dies doctorandorum
BOOK OF ABSTRACTS
2019

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BOOK OF ABSTRACTS

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Foreword

Faculty of Medicine Osijek for the fifth time celebrates the Days of PhD candidates - Dies doctorandorum 2019., an annual event, which is mainly of a scientific nature but also opened to the general public, where the research results of our doctoral students and their mentors is opened up to a wider analysis.

As it was the case in prior years, the main purpose of this event is to give PhD students a platform to show their peers and others their research interests and data in a surrounding consisted of other experts. I have no doubt that the participants will greatly benefit from the multitude of questions, observations and comments this kind of environment can provide. There is no doubt, the event will also help them to better understand the inner functioning of the scientific community, as well as to strengthen their dissemination skills through valuable experience in communicating their research.

Of course, I also hope this event will provide the possibility of doctoral students to connect with other people who share their interests, possibly other experts in the same field. It would be a great opportunity to seize this moment which could later result in further productive collaboration.
Dies doctorandorum 2019 is imagined to be informal as much as possible in order to create a space of discussion contributing to the scientific progress and integrity of our PhD candidates.

Despite this informal nature of the event, we will use the time being to observe the professional development of our PhD students. Having this priority in mind, and in accordance, with previous years the best poster presentations will be chosen by the Members of the Committee for Doctoral Studies and awarded a Dean's award.
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. So, I would consider it positive if the audience could create an encouraging and inspiring atmosphere for our young candidates so they can feel satisfaction and pride related to their sometimes difficult and valiant efforts to further scientific development.

Professor Jure Mirat, M.D., Ph.D.
Dean, Faculty of Medicine Osijek

Abstracts of annual seminars

Dissertation Proposal Title: Changes in thickness of the neurosensory retinal layers in 5 defined macular areas after phacoemulsification cataract surgery in correlation with the amount of ultrasound energy used in healthy individuals and diabetics

PhD Candidate: Slaven Balog, M.D., Ophthalmologic clinic dr. Balog, Osijek, Croatia

Mentor: Assist. Prof. Antonio Kokot, M.D., University of Osijek, Faculty of Medicine Osijek, Osijek, Croatia

Introduction: Cataract surgery performed by ultrasound (phacoemulsification) is a common method of cataract surgery today(1).
It has been shown that a cataract surgery by ultrasound method causes structural changes in the neurosensory retina of the posterior segment of the eye, more precisely in the region of the macula at the posterior pole (2,3), which is responsible for both central visual acuity and part of the peripheral vision.
Any pathological event in the posterior pole of the eye results in visual impairment and may create serious difficulties in the day-to-day functioning of the individual as a person.
There are many published papers that show the relationship between the ultrasound method of cataract surgery and the neurosensory retina thickening in the center of the macula-(foveola) (2,3), however, the published references do not mention the interrelation of consumed ultrasound energy during phacoemulsification and stratification changes in the thickness of each retinal layer in the macula, nor display changes the thickness of the neurosensory retina and individual retinal layers outside the center of the macula in healthy subjects and patients with diabetes mellitus

Hypothesis: Thickness change of the neurosensory retinal layers in 5 defined macular areas after phacoemulsification cataract surgery correlates with the amount of ultrasound energy used in healthy individuals and diabetics

Aims: Define the amount of ultrasound energy delivered during cataract surgery by phacoemulsification method, Define the change in the total thickness of the neurosensory retina as well as each individual retinal layer within the 5 defined macular areas, Determine the relationship between the amount of ultrasound energy delivered and the change in thickness of each individual retinal layer in the macula, as well as outside the same, by defined areas in healthy subjects, Establish a relationship

between the amount of ultrasound energy delivered and the change in thickness of each individual retinal layer in the macula, as well as outside the same, by defined areas in subjects with diabetes mellitus but without retinal involvement

Research plan: The study is planned as a prospective study that compares the total thickness of the neurosensory retina, as well as each individual retinal layer in 5 defined macular areas and the amount of ultrasound energy delivered during cataract surgery by ultrasound method of phacoemulsification. The measurement of the total thickness of the neurosensory retina, as well as its individual layers, will be done by "optical coherence tomography-OCT" on the OCT Spectralis Heidelberg device, while the amount of delivered ultrasound energy will be read from the Oertli Faros ultrasound surgery device. Patients of the Dr. Balog Polyclinic, teaching base of the Osijek Medical Faculty, regularly scheduled for cataract surgery will be included in the study. The order of study: Hypothesis, Organization of the study, Data source, Method of sampling (according to the inclusion and exclusion criteria), Pattern formation, Grouping data by groups (age, gender), Archiving data, Statistical data processing, The results, Analysis of the obtained results

Materials/Participants and methods: The subjects will be patients in the regular program planned for ultrasound surgery according to the previously defined inclusion and exclusion criteria.
Neurosensory retinal thickness measurement data expressed in microns will be collected through a Spectralis OCT Heidelberg device, while ultrasound energy values during cataract surgery will be read on the device for cataract surgery Oertli Faros.
Inclusion criteria: the presence of senile cataract,

- structurally neat posterior segment of the eye
- diabetes mellitus without retinal or macular involvement

Exclusion criteria: Diabetic maculopathy, Diabetic retinopathy, VMT (vitreomacular traction), glaucoma, any other retinal pathology of the posterior segment of the eye, posttraumatic events of the eye, infection of the eye, systemic diseases with an impact on the eye other than diabetes

Significance/Expected scientific contribution: Complete insight and contribute to understanding the relationship between the amount of ultrasound energy delivered during the phacoemulsification process and changes in both; the total thickness of the neurosensory retina and the thickness variation of each retinal layer separately in the 5 defined areas of the macula, but also outside in healthy subjects as well as in subjects with diabetes without recorded diabetic maculopathy.

MeSH/Keywords: Cataract**,** Retina**,** Macula**,** Phacoemulsification**,** OCT**,** Diabetes mellitus

Abstract Title: Clustering of mental and physical comorbidity and the risk of frailty in patients aged 60 years or more in primary care

Part of the Dissertation Proposal: Classification of patients general/family medicine into subgroups based on differences in the degree of severity of frailty syndrome and affective and cognitive disorders

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Mentor: Assoc. Prof. Ljiljana Trtica Majnarić, M.D.,PhD., Department of Internal Medicine, Family Medicine and the History of Medicine, Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia; Department of Public Health, Faculty of Dental Medicine and Health, University of Osijek, Osijek, Croatia

Introduction: Many problems in clinical medicine are characterised by high complexity and non-linearity. Particularly, this is the case with ageing diseases, chronic medical conditions that are known to tend to accumulate in the same person. This phenomenon is known as multimorbidity. In addition to the number of chronic diseases, the presence of integrated geriatric conditions and functional deficits, such as walking difficulties, of frailty - a general weakness associated with weight and muscle loss and low functioning, are important for the prediction of negative health outcomes of older people, such as hospitalisation, dependency on others or pre-term mortality.

Aim: This study aimed to identify the clustering of comorbidities, cognitive and mental factors associated with increased risk of pre-frailty and frailty, in patients old 60 years and more, in a primary healthcare setting in Osijek region, eastern Croatia.

Materials/Participants and Methods: A total of 159 patients were included in the cluster analyses. They were ≥60 years old and underwent a four-month follow-up. A multicomponent dataset was used, with data collected in the primary healthcare setting in Osijek region, eastern Croatia. Frailty was determined using the five criteria of Fried's phenotype model. Levels of anxiety and depression were recorded using the Geriatric Anxiety Scale (GAS) and the Geriatric Depression Scale (GDS), and the Mini-

Mental State Examination (MMSE) score assessed cognitive impairment. Bar diagrams and ven diagrams were used to represent distributions of geriatric conditions, their overlaps and distributions of somatic comorbidities. Clustering with k-means algorithm was used to identify clinical phenotypes associated with pre-frailty and frailty. Logistic regression models were used to identify predictors of frailty and pre-frailty.

Results: The generated three clusters demonstrated a high degree of overlap, indicating three overlapping clinical phenotypes. The first cluster contained 50 patients, the second cluster contained 74 patients and the third cluster contained 35 patients. The identified phenotypes could be characterised as: Obesity and preserved renal function (cluster No.1), Multimorbidity with mental disorders and slightly impaired renal function (cluster No.2), and Low renal function, cognitive impairment, and physical frailty (cluster No.3). Pre-frailty and frailty, in clusters No.1 and No2., were found in about a half of patients. The highest proportions of pre-frail and frail patients, compared to non-frail ones, were found in the cluster No.3 (in about 80% of patients), with the predominance of frail patients. The predictors of outcome included increasing age, number of chronic diseases, inflammation, anemia, anxiety, and cognitive impairment, and reduced muscle mass.

Conclusions: The results have shown that in primary care patients old 60 years and more, the major predictors of pre-frailty and frailty are cognitive function and renal function decline.

MeSH/ Keywords: cluster analysis, chronic diseases, comorbidity, frail elderly people, chronic kidney failure

Dissertation Proposal Title: The coccoid form of Helicobacter pylori is correlated to histopathological characteristics of stomach mucosa in Helicobater pylori associated gastritis

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Mentor: Assist. Prof. Dražen Švagelj, M.D., Ph.D., Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia; General Hospital Vinkovci, Vinkovci, Croatia

Introduction: Helicobacter pylori (HP) causes number of gastrointestinal diseases in humans. Numerous studies have confirmed that about 90% of gastric cancer (intestinal type) and MALT lymphoma is associated with HP infection. It is estimated that around 50% of the world's population is infected with HP. The average HP seroprevalence rate in the population of Croatia in the age groups of 20 to 70 years is between 60.4% and 68%. The bacterium in special conditions can move to a latent coccoid form. Several different studies have shown that the coccoid form of HP can cause acute gastritis. The advantage of the histological method is the ability to grade gastritis according to the revised Sydney Classification. It is recommended to use an immunohistochemical method for exact HP testing.

Hypothesis: The coccoid form of HP in HP associated gastritis is correlated to age, gender and pathohistological characteristics of stomach mucosa

Aims: To evaluate by immunohistochemical method is it the coccoid form of HP correlated to age, gender or pathohistological characteristics of stomach mucosa. To evaluate is it there a difference in the incidence of the coccoid form of HP due to the place of gastric mucosa biopsy (antrum and corpus).

Materials/Participants and Methods: The material will be samples of gastric mucosa obtained by ambulatory gastroscopic examination of patients with dyspeptic disorders in the period January 2019.-January 2020. The study will analyze the samples taken according to the recommendations of the revised Sydney classification. This material will be collected from the archives at the Department of Pathology and Cytology of the General Hospital Vinkovci. Paraffin blocks of samples prepared by standard histological protocol and stained with hemalaun-eosin, will be stained by immunohistochemical method on HP antigen. A positive immunohistochemical

reaction to HP will be quantified: 0 (vegetative form, without the presence of coccoid HP); 1 (coccoid and vegetative form of HP) and 2 (coccoid form of HP only). Materials without the presence of coccoid form HP will be used as a control group.

Research plan: 1.2019.-1.2020. Collecting of gastric specimens.
2.-4.2020.- Immunohistochemical staining on HP antigen. Reading the findings. Entering data into an Excel table. 5.-7.2020.- Statistical data processing. Writing a concept for a scientific article.

