory diseases. Extraosseous calcifications, including BAC and particularly arterial media calcifications, are accompanying MBD on CHD. It would be established whether BAC verified on mammography were prevalent in that population of patients, did they correlate with PAD degree determined by color doppler imaging (CDI) of the lower extremity arteries (LEA), and whether BAC correlated with degree of MBD.

Hypothesis: Female patients on CHD with pronounced MBD have more frequent and more extensive BAC and BAC correlate with PAD degree determined by CDI of LEA.

Research plan and aims: In female patients on CHD:

- To determine the features of MBD (serum concentrations of parathyroid hormone, calcium and phosphate; treatment for hyperparathyroidism and complications).
- To examine the association of MBD with frequency and extent of BAC on mammography imaging.
- To examine the correlation of BAC on mammography imaging with degree of PAD by CDI of the LEA.
- To examine the association of MBD with degree of PAD by CDI of the LEA.
- To investigate the correlation of BAC on mammography imaging with degree of PAD by CDI of the LEA in relation with MBD.

Patients and Methods: The study will include around 100 female patients aged 50 - 70 years in the Region of Eastern Slavonia and Baranja. Breast imaging will be performed by mammography to detect BAC, and CDI of the LEA for arterial lesions. Medical data of MBD will be collected from patients' medical records.

Expected scientific contribution: To prove whether neglected diagnostic method and neglected significance of BAC on mammography imaging indicate the degree of PAD by CDI of the LEA in female patients on CHD, in relation with MBD, and could it contribute to a faster diagnostic and therapeutic procedure.

Keywords: Chronic hemodialysis (CHD), Breast arterial calcification (BAC), Peripheral arterial disease (PAD), Mineral and bone disorder (MBD), Color doppler imaging (CDI)

Abstract Title: Immunohistochemical analysis of the rat hippocampus in a high-fat, high-sugar diet model following treatment with metformin and liraglutide

Part of the Disertation Proposal: The effects of high-fat, high-sugar diet and early metformin and liraglutide treatment on the hippocampal lipidome of male and female Sprague-Dawley rats

PhD candidate: Marin Kuharić, M.D.; Department of Pathophysiology, Physiology and Immunology, Faculty of Dental Medicine and Health Osijek, Osijek, Croatia

Mentor: Prof. Aleksandar Včev, M.D., Ph.D., Department of Pathophysiology, Physiology and Immunology, Faculty of Dental Medicine and Health Osijek, Osijek, Croatia

Introduction: Metabolic syndrome has become one of the largest non-communicable burdens on health care systems worldwide. Insulin resistance that develops due to numerous metabolic disruptions affects the central nervous system as well, including the hippocampus. Clinically manifesting neurodegeneration occurs in part due to disturbances in insulin signaling pathways as well as structural changes in neurons and their myelin sheaths. Identifying the expression levels of commonly affected lipids and proteins within the hippocampus could provide information about the intricacies of insulin resistance pathophysiology and whether or not certain commonly used pharmacotherapeutics (metformin, liraglutide) have neuroprotective effects.

Aims: To perform an immunohistochemical (IHC) analysis of all animal group hippocampi to determine the expression of the most common gangliosides and proteins expressed in brain tissue related to the pathogenesis or effects of insulin resistance.

Materials and Methods: The research was carried out on the brain tissue archive of 40 adult Sprague-Dawley rats, which were randomly separated into four groups of 10 with an equal sex distribution – a control group and three groups on a high-fat, high-sugar diet (HFHSD), two of which were treated with metformin and liraglutide, respectively. Free-floating IHC analysis was performed to determine the expression levels of GM1, GD1a, GD1b and GT1b gangliosides, insulin receptor (IR), insulin-like growth factor receptor, APP, pTAU. Analysis of acquired digital micrographs was performed using the computer software Fiji.

Results: IHC analysis showed statistically significant expression level differences for GM1, GD1b, IR and APP. GD1b expression levels differed significantly among both sexes between rats on a HFHSD and those treated with metformin and liraglutide, while GM1 expression levels were significantly different only between males on a HFHSD and those treated with liraglutide. IR expression levels were significantly different between males on a HFHSD and those treated with both antidiabetics, while APP expression level differences were significant in both sexes, but only between the HFHSD group and the one treated with metformin. The differences were seen in all analysed hippocampal regions (DG, CA1, CA3).

Conclusion: Common antidiabetic drugs have an effect on cerebral ganglioside composition and insulin metabolism-related protein expression, specifically in hippocampal regions where a HFHSD showed significant alterations. Further investigation using more sophisticated methods is required to ellucidate these effects.

MeSH/Keywords: Lipidome, hippocampus, metabolic syndrome, diabetes mellitus

Acknowledgements: Supported through research project UNIOS ZUP2018-44 (Ivic V).

Dissertation Proposal Title: Clinical Significance of N1-Methyladenosin (m^1A) Modification of RNA Molecules in Development of Head and Neck 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: The genetic code is much more than a sequence of DNA bases wrapped around a protein scaffold. It is well known that modification of DNA molecule such as methylation can alter the level of expression of a gene, but recently, focus of research has been on modifications of RNA molecules. There are more than 100 different modifications of RNA molecule mediated by enzymes that introduce it as writers, readers or erasers. These modifications allow for a vast diversity of functions of RNA molecules in each living cell. One such modification is m^1A, or methylation of first nitrogen atom of adenosine. The m^1A modification has been studied in gastrointestinal, ovary, breast, prostate, pancreas, kidney, and non-small cell lung cancer. However, its role in the etiology of head and neck cancer (HNC) has not been studied yet.

Hypothesis: Since m^1A modification of RNA affects its stability and thus gene expression, it could be related to clinico-patological characteristics of HNC tumors.

Aims: The aim of this study is to determine gene expression level of most common m^1A reader, writer, or eraser enzymes from publicly available transcriptomic databases, select those with statistically significant differential expression in HNC tissue samples, determine levels of m^1A modification in tissue samples and corelate the findings with patient data.

Materials/Participants and Methods: The study will be conducted on fresh frozen tissue samples of patients with HNC treated in University Hospital Center Zagreb Department of Otolaryngology, Head and Neck 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 like cBioPortal, GEPIA and UCSC Xena Browser to discover which of so far known enzymes involved in m^1A processing are differentially expressed in HNC. Fresh and FFPE tissue samples will be collected from Croatian HNC patients, together with their clinico-pathological data. Gene expression levels of the selected m^1A processing enzymes will be determined by qPCR and m^1A levels by IHC. Obtained experimental results will be correlated with clinical data, with special emphasis on the assessment of prognostic and predictive significance of m^1A RNA modification and its processing enzymes for HNC patients.

Significance/Expected scientific contribution: Head and neck 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: Head and Neck Neoplasms, 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

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Mentor 1: Assoc. Prof. Robert Smolić, M.D. Ph.D., Faculti of Medicine Osijek, Faculty of Dental Medicine and Health Osijek, University of Osijek, Croatia

Mentor 2: 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 will include 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 will be 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 will be sampled from patients to measure calpain 1 activity (fluorometry), IL-33 concentration (ELISA), and acute inflammatory response markers (CRP, fibrinogen, IL-6). 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 is performed over a period of 6 months at the Respiratory Centre and the Department of Infectology. The samples are 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: Metabolic markers of autophagy and apoptosis in chronic lymphocytic leukemia therapy monitoring

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Mentor: Assoc. Prof. Željko Debeljak, M.D., Ph.D., Clinical Institute for Laboratory Diagnostics, University Hospital Centre Osijek; Faculty of Medicine Osijek, University of Osijek, Croatia

Introduction: Despite different chromosomal mutations and their impact on B cell metabolism, the same drugs are used to treat chronic lymphocytic leukemia (CLL). Bruton tyrosine kinase (BTK) inhibitors and B-cell lymphoma 2 (BCL-2) inhibitors affect cell survival by regulating autophagy and apoptosis, respectively. Despite efficacy in patient survival, BTK inhibitors cause cell stress that enhances protective autophagy and can lead to drug resistance. BCL-2 inhibitors, on the other hand, induce B-cell apoptosis, but acquired mutations lead to disease progression and treatment failure.

Hypothesis: Patients with CLL with different cytogenetic mutations, treated with the same therapy, have different B cell metabolism. Metabolic markers of autophagy and apoptosis are differently expressed in these patients and correlate with response to therapy.

Aims: to evaluate whether the type of cytogenetic mutation has an effect on up- or downregulation of autophagy and apoptosis in untreated patients; to evaluate metabolic pathways of autophagy and apoptosis in treated patients; to evaluate the signal correlation between drugs (BTK and BCL-2 inhibitors) and autophagy / apoptosis metabolites; to evaluate the correlation between the clinical response to therapy with autophagy and apoptosis markers.

Materials/Participants and Methods: This study will be conducted on three groups of participants at the Clinical Institute for Laboratory Diagnostics of the Osijek University Hospital: treated and untreated patients with known CLL mutations and healthy controls. Whole blood samples will be drawn in K3-EDTA tubes (Becton Dickinson, Franklin Lake, New Jersey, USA). 2 µl of blood will be smeared on an indium tin oxide glass slide (Merck, Darmstadt, Germany) on which a 9-aminoacridine matrix (Merck,

Darmstadt, Germany) will be applied. Single B cells as laser targets will be selected using an optical microscope integrated into the iMScope TRIO mass spectrometer (Shimadzu, Kyoto, Japan) and matrix assisted laser desorption ionization - time mass spectrometry imaging (MALDI TOF MSI) analysis will be conducted on the selected regions of interest. Targeted and differential m/z signal expression analysis and statistical analysis will be performed in IMAGEREVEAL 1.1 (Shimadzu, Kyoto, Japan) image analysis software.

Research plan: 1. upgrading and validation of the current method for single cell MALDI TOF MSI; 2. MALDI TOF MS imaging of blood smears from treated and untreated CLL subjects and healthy control group; 3. assessment of markers of autophagy (spermine, spermidine, putrescine) and apoptosis (cell membrane phospholipids) in metabolic pathways; 4. statistical analysis and interpretation of results.

Significance/Expected scientific contribution: Understanding the mechanisms and metabolic pathways of autophagy and apoptosis in treated patients with possible individualization of CLL therapy.

MeSH/Keywords: chronic lymphocytic leukemia therapy, autophagy, apoptosis, mass spectrometry, MALDI TOF imaging

Abstract Title: Effect of consumption of functionally enriched eggs on the microvascular function in patients with acute coronary syndrome

Part of the Dissertation Proposal: Effect of consumption of functionally enriched hen eggs on cardiovascular function and the immune system in patients with acute coronary syndrome

PhD candidate: Ana Marija Masle, M.D., Department of Rheumatology, Clinical Immunology and Allergology, University Hospital Centre Osijek, Osijek, Croatia

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

Introduction: Acute coronary syndrome (ACS) is the leading cause of death in men and women in industrialized countries. It is characterized by a series of clinical syndromes in which complete or partial coronary artery obstruction develops by rupture of atherosclerotic plaque and thrombus formation. N-3 polyunsaturated fatty acids (n-3 PUFAs) are known to lower total cholesterol and low-density lipoprotein (LDL) cholesterol while increasing skin microvascular reactivity in young healthy individuals. In addition, lutein, selenium and vitamin E have been shown to have antioxidant effects. This research allows for the consumption of functional product (hen egg) enriched with multiple ingredients.

Aims: The aim of this study was to investigate the effects of consumption of functionally enriched hen eggs (n-3 PUFAs, vitamin E, lutein, selenium) on lipid profile and microvascular reactivity in patients with ACS.

Participants and Methods: 28 patients with existing ACS were divided in two groups, Experimental group (N=15) who consumed three enriched hen eggs/daily during three weeks, and Control group (N=13) who consumed three regular hen eggs/daily during three weeks. Biochemical parameters and nutrient concentrations were measured before and after the dietary protocol. Skin microvascular blood flow in response to post-occlusive reactive hyperemia (PORH), iontophoresis of acetlycholine (AChID) and sodium nitroprusside (SNPID) was measured by laser-Doppler flowmetry.