Significance/Expected scientific contribution: Because the HP is carcinogenic, the immunohistochemical method for HP detection should become routine to make an accurate diagnosis. Our assumption is that with more accurate diagnosis of the HP infection, a decrease in the incidence of cancer and gastric lymphoma is expected.

MeSH/Keywords: Helicobacter pylori, biopsy, immunohistochemistry, gastritis

Dissertation Proposal Title: Epidural steroid injection as opposed to percutaneous laser disc decompression in treating lumbar radicular pain

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Mentor: Assist. Prof. Ivan Radoš, M.D., Ph.D., Department of Anesthesiology, Reanimatology and Intensive Medicine, University Hospital Centre Osijek/Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Introduction: Lumbar pain is defined as pain in the lumbar spine, with or without propagation to the legs. It is a major public health, social and economic problem in modern society. It is estimated that 80% of the world's population experiences pain in the lumbar spine at least once during their life. If left untreated, acute lumbar pain goes into chronic pain syndrome. The mechanism of pain has several causes: mechanical stimulation of nerve endings in the outer part of the fibrosus annulus, direct pressure on the nerve root, and/or a chemical inflammatory cascade triggered by a protruding nucleus pulposus. Clinical examination, Lasegue test and magnetic resonance imaging are used to diagnose lumbar radicular pain. In order to avoid systemic and side effects of analgesics, undergoing general or regional anesthesia and long and extensive operations, minimally invasive procedures are used in the treatment of these pain. Epidural steroid administration (ESI), as well as percutaneous laser disc decompression (PLDD), are some of these procedures. Both methods are performed under the control of a fluoroscope. A corticosteroid solution, topical anesthetic, and saline solution is used for ESI. There are multiple anatomical approaches to the epidural space such as the transforaminal, interlaminar, and caudal approaches. Percutaneous laser disc decompression is a minimally invasive method of treating lumbar radicular pain using laser energy. When applied at the herniation site of the intervertebral disc, laser energy causes structural changes in the disc and water evaporation, thereby reducing the pressure in the intradiscal space and achieving disc decompression. Later, a stable scar is created at this site, which prevents the re-herniation of the intervertebral disc.

Hypothesis: Both treatment methods will be equally effective for intervertebral disc herniation without discoradicular contact at the follow-up examination one month

after surgery, but PLDD will be a more effective option in treating patients with radicular contact as a result of disc herniation.

Aims: To compare the efficacy of ESI and PLDD in patients with lumbar radicular pain whose radicular pain is caused by herniation of the intervertebral disc.
To determine whether there is a difference in efficacy between ESI and PLDD in intervertebral disc herniation with and without discoradicular contact.

Materials/Participants and Methods: The survey will be designed as a randomized prospective cohort study. The study subjects will be patients, divided into two groups where ESI or PLDD will be done.

Research plan: Basic demographic, social data and MR findings will be collected during the course of the research. Visual Analogue Scale (VAS), Pain detect, Lasegue test, Oswestry Low Back Disability Questionnaire, Hospital Anxiety and Depression Scale, SF - 36 Questionnaire will be measured at baseline and 15 days, 1, 3 and 6 months post-procedure.

Significance/Expected scientific contribution: In addition to minimally invasive procedures, such as ESI and PLDD, in the treatment of chronic pain, which is a major public health, social and economic problem, we have new treatment options that can help avoid general anesthesia, prolonged and extensive surgery.

MeSH/Keywords: epidural steroid injection, lumbar disc radicular pain, percutaneous laser disc decompression

Dissertation Proposal Title: Characteristics of patients detected in the National Program for Early Detection of Colorectal Cancer in Osijek-Baranja County (O-B County)

PhD candidate: Maja Čebohin, Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Mentor: Assist. Prof. Senka Samaradžić, M.D., Ph.D., Department of Public Health, Institute of Public Health for the Osijek -Baranja County, Osijek, Croatia

Introduction: Colorectal cancer is the third most common among malignant neoplasms in the world, but it is one of the rare tumours that allows for timely prevention. It is known as a 'silent disease' because many people lack clinical symptoms until the disease becomes difficult to treat. It is an important public health problem due to its growing incidence.

Hypothesis: Participants in the *National Program* in O-B County diagnosed with advanced adenomas and carcinomas have lower risk factors for disease development than the patients who underwent colonoscopy examination on a regular basis, due to the presence of disease symptoms.

Aim: The aim of the research is to compare the proportion of pathologic findings (advanced adenomas and carcinomas) in the *National Program* to the findings of patients who were regularly ordered for colonoscopy screening, with special reference to the proportion of risk factors for disease onset in both groups.

Participants and methods: The planned sample size are 692 people between the ages of 50 and 74, who were found positive and invited for a colonoscopy examination. The control group are the patients between the ages of 50 and 74 who were regularly ordered for a colonoscopy examination during the same period. Each respondent from the *National Program* will be joined by a subject from the control group of the same characteristics (according to gender, age, and the time of undergoing a colonoscopy). Screening method for early detection of colorectal cancer is the stool guaiac test for occult bleeding.

Research plan: Study participants received a notification letter and a consent form sent by the Croatian Institute of Public Health. After the receipt of the signed consent, three samples of the test were delivered to the participants' home address with guidelines for use. The tests were returned by regular mail to the Institute of Public Health of O-B County and analysed. Colonoscopy exam was performed on the participants with positive occult blood test at the Clinical Hospital Osijek. Control group consisted of patients aged 50-74 who did not undergo colonoscopy examination under the *National Program* but were ordered to a colonoscopy screening on a regular basis.

Significance/Expected scientific contribution: This study will show that the participants who underwent a colonoscopy screening as part of the *National Program* have lower risk factors for the onset of colorectal cancer than the patients who have come for a colonoscopy examination because of symptoms of the disease. Risk factors in the population of O-B County that favour the onset of colorectal cancer will be detected and further prevention programs will be planned based on the results of this study, with the aim of reducing the incidence and prevalence of colorectal cancer.

MesH/Keywords: National programme, selection, colon cancer, occult bleed test, colonoscopy

Dissertation Proposal Title: Improving the quality of life in patients with COPD using educational audiovisual media

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Mentor: Prof. Sanja Popović-Grle, M.D., Ph.D., Clinical Department for Lung Diseases Jordanovac, University Hospital Centre Zagreb, Zagreb, Croatia

Introduction: Chronic obstructive pulmonary disease (COPD) is characterized by permanent, most commonly progressive airway obstruction and is associated with chronic inflammation caused by airway exposure to harmful particles and gases. It is the leading cause of morbidity and mortality worldwide and together with respiratory infections of the lower respiratory tract, the third leading cause of death behind ischemic heart disease and stroke. The prevalence of COPD is steadily increasing. The latest estimates predict that by 2030 COPD alone will becomes the fourth cause of death worldwide. The most important and common risk factor for the development of respiratory disease is smoking. It is estimated that 20-40% of smokers suffer from COPD. The GOLD (Global Initiative for Chronic Obstructive Lung Disease) guidelines specifically highlight the enormous socioeconomic importance of COPD, based on high and increasing incidence, progressive course, diminished quality of life, reduced work capacity, work disability and increased mortality. If you take into account that COPD is a preventable and treatable disease, it is clear that the priority in COPD patient care should be procedures slowing down the progression of the disease, reducing the number of exacerbations, preventing development of complications and thereby reducing mortality rate. One step forward in problem solving and also one of the biggest challenges itself in healthcare is educating and enabling patients to understand the nature and course of the disease and to administer the necessary medication in an adequate manner. Education is the key part in COPD patient treatment. It is therefore a worrying fact that numerous studies report insufficient patient awareness. Health information provided to patients during medical consultations is often poorly explained and confusing to the patient. In many studies, poor education about the disease itself has been identified as one of the key reasons for patients' low compliance in treatment. This problem is growing to the extent that the World Health Organization has proclaimed it to be the "new pharmacological problem". Therefore, quality patient education through audiovisual

material enables them to better understand and manage the disease process itself, resulting in better treatment compliance, better disease control and improved quality of life.

Hypothesis: Patient education using educational audiovisual media is more effective than informing patients through medical consultations.

Aims: Examine the change in disease control after patient's education through audiovisual material. Examine the difference in patients quality of life between those educated through audiovisual material and those educated during medical consultation, Examine the difference in treatment compliance between patients educated through audiovisual material and those during medical consultations, Examine the difference in number of exacerbations and hospitalizations in patients educated through audiovisual material and those trained during medical consultations.

Participants and Methods: The study will include COPD patients older than 45 years. Participants will be randomly divided into 2 groups, one group will be educated about the disease through educational audiovisual material and the other will be provided with health information during standard clinical education practice. Inclusion criteria: participants aged over 45 who were diagnosed with COPD at the time of study enrollment, were treated by a pulmonologist over 2 years and had at least one recorded COPD exacerbation within a year treated with antibiotics and / or systemic corticosteroids. Exclusion criteria: 1) if they are treated for another serious chronic illness (cardiovascular - myocardial infarction, cerebrovascular, cancer) that significantly affects the quality of life, 2) if they are unable to perform a diagnostic protocol and understand the content of the audiovisual material.

Research plan: The study is designed as a prospective randomized controlled trial. The patients eligible for the study have a diagnosis of COPD, were older than 45 years and gave consent for the participation in the study. The study will be conducted in the Department of Pulmonology of the University Hospital Centre Osijek. At the initial visit to the pulmonologist a detailed examination of the patients will be carried out: history with an emphasis on smoking experience, respiratory problems and the main symptoms of COPD (cough, chronic expectoration and shortness of breath). Shortness of breath will be graded using the mMRC scale. Clinical examination and spirometry will be performed with a bronchodilator test according to ATS and ERS standards. Participants will complete 4 self-assessment questionnaires independently: 1. St. George's Respiratory Questionnaire, 2. CAT test - COPD assessment test, 3. Baseline / Transition Dyspnea Index (BDI / TDI), 4. Morisky Adherence Scale. One group of

patients will be educated about the disease through audiovisual material. Educational audiovisual material will contain information about smoking as a harmful risk factor for the onset and progression of the disease, the progressive course of the disease, the importance of taking inhaled therapy regularly and with correct inhalation technique, regular and adequate physical activity, pulmonary physical therapy methods and ways to prevent exacerbation. At the final visit to the pulmonologist, 6 months after audiovisual education, a clinical examination of the patient, spirometry with a bronchodilator test and completion of self-assessment questionnaires will be performed again.

Significance/Expected scientific contribution: Improve the education and significantly clinical outcomes (adherence, quality of life, disease control) of COPD patients by using educational audiovisual material relative to standard clinical education practice during regular clinical patient visits. The advantage of professional audiovisual material would be manifested by the possibility of daily use of the quoted audiovisual material through the media most acceptable to the individual patient (smartphones, web, television, DVD).