Results: LDL cholesterol was significantly decreased in experimental group compared to the control group. There was a statistically significant increase in vitamin E in the experimental group after the dietary protocol. PORH, AChID, SNPID were significantly increased in the experimental group while they remained unchanged in the control group.

Conclusion: Consumption of functionally enriched hen eggs for three weeks has favorable effect on lipid profile and has positive effect on microvascular reactivity in ACS patients.

Keywords: acute coronary syndrome; antioxidants; endothelial dysfunction; functional food; microvascular function

Abstract title: Proton pump in cartilage chondrocytes of the locomotor system

Part of Disertation Proposal: The existence of a proton pump in the chondrocytes of the locomotor system and its influence on the pathophysiology of bone and joint cartilage

PhD candidate: Tomislav Matejić, M.D., Department of Traumatology, Clinical Hospital "Sveti Duh" Zgreb, Croatia

Mentor: Assist. Prof. Egon Biuk, M.D., Ph.D., Clinic for Orthopedics and traumatology, University Hospital Centre Osijek; Faculty of Medicine Osijek, University of Osijek, Croatia

Co-Mentor: Assist. Prof. Željka Perić Kačarević, PhD, M.Biol., Department of Anatomy, Histology, Embryology, Pathological Anatomy and Pathological Histology, Faculty of Dental Medicine and Health Osijek, University of Osijek, Croatia

Introduction: In an undamaged joint, there is a balance between building and decomposition of molecules. In pathophysiological disorders of cartilage, the balance of water and proteoglycans are lost. The enzyme responsible for this active transport is the proton pump (H^+/ K^+ ATPase). Its beta subunit has a function in catalysis and ion exchange that is still unclear.

Goals: In the study, we will show the presence of the beta subunit of H^+/ K^+ ATPase in the chondrocytes of the bone and joint cartilage and investigate its influence on pathophysiological changes in the cartilage itself. We will also show the presence of the beta subunit in the bone and articular cartilage chondrocytes depending on the sex of the subjects and age distribution, also in healthy bony articular cartilage in subjects, related to age and sex and pathomorphologically altered bony articular cartilage, related to age and sex.

Materials / participants and methods: Pathomorphologically altered cartilage patterns of the femoral head and femoral and tibial condyles, obtained during implantation of a total hip or knee endoprosthesis were examined and compared with healthy samples cartilage of patients with trauma to the meniscus of the knee and its partial extirpations. All samples were obtained from patients treated at the

Orthopedic Clinic and traumatology of the Clinical Hospital Center in Osijek and processed in Clinical Institute of Pathology and Forensic Medicine of the Clinical Hospital Center in Osijek. Tissue is fixed in 10 % formalin, processed in an automated tissue processor and incorporated into paraffinic blocks, cut into layers 5 μ thick and colored conventional by staining (hemalaun-eosin) and immunohistochemically, to a beta subunit of a proton pump. The proton pump signal obtained is then histologically examined and quantified immunohistochemical staining.

The results: The results indicate the presence of the enzyme H^+/ K^+ ATPase on the beta subunit of the proton pumps in the chondrocytes of healthy and pathomorphologically altered bony articular cartilage.

Conclusion: The results indicate the existence of proton H^+/ K^+ ATPase on the beta subunit
proton pumps in the chondrocytes of healthy and pathomorphologically altered bony joints
cartilage and this research will help us better understand the processes that lead to pathomorphological changes in bony articular cartilage.

Keywords: Proton pump beta subunit (H^+/ K^+-ATP), chondrocytes, bone and joint cartilage, pathomorphological changes

Acknowledgement: Ass. professor Željka Perić Kačarević, Ph.D., M. Bio., and Assist. Prof. Egon Biuk, M.D.Ph.D., on support and assistance in work.
Assoc. Prof. Martina Smolić, M.D. Ph.D., on the provided knowledge, help and guidance during this study.

Dissertation Proposal Title: Effect of the Vojta therapy in the treatment of severe functional constipation in children

PhD candidate: Marko Mesić, M.D., Pediatric Surgeon, Children's Hospital Zagreb, Zagreb, Croatia

Mentor: Assoc. Prof. Mario Kopljar, M.D., Ph.D., General and Abdominal Surgeon, University Hospital "Sestre milosrdnice", Zagreb, Faculty of Medicine Osijek, University of Osijek, Croatia

Introduction: More than 25 % patients followed by pediatric gastroenterologists are constipated. Constipation can be defined as an infrequent bowel movement or a difficult passage of stool that persists for several weeks or longer.
Frequency of normal bowel movements varies with age, from 2 to 4 stools per day in newborns, to 1 stool per day in the first years of life.
Constipation can be devided into functional (95 %) and organic (5 %). „Slow transit" and „outlet obstruction" are two main forms of functional constipation. The first one is a reflection of colonic motility disorders. The second one is due to uncontrolled function of the pelvic muscles.
Organic causes are specific structural, neurologic, toxic/metabolic or bowel disorders. They are rare, but very important to be recognized. The most common is Hirschprung disease. Other organic causes presented in newborn period or later are anorectal malformations, cystic fibrosis, metabolic disorders like hypothyroidism, hypercalcaemia, hyperkalemia and vertebral disorders.
Early diagnostic is very important in order to prevent unnecessary, unpleasant and harmful procedures and to develop the strategy of treatment. Beside the laboratory and biochemical tests, anorectal manometry, rectal biopsy, colonic transit study, colonic manometry and contrast enema represent very important diagnostic procedures in defining the form of constipation.
Specific organic causes should be treated. Functional constipation is treated with specific dietary measures and changing the habits which include meals with the right ammount of fibers, plenty of water and toilet training. Inefficiency of the above mantioned measures demands stool softeners and rectal enemas.
Chronic constipation presents a great burden in everyday life in both children and their parents. Many of them, depending of etiology, have problems regarding social life and everyday activities.

They suffer from soiling, pain and need rectal enemas on a daily base and very often manual desimpaction of faecaloma. Eventually, some of them can be cured only with surgical intervention, with or without colostomy formation.
Vojta therapy or reflex locomotion was initially used in the treatment of children with cerebral palsy. Indication has been expanded in all kinds of motoric deviations. Taking an initial and specific body positions associated with pressure on defined points, unconscious congenital pattern of reflex locomotion is activated. In all people, no matter of age, this kind of stimulation provokes one of two patterns of locomotion basic for all other types of human locomotion: reflex creeping and turning.
Reflex locomotion is provoked within three main positions: on the stomach, on the back and on the side using 10 pressure areas on the body and extremities. This kind of therapy activates intensive contractions of abdominal wall muscles in coordination with the diaphragm which increases intraabdominal pressure affecting on bowel and bladder emptying. During that period, pelvic floor muscles are in the state of stretching which contributes to urethral and anal sphincter contractions. Education of patients and their parents provides home-based therapy.
Health related quality of life (HRQOL) describes the patient's perception of health. It measures the impact of a chronic disease on the physical, social and emotional behavior in both children and parents. It has a huge clinical interest especially in children. Disability or limitation in children represents a huge stress for parents, which can have a great impact on both physical and mental health.

Hypothesis: Vojta therapy increases stool number and decreases the need for a laxative therapy in children with severe functional constipation. It affects the quality of life of children and their parents.

Aims: The aim is to determine whether Vojta therapy increases the number of defecations in children with severe constipation and whether it decreases the need for a laxative therapy.
Specific objectives are to determine does Vojta therapy affect the quality of life in constipated children and their parents, to define the difference in therapy results in patients treated with laxatives and toilet training in comparisson with combined Vojta and laxative therapy, and to determine the difference in the quality of life in children and their parents depending on the type of the therapy.

Materials/Participants and Methods: Research will be designed as a prospective, randomized and open study. Participants will be children with diagnosed functional constipation according to Rome IV criteria, suffering from severe functional constipation defined as the one with the failure after 4 weeks of standard laxative therapy. Laxative therapy failure is defined as the absence of stool in more than 3

days, repeated two times during period of 4 weeks of laxative therapy treatment or need for desimpaction during above mentioned 4 weeks period.
The study will include the parents of constipated children.
Standard laxative therapy includes polyethylene glycol (PEG) with the dose of 0.2 - 0.4 g/kg/day. Children will be divided in two groups by random selection. First group will be treated with the standard laxative therapy PEG with the dose of 0.2 - 0.4 g/kg/day and toilet training within 3 months. The second group will be treated with a combined laxative therapy with the dose of 0.2 - 0.4 g/kg/day and Vojta therapy within 3 months. The quality of life will be determined before starting the therapy and at the end of therapy.
Including criterias are children at the age of 5 to 18 years of life, diagnosed with severe functional constipation defined as the one with failure of standard laxative therapy (PEG, 0.2-0.4 gr/kg/day) within 4 weeks of treatment, signed informed consent of parents and children older than 9 years of age.
Excluding criterias are children younger than 5 year of age, children who are not capable to fill the information form due to their mental status or educational problems and refusal to participate.
To observe the mean effect in the difference of numerical variables between two independent groups of subjects, with 10 % expected decrease of mean value of quality of life with a significance level of 0.05 and a strength of 0.9, the minimum required sample size is 47 subjects per group. Including the unresponsive and fallout rate of 25 %, the estimate number of 60 participants in each group should be enough to draw conclusions.

Research plan: Using including criterias and confirming the diagnose of chronic constipation, the initial value of quality of life in children and their parents will be determined using PedsQL 4.0 and PAC-QOL questionnaires with special versions for children (depending on age) and parents. Anorectal manometry will be done at the beginning and at the end of the study as a standard procedure in order to define pelvic muscle and anal sphincter function and to exclude organic constipation.
Using randomized software the children will be divided into two groups, children with laxative terapy and toilete training and children with laxative and Vojta therapy.
Bristol stool chart will be used on a daily base to follow the number and quality of stools. Every day, laxative dose will be presented in the table.
Quality of life will be determined after first, second and third month of treatment during regular clinical controls.
After defining the normal data distribution (Kolmogorov- Smirnov), identifying differences between two groups will be based od parametric (Student t-test, ANOVA) and nonparametric tests (Mann-Whitney, Kruskal-Wallis, ANOVA). Correlation will be determined using Pearson or Spearman methods. X2 and Fischer test will be used

for table contingency analysis. P values less than 0.05. will be considered statistically significant.

Significance/Expected scientific contribution: The results of the study will show the effect and peculiarities of Vojta therapy in the treatment of patients with functional constipation. Eventually, the results will contribute to taking a stand, based on scientific evidence, about Vojta therapy and questionnaires used for quality of life prediction and reduce negative impact of chronic constipation on children health.

Keywords: chronic constipation, idiopathic constipation, children, parents, quality of life

Dissertation Proposal Title: The correlation between the transcription Nrf2 Factor and the heme oxygenase-1 serum concentrations in patients with coronavirus disease 2019 (COVID-19)

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Mentor 2: Assoc. Prof. Robert Smolić, M.D. Ph.D., Faculti of Medicine Osijek, Faculty of Dental Medicine and Health Osijek, University of Osijek, O Croatia

Introduction: Coronavirus disease 2019 (COVID-19) is caused by a novel SARS-CoV-2 virus that is most presented as a flu-like disease, but with some patients it can take a more severe clinical course and cause severe clinical forms which are associated with an enhanced immune response. The nuclear factor erythroid 2-related factor 2 (Nrf2) and the heme oxygenase-1 (HO-1) are becoming interesting in terms of the development of more severe forms of COVID-19 disease due to their antiinflammatory effect. Endogenous and/or exogenous induction of the Nrf2/HO-1 pathway could have a beneficial effect on the course of the COVID-19 disease. The protective effect of Nrf2/HO-1 pathway has already been proven in some diseases such as pneumonia, asthma, COPD and pulmonary fibrosis.

Hypothesis: Decreased Nrf2 i HO-1 serum concentrations have been associated with an enhanced immune response and severe clinical forms of COVID-19 disease.

Aims: The primary aims of this study are to examine the relationship between Nrf2 and HO-1 serum concentrations in patients with COVID-19 disease, their association with the indicators of inflammatory response severity (CRP, ferritin, IL-6) and the clinical severity (MEWS criteria). The secondary aims are to examine the effect of oxygen therapy, 1,25-dihydroxyvitamin D3 values, age and comorbidities on their values.

Participants and Methods: This cross-sectional study included 80 patients with COVID-19 disease with the following criteria: 1) between 18 and 80 years of age; 2) positive PCR test for SARS-CoV-2; 3) the presence of symptoms of COVID-19 disease

for at least 7 days and 4) the absence of diseases in which an association with lower Nrf2 values has been previously proven (malignant and autoimmune diseases). After collecting patient data and disease information (age, sex, comorbidities, the results of laboratory and radiological examinations, the method and the outcome of the treatment), peripheral venous blood was sampled to measure Nrf2 and HO-1 serum concentrations by ELISA method. The severity of the inflammatory response was assessed on CRP, ferritin and IL-6 values, while the clinical severity was based on MEWS criteria.

Research plan: This study is conducted at the University Hospital Osijek (collecting patient data and blood sampling) and the Faculty of Medicine Osijek (laboratory processing). Blood samples were collected from January to June 2021 from hospitalized patients whose diseases' course lasted at least 7 days (the first day being the day the symptoms first appeared). The study was approved by the Ethics Committee.

Expected scientific contribution: The contribution of this study is to establish the correlation between Nrf2 and HO-1 serum concentrations and the severity of immune response and clinical course in patients with COVID 19 disease.

Keywords: COVID 19, Heme oxygenase-1, Nrf2 factor, SARS-CoV-2 virus, 1,25 Dihydroxyvitamin D3

Dissertation Proposal Title: Brain SPECT with ^{123}I-ioflupane as a diagnostic biomarker of Parkinson's disease type

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Mentor 2: Assit. Prof. Svetlana Tomić, M.D., Ph.D., Faculty of Medicine Osijek, University of Osijek, Department of Neurology, University Hospital Centre Osijek, Croatia

Introduction: Parkinson's disease (PD) is a chronic, multisystem and a progressive neurodegenerative disease. Older age, gender, genetics and the influence of environmental factors are the most significant risk factors. PD is clinically manifested by a number of motor and non-motor symptoms as a consequence of neuronal degeneration of the dopaminergic, serotonergic, noradrenergic, and cholinergic systems. The presence of Lewy bodies and neurite outgrowth is the basic pathohistological feature of this disease. Dopaminergic neurons make up three important dopaminergic pathways: nigrostriatal, mesocorticolimbic, and tuberohypophyseal. In patients' with PB, the nigrostriatal pathway is the most affected one. The nigrostriatal pathway connects the substantia nigra pars compacta with the caudate nucleus and putamen. The process of nucleus activation takes place through two pathways: direct and indirect. The direct pathway is involved in facilitating the desired movements, while the indirect pathway participates in the suppression of unwanted movements. Putamen structures are important in the regulation of motor symptoms, while caudate nucleus structures are responsible for nonmotor symptoms. It is known that the course of PD is not uniform and that there are different subtypes of the disease depending on the pronounced motor and non-motor symptoms, the onset of the disease or on genetic factors. Subtypes differ from each other in the rate of progression, severity of clinical picture and the final outcome. PD is divided into three subtypes: mild motor-predominant, difuse-malignant, and intermediate.

Hypothesis: The difference in the accumulation of ^{123}I-ioflupane in putamen and caudate is a diagnostic biomarker of the PD type and it also enables the differentiation of PD into motor, non-motor and intermediate type of the disease.

Aim: To demonstrate and compare the difference in the accumulation of ^{123}I-ioflupane with the results of the tests of motor and non-motor functions, which will allow the division of patients into PD types.

Materials/Participants and Methods: The study will involve 150 PD patients divided into 3 groups. Monitoring the accumulation of ^{123}I-ioflupane in dopaminergic neurons using single-photon emission computed tomography (SPECT) will provide insight into the functionality / damage of neurons in the striatum. The division of PD into mild-motor, intermediate and diffuse-malignant type will be performed based on the findings of cognitive tests, the presence of orthostatic hypotension, anamnestic data on REM sleep disorder and the severity of motor and non-motor symptoms.

Research plan: To investigate by visual interpretation the differences in the accumulation of radiopharmaceuticals in striatum.
To prove by semiquantitative analysis the difference in the accumulation of radiopharmaceuticals in the regions of interest that correlate with the test scoring results.

Significance/Expected scientific contribution: To determine the contribution of the brain SPECT with ^{123}I-ioflupane as a diagnostic biomarker of PD type and to predict the course of the disease and the patients' survival prognosis.

Keywords: Parkinson's disease, striatum, dopaminergic neurons, brain SPECT, ^{123}I-ioflupan

Dissertation Proposal Title: The impact of sleep apnea on maintaining the target values of LDL cholesterol after percutaneous coronary intervention in patients with acute coronary syndrome

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Mentor: Assist. Prof. Irzal Hadžibegović, M.D., Ph.D., UH Dubrava, Zagreb, Croatia

Introduction: Obstructive sleep apnea (OSA) is the most common breathing disorder during sleep. The syndrome is underestimated and often unrecognized. The diagnosis of OSA is made by polysomnography. OSA is associated with chronic diseases including coronary heart disease. In persons diagnosed with OSA, the incidence of major adverse cardiovascular events after percutaneous coronary interventions (PCI) is higher. Given the negative impact of OSA on cardiovascular mortality and morbidity, the thesis that OSA exacerbates dyslipidemia is logical. Acute coronary syndrome (ACS) requires urgent treatment, and the gold standard is PCI. Statins are part of standard chronic therapy after PCI. However, there is a lack of research focused on the influence of OSA on the concentration of LDL cholesterol in persons who have a statin in therapy at the maximum dose.

Hypothesis: The proportion of patients with ACS and successful PCI who do not achieve LDL cholesterol target values is higher among patients with OSA compared to patients without OSA.

Aims: The primary objective of the study was to determine the prevalence of OSA among patients with ACS, to assess the impact of OSA on possible differences in LDL cholesterol concentration and the success of maintaining target values during treatment between the test and control groups.

Materials/Participants and Methods: Patients with ACS who have had successful PCI within 24 hours and who have no contraindication to taking the maximum dose of statins.
Patients with ACS, after a successful PCI, will have a polysomnography during their initial hospital stay. Patients who have an apnea-hypopnea index (AHI) greater than 5 after polysomnographic testing will be assigned to the test group. Patients who

have an AHI of less than 5 after polysomnographic examination will be assigned to the control group. All subjects will have their lipid values analyzed upon arrival at the hospital and 1, 2, and 12 months after PCI.

Research plan: The prospective cohort study should start in October 2021. Respondents will be included in a period of one year and LDL cholesterol levels will be monitored 1, 2 and 12 months after PCI.

Significance/Expected scientific contribution: Diagnosis of OSA by polysomnography is performed in a small number of centers in Croatia, and the average time from diagnosis to the beginning of the use of the continuous positive airway pressure (CPAP) mask is more than 30 days. CPAP therapy has been shown to have a positive effect on comorbidities associated with OSA. If the study hypothesis is confirmed, further OSA therapy and lipid-lowery therapy should be considered to reduce LDL cholesterol and treatment optimization after ACS.

MESH: Sleep apnea, obstructive (OSA); Acute coronary syndrome (ACS); Percutaneous coronary intervention (PCI); Polysomnography; Cholesterol, LDL

Dissertation Proposal Title: A comprehensive evaluation of microfragmented adipose tissue and concentrated platelet-rich plasma effects in the therapy of knee osteoarthritis: a prospective randomized study

PhD candidate: Vilim Molnar, St. Catherine Specialty Hospital Zagreb, Croatia

Mentor: Prof. Dragan Primorac, M.D., Ph.D., St. Catherine Specialty Hospital

Introduction: Osteoarthritis (OA) is the most common progressive musculoskeletal condition that can affect the joints and novel treatment options such as microfragmented adipose tissue containing stromal vascular fraction (MFAT) with mesenchymal stem cells (MSCs) can slow disease progression. There is a need for more structured clinical research related to the preparation and application of MSCs, but also better patient selection and objectification of treatment effects

Hypothesis: There are significant differences in the concentrations of certain cytokines, chemokines, N-glycans, and miRNAs in synovial fluid and blood of patients with knee OA before and after administration of MFAT in combination with concentrated platelet-rich plasma (cPRP).

Aims: The aim of this study is to objectify the effects of MFAT in combination with cPRP in the treatment of knee OA and to compare the effects of this therapy with the intraarticular application (i.a.) of hyaluronic acid (HA).

Materials/Participants and Methods: In the first part of the research, the selection and stratification of patients with knee OA will be performed to divide patients into detailed groups, i.e. phenotypes, according to data obtained from patient history, clinical examination, X-rays and magnetic resonance imaging of the knee. Sixty patients of the selected phenotype will receive additional gadolinium-enhanced MRI of cartilage (dGEMRIC) to measure glycosaminoglycan (GAG) levels within the hyaline cartilage. Patients will be randomly divided into two groups. One group will be treated with the i.a. of MFAT and cPRP (n=40), while HA will be administered to the second group of patients with knee OA (n=20). Furthermore, patients will complete clinical questionnaires and analysis of cytokines, chemokines, N-glycans and miRNAs from synovial fluid and blood will be provided, as well as serum phenylalanine (PA) analysis. The effects of two therapeutic methods will be objectified and compared by

repeating previous diagnostic procedures: patient history, orthopedic examination, clinical questionnaires, dGEMRIC, serum PA analysis, cytokines, chemokines, N-glycans and miRNAs analysis in synovial fluid one month after and in blood six months after therapeutic intervention.

Research plan: The next step in the research is to obtain the approval of the Institutional Review Board. Upon approval, the selection and stratification of patients with knee OA will begin, as well as the intervention study, which will last another year and six months. Upon completion of the study, a statistical analysis of all parameters will be performed, as well as the preparation of publications and PhD thesis.

Significance/Expected scientific contribution: This is the first study to objectify the effects of MFAT + cPRP therapy by analyzing cytokines, chemokines, N-glycans, miRNAs, and by measuring GAG levels within the hyaline cartilage of the knee.

MeSH/Keywords: Knee osteoarthritis, Mesenchymal stem cells, Platelet-Rich Plasma, Hyaluronic acid, Cytokines

Dissertation Proposal Title: Caring for your own oral health between healthy, schizophrenic and depressed

PhD candidate: Ivana Pavličević Tomas, M.D.

Mentor 1: Prof. Dunja Degmečić, M.D., Ph.D., University Hospital Centre Osijek; Faculty of Medicine Osijek, University of Osijek, Croatia

Introduction: Everyone has the right to health care and the opportunity to achieve the highest possible level of health in accordance with legal regulations. Health care in the sense of the Health Care Act includes a system of social services and activities for the preservation and improvement of health, disease prevention, early detection of disease, timely treatment and health care and rehabilitation. Mental health is defined by the World Health Organization as a state of well-being in which an individual realizes his potential, can cope with normal life stress, work productively and fruitfully, and is able to contribute to the community. People with mental disorders find it harder to function in their environment, and their stigmatization, which includes negative labeling, marginalization, and avoidance because they have a mental illness, becomes a problem in itself. Therefore, it is concluded that people suffering from a mental disorder inadequately care about their own health compared to the population without mental illness. Mental illness can have a variety of strong consequences on a person's lifestyle, including social and financial status under which physical health and overall functionality can be compromised. The association of impaired physical health with psychiatric disorders is an increasing subject of numerous studies, but is less focused on oral health issues although it is an important part of physical health. Many psychiatric disorders are associated with a number of dental diseases. Poor oral health can also affect diet, speech, and other social and psychological areas of life.