MeSH/Keywords: Pulmonary Disease, Chronic Obstructive, Video-Audio Media, Quality of Life, Education

Abstract Title: The association between brain derived neurotrophic factor and cognition in veterans with posttraumatic stress disorder

Part of the Dissertation Proposal: The association between brain derived neurotrophic factor and cognition in veterans with posttraumatic stress disorder

PhD candidate: Sandra Domitrović Spudić, General hospital Karlovac, University of Osijek, Faculty of Medicine Osijek, Osijek, Croatia

Mentor: Assist. Prof. Suzana Uzun, M.D., Ph.D., University Psychiatric Hospital Vrapče, Vrapče, Croatia

Introduction: Posttraumatic stress disorder (PTSD) is a severe and disabling trauma- and stress-related disorder, which develops after experiencing or witnessing traumatic events. Combat-related posttraumatic stress disorder (PTSD) is frequently comorbid with cognitive decline and various cognitive symptoms. Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin growth factor family, which regulates neuronal differentiation, survival, and synaptic plasticity. It is expressed throughout the developing and mature brain with highest levels in the prefrontal cortex and hippocampus. BDNF has been implicated in the biology of psychiatric disorders as well as learning and memory and response to stress, all processes that are altered in PTSD.

Aims: determine BDNF plasma concentration and determine the distribution of BDNF Val66Met and BDNF C270T genotypes in veterans with PTSD and control subjects; determine cognitive impairment in veterans with PTSD; determine plasma BDNF concentration and determine the distribution of genotypes BDNF Val66Met and BDNF C270T in PTSD subjects who will be divided by cognitive scales on cognitively impaired subjects with PTSD and PTSD subjects who do not show cognitive impairment

Materials/Participants and Methods: 350 male war veterans with combat-related PTSD and 350 age-grouped male control subjects were included. They were consequtively sampled in Department for Biological Psychiatry and Psychogeriaric University Hospital Vrapče, Zagreb, Croatia.
Evaluations were done using diffrents psychological scales measuring cognition and were used to determine cognitive deterioration in veterans with PTSD : PANSS,

Clinician Administred PTSD Scale (CAPS); cognitive tests: MMSE2, The Montreal Cognitive Assesment.

Results: conformation with BDNF C270T polymorphism; TT cariers had the most severe symptoms and pronounced cognitive decline vs. Carriers of the CT and CC genotypes

Conclusion: These preliminary data confirmed our hypothesis that BDNF is related to cognition and also revealed for the first time a significant association between BDNF C270T and cognition in PTSD, which is also confirmed with diffrents psychological scales measuring cognition. Such an approach will offer integration and understanding of multidimensional databases and identify new diagnostic and prognostic biomarkers of PTSD as well as new targets for therapeutic interventions in PTSD

MeSH/Keywords: Brain deriveded neurotrophic factor, cognition, PTSD, stress, polymorphism, cognition scales

Dissertation Proposal Title: Personality structure and insight into illnes in early-stage psychosis patients and their parents/caregivers

PhD candidate: Lada Goršić, M.D., Psychiatric Hospital „Sveti Ivan" , Zagreb, Croatia

Mentor: Assoc. Prof. Branka Restek Petrović, M.D., Ph.D., Department of Psychiatry, Psychiatric Hospital „Sveti Ivan" , Zagreb, Croatia

Introduction: Impaired insight into illnes is common during early stage of psychosis and is associated with treatment delay, poorer treatment adherence, poorer social functioning, agressive behavior and poorer long-term outcomes. Approximately 50%-80% of patients with psychosis exhibit poor insight, even when in remission.
Insight is defined as a multidimensional construct comprising awareness of having a mental illness, its consequences, compliance with treatment and ability to label psychotic symptoms as pathological. As it is a relational concept, it can only be meaningfully evaluated in a person with experience of an illness. Therefore, insight is considered a multifaceted concept that reflects complex interaction of factors: personal, cognitive, environmental and biological, but it is still unclear to what extent personality may play a role in that concept. Given that these are mostly young people who still live with their parents, the role of the parents/caregivers is very important in recognizing the first symptoms of psychosis. Higher parental involvement during early psychosis is associated with greater therapeutic co-operation and shorter hospitalizations, while lower parental involvement is associated with longer untreated psychosis (DUP) and less therapeutic co-operation. In that context, the study of how personality may influence on insight into illness seems relevant.

Hypotheses: Insight into illness is related to the personality traits of patients in the early stages of psychotic disorder and their parents/caregivers. The insight of parents/caregivers is related to the insight of those patients.

Aim: To examine the correlation of insight into illness with the personality traits of patients in the early stages of psychotic disorder and their parents, as well as relatedness between parental and patient's insight.

Material/Participants and Methods: The target population will consist of patients in the early stages of psychosis hospitalized at Psychiatric Hospital ''Sveti Ivan" and

their parents /caregivers. The inclusion criteria will be diagnosis of the early stage of psychosis from the psychotic spectrum (F20-29) according to the ICD 10th Revision, signed informed consent, hospital treatment, both sexes, age 18 to 35 and ability to complete the questionnaires without assistance. Non-inclusion criteria will be: severe brain damage, mental retardation, severe drug dependence, acute suicidality and inability to complete the tests without assistance.

Research plan: A cross-sectional study will be conducted on a consecutive sample of early-stage psychosis patients (disease duration <5 years) hospitalized at the Psychiatric Hospital "Sveti Ivan" in Zagreb and their parents/caregivers. The study will be conducted in accordance with ethical principles and with the approval of the Hospital Ethics Committee. The purpose and objectives of the research will be explained to all participants and as they sign informed consent, they will be subjected to a clinical interview, questionnaires and measurement scales. Instruments to be used : the Scale to assess Unawareness of Mental Disorder (SUMD), the Positive and Negative Syndrome Scale (PANSS), the Clinical global impression scale - severity (CGI-S) for patients and modified version of the SUMD for caregivers. The Zveltc G. Object Relations Test (TOO) and the Kernberg Inventory of Personality Organization (IPO) will be given both to patients and their parents/caregivers.

Significance/Expected scientific contribution: to improve understanding of insight in patients and their families, as this in turn might improve overall outcome and their quality of life.

MeSH/Keywords: insight into illness, personality structure, early-stage psychosis

Dissertation Proposal Title: The Correlation between the professional identity level and the mentoring support satisfaction level of nursing students with respect to different mentoring models throught Clinical teaching

PhD candidate: Ivana Gusar, Department of Health Studies, University of Zadar

Mentor 1: Assist. Prof. Robert Lovrić, Ph.D., MSN, RN, Faculty of Dental Medicine and Health Osijek, University of Osijek, Osijek, Croatia
Mentor 2: Assist. Prof. Andrea Tokić, Ph.D., Department of Psychology, University of Zadar, Zadar Croatia

Introduction: There is no single universal form and agreement on the mentoring of nursing students (NS) in Europe. Mentoring form is an important factor that affects students' satisfaction, the achievement of the final learning outcomes and professional development of students. Professional identity (PI) implies knowledge, skills, attitudes, values and beliefs that we share with the members of a particular group of people and that, at the same time, distinguish us from other groups. PI develops during education and the preparation of students for future work. The professional identification of nurses is a precondition for quality health care.

Hypothesis: The level of PI is related to the mentoring support satisfaction level of NS with respect to different mentoring models thought clinical teaching.

Aims: The aim is to gain insight into the correlation between the PI level and the mentoring support satisfaction level of NS with respect to different mentoring models thought clinical teaching.
It will be measured: level of PI of NS, level of satisfaction of NS with mentoring support during clinical teaching, differences in the level of satisfaction with mentoring support among NS mentored with an individual and group form of mentoring, differences in the level of PI among NS mentored with an individual and group form of mentoring, correlation between the students' satisfaction with mentoring support and the level of PI with respect to the individual and group form of the mentoring.

Materials/Participants and Methods: For the purpose of measuring PI, three instruments were validated on 432 NS and the most appropriate was selected. For the measure students' satisfaction with mentor support *Questionnaire for validation of*

mentoring support will be used on NS in University of Zadar. The research will include first, second and third year students. The students of each year will be divided into two groups. Each group of students will have a different form of mentoring (individual or group) at first semester and another form in second semester.

Research plan:
This research will be conducted longitudinally during one academic year:

- Phase 0: psychometric analysis of three questionnaires for the PI level assessment. Selection of the most reliable questionnaire.
- Phase 1: (beginning of the year) the first PI level assessment of the NS.
- Phase 2: (first semester) division of first, second and third year students into two groups measuring students' satisfaction with mentor support and PI level assessment of the NS after performing clinical practice in individual or group mentoring form
- Phase 3: (second semester) replacement groups measuring students' satisfaction with mentor support and PI level assessment of the NS after performing clinical practice in individual or group mentoring form.

Significance/Expected scientific contribution: This research will provide a reliable instrument for the PI level assessment of NS in the Croatian. The results could point out the significant contribution of mentoring form and students' satisfaction with mentor support to the development of the students' PI during their studies, as well as the possible need for the implementation of a certain form of mentoring in the undergraduate nursing education system.

MeSH/Keywords: mentoring form, students' satisfaction, professional identity, nursing, education

Disertation proposal title: Differences in the incidence of hypoglycaemia after strength training and aerobic type exercise in patients with type 1 diabetes

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

Introduction: Physical activity is an integral part of diabetes treatment. Over the past few years, the importance of the strength training has been increased in changing body composition and better glucose utilization during physical activity and rest period with patients both type 1 and type 2 diabetes. Increased risk of developing hypoglycemia and worsening of glycemic control in exercise are the main reasons of concerns of patients with type 1 diabetes.

Hypothesis: Strenght training is associated with better glucose stability then continuous moderate intensity aerobic exercise, both immediately after exercise and during nightime.

Aims: The aim of this study is to evaluate glycemic control during and after exercise and the specificity of the glucose response in different forms of exercise (aerobic exercise and strenght exercises) using a sensor for continuous measuring glucose in patients with type 1 diabetes. The utility of predefined hypoglicaemia avoidance protocol will be evaluated, as well as hypoglycemia induced neurohumoral response and markers of chronic inflammation depending on type exercise.

Materials/Paticipans and Methods: In this study 40 patients will br included with at least 1-year duration of type 1 diabetes, HbA1c less than 9% and stable insulin regimen for 3 months. The study was designed as randomized prospective cross-over study. Patients will be randomized according to the type of exercise (aerobic or strength training) with a „washout" period of three days before the groups change.

Research plan: In the initial part of study patients will be selected based on the inclusion criteria. Multiple neurohormonal, inflammatroy and biochemistry laboratory parameters will be determined both before and after exercise training. Patients will

also carry out glycemic self-monitoring using a glucometer 8 times a day and will be provided with self-control logs and hypoglycemic event evaluation questionnaires. All subjects will be educated on the protocol for avoiding hypoglycemia, and they will also record in their diaries the type and amount of ingested carbohydrates. There will be two types of training – one will contain strength exercises and the other will be aerobic, training protocol will be created by kinesiologist.

Significance/Expected scientific contribution: The practical application of this research is aimed at prevention of physical activity induced hypoglycemia in people with type 1 diabetes as well as finding out optimal types and intensity of exercise for the purpose of favorable effect on inflammatory and neurohumoral markers.

MeSH/Keywords: type 1 diabetes, exercise, continuous glucose monitoring, hypoglycemia, glucovariability, strength training, aerobic activity

Abstract Title: The effect of simvastatin and metformin on the accumulation of lipid droplets in the 3T3-L1 cell culture model of adipogenesis

Part of the Dissertation Proposal: The effect of simvastatin and metformin on the regulation of adipogenesis in vitro

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Mentor 1: Prof. Aleksandar Včev, M.D., Ph.D., Faculty of Dental Medicine and Health Osijek; Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia
Mentor 2: Assoc. Prof. Martina Smolić, M.D., Ph.D., Faculty of Medicine Osijek; Faculty of Dental Medicine and Health Osijek, University of Osijek Osijek, Croatia

Introduction: Obesity is a worldwide epidemic as the prevalence of obesity has increased rapidly along with the concurrent rise in type 2 diabetes mellitus (T2DM) and cardiovascular disease. Metformin has long been used for its glucose-lowering effects but has also gained attention for its pleiotropic effects, such as lowering the body weight of obese T2DM patients, which was shown to be more effective than in patients with lower baseline body mass. The underlining mechanism responsible for this difference is unknown. Furthermore, there are very limited data available from in vitro or in vivo studies examining the effects of metformin on adipogenesis. Coronary heart disease (CHD) due to impairment of lipid metabolism is the major cause of mortality in obese patients. The correction of dyslipidaemia by statins significantly decreases the risk of CHD events. Statins are thought to have additional benefits and effects in nonhepatic tissues and cell types, such as adipose tissue. Given the fact that a large number of obese patients have comorbidities such as type 2 diabetes mellitus and dyslipidaemia, and adipocytes play a central role in regulating glucose and lipid metabolism, the effect of metformin and simvastatin on adipogenesis should be thoroughly investigated.

Aims: The aim of this study is to establish a model of adipogenesis in vitro and to evaluate the effect of simvastatin and metformin therapy on the lipid accumulation during the process of adipogenesis.

Materials/Participants and Methods: Murine 3T3-L1 cells (Sigma Aldrich, Missouri, USA) is a cell line of preadipocites that can adopt a rounded adipocyte phenotype and accumulate lipid droplets of triacylglycerols after appropriate stimulus. A combination of dexamethasone (DEX) (Sigma Aldrich, Missouri, USA), isobutylmethylxanthine (IBMX) (Sigma Aldrich, Missouri, USA) and insulin (Sigma Aldrich, Missouri, USA) was used for the induction of adipogenesis. Simvastatin (Sigma Aldrich, Missouri, USA) and metformin (Sigma Aldrich, Missouri, USA) were used to reduce adipocyte differentiation. Methods: Murine 3T3-L1 preadipocytes were grown in 10% CS/FBS-DMEM with 1% antibiotic solution (penicillin/ streptomycin) at 37 °C in a humidified atmosphere of 5% CO2 in air. To establish the model of adipogenesis, cell differentiation was induced after full confluence by adding 0.5 mM IBMX, 0.25 µM DEX and 1 µg/ml insulin to DMEM with 10% FBS. The influence on cell proliferation of metformin (10, 20, 30 mM), simvastatin (5, 10, 20 µM) and the concomitant treatment (5 µM simvastatin + 10 mM metformin, 10 µM simvastatin + 20 mM metformin, 20 µM simvastatin + 30 mM metformin) was assessed by MTT assay after 24, 48 and 72 h. Metformin at 20 mM, simvastatin at 20 µM and the combination of 5 µM simvastatin + 10 mM metformin was used for morphological examination of adipocyte differentiation inhibition. Adipocyte differentiation was visualised by the Oil Red O staining.

Results: The 12-day differentiation protocol using insulin, dexamethasone and IBMX resulted in morphological change and the accumulation of lipid droplets in mature adipocytes visible by Oil-Red-O staining. MTT assay showed significant inhibition of cell viability of 3T3-L1 preadipocytes using metformin at 30 mM ($p<0.001$, t-test with Bonferroni correction), simvastatin at 20 µM ($p<0.001$, t-test with Bonferroni correction), and the combination of 20 µM simvastatin + 30 mM metformin ($p<0.001$, t-test with Bonferroni correction) after 72 h, compared to control untreated predipocytes. During the 12-day differentiation period, metformin and simvastatin reduced the intensity of Oil-Red-O staining compared to control differentiated adipocytes. The concomitant treatment 5 µM simvastatin + 10 mM metformin significantly reduced the number of lipid droplets in one cell compared to the control differentiated adipocytes ($p<0.05$, Anova with post-hoc Tukey test).

Conclusion: This study assessed the effects of metformin and simvastatin on lipid accumulation during adipogenesis, finding that monotherapy and concomitant therapy reduces morphological change of preadipocytes in mature adipocytes. However, more research is needed to define the role of metformin and simvastatin in adipocyte differentiation, their molecular targets and possible impact on the weight loss.

MeSH/Keywords: adipogenesis, 3T3 cells, simvastatin, metformin, lipid droplets

Dissertation Proposal Title: Effects of Tranexamic Acid Use on Blood Loss, Risk of Thromboembolism, and Functional Outcomes in Hip Fracture Surgery in Elderly Population in Croatia

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Mentor: Prof. Roman Pavić, M.D., Ph.D., Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia; Department of Traumatology, Sestre Milosrdnice University Hospital Center, Zagreb, Croatia

Introduction: There is a global increase in percentage of elderly population (>65 years old). Hip fracture is very common in this population, it is associated with a high morbidity and mortality, impaired mobility, and poor performance of activities of daily living (ADL) afterwards. Tranexamic acid (TXA) has been widely used in elective orthopaedic surgery, mainly in joint replacement, to reduce blood loss and transfusion requirement. Usually, the TXA is administered either intravenously (IV) or topically.

Hypothesis: Main hypothesis is that there are differences in blood loss and transfusion requirement regarding different routes of TXA administering during hip fracture surgery. Secondary hypothesis is that there are differences in functional outcomes regarding different routes of TXA administering during hip fracture surgery.

Aims: Our primary aim is to determine efficacy and safety of different routes of administrating TXA use in hip fracture surgery regarding blood loss, transfusion requirement, and postoperative thromboembolic incidents. Secondary aim is to determine the influence of TXA use and route of administering on functional outcomes of hip fracture surgery.

Materials/Participants and Methods: Randomized controlled trial including elderly first-time hip fracture patients, surgically treated in County General Hospital Vinkovci. Inclusion criteria will be: age >65 years, X-ray confirmed hip fracture, first-time hip fracture, surgical treatment. Exclusion criteria will be: contraindications to TXA, previous hip fracture(s), periprosthetic fracture(s), other fractures at time of admission, pathologic fracture (metastatic cancer), inability to walk previous to fracture, cognitive impairment compromising communication. Expected sample size

is 160. Statistical analysis will be applied to compare patients' pre- and postoperative variables and outcomes.

Research plan: Enrollment in the study will be on admission to hospital, after giving informed consent. Follow-up time will be 9 months. On enrollment medical history will be noted, ADL (Rapid Disability Rating Scale version 2, Barthel Index of Activities of Daily Living), quality of life (Short Form 36), and baseline haemoglobin values. Patients will be randomly assigned to groups: IV TXA (10mg/kg IV preoperatively and placebo topical), topical TXA (placebo IV and topical 10mg/kg intraoperatively), combined IV and topical TXA (10mg/kg IV preoperatively and topical 10mg/kg intraoperatively) and control group (placebo IV and topical). Perioperative variables will be noted (type of fracture, postoperative haemoglobin values, postoperative drainage output, transfusion requirement). On 3rd and 9th month follow-up we will again assess thromboembolic incident rates, mortality rates, ADL, quality of life, as well as measure outcome of hip fracture treatment (Harris Hip Score).

Significance/Expected scientific contribution: To the best of our knowledge, this is the first study of this kind on elderly population in Croatia. The results should further improve care for the elderly, reducing the cost and improving the outcome of surgical treatment.

MeSH/Keywords: hip fracture, proximal femur fracture, tranexamic acid, outcomes, complications

Abstract Title: Eye biometry of healthy adult prepresbyopic people in Zagreb, Croatia Eye biometry of prepresbyopic people in Zagreb, Croatia

Phd candidate: Štefanija Kolačko, RN, MD in Nursing

Mentor: Jurica Predović, M.D., Ph.D., University Eye Clinic, Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia; University Hospital "Sveti Duh", Zagreb, Croatia

Introduction: The technological progress in ophthalmology enables more accurate assessment of biometrical properties of human eye. The biometrical characteristics of human eye differ among various populations and are known to change throughout the life time as a consequence of physiological ageing processes. It is crucial to know exact biometrical values for each population since they have an impact on various therapeutic strategies. There are no biometrical surveys of healthy adult human prepresbyopic eyes in Zagreb County.

Aim: The aim of this study is to measure near emmetropic healthy eyes biometrical values of healthy middle aged (prepresbyopic) people in Zagreb County using modern technologies and to compare these data with data obtained by previous technologies.

Participants and Methods: Subjects from Zagreb County, aged 18-39 years, were recruited for this prospective study. They underwent complete ophthalmic examination prior to inclusion in the study and only those having normal visual acuity were included. All subjects with pathologic conditions and with spherical component of more than -1.00 diopters (D) or more than +2.50 spherical D and oblique astigmatism of more than 1.00 D and more than 1.50 D of with or without the rule astigmatism were excluded. Gender and date of birth were recorded. Body weight and body height were measured. Optical low coherence reflectometry (Lenstar LS 900®) and optical coherence tomography (Canon HS-100 SD-OCT) of the macular region and optical disc were used to measure biometrical properties of both eyes of each subject, including: axial length (AL), the flattest (K1) and steepest (K2) keratometry,central corneal thickness (CCT), anterior chamber depth (ACD), lens thickness (LT), corneal white to white (WtW) diameter, pupil size, minimal macular thickness, average macular thickness and volume in central 6 mm zone,disc area surface, rim area surface, cup volume, rim volume, peripapilar retinal nerve fibre layer (RNFL) thickness.

Descriptive statistical methods were used to present the data. The metric data were presented as mean +/- Standard Deviation. Group differences were compared using paired samples t-test. Data correlations were analysed using bivariate Pearson correlations with indicated correlation ratio (r) and significance level (p). Results with two tailed $p<0.01$ were considered as statistically significant. Linear regression analysis was also performed. Analyses were conducted in SPSS Statistics for Windows v.17.0.

Results: 152 females (aged 22+/-5 years and 84 males (aged 25+/-6 years) were included in the study. AL was 23.65mm in males and 23.45in females. CCT was 568µm in males and 546 µm in females. ACD was 3.12 mm in male and 3.06 mm in female. LT was 3.62 mm in male and 3.65 mm in female. K1 was 42.77 D in male and 43.14 D in female, K2 was 43.50 D in male and 43.95 D in female. Minimal macular thickness was 223 µm in male and 222 µm in female.

Conclusion: This is the first survey of biometrical properties of healthy adult human near emmetropic prepresbyopic eyes in Zagreb County measured by optical instruments. These data will contribute to better understanding in ocular morphology and ocular diseses.