Hypothesis: The level of self-care with an emphasis on the oral health of patients with schizophrenia and depressive disorder is lower and inadequate compared to the healthy population.

Aims: The aim of this study was to determine whether there are specifics of oral health disorders in people with schizophrenia and depression compared to the population who do not suffer from mental illness. Emphasis should be placed on the periodontal status of patients and the comparison between these groups of patients.

Take into account the diagnosis, the duration of the disease, the pharmacotherapy that the patient is taking, laboratory findings, orthotympanogram and psychological and psychiatric assessment of the current state of the disease. Compare the quality of life and the level of functioning with regard to lifestyle and self-care in healthy, schizophrenic and depressed people. Identify the existence of periodontal specifics that might help in diagnosing and monitoring the course of psychiatric illness.

Research plan: Cross-sectional study will be used in this paper.

Materials/Participants and Methods: The study will be conducted on three separate groups consisting of healthy people, people with schizophrenia and people with depressive disorder. These three groups of people will be examined separately, examined and compared by a psychiatrist and a dentist, a periodontology subspecialist.
The data to be used in this study will be collected in such a way that each respondent from the study groups will complete a questionnaire and specific rating scales under the supervision of a psychiatric specialist and access to medical records. The survey questionnaire will be structured and designed for research purposes and will include data indicating the course of the disease, pharmacotherapy and physical health care of the study group with emphasis on lifestyle (smoking, alcohol consumption, drug abuse, diet, exercise, personal hygiene) and dental health. Mental health as an important segment of general health will be assessed on the basis of filling in the questionnaire and rating scales and by reviewing the current medical documentation. The level of functioning will be assessed separately with regard to social and professional functionality (employment, existence, working conditions, social interactions, way of spending free time). Each patient will be examined by a dentist with a narrower specialty of periodontology with recording the specifics of oral status. They will be involved in diagnostic methods (laboratory examination, orthotympanogram, psychological diagnostics).

Significance/Expected scientific contribution: The key findings of the dissertation will be reflected in a comprehensive and systematic presentation of oral health care among the healthy, schizophrenic and depressed. Knowing that oral health has significant consequences on the quality of life of patients, we will study in detail how oral and mental illnesses affect the quality of life of mentally ill patients. We will study periodontal specifics that could help diagnose and monitor the course of mental illness. Based on the obtained results, the manner and development of primary, secondary and tertiary preventive measures can be considered in order to ensure the improvement of mental health in the Republic of Croatia.

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

PhD candidate: Oliver Pavlović, Osijek University hospital, University of Osijek, Osijek, Croatia

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

Aims: 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.

Immunohistochemistry
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.

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

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: Cervical spine degenerative conditions effect up to two-thirds of the population and are the most common cause of acquired disability in patients over the age of 50(1). These disorders commonly present with axial pain, myelopathy, radiculopathy or a combination of these symptoms. Treatment options can be classified as non-operative or operative. Non-operative treatments include analgesia and physiotherapy, and are useful primarily in cases without significant cord compression. Surgical intervention is generally indicated in with failure of conservative management or with evidence of cord compression or myelopathy(2). Factors like age, comorbidities and radiological findings are also taken into consideration when planning treatment(3, 4).

Anterior cervical discectomy and fusion (ACDF) is considered the gold standard for many degenerative diseases of the cervical spine because of its relative simplicity, minimal risk, and reliability.(5) It was first described by several authors in 1958,(6, 7) and became established as one of the most common spine procedures. A recent population-based observational study revealed a steady increase in cervical spine surgeries in both inpatient and outpatient settings.(8)

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% (Class III)(9).

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.

Standard surgical approach includes surgery in supination with mild reclination of the neck. Transverse incision on the right or the left side of the neck in the height of the level of the disc with determination of the level using C-arm. Anterior approach is performed through preparation between the carotid artery, oesophagus and trachea. M.longus colli are coagulated and the surgical field is exposed using retractors. In microsurgical technique the complete disc is removed and in this way the pressure of the nerve and cervical spine is released. Empty space left following discectomy can be replaced with osseous autograft, osseous allograft, titanium or PEEK cage, following the implantation of ventral plate, which is fixed on the vertebra using screws.

In multiple studies, overall morbidity rates for ACDF varied from 13.2% to 19.3%. These included in descending order; dysphagia (1.7%-9.5%), postoperative hematoma (0.4%-5.6% (surgery required in 2.4% of 5.6%), with 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%), increased radiculopathy (1.3%), 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(12).
It has been postulated that the number of complications in the cervical spine surgery rises with number of operated segments(13). There are different data in the literature regarding this question. 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). In appropriate patients, 4-level ACDF is a safe, efficacious method for treating multilevel cervical spinal cord compression, with acceptable complication rates and the ability to achieve neurological improvement and high fusion rates(15). 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). Dysphagia is a relatively common complication in the early postoperative period following ACDF and may cause patients significant discomfort and distress. 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). Recent study has shown that 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, and the authors' analysis demonstrates an equivalent rate of fusion and time to fusion between 3- and 4-level surgeries(18).
Clinical outcomes of multilevel anterior cervical fusion can be optimized through the use of biologics and graft selection, the evaluation of pre-existing deformity, the assessment of comorbidities, and the selection of fusion levels. Meticulous surgical technique in conjunction with modern surgical tools, such as instrumentation and biologics, allow surgeons to address complex cervical problems while limiting morbidity and enhancing clinical outcomes(19).

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, 172 patients (15.3%) had diabetes mellitus, which affected patients equally by sex, and 389 patients (34.6%) used tobacco. 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. Only 21 patients stayed in the hospital longer 3 nights or more. Portion of the results regarding comparison between inpatient and outpatient surgery have already been published(20).
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. One- and two-level surgeries were done significantly more often in the outpatient setting while the operations for three and four levels were more often performed as inpatient procedures. Outpatients had more complications for one- and two-level surgery compared to inpatients, and inpatients having three-level surgery had significantly more complications. Four-level surgery patients showed no difference in the number of complications between inpatient and outpatient groups. Comparison of pain reduction, neurological outcome, operative time, the concrete level as well as inpatient/outpatient surgery setting in regard to single-level and multiple-level surgery are currently in the process of data collection and statistical analysis.

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.

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

References:

1. Fehlings MG, Arvin B. Surgical management of cervical degenerative disease: the evidence related to indications, impact, and outcome. J Neurosurg Spine. 2009;11(2):97-100.
2. Quinn JC, Kiely PD, Lebl DR, Hughes AP. Anterior surgical treatment of cervical spondylotic myelopathy: review article. HSS J. 2015;11(1):15-25.
3. Oh LJ, Ong S, Ghozy S, Dmytriw AA, Zuccato J, Mobbs R, et al. Dysphagia rates in single- and multiple-level anterior cervical discectomy and fusion surgery: a meta-analysis. J Spine Surg. 2020;6(3):581-90.
4. Kuijper B, Tans JT, Schimsheimer RJ, van der Kallen BF, Beelen A, Nollet F, et al. Degenerative cervical radiculopathy: diagnosis and conservative treatment. A review. Eur J Neurol. 2009;16(1):15-20.
5. Fowler SB, Anthony-Phillips P, Mehta D, Liebman K. Health-related quality of life in patients undergoing anterior cervical discectomy fusion. J Neurosci Nurs. 2005;37(2):97-100.

6. SMITH GW, ROBINSON RA. The treatment of certain cervical-spine disorders by anterior removal of the intervertebral disc and interbody fusion. J Bone Joint Surg Am. 1958;40-A(3):607-24.
7. Cloward RB. The anterior approach for removal of ruptured cervical disks. 1958. J Neurosurg Spine. 2007;6(5):496-511.
8. Baird EO, Egorova NN, McAnany SJ, Qureshi SA, Hecht AC, Cho SK. National trends in outpatient surgical treatment of degenerative cervical spine disease. Global Spine J. 2014;4(3):143-50.
9. Matz PG, Holly LT, Groff MW, Vresilovic EJ, Anderson PA, Heary RF, et al. Indications for anterior cervical decompression for the treatment of cervical degenerative radiculopathy. J Neurosurg Spine. 2009;11(2):174-82.
10. Epstein NE. A Review of Complication Rates for Anterior Cervical Diskectomy and Fusion (ACDF). Surg Neurol Int. 2019;10:100.
11. Zaki O, Jain N, Yu EM, Khan SN. 30- and 90-Day Unplanned Readmission Rates, Causes, and Risk Factors After Cervical Fusion: A Single-Institution Analysis. Spine (Phila Pa 1976). 2019;44(11):762-9.
12. Buttermann GR. Anterior Cervical Discectomy and Fusion Outcomes over 10 Years: A Prospective Study. Spine (Phila Pa 1976). 2018;43(3):207-14.
13. Koller H, Hempfing A, Ferraris L, Maier O, Hitzl W, Metz-Stavenhagen P. 4- and 5-level anterior fusions of the cervical spine: review of literature and clinical results. Eur Spine J. 2007;16(12):2055-71.
14. Kim S, Alan N, Sansosti A, Agarwal N, Wecht DA. Complications After 3- and 4-Level Anterior Cervical Diskectomy and Fusion. World Neurosurg. 2019;130:e1105-e10.
15. Jack MM, Lundy P, Reeves AR, Arnold PM. Four-level Anterior Cervical Discectomy and Fusions: Results Following Multilevel Cervical Fusion With a Minimum 1-Year Follow-up. Clin Spine Surg. 2021;34(4):E243-E7.
16. Rajan PV, Emara AK, Ng M, Grits D, Pelle DW, Savage JW. Longer operative time associated with prolonged length of stay, non-home discharge and transfusion requirement after anterior cervical discectomy and fusion: an analysis of 24,593 cases. Spine J. 2021.
17. Oh LJ, Dibas M, Ghozy S, Mobbs R, Phan K, Faulkner H. Recurrent laryngeal nerve injury following single- and multiple-level anterior cervical discectomy and fusion: a meta-analysis. J Spine Surg. 2020;6(3):541-8.
18. McClure JJ, Desai BD, Shabo LM, Buell TJ, Yen CP, Smith JS, et al. A single-center retrospective analysis of 3- or 4-level anterior cervical discectomy and fusion: surgical outcomes in 66 patients. J Neurosurg Spine. 2020:1-7.
19. McCarthy MH, Weiner JA, Patel AA. Strategies to Achieve Spinal Fusion in Multilevel Anterior Cervical Spine Surgery: An Overview. HSS J. 2020;16(2):155-61.

20. Mullins J, Pojskić M, Boop FA, Arnautović KI. Retrospective single-surgeon study of 1123 consecutive cases of anterior cervical discectomy and fusion: a comparison of clinical outcome parameters, complication rates, and costs between outpatient and inpatient surgery groups, with a literature review. J Neurosurg Spine. 2018;28(6):630-41.

Dissertation Proposal Title: Klotho expression in the early pathogenic development of vascular calcification in CKD (uremic rats)

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Mentor: Prof. Josip Španjol, Clinical Hospital Center Rijeka, Rijeka, Croatia

Introduction: Chronic kidney disease (CKD) is estimated to affect more than 10 % of the world population. Cardiovascular diseases are the most important complication and the primary cause of death in patients with CKD. Most patients with CKD also show abnormal mineral metabolism defined as chronic kidney disease – mineral and bone disorder (CKD-MBD). CKD-MBD includes changes in mineral homeostasis, bone quality, cardiovascular and soft tissue calcifications. Vascular calcification (VC) has been associated with morbidity and mortality and as a strong predictor of cardiovascular risk in patients with CKD. The prevalence of VC and the risk of CKD have been shown to increase as the glomerular filtration rate (GFR) decreases. Calcification of the cardiovascular system is a highly regulated process of calcium phosphate mineral deposition in the intimal and medial layers of the blood vessel and in the heart valves.

Hypothesis: We hypothesize that Klotho protein plays an important role in formation of VCs. In our research we will investigate how is Klotho expressed in VCs of uremic rats.