MeSH/Keywords: biometry, eye, cornea, lens, macula lutea

Abstract Title: Positive effect of *n*-3 PUFAs on reducing oxidative stress level in young healthy individuals

Part of the dissertation proposal: Influence of omega-3 enriched eggs on immune system and level of oxidative stress in young healthy people

PhD candidate: Nikolina Kolobarić, mag. prot. nat. et amb.; Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Mentor: Assist. Prof. Anita Matić, Ph.D., Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Introduction: Oxidative stress plays a role in development of cardiovascular diseases due to its direct action on the function of blood vessels and immune response activation. Our previous study showed positive effect of *n*-3 polyunsaturated fatty acids (PUFAs) on microvascular reactivity, reducing oxidative stress, repairing the lipid profile and consequently reducing the risk of cardiovascular diseases in the future. Most of these types of research has been done with diet supplements (capsules) and mainly on cardiovascular patients, therefore it is of our interest to observe the effect of *n*-3 PUFAs in healthy young subjects.

Aims: Determine the effect of consumption of *n*-3 PUFA enriched eggs on systemic oxidative stress level in young healthy individuals without previous endothelial damage or chronic inflammatory processes.

Materials/Participants and Methods: 33 healthy participants (18 men and 15 women, age: 20-26), were included in the protocol and divided into two groups: one group consumed *n*-3 PUFA enriched chicken eggs (OE group; n=18, 2 eggs/day, n-3 PUFA=403.10 mg/day) while the other one consumed regular chicken eggs (RE group; n=15; 2 eggs/day, n-3 PUFA=74.63 mg/day) for 3 weeks. Study was randomized double blind placebo controlled. First part of the study involved determination of oxidative stress markers Ferric Reducing Ability of Plasma (FRAP), Thiobarbituric Acid Reactive Substances (TBARS) and antioxidative enzymes' activity in serum samples before and at the end of the protocol. In the second part, peripheral leukocytes' oxidative stress was measured by flow cytometry with DCF-DA (2',7' - dichlorofluorescin diacetate) assay at the beginning and at the end of the study. Levels of intracellular ROS were

assessed prior and 30 minutes after PMA (phorbol-12-myristate-13-acetate) mediated induction of ROS production.The study protocol and procedures are conformed to the standards of the Declaration of Helsinki and were approved by the Ethical Committee of the Faculty of Medicine, University of Osijek. To test differences among groups student t-test was used, and intra-group differences were tested by paired t-test; $p<0.05$ was considered significant.

Results: In female OE group, TBARS was significantly decreased and GPx activity significantly increased after dietary protocol. GPx activity was significantly increased in female OE group after dietary protocol compared to RE group. In male group, changes in the level of oxidative stress markers and enzyme activity are not expressed due to significantly higher antioxidative capacity when compared with women group.Flow cytometry measurements have shown that *n*-3 PUFA enriched eggs consumption reduced levels of oxidative stress upon PMA stimulation in granulocytes of men OE men had significantly lower level of oxidative stress in granulocytes prior and upon PMA stimulation compared to RE men. This effect seems to be restricted to male subjects.

Conclusion: Results suggest the positive effect of *n*-3 PUFAs on reducing systemic oxidative stress in young healthy individuals due to increased blood antioxidative activity.

MeSH/Keywords: antioxidative activity; oxidative stress; *n*-3 PUFA enriched eggs; flow cytometry; healthy participants

Acknowledgement: The work is supported by grant to Scientific Centre of Excellence for Personalized health care, University of Osijek, Croatia

Abstract Title: Antioxidant pre-treatment of garlic extracts reduces the toxic effects of sodium taurocholate in a cell culture model of ulcer disease

Part of the Disertation Proposal: The activity of garlic and ginger extracts to the epithelial damage caused by sodium taurocholate in a cell culture model of ulcer disease

PhD candidate: Lucija Kuna**,** MSc, Faculty of Dental Medicine and Health Osijek, University of Osijek, Osijek, Croatia

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

Introduction: Peptic ulcer is the most frequent gastrointestinal disorder affecting 10% of the world population. Pharmacotherapy of ulcer disease includes several groups of drugs, but proton pump inhibitors (PPI) and supression of Heliobacter pylori infection represent the gold standard. Although conventional regimens are effective, side effects can limit their prolonged clinical utility. On the other hand, studies have demonstrated that herbal medicines exhibit therapeutic benefit for gastric ulcer with fewer side effects. The major role of *Allium sativum* extract has been observed in antioxidant effect by scavenging reactive oxygen species (ROS), inhibiting lipoprotein oxidation and lower serum glucose induction of antioxidant enzymes. Although, its effect in gastroprotection of gastric ulceration has been demonstrated, the study of the effect and mechanism of garlic in the cell model of ulcer disease are not fully elucidated.

Aims: The aim was to establish a cell culture model of ulcer disease, measure the toxic effect of sodium taurocholate (NaT) and to determine possible inhibition of NaT caused oxidative stress by antioxidant treatment of garlic extracts (GE).

Materials and Methods: Human gastric AGS cell line (ATCC, Germany) was used to develop the cell culture model of ulcer disease by sodium taurocholate (NaT), (Sigma Aldrich, USA) exposure. To show effects of garlic extracts (Sigma Aldrich, USA) on cell survival, cells were treated with increasing concentrations of GE 350, 500, 1000 and 2000 µg/ml. To induce oxidative stress 3 mM NaT was added to the RPMI 1640 medium (Thermo Fischer Scientific, USA) without FBS. This concentration of NaT causes death

in 50% of cells if treated 30 minutes. Different concentrations of GE 100, 150, 250 and 350 µg/ml were used for pretreatment of cells prior to exposure to NaT and evaluating oeffects on cell survival. GE was dissolved at appropriate concentrations in ethanol. The extent of cytotoxicity and apoptosis was evaluated by cell counting using Neubauer hemocytometer and Trypan blue dye.

Results:. Cells treated with 100, 150, 250 and 350 µg/ml of GE showed a statistically significant higher survival rates compared to NaT-alone treated controls. NaT-alone treated cells had 48,4% survival compared to 100 µg/ml GE pretreated cells which had 64,4% survival (p =0.0184), 150 µg/ml had 65,9% survival (p = 0.0200), 250 µg/ml had 70,37% (p =0.0091) and 350 µg/ml pretreated cells had 81,48% survival (p =0.0033). However, concentrations of GE higher than 350 µg/ml showed significant decreased in cell viability.

Conclusion: Antioxidant pre-treatment with lower concentrations of GE is able to hamper NaT induced oxidative stress in gastric epithelial cells and as such could play a significant role in prevention of ulcer disease. However, more experiments are needed to elucidate effect of garlic extracts in gastric ulcer disease.

MeSH/Keywords: gastric ulcus, sodium taurocholate, antioxidant, garlic, AGS cell line

Dissertation proposal title: Characteristics of mucosal vibration in oesophageal and tracheoesophageal speech in laryngectomy patients

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Mentor: Assist. Prof. Ana Đanić Hadžibegović, Ph.D., ENT and Head and Neck Surgery Clinic, University Hospital Centre Zagreb, Zagreb, Croatia

Introduction: Laryngeal imaging provides visual indicators of tissue health and function, which are critical for accurate diagnosis and outcome measures for determining the effectiveness of treatment. High-speed videoendoscopy (HSV) provides the most reliable and accurate objective quantification of the vocal mucosa vibratory behaviour regardless of whether this behaviour is periodic or aperiodic. Although HSV is the only method that visualizes and measures vibration of oesophageal and hypopharyngeal segment (neoglottis) after laryngectomy, there is lack of clinical data about it in current literature.

Hypothesis: Characteristics of mucosal vibration of pharyngoesophageal segment in alaryngeal speech differ from characteristics of mucosal vibration of laryngeal speech in healthy individuals. Characteristics of mucosal vibration are different in tracheooesophageal and oesophageal speech, between patients who have had a cricopharyngeal myotomy and those who have not, and also depending on postoperative radiotherapy.

Aims: 1. Describe the characteristics of mucosal vibration in oesophageal and tracheoesophageal speech production. 2. Determine whether radiotherapy affects the characteristics of mucosal vibration. 3. Determine whether cricopharyngeal myotomy affects the characteristics of mucosal vibration. 4. Assess the impact of loss of voice in terms of psychological and socioeconomic problems in laryngectomy patients, and make an assessment of their quality of life.

Material/Participants and Methods: Research will involve laryngectomy patients of both sexes, between 18 and 80 years of age, who underwent total laryngectomy and voice rehabilitation, and who completed oncological treatment. Non-eligibility criteria: acute respiratory infection, other primary cancer. Subjects will undergo a voice

assessment, videostroboscopy, functional endoscopic evaluation of swallowing, HSV during voice production. Analysis will involve the use of special computer software of mucosal vibration assessment.

Research plan: Each subject will undergo an ENT examination and subjective and objective perceptual evaluation of voice quality. Voice production time will be measured and an MDVP analysis will be made, as well as endoscopic evaluation of swallowing, and HSV during voice production.

Significance/Expected scientific contribution: Description would be provided of still undescribed mucosal vibration characteristic, parameters would be proposed for potential use in creating an alaryngeal speech evaluation algorithm. An optimal method of neopharynx formation during laryngectomy could potentially be proposed.

MeSH/Keywords: mucosal wave, high-speed videoendoscopy, total laryngectomy, alaryngeal speech, neoglottis

Dissertation Proposal Title: Correlation between Autism Spectrum Disorder (ASD) and Electroencephalography (EEG) abnormalities in Children

PhD candidate: Ivana Lenz, Department of Pediatrics, Clinical Medical Center Osijek, Osijek, Croatia

Mentor: Assist. Prof. Silvija Pušeljić, M.D., Ph.D., Department of Pediatrics, Clinical Medical Center Osijek, Osijek, Croatia

Introduction: Autism spectrum disorder (ASD) is defined by a heterogeneous constellation of behavioral symptoms that emerge over the first years of life. It refers to a group of complex neurodevelopmental disorders, characterized by deficits in social communication and interaction and demonstrating restricted, repetitive, and stereotyped patterns of behavior. The symptoms are present from early childhood and are impairing to everyday functioning. People with autism spectrum disorder (ASD) have language disorder, intellectual disability, and epilepsy at higher rates than the general population.
There is good evidence that electroencephalographic (EEG) changes are common in children with autism and these EEG changes are considered to be signs of cerebral dysfunction. Electroencephalography (EEG) can measure neural activity and may provide a useful tool to detect children at risk of developing autism spectrum disorder (ASD) and, thus, provide an opportunity for early intervention. Electroencephalogram (EEG) epileptiform activity is also present at a substantially higher rate in children with autism than normally developing children.

Hypothesis: Correlation between children who have autistic spectrum disorder (ASD) with incidence of epilepsy.

Aim: The aim of this study is to study the characteristics of EEG findings and epilepsy in autistic spectrum disorders (ASD) and the associated neurophysiological symptoms.

Participants and Methods: The study will be conducted at the Institute of Child Neurology, Genetics, Endocrinology, Metabolic Diseases and Rheumatology in Clinical Hospital Center, Osijek. It will include all children that have been treated and followed up at Department of Pediatrics, Osijek, from January 2010 till the end of December 2019. The age range will be from three to seven years. Each participant's

EEG had been recorded. And all of the children included in this study are having some kind disorder from autistic spectrum, which includes autistic disorder, Asperger syndrome, childhood disintegrative disorder, and pervasive developmental disorder-not otherwise specified (PDD-NOS). Written, informed consent was provided by the parents or guardians prior to their child's participation in the study.