Aims:
1. Analyze the expression of Klotho protein and mRNA in the aorta of uremic rats and in *in vitro* cell culture (VMSC, HUVEC)
2. Analyze the development of aortic calcification 10 and 20 weeks after 5/6 nephrectomy
3. Examine tissue co-expression of Klotho protein with other factors that contribute to development or inhibit VC

Materials/Participants and Methods:
1. experimental model on animals - 5/6 nephrectomy
2. collection of abdominal aortic samples after 10 and 20 weeks
3. analysis of aortic calcification

4. immunohistochemical analysis of Klotho protein expression in healthy aortas and after 10 and 20 weeks of nephrectomy.
5. Analysis of co - expression of Klotho and fetuinA, OPG, alpha-SMA, OPN, Runx2 and BMP-2 in healthy aortas, and after 10 and 20 weeks of nephrectomy.
6. Klotho protein expression on *in vitro* aortas under different conditions of Pi and Klotho
7. Determination of Klotho mRNA expression in VSMC and HUVEC cell cultures at different Pi conditions.

Research plan:
establishment of the uremic rat model
collection of tissue samples
immunohistochemistry, Western Blot, PCR, cell culture
statistical analysis
publication

Significance/Expected scientific contribution: Although the relevance of VC assessment is recognized in clinical practice, quantitative methods are still associated with radiography or ultrasound, with many drawbacks, such as cost and time, radiation exposure and lack of standardized results. Therefore, molecular event analysis and consequent development of biomarkers for early VC detection is crucial for prevention of CVD outcome in patients with CKD, enabling preventive measures to reduce development and progression of VC.

MeSH/Keywords: chronic kidney disease, atherosclerosis, Klotho, mineral and bone disorder, cardiovascular diseases

Dissertation Proposal Title: Validation of the Croatian translation of LPR-HRQL questionnaire and the assessment of quality of life in patients with laryngopharyngeal reflux using various therapeutic methods

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Mentor: Prof. Andrijana Včeva, M.D., Ph.D., ENT Clinic, University Hospital Centre 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; laryngopharyngeal reflux health-related quality of life questionnaire; pantoprazole; esomeprazole; eating habits

Dissertation Proposal Title: Relation of inflammatory markers of intestinal disease with the severity of motor and non-motor symptoms in Parkinson`s disease

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Mentor: Assist. Prof. Svetlana Tomić, M.D., Ph. D., Department of Neurology, Clinical hospital center Osijek, Croatia

Introduction: Parkinson's disease is a neurodegenerative disease that affects numerous structures of the central nervous system with consequent neuronal degeneration and decreased neurotransmitter levels.One of the most important structures affected is substantia nigra which produces dopamine. The disease is characterized by the appearance of motor and non-motor symptoms and the digestive system is often affected. According to the results of previous studies, there is a relationship between the gut microbiome, intestinal inflammation and increased intestinal permeability in the pathogenesis of disease. Several studies indicate an increased expression of proinflammatory cytokines in biopsies of the colonic mucosa and the presence of intestinal inflammation in patients with Parkinson's disease when compared to healthy subjects.

Hypothesis: The hypothesis states that patients with Parkinson 's disease have elevated levels of intestinal inflammation markers in stool sample relative to healthy subjects and that levels of these markers are associated with the severity of motor and non-motor symptoms of the disease.

Aims: The aim of the study is to examine levels of intestinal inflammation markers in a stool sample of patients compared to healthy subjects and to determine are these levels associated with the severity of motor and non-motor symptoms.This will include the determination of calprotectin, zonulin, lactoferrin, alpha-1-antitrypsin, beta-defensin, lysozyme and secretory immunoglobulin A levels.

Materials/Participants and Methods: Study will include 64 patients with Parkinson 's disease and 64 healthy controls. The analytes will be determined by ELISA method in the stool extract. The presence of motor and non-motor symptoms will be assessed using standardized questionnaires.

Research plan: This is a case-control study which will last for one year starting from July 2021.

Significance/Expected scientific contribution: Results of this study could indicate the digestive system as the entry point of environmental factors and its contribution to the onset of the disease. This could open the possibility of further examination of the disease etiology and possible prevention of its severe forms by early treatment of inflammatory processes in the intestine.

MeSH/Keywords: Parkinson; inflammation; intestinal; motor; nonmotor.

Abstract Title: Methodology, characteristics, and clinical validity of posterior root muscle reflex (PRMR) in the muscles of low extremities

Part of the Dissertation Proposal: Methodology, characteristics, and clinical validity of posterior root muscle reflex (PRMR) in the muscles of low extremities

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Mentor: Prof. Davor Jančuljak, M.D., Ph.D., Clinical hospital center Osijek, University of Osijek Faculty of Medicine, Osijek, Croatia

Introduction: The existing neurophysiological methodologies do not allow us to document lesions of all sensory and motor nerve roots at their entry/exit to the spinal or within the cauda equina. Transcutaneous electric stimulation of the cauda equina and recordings of PRMR show strong evidence that this reflex is equivalent to the H-reflex. Both reflexes are elicited by the same afferents but at different sites of the afferent reflex arc, the H-reflex at the level of the peripheral nerve while PRMR at the root level. With a single transcutaneous electrical stimulus over the cauda equina, all roots could be selectively stimulated either motor or sensory, resulting in PRMR in all muscles of low extremities. Therefore eliciting PRMR becomes a promising neurophysiological diagnostic tool for the different kinds of pathology of cauda equina.

Aims: Our goal was to demonstrate the elicitability of PRMR, collect our normative data in lower extremity muscles of healthy subjects, and correlate them with results in a small group of patients diagnosed with lumbosacral spinal roots lesions.

Material/Participants and Methods: 11 healthy patients (6 males and 5 females) between 21 and 74 y.o. (mean 45) and 4 patients diagnosed with polyradiculoneuritis were included in this cross-section prospective study. For eliciting PRMR, we modified the technique described by Minassian et. al. (1). The strong stimulus was applied over the bony level of cauda equina roots using a pair of disk electrodes. For recordings of PRMR, we used a pair of surface electrodes placed bilaterally over the skin of low limb muscles. Latency, amplitude, and area of the PRMR responses were measured from the best selectively elicited PRMR.

Results: The PRMR was successfully recorded in all healthy subjects, in all muscle groups, bilaterally. The characteristics of recorded PRMR were consistent with results in the earlier study(1). The PRMR was very stable with consistent latency values with considerable interindividual variability of amplitudes and areas. The measurements of areas of PRMR responses more correctly represent its values than measurements of amplitude. In patients with nerve roots involvement, we detected a complete loss of PRMR, significant PRMR area decrement, or latency prolongation in comparison with our normative values obtained in healthy individuals. All patients had abnormal parameters of PRMR.

Conclusion: This relatively novel method of eliciting PRMR is simple, non-invasive, and safe. It can give specific and sensitive information about involved sensory and motor lumbosacral nerve roots. It is a reliable technique with a high potential to become a new diagnostic tool for the lumbosacral root's functional integrity.

MeSH/Keywords: posterior root muscle reflex, cauda equina stimulation, monosynaptic spinal reflex, polyradiculoneuritis, lumbosacral spinal roots.

References:1.Minassian, K., Persy,I., Rattay,f., Dimitrijevic,M. R., Hofer,C.,Kern,H. (2007). Posterior- root muscle reflexes elicited by trnascutaneous stimulation of the human lumbosacral cord.Muscle Nerve 35(3):332-336.

Abstract Title: Effect of alendronate and hop extract on blood and serum parameters of ovariectomized Wistar rats

Part of the Disertation Proposal: The effects of administration of hop extract, alendronate and their combination in rat ovariectomy model of osteoporosis

PhD candidate: Edi Rođak, Faculty of Medicine Osijek, University of Osijek, Croatia
Mentor: Assist. Prof. Nikola Bijelić, Ph.D., Faculty of Medicine Osijek, University of Osijek, Croatia

Co-mentor: Prof. Nada Oršolić, Ph.D., Faculty of Science, University of Zagreb, Zagreb, Croatia

Introduction: Osteoporosis (OP) is a common metabolic disease of the bone, often caused by a lack of estrogen in menopause, leading to the loss of bone mass and increased fragility. In modern OP therapy, bisphosphonates (drugs that slow down bone resorption by osteoclast inhibition) are mostly used. Their long-term use is related to different side-effects.
Novel research shows the potential of phytoestrogens as supplemental or replacement therapy. Main hop phytoestrogens are 8-prenylnaringenin (8PN) and 6-prenylnaringenin (6PN). Xanthohumol, a main ingredient in hop extract, is a precursor for both 8PN and 6PN production in liver and intestinal microbiota.

Aims: To examine the changes in hematological and biochemical parameters in ovariectomized rats treated with hop extract, alendronate and their combination

Materials/Participants and Methods: Seventy 6 month-old female Wistar rats were divided into 7 groups. Bilateral ovariectomy was performed on 6 groups and sham operation on one group. The treatment was administered 1 month after ovariectomy by daily intragastric doses of alendronate and/or hop extract for 2 weeks. Hematologic analysis was performed on whole blood, while biochemical parameters and bone turnover markers were analyzed from serum samples. Kruskal-Wallis test with Dunn's post hoc analysis and Bonferroni correction was used for statistical analysis.

Results: Hop extract group (X) had more white blood cells compared to control group (C) and higher MCHC than untreated ovariectomized group (OV). Group receiving

lower dose of alendronate with hop extract (AL-X) had more red blood cells and hemoglobin and higher hematocrit compared to C, while higher dose of alendronate combined with hop extract (AH-X) lowered platelet count compared to C. Groups receiving high and low dose of alendronate (AH and AL) had lower platelet count compared to C. AH also had higher MCHC than both C and OV and AL had higher hematocrit than C. AH-X and OV both had lower albumin and total serum protein levels than C, while X had higher creatinine levels than AH group. AH-X group had higher values of P1NP compared to control. No significant difference was observed in CTX1 levels.

Conclusion: All tested groups showed some effect on blood and serum parameters. Changes in P1NP levels show that higher dose of alendronate with hop extract promotes bone formation more than alendronate alone. Further research on usage of hop extract in osteoporosis is warranted.

MeSH/Keywords: phytoestrogens, hops, osteoporosis, bone metabolism, 8-prenylnaringenin

Abstract Title: Molecular epidemiology of the West Nile virus infections in Osijek Baranja County

Part of the Disertation Proposal: Clinical characteristics and molecular epidemiology of West Nile virus infections in eastern Croatia

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CoMentor: Tatjana Vilibić-Čavlek, M.D., Ph.D., Croatian Institute of Public Health, Zagreb, Croatia, School of Medicine, University of Zagreb, Zagreb, Croatia

Introduction: West Nile virus (WNV) is a single-stranded RNA virus that belongs to the genus Flavivirus of the family Flaviviridae. The virus was isolated in 1937. from the blood of a febrile woman in the West Nile region of northern Uganda. Today, WNV is one of the most widespread arboviruses. Based on phylogenetic analyzes, 7 gene lines have been described so far, of which the most significant are lines 1 and 2. WNV infection is a zoonosis that occurs seasonally (summer, early autumn) related to the activity of mosquitoes (genus Culex). The primary hosts and reservoirs of the virus are different species of birds and the virus is maintained in a bird-mosquito-bird cycle. Also, other animals (horses) as well as humans can become infected.
The incubation period for infection is 2 - 6 (14) days. The clinical manifestations of WNV infection in humans are asymptomatic in 80 %, in about 20 % it manifests as WNV fever. Less than 1 % of patients develop a neuroinvasive form of the disease (meningitis, encephalitis, myelitis). Risk factors for the development of severe neuroinvasive forms of the disease are older age and immunosuppression, while the role of other underlying diseases such as arterial hypertension, diabetes, and cerebrovascular disease in the pathogenesis of WNV infection has not been fully elucidated. In such patients, the mortality rate can be 10 to 15 %, and survivors often have permanent neurological damage.
Although the onset of symptoms is preceded by a viremic period, viremia in humans persists for only a short period and the disease usually manifests after the virus is no longer detectable. Therefore, the diagnosis of WNV infection is usually based on serological methods. There is no specific drug and the therapy is symptomatic.

In the last two decades, cases of WNV infection in humans and animals have been continuously recorded in Europe. In Croatia, the first clinical cases of neuroinvasive WNV infection were described in 2012 in Osijek-Baranja County, after which the disease appeared sporadically or epidemically in subsequent transmission seasons in eastern and northwestern counties. Because a large number of infections are asymptomatic, the exact prevalence of WNV infection is unknown. Furthermore, so far in Croatia, only two strains of WNV detected during the 2013 epidemic have been sequenced and genetically characterized in the area of Zagreb which belonged to line 2.
According to the data of the Reference Center of the Ministry of Health for Epidemiology, around 200 cases of aseptic meningitis/encephalitis are reported annually in Croatia (50 cases in the eastern Croatian counties), of which 80 – 90 % have no proven etiology. Because WNV is not included in routine diagnostic algorithms, the significance of this virus in the etiology of neuroinvasive diseases, clinical and laboratory features, as well as molecular epidemiology, have not been fully elucidated. Also, the impact of underlying diseases on the severity of the clinical manifestations and the outcome of WNV infection has not been elucidated.

Aims: Determine the incidence of WNV infection in patients with neuroinvasive disease, define the clinical features of WNV neuroinvasive disease, define laboratory characteristics of WNV neuroinvasive disease, genotyping, and phylogenetically characterizing detected virus strains.

Materials and Methods: The study presents 24 patients with central nervous system infection caused by West Nile virus, hospitalized at the Clinic for Infectious Diseases, Osijek in the period 2012 – 2021. Serum, cerebrospinal fluid (CSL) and urine samples were taken from patients with neuroinvasive infection. Clinical symptoms and laboratory parameters were collected using a survey questionnaire. CSL and urine samples were screened for the presence of WNV RNA by real-time RT-PCR. Positive samples were further tested by classical and "nested" RT-PCR method to obtain genome segments of sufficient length for genotyping. All samples were extracted after gel electrophoresis from the gel and purified, and sequencing and phylogenetic analysis will be performed in the following period. Serological testing of serum samples and CSL (IgM and IgG antibodies, the avidity of IgG antibodies) have been made with commercially available enzyme-linked immunosorbent assays, and in case of cross-reactions, a confirmatory test by neutralization test was done. All nested PCR reactions were performed under the same PCR conditions as for the RT-PCR, but with exclusion of the RT step. Conventional PCR was carried out in Biometra T3000 PCR Cycler (Biometra, GmbH). Amplified products were visualized on 1% agarose gel. DNA samples extracted from excised gel fragments were Sanger sequenced in both

directions by Humanizing Genomics, Macrogen Inc. with the use of internal primers. After sequencing, the raw nucleotide sequences were assembled and the primer sequences were trimmed off. Genotyping and phylogenetic grouping of obtained sequences were based on comparison with strains retrieved from the GenBank and obtained using BLAST algorithm (http://www.ncbi.nlm.nih.gov). Maximum likelihood phylogenetic analysis was conducted and the evolutionary analyses were performed by using MEGA7 (Kumar, Stecher, & Tamura, 2016).

Results: In the period from 2012 to 2020, 24 patients (14 women and 10 men) with WNV neuroinvasive form of the disease were hospitalized. The mean patient's age was 65. WNV diagnosis was confirmed by the detection of WNV IgM and low avidity IgG antibodies in serum and CSF. Molecular and phylogenetic WNV analysis: five sequenced strains (phylogenetic neighbour-joining analysis of a 222 nucleotide fragment of the WNV NS5 gene) showed circulation of WNV lineage 2.

Conclusion: Detection of WNV in humans in Croatia highlights the importance of a continuing integrated human, animal and vector surveillance ('One health') of these emerging zoonoses. Since WNV became endemic in many Croatian counties (especially in Osijek-Baranja County), it is important that clinicians are reminded to include WNV in the differential diagnosis of aseptic meningitis during the arbovirus transmission season.

MeSH/Keywords: West Nile virus, clinical characteristics, molecular epidemiology, eastern Croatia, neuroinvasive infection.

Acknowledgment: This study was supported in part by the Croatian Science Foundation, Project No. IP-2016-06-7456: "Prevalence and Molecular Epidemiology of Emerging and Re-emerging Neuroinvasive Arboviral Infections in Croatia" (to Tatjana Vilibic-Cavlek).

Dissertation Proposal Title: The role of CGRP (calcitonin gene-related peptide) as a migraine biomarker in children

PhD candidate: Jadranka Sekelj Fureš, M.D., Children's Hospital Zagreb, Zagreb, Croatia

Mentor: Assist. Prof. Andrea Šimić-Klarić, M.D., Ph.D., Department of Pediatrics, Faculty of Medicine, University of Osijek, Croatia, Department of Pediatrics, County Hospital Požega, Croatia

Introduction: The most common symptom in neuropediatric clinic is a headache. It is known that the incidence of headaches increases with the age of the child, and by the age of 18 more than 90 % of adolescents had at least one headache attack in their lifetime.

The prevalence of headaches in children is 54.4 %, and the prevalence of migraine is 9.1 %. Headaches are classified as primary (the cause is intrinsic and located within the central nervous system) or secondary (headache is just a symptom of some other underlying disease).

Detailed diagnostic criteria for primary headaches, secondary headaches and painful cranial neuropathies are provided by the International Classification of Headache Disorders, 3rd edition (ICHD-3b).

The most common primary headaches in children are migraine and tension headaches, while trigeminal autonomic headaches are rare in children younger than 10 years of age.

Of all the primary headaches, migraine requires the greatest attention due to its acute-episodic occurrence characterized by recurrent attacks of moderate to severe throbbing pain lasting 2-72 hours. In addition to headache, nausea, vomiting, photophobia and / or phonophobia may also occur. The characteristics of migraine in children, in contrast to migraine in adults, are shorter duration of attacks and more frequent bilateral (bifrontal or bitemporal) pain.

Approximately 10 % of children with migraine suffer from migraine with aura where the aura includes visual, sensory, motor, retinal symptoms, speech disorders, or brainstem symptoms. Chronic migraine is the most common chronic headache in children and adolescents, and is characterized by a headache that occurs over 15 or more days during the month.

The diagnosis of primary headache is made clinically. According to the ICHD

classification of headaches in children, migraine can be diagnosed after five attacks of headache with migraine characteristics without aura or after two attacks of headache with migraine characteristics with aura, so sometimes the diagnosis requires months or even years of monitoring.
It is for this reason that there is a need to identify a specific and sensitive migraine biomarker that would be easy to determine even outside of a headache attack.
In the past 20 years CGRP (calcitonin gene related peptide) has been defined as a neuropeptide that has a clear role in the pathophysiology of migraine. It is secreted from the trigeminal ganglion and acts as a potent vasodilator of cerebral and dural blood vessels. Although the mechanism by which CGRP acts as a migraine trigger has not been fully elucidated, it is known that CGRP acts as a mediator of trigeminal vascular pain transmission from intracranial vessels to the central nervous system and is a vasodilating component.
It has been shown that the stimulation of the trigeminal ganglion induce CGRP release, and iatrogenic CGRP promotes migraine attack in migraine sufferers.
Elevated serum CGRP levels during migraine attacks have been found in both adults and children.
Further research has shown elevated serum CGRP values beyond the migraine attack itself, while this was not the case in primary tension-type headaches.
Elevated CGRP values normalized in patients with acute migraine after the introduction of sumatriptan therapy suggesting that triptans block the release of CGRP and thus lead to a reduction of discomfort.
CGRP has been defined as a new target for therapeutic options but also for migraine prevention, and monoclonal antibodies to CGRP and its receptors have been developed that show promising results in migraine prevention.

Hypothesis: CGRP can be a reliable biomarker of childhood migraine and thus can shorten the time of migraine diagnosis through detection of children which will suffer from migraine even after the first headache attack.

Aims: The main goal of this study is to answer the question of whether CGRP can be a reliable biomarker of childhood migraine and whether we can thus shorten the time of migraine diagnosis, and ultimately detect the group of children that would be the target group for CGRP antagonist therapy.As a secondary goal, it is important to mention the determination of reference values of CGRP in the group of children aged 5 - 18 years.

Materials/Participants and Methods: The subjects would be children aged 5 - 18 who were hospitalized or referred for examination to the Neuropediatric Department in Children's Hospital Zagreb due to primary headache.

To observe the mean effect in the difference of numerical variables between the three independent groups (control group, migraine headache, tension headache), with a significance level of 0.05 and strength 0.8, the minimum required sample size is 159 subjects (53 subjects per group).
All must meet the clinical and diagnostic criteria of primary headache, and would be divided into two main groups:
Group 1 - children with clinical criteria for migraine headache and
Group 2 - children with clinical criteria for tension headache.
After the diagnosis of primary headache, the serum CGRP value of both groups of subjects would be determined.
In order for a certain value of CGRP in serum to be informative, it is necessary to determine the reference values of the test depending on the age of the child, so it is necessary to have a control group of children in this study who have no anamnestic data on neurological problems.
Methods:
The following data will be collected from the included subjects:

- Clinical characteristics of headache, age of onset of first headache attack, associated diseases and disorders, results of common clinical and paraclinical methods that are otherwise routinely used in the diagnosis of children with primary headaches (e.g. TCCD, MRI / MRA of the brain).
- All subjects would be tested for serum CGRP by ELISA using one of the commercially available kits. The measurements would be performed in the Biochemical Laboratory of the Children's Hospital Zagreb
- The CGRP values of children with migraine and children with tension headache would be compared with the values of CGRP in the control group, and we expect significantly higher values of CGRP in the group of children with migraine.

Blood samples for CGRP determination will be taken after obtaining informed consent from the parent / legal guardian and the child older than 9 years, and no additional venipunctures will be performed for blood collection in addition to the venipunctures indicated and necessary as part of normal diagnostic processing.

Research plan:

1. Examine the values of CGRP biomarker by ELISA method in the Biochemical Laboratory in Children's Hospital Zagreb on blood samples of patients diagnosed with primary headache
2. Investigate the reference values of CGRP biomarker by ELISA method in the Biochemical Laboratory in Children's Hospital Zagreb on blood samples of the control group of patients
3. Compare the values of serum concentrations of CGRP in three groups of patients (control group, patients with tension headache, patients with migraine headache)

4. To examine whether there is a statistically significant difference in CGRP values in the group of patients with migraine compared to the other two groups.

Significance/Expected scientific contribution: We expect that the results of this study will show that the value of CGRP in the blood of children after the first attack of migraine headache is significantly elevated compared with the control group and the group of children with tension headache. Such a result would mean that the determination of CGRP can be introduced into the diagnostic algorithm in children with migraine and thus significantly shorten the scope of diagnostic processing as well as the time to diagnosis.

MeSH/Keywords: CGRP, Headache disorders, Children, Migraine, Tension-type headache

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.

PhD candidate: Igor Šesto, M.D., Magdalena - Clinic for Cardiovascular Diseases of the Faculty of Medicine, J. J. Strossmayer University of Osijek, Krapinske Toplice, Croatia

Mentor: Prof, Jure Mirat, M.D., Ph.D., J. J. Strossmayer University of Osijek, Faculty of Medicine Osijek, Osijek, Croatia

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: Effect of blood adiponectin levels on infflamatory response, severity of Clinical illness and final outcome in hospitalized COVID-19 patients

PhD candidate: Sonja Škiljić, M.D. University Hospital Centre Osijek, Osijek, Croatia

Mentor: Prof. Slavica Kvolik, M.D., Ph.D., University Hospital Centre Osijek; Faculty of Medicine Osijek, University of Osijek, Croatia

Introduction: Adiponectin is an adipocyte hormone with anti-inflammatory effects. The adiponectin levels are decreased in obese patients. Hypoadiponectinemia is speculated to play a key role in the relationship between obesity and COVID-19 respiratory failure.