Research plan: We will study specific electroencephalography (EEG) patterns in autism and to correlate severity of autism to EEG patterns. We will check all the EEG records from all the children involved in our study, and then we will compare the clinical status of the child and the severity of their condition.

Significance/Expected scientific contribution: The results of the study will help to ease the incidence of EEG epileptic discharges in children with autism spectrum disorder (ASD)

MeSH/ Keywords: Autism spectrum disorder (ASD), Electroencephalography (EEG), Epilepsy

Abstract Title: Individual factors influencing the duration of untreated psychosis

Part of the Dissertation proposal: Association of childhood trauma and personality traits with the duration of untreated psychosis in early-course psychosis

PhD Candidate: Vanja Lovretić, M.D., Psychiatric Hospital „Sveti Ivan", Zagreb, Croatia

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Introduction: Duration of untreated psychosis (DUP), or the time between onset of psychosis and treatment initiation, is a prognostic factor of schizophrenia: a longer DUP has been associated with poorer outcome. Childhood trauma is strongly linked to an increased risk of developing a psychotic disorder. The ways in which personality and psychosis influence each other remain unclear.

Aims: To investigate the association between childhood trauma and personality traits with the DUP.
Secondary aims: To investigate the association between childhood trauma and the DUP, To investigate the association between childhood trauma and personality traits, To investigate the association between specific personality traits and the DUP, To investigate the association between childhood trauma and personality traits with psychotic symptoms, To determine the prevalence of childhood trauma in total sample

Participants and Methods: The study included 64 participants, 38 males (59.4%) and 26 females (40.6%), diagnosed with early-course psychosis (less than 5 years duration of illness) who were hospitalized in Psychiatric Hospital "Sveti Ivan". Mean age was 27.94 years (SD=7.17). The median DUP was 52.5 days (min.-max. 2-735 days). Fifty five participants (85.9%) confirmed experiencing childhood trauma, while 39 participants (60.9%) experienced two or more traumatic events during their childhood.
All participants were subjected to a clinical interview and answered the questions from self-report questionnaires and measurement scales. The actual psychopathological symptoms were assessed with Positive and Negative Symptoms Scale (PANSS). Existence, number and severity of childhood trauma were measured by Childhood

Trauma Questionnaire, while personality traits were measured by The Inventory of Personality Organization (IPO).

Results: Considering DUP, there was no significant association found between patients who experienced childhood trauma and those who did not. Furthermore, there was no association found between DUP and examined personality traits (primitive defences, identity diffusion and reality testing). Participants with longer DUP had more prominent positive symptoms. Participants who experienced childhood trauma had more prominent primitive defences and positive symptoms. Furthermore, participants with more prominent primitive defences and identity diffusion, as well as those with more difficulties with testing the reality, had more prominent positive symptoms.

Conclusion: Preliminary results showed that there is no association between patients who experienced childhood trauma and those who did not when considering DUP. On the other hand, participants who experienced childhood trauma had more prominent primitive defences and positive symptoms. Therefore, focusing therapeutic interventions on childhood trauma could lead to better outcomes in treating that segment of the population of patients with psychotic disorders.

MeSH/Keywords: childhood trauma; personality traits; duration of untreated psychosis; early-course psychosis; psychopathology

Abstract Title: Is there effect of GERD on hospital readmission in schizophrenia and recurrent MDD

Part of Dissertation Proposal: Association of GERD with outcome of antypsychotic therapy and the quality of life

PhD Candidate: Katarina Matić, M.D., Psychiatric hospital "Sveti Ivan", Zagreb, Croatia

Mentor: Prof. Igor Filipcic, M.D., Ph.D., Head of Psychiatric Hospital „Sveti Ivan", Zagreb, Croatia

Introduction: Many studies have demonstrated the bidirectional association of gastroesophageal reflux disease (GERD) and major depressive disorder (MDD). The relationship between schizophrenia and GERD is largely uninvestigated.

Aims: To investigate whether GERD is associated with poorer MDD and schizophrenia treatment outcomes, as indicated by a higher rate of psychiatry readmission, independent of psychiatric comorbidities and other clinical and sociodemographic parameters.

Participants and Methods: The targeted populations were patients diagnosed with recurrent MDD (ICD 10 F33) or schizophrenia (ICD 10 F20). The inclusion criteria were both genders, age ≥18 years, treated or controlled in a psychiatric hospital as either an inpatient or outpatient, and being able to answer the questionnaire. The exclusion criteria were acute suicidality, dementia, mental retardation, acute psychosis, and intoxication. We chose a consecutive sample of outpatients by the order of their arrival at the exam as well as all patients who were hospitalized during the enrollment period.

Results: We analyzed a sample of 193 patients who had been diagnosed with recurrent MDD (ICD-10 F33) and 117 patients who had been diagnosed with schizophrenia (ICD-10 F20) . The samples from these two populations were relatively comparable according to sex, education, number of household members, body mass index, consumption of fruits and vegetables, and physical activity. In all other sociodemographic, lifestyle and clinical characteristics, two samples were markedly different.

The GERD point prevalence was 235/1060 (22.2 %; 95 % CI 19.9 % to 24.8 %) in the total sample from the general psychiatric population collected for the main prospective study. The prevalence of GERD was three times greater in recurrent MDD patients than in schizophrenia patients. The relative risk for GERD was RR = 3.03 (95% CI 1.73 to 5.59) in recurrent MDD patients compared to schizophrenia patients. Patients who had been diagnosed with recurrent MDD had GERD in 65 / 193 (33.7 %; 95 % CI 26.7 % to 40.4 %) of the cases. Patients who had been diagnosed with schizophrenia had GERD in 13 / 117 (11.1 %; 95 % CI 5.9 % to 17.4 %) of the cases. This difference in prevalence was statistically significant ($\chi^2 = 23.0$; df = 1; $p < 0.001$; $\varphi = 0.25$). Robust regression was performed in 22 iterations in recurrent MDD and in 30 iterations in schizophrenia analysis. The distribution of residuals in the schizophrenia analysis was not significantly different from the normal one: Shapiro Wilk test, $p = 0.134$; D'Agostino skewness, $p = 0.087$; Kurtosis, $p = 0.407$; and Omnibus K^2 test, $p = 0.168$. In the recurrent MDD analysis, the D'Agostino Omnibus K^2 test revealed significant departures of the residual distributions from the normal ones, although residual and normal distributions were symmetric. We did not detect a serial correlation of residuals in any case (Durbin-Watson test statistics were 2.0 and 2.3, respectively). No multicollinearity among the included variables was detected. After adjusting for all pre-planned possible confounders, GERD was significantly associated with the number of psychiatric rehospitalizations in recurrent MDD patients but not in schizophrenia patients (Table 3). The adjusted mean (95 % CI) number of psychiatric rehospitalizations in recurrent MDD patients with and without GERD was 5.1 (95 % CI 3.9 - 6.4) and 3.9 (95 % CI 3.1 - 4.8), respectively (Figure 2). The adjusted mean (95 % CI) number of psychiatric rehospitalizations in schizophrenia with and without GERD was 5.2 (95 % CI 1.4 - 9.0) and 6.2 (95 % CI 4.9 - 7.4), respectively.

Conclusions: GERD seems to be associated with higher rates of psychiatric rehospitalization in patients with MDD than in patients with schizophrenia. It is understood that antipsychotics have a protective effect on GERD and that anti-ulcer drugs combined with antipsychotics have a beneficial effect on the outcome. However, further studies should examine the causal relationship between psychiatric disorders and GERD.

Keywords: Depressive Disorder, Major, Schizophrenia, Gastroesophageal Reflux, Treatment

Dissertation Proposal Title: Prediction of RDS development using newborn's metabolomic profile

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Mentor: Assoc. Prof. Silvija Pušeljić, M.D., PhD., Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Introduction: Respiratory distress syndrome is most common cause of morbidity and mortality among preterm infants. Symptoms of the condition are: shallow breathing, apnea, grunting, inspiratory stridor, nasal flaring, poor feeding and tachypnea. Neonates with resiratory distress (RD) are several times times more likely to die than those without RD. The manifestations of the disease are caused by diffuse alveolar atelectasis, edema and cell injury. There are many causes for RD development of which about 20% is still unknown. Liquid chromatography–mass spectrometry is an analytical chemistry technique that combines the physical separation capabilities of liquid chromatography with the mass analysis capabilities of mass spectrometry. Coupled chromatography systems are popular in chemical analysis because the individual capabilities of each technique are enhanced synergistically.

Hypothesis: RDS in neonates GD ≥32 weeks and/or RM ≤2500g can be predicted prior to development of clinical simptoms by deviation of individual metabolites in metabolomic profil

Aims: to investigate whether there are certan metabolites at birth that are predictors of RDS development, if that's the case, determine the exact time when can we expect their appearance, to investigate are there any metabolites present in mother during labor that are indicative to RDS development, try to prevent the need for invasive ventilation, to investigate connections between standard laboratoty markers (CRP, PCT, KKS, AST, ALT, GGT, LDH, ALP, CK, Mgb, ABS, etc.) and metabolomic profile

Participants and Methods: The research will be preformed during the current year. The participants will be all children accepted for treatment at the Department of neonatology, KBC Osijek at a gestatonal age between 32 and 38 weeks, weighting ≤2500 g. Three blood samples will be collected. First blood sample will be collected

from mother entering first stage of labor (latent phase). Second blood sample will be umbilical blood (umbilical vein), and third blood sample will be from child that has developed RDS. Blood samples will be obrained from all study participants into EDTA-containin tubes. Immediately after collection, blood will be frozen and stored at -20°C until the time of analysis. Blood samples will be analysed liquid chromatography–tandem mass spectrometry (LC–MS-MS). Results will be quantitatively expresed for each single metabolite. RDS will be diagnosed according to the following criteria: respiratory distress begining in the first hours of life and lasting at least 24h, need for mechanical ventilation including continous positive airway preassure, presence of typical radiological findings in chest X-ray, and abnormal arterial blood gas results. This will be case - control study due to insufficient funds to do cohort study. Newborn child that developes RDS will be compared with next newborn child that is healthy (control).

Significance/Expected scientific contribution: Finding certan metabolites that are highly associated with RDS development in preterm neonates GD between 32-38 weeks and/or ≤2500g. Prediction of RDS development would prevent unnecessary examinations and avoid the long-term consequences of invasive mechanical ventilation

MeSH/Keywords: metabolomics, prematurity, newborn, mass spectrometry, RDS

Dissertation Proposal Title: Probiotics use in hen's egg allergen specific immunotherapy - influence on development of allergen tolerance

PhD candidate: Iva Mrkić Kobal, Children's Hospital Srebrnjak, Zagreb, Croatia

Mentor: Assoc. Prof. Mirjana Turkalj, M.D., Ph.D., Children's Hospital Srebrnjak, Zagreb, Croatia; Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia; Croatian Catholic University, Zagreb, Croatia

Introduction: Standard treatment for food allergy is food avoidance which leaves the patient susceptible to ingestion of small amount of hidden food and development of life-threatening allergic reactions. Oral immunotherapy (OIT) has evolved as the only etiological treatment of food allergy. As an additional possibility ofimprovement of OIT, different adjuvants, per example probiotics, have been introduced.