Hypothesis: Lower blood adiponectin levels are associated with higher infflamatory response, severe illness and worse outcome in COVID-19 patients.

Aims: Aim of this study is to investigate correlation between blood levels of adiponectin, infflamatory response, severity of illnes and clinical outcome in hospitalized COVID-19 patients.

Materials/Participants and Methods: Single-center prospective observational study.Inclusion criteria: age >18 yeras, COVID 19- respiratory failure, hospitalization.
Exclusion criteria: age<18 yers, non COVID-19 respiratory failure, coexisting superinfection, acute surgical condition.
After Ethical approvement and patient informed consent sixty patients will be included. We will investigate: gender, age, body mass index (BMI), waist circumference, waist to hip circumference ratio, co-existing type 2 diabetes mellitus (T2DM) and hypertension. Adiponectin blood levels, Complete and Differential Blood Count, Neutrophil-Lymphocyte Ratio, IL-6, C-reactive protein (CRP), ferritin, plasma cholinesterase, procalcitonin (PCT), D-dimers, fibrinogen level, will be obtained from blood samples in time on admission to hospital (0 hour), 72 and 96 hour later and in time of intubation for patients who will be intubated and mechanically ventilated. Severity of illnes will be estimated by using Sequential organ failure assesment score (SOFA score), severity of acute respiratory distress syndrome (ARDS) according to PaO2/FiO2 and a need for intubation, MV and admission to ICU.

Research plan: Patients will be divided in two groups. GROUP A: non intubated, GROUP B: intubated, MV,admission to ICU. We will estimate days of hospitalization and MV, number of ICU days and final outcome. We will compare correlations of collected data between two groups using appropriate stastistic methods.

Significance/Expected scientific contribution: The adipocytokines have important role in many aspects of inflammation and immunity. Several recent studies indicate connection between adipocytokines and severity of COVID-19. Understanding of the underlying mechanisms which contributes to the severity of COVID-19 is important for developing more predictive diagnostics and possible treatment options.

Abstract Title: Elements of motivation, work environment and job satisfaction as predictors of turnover intention amoung nurses

Part of Dissertation Proposal: The effect of job satisfaction, work motivation, nursing practice environment and personal characteristics on job resigning

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Mentor: Assoc. Prof. Boštjan Žvanut, University of Primorska, Faculty of health sciences, Izola, Slovenija

Introduction: Many East European Union countries are facing severe problems with staff turnover, especially that of its nurses. In fact, job leaving is regarded as a major contributor to the shortage of nurses. As this represents a severe problem at both macroeconomic and microeconomic levels, policymakers and healthcare managers should undertake all necessary activities to diminish the incidence of this phenomenon.

Aims: The aim of this study is to examine, identify and describe the factors that influence turnover intenion of nurses in Croatia.

Materials/Participants and Methods: A quantitative, non-experimental research design was used. An anonymous survey was performed, where a closed-ended questionnaire was used as an instrument for collecting the data. The data were collected between 1st February and 31st March 2017 in Croatian towns: Pula, Rijeka and Karlovac, where 125 registered nurses (RN) voluntary responded to the questionnaire in paper form. The translated Multidimensional Work Motivation Scale (MWMS) and Practice Environment Scale of the Nursing Work Index (PES-NWI) was used.

Results: The results indicate that nurses' job dissatisfaction, combined with a higher rate of absenteeism, represents a clear indication of their future turnovers. Nursing practice environment and personal motivation do not have a significant direct effect on the Intention to leave the job, but do have an indirect one through job satisfaction.

Conclusion: Recognizing nursing challenges in healthcare system and the factors which influence the intention of nurses to leave can help to development of a clear strategy and retention measures according to factors that influence their turnover intention.

MeSH/Keywords: Motivation; Work environment; Personal autonomy; Intention to leave; Self determination Theory

Acknowledgement: We would like to thank: Marylène Gagné, BA McG., MA PhD Roch., School of Psychology, University of Western Australia for her consent. Finally, we would like to thank all the participants of the study.

Dissertation Proposal Title: 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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Mentor: Prof. Davor Plavec, M.D., Ph.D., Srebrnjak Children's Hospital, Zagreb, Croatia

Co-mentor: Assist. Prof. Sanda Škrinjarić-Cincar, M.D., Ph.D., Faculty of Medicine Osijek, University of Osijek, Osijek-Baranja County Health Center, Osijek, Croatia

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. Mortality from COPD is low at age 45, but increases significantly at age 65 with a significant comorbidity. COPD is a huge economic and public health problem, especially related to severe illness and exacerbation. The main risk factor for developing COPD is tobacco smoke, but only < 1/3 of smokers develop COPD during their lifetime. Due to the progressive nature of the disease, COPD leads to premature disability and death. Stopping or slowing down the progression of the disease is still unavailable to therapeutic interventions although their effect has been shown to be more significant in the early stages of the disease. Early diagnosis should allow an early intervention and thus possible prevention of COPD progression. The only existing method of early diagnosis suitable for screening is spirometry (which is also used as the 'gold standard' for the diagnosis of COPD). Although global screening is not recommended by the guidelines, recommendations for early diagnosis (finding cases) is advocated. The established innovations in the research of the genetic basis for the development of COPD based on large cross-sectional studies through the population require their confirmation on a global level through appropriate prospective studies. In contrast, symptoms and health-related loss of quality of life (HRQoL) often precede diagnostically significant loss of lung function (FEV1/FVC < 0.7 or < lower limits of normal [LLN]). Therefore, we need new simple tools to identify patients in the early (pre)symptomatic phase of the disease before the onset of significant damage to the target organ. Based on the assumptions made; the possibility of identifying a population at risk and the possibility of significant benefit to patients if COPD is detected early, we decided to develop and test a new tool (to be used alone or in combination with other markers) in a prospective cohort

study of at-risk populations (active smokers with significant cumulative exposure to tobacco smoke, without a previous diagnosis of COPD). Newly developed tools should be based on screening tool assumptions; possibility of use globally, inexpensive, self-applicable, moderate to high sensitivity and high specificity (no false positive results) for COPD. 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 (Clinical COPD Questionnaire - CCQ, 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).

Hypothesis: The MARKO questionnaire score at inclusion and/or its individual domains will be statistically significantly associated with the risk of incident COPD.

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 5 years monitoring period. Secondary objectives: (a) to determine the cumulative incidence of GOLD I-III COPD in subjects enrolled in follow-up (active smokers without COPD) over 5 years of follow-up; (b) identify the diagnostic parameters that are sensitive for early changes in the development of COPD; (c) compare the predictability of the MARKO questionnaire for the development of COPD during 5 years of follow-up with other diagnostic tests (SGRQ, CAT, lung function, 6MWT).

Participants and Methods: In the study, according to the inclusion and exclusion criteria subjects were selected at a GP office and referred to pulmonologists (N = 450). At the initial examination by a pulmonologist, 58 subjects were diagnosed with COPD (53 GOLD I and 5 GOLD II grade) and 392 of them were selected for further monitoring (of which 171 in grade GOLD 0). The first follow-up visit to the pulmonologist after 2 years was attended by 320 subjects (72 lost from follow-up, refused to come for a check-up or died), and the second after 5 years by 260 subjects (60 lost from follow-up, refused to come for check-up or died).
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 (cardiovascular, cerebrovascular, diabetes, hepatitis, nephropathy, chronic dialysis, systemic disease, cancer) that significantly affects quality of life; (b) on immunosuppressive therapy; (c) significant acute respiratory illness (pneumonia, TB, influenza, rhinosinusitis) 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: they completed 3 self-assessment questionnaires - MARKO questionnaire, SGRQ and CAT, ECRHS II Respiratory Questionnaire, medical history (with comorbidities) and physical, laboratory (CBC, hsCRP), lung function tests (spirometry with bronchodilator test, COPD6 measurements, 6MWT), and EBT.

At the follow-up examinations at the pulmonologist after 2 and 5 years, the following workout was performed: 3 self-assessment questionnaires - MARKO, SGRQ and CAT, history (with comorbidities) and physical, lung function tests (spirometry with bronchodilator test, 6MWT), and EBT.

1. History and physical: Detailed history with an emphasis on respiratory problems and the main symptoms of COPD: cough, chronic expectoration and shortness of breath, and smoking history was taken. The feeling of shortness of breath was graded according to the mMRC scale, a 5-point scale (0-4) that only grades the severity of dyspnea. Other symptoms indicative of COPD were examined: nocturnal awakenings, chest pain, and lack of energy. The presence of associated chronic diseases as well as the use of chronic therapy were determined. A clinical examination with vital signs and auscultation of the lungs was performed, distinguishing respiratory noise according to intensity (normal/weakened), expiration with regard to duration (normal, prolonged) and the presence of bronchial sounds (yes/no).

2. The MARKO questionnaire was designed and developed by a group of experts (three physicians pulmonologists and two psychologists) and contains 18 questions covering the manifestations and frequency of symptoms that occur in the initial stage of COPD and can affect quality of life. The overall score ranges from 0 to 57, a higher score indicates a worse HRQoL level.

3. The CAT is a validated, short (8-question), simple self-administered questionnaire with good discriminant properties, developed for use in routine clinical practice to assess the health status and HRQoL of patients with COPD.

4. The SGRQ is a standardized and self-administered questionnaire specializing in respiratory diseases divided into three domains: symptoms (8 questions), activities (16 questions), and impact on the patient (26 questions). For each domain and for the entire questionnaire, the score range is from 0 (no problems) to 100 (maximum problems).

5. Vitalograph COPD6: The device allows fast, easy and accurate detection of COPD without the risk of false negative results, displaying values of forced expiratory volume in 1 and 6 seconds (FEV1, FEV6), the ratio and % of expected values, obstruction

index and lung age. Based on the measured parameters, the device classifies patients according to the GOLD guidelines.
6. Spirometry with bronchodilator test: Lung function was measured by the standardized spirometry in accordance with the official recommendation of the European Respiratory Society (ERS). Spirometric parameters were measured: forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1), peak expiratory flow (PEF) - expressed in L and L/min and as % of expected, and FEV1/FVC ratio (Tiffenaeu index) expressed as a percentage. The bronchodilator test was performed 20 minutes after inhalation of 400 mcg of salbutamol.
7. 6-minute walk test with measurement of blood oxygen saturation (6MWT): A practical and simple test to assess the functional capacity of exercise done according to ERS recommendations. The test performance time was measured, and the distance the subject walked was expressed in meters. This test assesses the submaximal level of functional capacity, at which most daily physical activities are performed.
8. Exhaled breath temperature (EBT): As a consequence of inflammation and remodeling/destruction of the airways, changes in blood flow through the airway system occur affecting the temperature of the exhaled air. EBT was measured before and after smoking a cigarette using an X-halo device (Delmedica Investments, Singapore). It is a simple device in which air inhaled through the nose is exhaled through the mouth directly into the device for one to five minutes until a stable exhalation temperature is reached.
9. Blood biomarkers: A peripheral blood sample (1 tube, 5 ml) was taken in the morning on an empty stomach and a complete blood count (CBC) and highly sensitive C-reactive protein (hsCRP) were determined.
10. Statistical analysis: For the purposes of this doctoral dissertation, 260 subjects (205 without disease progression and 55 with disease progression) who were monitored and evaluated after 5 years will be included in the analysis. Sample size was calculated based on the initial study which estimated the incidence of COPD 18% over 5 years of follow-up, with an estimated mean (SD) difference of the MARKO score of 3 (5) with a statistical strength of 80% and bilateral $\alpha = 0.05$. The risk of developing the disease will be assessed in subjects without an initial diagnosis of COPD by comparing those diagnosed with COPD after 5 years of follow-up with those who do not meet the criteria for a diagnosis of COPD. The risk of disease progression will also be assessed according to the following criteria: (1) a subject who changes the severity of COPD (from GOLD 0 to GOLD I-III) at the end of 5 years of follow-up; (2) a subject who has accelerated loss of pulmonary function (annual rate of loss of postbronchodilator value FEV1> 70 mL). Numerical data will be expressed by arithmetic mean (AS) and standard deviation (SD) or as median and interquartile range (IQR) depending on the distribution, and categorical data will be expressed by number and percentage. Variables not having a normal distribution will be normalized and as such used in

later analyzes. Differences in distribution between groups will be compared by chi-square test or Fisher's exact test, and values of numerical data by Student's t-test or analysis of variance. Bonferroni correction will be used for multiple comparisons. The analysis of the MARKO questionnaire will focus on secondary validation to determine the validity of the construct by factor analysis and the predictability for the incident COPD. Factor analysis will select those issues that alone or in combination with other variables further contribute to the multivariate model. The correlation of individual variables and their number in the final model will be determined by univariate regression analysis, and the final outcome will be presented binary as the odds ratio (OR). Outcome-related variables in univariate analysis at the $p < 0.20$ level will be analyzed using multivariate regression analysis. ROC analysis will determine the predictive value (specificity, sensitivity, positive and negative predictive value) of the MARKO questionnaire. Statistical analysis will be performed using the statistical software package Statistica version 12 (StatSoft, Inc. Tulsa, OK). The results with the significance level $p < 0.05$ will be considered statistically significant.

Research plan:

Prospective cohort research

The doctoral dissertation will be done as part of the "MARKO" study registered at https://clinicaltrials.gov/ (Early Detection of COPD Patients in GOLD 0 (Smokers) Population (MARKO), number: NCT01550679), a multicenter prospective study of a cohort of subjects at risk for developing COPD (aged 40 to 65 years with a smoking experience of at least 20 pack-years). The study was conducted through 40 family medicine practices and in 7 hospitals (Pulmonology departments) in the Republic of Croatia. In the first phase through the family doctor's offices (GPs), subjects at risk for developing COPD were recruited, according to the inclusion and exclusion criteria (N = 450). The respondents filled in the MARKO questionnaire and their lung function was measured using the COPD6 device. After 2-4 weeks they were referred to a pulmonologist where they were underwent the diagnostic protocol to rule out or confirm a possible diagnosis (and stage of COPD). Pulmonologists performed a detailed workout: medical history and physical, the respondents completed 3 self-assessment questionnaires - MARKO questionnaire, Saint George Respiratory Questionnaire (SGRQ) and the COPD Assessment Test (CAT), and the Respiratory Questionnaire from the European Community Respiratory Health Study II (ECRHS II), comorbidities were determined, laboratory tests (CBC, hsCRP), lung function tests (spirometry with bronchodilator test, 6-minute walk test (6MWT)), exhaled breath temperature (EBT) before and after smoking a cigarette. The pulmonologist ruled out or confirmed the diagnosis of COPD (N = 58) and assessed the severity (GOLD I, N = 53; GOLD II, N = 5). The second phase of follow-up over 2 years included subjects without a diagnosis of COPD and those in grade GOLD 0 (symptomatic smokers with

postbronchodilator FEV1/FVC ≥0.7, N = 171) (392 in total). Subjects included in the second phase of follow-up were re-evaluated by a pulmonologist after 2 and 5 years with regard to the presence of diagnosis and severity of COPD (3 self-assessment questionnaires - MARKO, SGRQ and CAT, history and clinical examination, lung function tests (spirometry with bronchodilation test, 6MWT), and EBT).

Expected scientific contribution: New knowledge about the risk of development of COPD in the exposed population and the predictability of the newly constructed MARKO questionnaire as a standalone tool or in combination with other markers and characteristics of subjects such as EBT, lung function, 6MWT and systemic inflammatory markers (hsCRP, WBC). We expect that the results of this research will improve our understanding of COPD development and offer sensitive and simple markers of incidence of COPD, thus providing people at risk (sensitive to tobacco smoke) the possibility of an early risk detection, early intervention and secondary prevention and improved care for the long-term consequences of COPD.

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

Abstract Title: Consumption of nutritionally enriched eggs enhances endothelial function in healthy young persons

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

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Introduction: Functional food, in addition to the appropriate nutritional effects, also has a beneficial effect on one or more targeted functions in the body, improves health and well or reduces the risk of disease. Recent research within the Scientific Center of Excellence for Personalized Health Care has shown that consumption of n-3 polyunsaturated fatty acids (n-3 PUFAs) - fortified eggs had a positive effect on microvascular reactivity, and the level of oxidative stress and mild anti-inflammatory effects in healthy young subjects of both sexes. The next step in functional food research is to further examine eggs enriched with other antioxidants.

Aims: The aim of this study was to determine the effect of consumption of hen eggs enriched with four nutrients (n-3 PUFA, lutein, vitamin E and selenium) on microvascular reactivity in healthy young subjects and to assess potential role of metabolites of COX1 and COX2 in that response.

Participants and Methods: This was a randomised, 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=15; consumed regular hen eggs (approximately 595 mg of vitamin E, 110 mg of lutein, 0.181 mg of selenium and 438 mg of n-3 PUFAs) and Nutri4 group (n=20)

who consumed enriched eggs (daily in total approximately 1098 mg of vitamin E, 616 mg of lutein, 0.191 mg of selenium and 1026 mg of n-3 PUFA). All subjects took 3 hard boiled hen eggs per day for 3 weeks. The concentration of free-fatty acids and vitamin E in serum was measured before and after dietary protocols.
Skin microvascular blood flow in response to post-occlusive reactive hyperemia (PORH) and iontophoresis of acetylcholine (AChID) and sodium nitroprusside (SNPID; endothelium-independent) were assessed by laser Doppler flowmetry before and after dietary protocols. In separate sets of experiments, participants took per os a 100 mg of indomethacin (a non-selective cyclooxygenase inhibitor) before functional (LDF) measurements. Protein expression of enzymes important in microvascular reactivity (COX1, COX2, iNOS, eNOS and nNOS) was assessed from peripheral blood mononuclear cells (PBMC) by Western blot before and after respective dietary protocol. Arterial blood pressure (BP), heart rate (HR) and body composition, body fluid status, anthropometric measurements were measured before and after respective dietary protocol. In addition, lipid profile, concentration of urea, creatine, urate, AST, ALT, GGT, sodium, potassium, iron, transferrin, glucose, calcium, and hsCRP were measured in serum. 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 Science Center of Excellence, 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: PORH and AChID were significantly enhanced, and SNPID remained unchanged in Nutri4 group, while none was changed in Control group following respective diet. After administration of 100 mg indomethacin, decreased response to PORH was observed in the Nutri4 group compared with measurements before egg consumption. In protein expression, the COX2 enzyme was significantly higher in the Nutri4 group, while expression of other enzymes remained unchanged. LDL cholesterol was significantly increased in Control group compared to Nutri4 group. Urea and glucose were significantly increased in the Nutri4 group compared to Control group after protocol, but still within the reference range. Vitamin E, pentaenoic acid and docosahexaenoic acid was significantly higher in the Nutri4 group after the dietary protocol. After dietary protocols, there was no significant statistical difference in body composition, body fluid status, BP and HR.

Conclusion: Consumption of functionally enriched eggs improves microvascular endothelium-dependent vasodilation in healthy young subjects. The observed changes may be attributed to altered production of vasoactive metabolites of COX pathways.

MeSH/Keywords: microcirculation, endothelium, vasodilation, cyclooxygenases, postocclusive hyperemic response, functional food, hen eggs

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: Serum periostin as a potential biomarker for asthma symptoms in children with history of respiratory syncytial virus infection

Part of the dissertation proposal: Risk factors for allergic sensitization and atopic diseases development in children after infection with respiratory syncytial virus

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Introduction: Periostin is a matricellular protein that have a role in development of allergic diseases, up-regulated in response to IL-4 and IL-13. Previous studies reported that periostin can be a non-invasive biomarker of T2-driven inflammatory response in asthma in adults, with inconsistent results in children.

Aims: to determine the usefulness of serum periostin levels as a potential biomarker for asthma, especially recent asthma symptoms in children who have been infected with respiratory syncytial virus (RSV) in the first two years of life.

Material/participant and methods: This prospective study observed 72 children from birth. RSV infection was confirmed with positive serum specific RSV Immunoglobulin G (IgG) at one and/or two years of age. Asthma was diagnosed according to International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire at 10 years of age. Fractional exhaled nitric oxide (FeNO), lung function, skin prick test and blood samples for analysis of specific immunoglobulin E (sIgE) on standard pallet of inhalant allergens, total IgE (tIgE) and periostin were provided.

Results: At 10 years of age, asthma was diagnosed in 23 (31.9 %) of the observed children. In 15 (20.8 %) of them who reported asthma symptoms during the last 12 months, median serum periostin levels were 40.04 ng/ml. In 57 (79.2 %) children who were free of asthma symptoms in the last 12 months, mean serum periostin levels

were 30.57 ng/ml. Serum periostin levels correlated significantly with recent asthma symptoms (Kendall's Tau-B = 0.236, p = 0.016).

Conclusion: Serum periostin levels significantly correlated with asthma symptoms during the last 12 months in 10-year-old children who were infected with RSV during their first two years of life. Our preliminary results suggest that serum periostin level may be useful in diagnosing asthma in children, especially those with recent asthma symptoms and history of RSV infection in infancy.

MeSH/Keywords: biomarkers, periostin, pediatrics, asthma, allergic rhinitis, atopic dermatitis

Abstract: Influence of motivational interviewing and intensive education of insulin titration patients on basal oral therapy

Part of the Disertation Proposal: The impact of intensive education and motivational interview on glycemic levels and quality of life

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Introduction: The prevalence of diabetes has reached global proportions with 202 million cases projected by 2035. Croatian guidelines for the treatment of type 2 diabetes emphasize education as the cornerstone of treatment. Studies show that 16 – 28 % of patients achieve adequate insulin titration at the beginning of therapy. The introduction of insulin therapy presents a risk for laps in titration and application, which can lead to development of complications and fatal outcomes. Poor patient motivation and uncertainty in titration is an important factor of poor glycemic control and the reduction in the number of hypoglycemic events in patients on basal oral therapy (BOT). A unanimous agreement of healthcare professionals and patients exist to develop additional tools and models to help patients achive better glycemic control and prevent short and long term complications. In this intervention study, regular education and motivational interviewes will be performed every two weeks for 6 months by a nurse

Aims:
1. Examine the effect of motivational interview and intensive education on glycemic control and the reduction in the number of hypoglycemic events compared to the standard form of education after 3 and 6 months
2. Examine the impact of motivational interview and intensive education, performed every two weeks, on the subjects' quality of life compared to the standard form of education after 3 and 6 months

Materials/Participants and Methods: Patients at the County hospital Čakovec meeting following criteria will be included in the study: diagnosis of type 2 diabetes for at least a year, age 18 years or older, treated with oral hypoglycemics and basal insulin. Hospital information system will be used for collecting HbA1c values. Patient self control diary will be used for evaluation of hypoglycemic events. To observe the mean effect in the difference of numerical variables between two independent groups of subjects, with a significance level of 0.05 and a strength of 0.9. Sample size is 10 subjects per group, a total of 20 subjects

Results: HbA1c was significantly reduced in the intervention group after 6 months. In both groups, intervention and standard, there was significantly less hypoglycemia after 3 and 6 months (*Mann Whitney U test, P = 0,01*; P<0,05). Analyzing the WHOQOL-BREF questionnaire there are no significant differences between two groups in all areas of quality of life

Conclusion: Intensive education and motivational interview regarding insulin titration of patients on BOT conducted by a nurse every two weeks, leads to better glycemic control , especially after 6 months.
Also intervention leads to less hypoglycemic events, especially after 3 months. There is no difference in quality of life after intervention

Keywords: basal oral therapy, diabetes, education, hypoglycemia, nurse

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 Disertation 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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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

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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 why 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

The list of PhD Candidates, Mentors and Titles of abstracts of annual seminars

ISBN: 978-953-7736-57-6