Hypothesis: The use of probiotics together with OIT is more effective in tolerance and desensitization induction than the use of OIT alone, providing more safety (fewer side effects).

Aims: The aim of our study is to investigate the development of hen's egg tolerance due to OIT and probiotics use. We also want to investigate the role of skin prick test (SPT), specific IgE (sIgE) to egg white, ovomucoid and basophil activation test (BAT) as potential markers in monitoring therapy success and evaluate the difference of side effects between groups.

Participants and Methods: This is a prospective, randomized, double blind and placebo-controlled study involving participants aged 5-18 with confirmed allergy to hen's egg. These participants will be randomized into two groups, one receiving OIT + probiotic and other receiving OIT +matching placebo. The size of sample is calculated with assumption that 20% of participants won't finish the study per protocol with statistical power of 80% and statistical error α 0.05%. Inclusion criteria: age 5-18, positive double-blind placebo controlled oral food challenge (DBPCOFC) to hen's egg. Exclusion criteria: uncontrolled and severe asthma, malignant disease, active autoimmune disease, eosinophilic esophagitis and other eosinophilic digestive disorders, difficult compliance, and treatment with β blockers and ACE-inhibitors. All participants will have confirmed written information consent. All participants included

in the study will receive OIT according to protocol for 12 months period. At the end of treatment, they will be assessed for desensitization using DBPCOFC and screened for SPT to hen's egg, sIgE to egg white, ovomucoid and BAT to egg white. After one-month period of egg elimination diet they will be reassessed with DBPCOFC. The *in vivo* and *in vitro* test and DBPCOFC for assessment of tolerance will be done 3 months after cessation of treatment. A control group of participants with hen's egg allergy will be monitored for the same period with the exception of DBPCOFC at T2. Data analysis will be done using STATISTICA ver. 12 in a blinded fashion. Two sided chi-square test and analysis of variance for repeated measurements will be used with $p<0.05$ as statistically significant.

Research plan: Research will be conducted in Children's Hospital Srebrnjak in period from 2019 - 2022. We plan to receive Ethical Committee and Medical Faculty approval and start participants recruitment by 11/2019. The recruitment will last till 11/2020. The duration of study with till last patient, last visit is 15 months. We plan to do all statistical workup in the 02/2022.

Significance/Expected scientific contribution: Our research will contribute the evidence of probiotics use as adjuvants in OIT inducing food desensitization and tolerance.

MeSH/Keywords: food allergy, oral immunotherapy, probiotics, desensitization, tolerance

Abstract Title: The influence of hyperthyroidism and it's treatment by antithyroid drugs on changes of bone metabolism, bone density and Wnt inhibitors sclerostin and dickkopf 1

Part of the Disertation Proposal: The influence of Graves'disease and it's treatment by antithyroid drugs on changes of bone metabolism and bone density

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Introduction: Graves' disease (GD) is an autoimmune disease characterized with hyperthyroidism which can cause a decrease in bone mineral density (BMD). The first line therapy is antithyroid drugs (ATD) which can lead the disease remission and improvement in BMD. Osteopenia and osteoporosis are becoming a major health burden leading to research of various pathophysiological mechanisms responsible for bone remodeling. Wnt (wingless integrated) signaling pathway is a very important factor in bone homeostasis, especially canonical pathway. Present data indicate that stimulation of Wnt pathway leads to bone mass increase and opposite, its inhibition to bone mass decrease. Hence, inhibitors of canonical Wnt pathway became focus of interest, in particular sclerostin and dickkopf 1 (Dkk 1).

Aims:

1. to determine a concetration difference of bone formation marker osteocalcin (OC) and bone resorption marker deoxypyridinoline (Dpd) and Beta-Crosslaps (β-CTx) at the time of diagnosing GD and beginnig of treatment with ATD and after a period of 12 months

2. to determine a difference in BMD at the time of diagnosing GD and beginnig of treatment with ATD and after a period of 12 months
3. to determine a concetration difference of sclerostin and Dkk 1 at the time of diagnosing GD and beginnig of treatment with ATD and after a period of 12 months

Materials/Participants and Methods: Participants are patients with newly diagnosed GD based on elevated concetrations of free triiodothyronine (FT3) and free thyroxine (FT4), suppressed thyroid stimulating hormone (TSH), positive TSH receptor autoantibodies (anti TSH–R), positive thyroid Tc99m pertechnetate scan and thyroid ultrasound appearance. BMD at lumbar spine and left hip is determined. From 8 – 10 a.m. patients gave their serum and urine samples in oder to determine OC, Dpd and β-Ctx, sclerostin and Dkk 1. They filled a query about their diet habits. Patients are treated with ATD. After twelve months control BMD is done and patients gave new samples of serum and urine to do control concetration measurments of the above mentioned bone markers and Wnt inhibitors.

Results: The research included 43 patients. Five of them (12%) are male and 38 (88%) are female. Average age is 47 years, ranging from 20 to 75 years. Control tests done in 16 patients show significantly lower values of FT3, FT4, anti TSH–R, OC, β–CTx and Dpd, while significantly higher values of TSH and BMD of lumbal spine. There is no significant difference in BMD of the left hip. In this stage of the study we did'n analyze concetrations of sclerostin and Dkk 1.

Conclusion: These findings suggest that in our patients decreased BMD of lumbar spine and bone remodeling changes are of secondary etiology, due to GD. By achieving euthyroid state with ATD treatment, control findings significantly improved so additional management and/or treatment of transitory osteopenia/osteoporosis are not necessary.

MeSH/Keywords: hyperthyroidism, bone metabolism, bone density, sclerostin, dickkopf 1

Abstract Title: Collagen deposition and adipose tissue morphology in subcutaneous and visceral adipose tissue of young male children

Part of the Dissertation Proposal: Proinflammatory and remodeling processes in visceral and subcutaneous adipose tissue defined through the activity of immunomodulatory adipocytokines

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Mentor 2: Assist. Prof. Dalibor Divković, M.D. Ph.D., University Hospital Osijek, Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Introduction: Adipose tissue (AT) has been extensively studied for its role in the etiology of the metabolic syndrome. AT dynamically responds to increased or decreased caloric intake by adipocyte hypertrophy and hyperplasia and thus maintaining the energy homeostasis of the body. In response to changes in the nutritional status, the AT undergoes through dynamic remodeling, including quantitative and qualitative alternations in adipose tissue-resident cells that indicates that AT remodeling in obesity is closely associated with AT function. AT remodeling is characterized by increased deposition of extracellular matrix (ECM) proteins, and collagens form the largest group of ECM proteins which structure and density are strictly regulated. The ECM is a crucial component of AT that is necessary for maintaining cellular and structural integrity. Furthermore, changes in the number and size of adipocytes affect the microenvironment of expanded fat tissue accompanied by alternations in adipokine secretion. An understanding of how AT remodeling differs between visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) fat depots may unlock the links between visceral adiposity and metabolic disease.

Aims: Measure body weight and height, waist and thighs circumference, calculate body mass index and Z-score of subjects, Determine the surface area of adipocytes in VAT and SAT in subjects, Compare the surface area of adipocytes in VAT and SAT in subjects dependent on age, Determine the collagen content in VAT and SAT in subjects dependent on age, Compare the collagen content in VAT and SAT in subjects dependent on age;

Participants and Methods: The research is based on young male children, up to 27 subjects for this preliminary part of the research, who were hospitalized for elective inguinal hernia surgery at the Department of Pediatric Surgery of the University Hospital Osijek. Prior to the beginning of the research every parent of the subject that meets the criteria for the research was given written explanation of the purpose of the research and signed informed consent. Samples of SAT and VAT were gained during surgical procedure. Histological staining of extracellular components was performed with Masson's trichrome stain using standard protocol, and histomorphometric analysis of digital images of histological tissue sections was conducted using the free online image analysis program Fiji, a distribution of ImageJ. All images were acquired under the same conditions. Quantification of collagen content on Masson's trichrome stain tissue sections was performed with „Color Transformer 2" plug-in and then proceeded manually with Threshold tool. Adipocyte surface area was measured with "Adiposoft" plug-in with manual corrections. Data analysis was performed using IBM SPSS Statistics software (version 21, IBM Corporation, NY, USA).
Inclusion criteria for the research: anamnestically healthy subjects who do not suffer from diabetes, cardiovascular, neurovascular, genetic, hormonal and malignant diseases.
Exclusion criteria for the research: the presence of a positive history of severe and chronic disease, and of previously known diseases (e.g., malignant and genetic disorders). Excluded are individuals who were operated due to entrapped ventral hernia.

Results: Tissue sections staining with Masson's trichrome stain for ECM content such as collagen showed increased collagen deposition in the group where subjects were over 5 years of age which was statistically significant when compared to the group up to 5 years of age; p=0,021 (Fig. 3). There was no statistical significance among groups when comparing adipocyte surface area in SAT and VAT (Fig. 3). For more and detailed conclusions, further analytical methods need to be done such as RT-PCR, IHC and ELISA on larger sample size.

Conclusion: In healthy, growing young male children, increased deposition of collagen content is age related.

Keywords: adipose tissue, collagen, adipocytes, adipose tissue remodeling, extracellular matri

Abstract Title: Effects of high glucose and hydrogen peroxide on cellular survival in the HK2 cell line

Part of the Disertation Proposal: Molecular mechanisms of renoprotective effect of empagliflozin and liraglutide on cellular model of proximal tubular cells.

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Introduction: Diabetic nephropathy (DN) as a complication of type I and type II diabetes mellitus causes changes in microvasculature, leading to end-stage renal disease and cardiovascular disease. Hyperglycemia, proteinuria, hypoxia, and inflammation contribute to proximal tubular damage in DN by shifting the hormone-induced release of cytokines (TGF-β), the renin-angiotensin system, etc. Hyperglycemia promotes cellular hypertrophy and enhances collagen transcription in tubular cells. Hypoxia causes tubular damage through oxidative stress, inflammation, and matrix production. Inflammation and chronic high levels of circulating glucose and its end metabolites are major causes of tissue damage in DM by, among other, making high levels of oxidative and nitrosative stress in kidneys.

Aims: The aim of our study was to determine the viability of cells of tubule damage in *in vitro* model of DN in HK2 cell line.

Materials and Methods: HK2, an immortalized proximal tubule epithelial cell line from normal adult human kidney was exposed to pathophysiological mediators that mimic DN: high glucose and oxidative stress. The cells were cultured in DMEM/ Hams-F12 with GlutaMAXTM I medium containing Heat-inactivated fetal calf serum, Hydrocortisone 21-hemisuccinate sodium salt, Epidermal Growth Factor (hEGF), Supplement of insulin, transferrin and selenium (ITS 500x), Triiodothyronin, 1 % Penicillin (100 units/ml) -Streptomycin (10,000 lg/ml). HK-2 cells were grown in cell incubator at 37 °C with 5% CO_2 (v/v). To examine the role of oxidative stress in HK2 induced by high glucose (HG) and hydrogen peroxide (H_2O_2) the cells were treated

with different concentrations of glucose (4.5, 15, 30, 45 mM), H_2O_2 (0.5, 1 mM) and the combination of glucose and H_2O_2 (30/0.5mM, 45/0.5mM and 45/1mM). A Trypan blue test and MTT assay were used for the assessment of cell viability after 24 hours of the treatment.

Results: To assess oxidative stress, HK-2 cells were treated with different concentrations of HG and H_2O_2. Trypan Blue test showed significant decrease in survival of cells treated with HG at 15,30 and 45mM ($p<0.001$, t-test with Bonferroni correction), H_2O_2 at 1mM ($p<0.001$, t-test with Bonferroni correction) and the combination of 45 mM HG + 1 mM H_2O_2 ($p<0.001$, t-test with Bonferroni correction) after 24 hours, with untreated cells as a control. MTT assay demonstrated significant inhibition of cell viability of HK2 cells using HG at 45mM ($p<0.01$, t-test with Bonferroni correction), H_2O_2 at 1mM ($p<0.01$, t-test with Bonferroni correction) and the combination of HG at 45 mM+ H_2O_2 at 1 mM ($p<0.001$, t-test with Bonferroni correction) after 24 hours, compared to untreated control.

Conclusion: These results demonstrate that HG and H2O2 increase apoptosis in human tubular cells via stimulation of oxidative stress. Further i*n vitro* studies should be made to clarify the underlying molecular mechanisms of empagliflozin and liraglutide protective effects on renal tubular epithelial injury which would potentiate the development of new specific therapeutic options for preventing tubulointerstitial injury in diabetic nephropathy.

MeSH/Keywords: diabetic nephropathy, proximal tubule, hydrogen peroxide**,** high glucose, HK2

Dissertation Proposal Title: Mental disorders among overweight and obese children and adolescents

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Mentor 2: Assist. Prof. Ivan Miškulin, Ph.D., Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Introduction: Obesity among children becomes one of the most significant public health problems in the whole world. Altought it stagnates in some countries, obesity prevalence rises globally, as well as specific commorbidities. It is not examined how mental disorders coexist with overweight and obesity, as well as how are this disorders associated, but there is consensus that obesity threatens mental health. It is necessary to systematically review literature and to meta-analyse it to determine the prevalence and the most common mental disorders and to determine potential sources of heterogenity among existing studies.

Hypothesis: Overweight and obese children and adolescents are more often affected by mental disorders than children and adolescents from general population. Prevalence of mental disorders is between 40% and 50% and most common is anxious disorder.

Aims: (1) to estimate prevalence and association of mental disorders and overweight and obesity among children and adolescents, (2) to estimate types of mental disorders in this population, (3) to compare mental disorders prevalence within this and the general population, (4) to estimate differences in the prevalence acording to gender, (5) to estimate association of mental disorders and obesity severity, (6) to estimate the studies quantitatively and qualitatively, (7) to estimate heterogenity and sources of the heterogenity in the estimation of prevalence and association, (8) to estimate the need for new studies and their characteristics.

Participants and Methods: Study material will be published research and available unpublished publications which present prevalence and association in their results or from which they can be calculated.

Research plan: Study will be conducted in online databases MEDLINE, EMBASE and PsycINFO via OVID, OpenGrey and ProQuest Dissertations & Theses according to pre-planned strategy. Two authors will conduct databases research independently in two phases. Study quality assesment is going to be conducted according to STROBE (Strengthening the reporting of observational studies in epidemiology) guidelines by two authors independently, as well es data extraction.

Significance/Expected scientific contribution: Study will discover proportion of overweight and obese children and adolescents with mental disorders, mental disorder types by frequency. Study will show are there differences in the prevalence of mental disorders considering the gender, the age or the origin of the child. This study will encourage the introduction of the mental disordes screening among overweight and obese children and adolescents. Study will analyse quality of the available research and the sources of heterogenity among them. It will detect especially at-risk groups among study population and serve for the planning of new research and metodology.

MeSH/Keywords: children, adolescents, overweight, obesity, mental disorders, systematic review

Dissertation Proposal Title: Application of array-comparative genomic hybridisation in semen analysis

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Mentor: Assoc. Prof. Jasenka Wagner Kostadinović, Ph.D.,, Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Introduction: Infertility in humans is common problem occurring in approximately 12% of the reproductive population. Recent data has showed that in every third infertile case, reason is male infertility and that 50% of male infertility cases are caused by genetic factors. Routine laboratory testing for male infertility include hormone and semen analysis (spermiogram). Sperm fluorescent in situ hybridization (FISH) is a widely used screening tool to aid in counseling couples with severe male factor infertility, especially in cases of prior repeated in vitro fertilization (*IVF)*/intracytoplasmic sperm injection (ICSI) failure or recurrent pregnancy loss. With this method it is possible to detect specific chromosomal aberrations in ejaculate. Array-Comparative Genomic Hybridization (aCGH) involves screening of the entire chromosome complement by DNA microarray. It is a new method of molecular karyotyping that could serve in detection of male infertility, determine the cause, help in prediction of IVF result and help in prenatal diagnosis of Y chromosome diseases.

Hypothesis: aCGH on single human sperm can help in understanding the biology and pathophysiology of spermatogenesis and sperm chromosome aberrations in men undergoing IVF/ICSI. Using this method new genetic abnormalities can be found and existing confirmed, which should at the end lead to better success of assisted reproductive techniques (ART).

Aims: Investigate frequency of structural and numerical chromosomal changes in ejaculate of men undergoing IVF and ICSI compared to healthy fertile individuals, investigate relation of chromosomal changes and efficacy of IVF and consequently number of healthy newborns, examine influence of chromosomal aberrations on spermiogram results and investigate whether aCGH can help to choose the best sperm for IVF.

Participants and Methods: Participants will be infertile patients with oligozoospermia undergoing ICSI. Healthy fertile individuals will serve as a control. Conventional semen analysis will be performed. Single sperm will be isolated using micromanipulator, whole genome amplification (WGA) will be performed before aCGH. Data analysis will be performed using various genetic databases.

Research plan: Additional studying the literature with emphasis to technical data and experiment design, writing the research project and applying for the funds, application to ethical committees, public defense of topic of doctoral thesis, collection of samples, performing the analysis, data analysis, writing and publishing the manuscript, public defense of doctoral thesis.

Significance/Expected scientific contribution: This would be one of the few studies of chromosomal abnormalities in ejaculate using aCGH. By applying this method, we will try to improve current IVF methods. Detection of chromosomal aberrations in sperm would contribute to understanding of male infertility and bring us new knowledge for successful ART.

MeSH/Keywords: infertility, sperm, semen analysis, reproduction, array comparative genomic hybridization

Dissertation Proposal Title: The anti-inflammatory effect of intravenous anesthetics in abdominal surgery

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Introduction: Clinical studies have shown that IV administration of anesthetics, lidocaine and ketamine with their anti-inflammatory properties, modulates the acute immune response associated with surgical tissue injury, and in this manner they are able to reduce postoperative pain. Lidocaine has anti-inflammatory effects on polymorphonuclear granulocytes, IL-6 and IL-8 cytokines, complement component C3a and IL-1ra in serum. Ketamine produces its anti-inflammatory effects by reducing CRP and IL-6 in serum and by inhibiting NF-kB, which regulates gene transcription responsible for the production of proinflammatory factors.

Hypothesis: Perioperative combinend IV administration of lidocaine and ketamine could have a more favorable anti-inflammatory effect compared to anesthetic given alone or with placebo.

Aims: To investigate the effects of lidocaine and ketamine in patients undergoing abdominal surgery on: Acute immune response by following the serum levels of leukocytes and proinflammatory factors (CRP,IL-6, IL-8), Postoperative pain management, Recovery of bowel function, Length of stay in the ICU and length of hospitalization, Reduced administration of opioids, Reduction of total treatment costs

Materials/Participants and Methods: A double-blind, placebo-controlled study will include 120 patients undergoing conventional colorectal surgery. Patients will be randomly assigned to one of four groups: lidocaine, ketamine, lidocaine-ketamine, and placebo. Lidocaine will be administered at a dose of 1.5 mg/kg prior to surgical incision followed by an infusion at a rate of 1.5–2 mg/kg/hr until the end of surgery. Ketamine will be administered at a dose of 0.5 mg/kg in a bolus prior to surgical incision followed by an infusion at a rate of 0.1–0.2 mg/kg/hr until the end of surgery.

Bolus and continuous placebo infusion (0.9% NaCl) will be administered identically as the aforementioned anesthetics until the end of the surgery.

Research plan: The intensity of pain will be measured using the VAS score 2 hours and 4 hours following surgery and then every 12 hours the following 48 hours. We will measure the consumption of opioids during and after surgery, the length of stay in the ICU, where pain control and analgesics use will be measured, as well as recovery of bowel function. Leukocytes and proinflammatory markers in serum (CRP, IL-6, IL-8) will be measured before induction of anesthesia, then 12 hours and 36 hours following the completion of surgery. The study was registered on ClinicalTrials.gov and was assigned identification number NCT03821545.

Significance/Expected scientific contribution: The modulation of the acute immune response to surgical stress following the administration of IV anesthetics during colorectal surgery results in faster recovery of patients, reduced time of hospitalization along with reduced treatment costs.

MeSH/Keywords: lidocaine, ketamine, anti-inflammatory effect, surgical stress, abdominal surgery

Dissertation Proposal Title: The effect of age, cigarette smoking and oral L-Arginine supplementation on hepatic blood flow in healthy subjects

PhD candidate: Tijana Pandurović, Department of Radiology, General Hospital Vukovar, Vukovar, Croatia

Mentor: Assoc. Prof. Lada Zibar, M.D., Ph.D., Faculty of Medicine, University of Osijek, Osijek, Croatia; Department of Nephrology, Clinic of Internal Medicine, Merkur Clinical Hospital, Zagreb, Croatia

Introduction: Arterial system is a blood distribution system adaptable to variations in blood flow in certain body parts, especially in muscles. One of the main causes of vascular dysfunction by arterial damage is atherosclerosis. Less attention is paid to blood flow disorders that occur distally from regional arteries (arterioles, capillary plexuses), to elasticity and to adaptability of parenchymal organs. There is not much research that observes several levels of circulatory system concurrently within a subject. There's a possibility of identifying three subgroups of disorders: dominantly arteriosclerosis, capillary dysfunction caused by parenchymal organ fibrosis and mixed forms. It is important to point out that a tissue with an inadequate perfusion often depends on local synthesis of nitric oxide (NO). Since an increased synthesis of NO occurs in such patients, a relative deficit of L-arginine as a crucial precursor is possible.

Hypothesis: Older age and nicotinism attribute to vascular dysfunction in subjects that do not suffer from circulatory or liver diseases and peroral L-arginine administration affects blood flows in tissues, including liver.

Aims: To determine liver circulatory and microcirculatory system within healthy subjects before and after L-arginine administration, To determine the effect of age and nicotine on vascular function, To determine the effect of L-arginine on vascular function

Participants and Methods: Four groups of subjects are formed, defined by their age and nicotine consumption. Each group consists of at least 30 subjects. The first two groups are made of people aged 30 or younger, defined as younger adults; one group is comprised of smokers, while the other of non-smokers. The next two groups are

made of people older than 30, defined as older adults, and are also divided into a smoker and a non-smoker group. A smoker is defined as a person who has, in the last year, smoked at least 10 cigarettes per day.
A Color Doppler on a Philips Affinity 70 ultrasound device will be used to observe arteries and to measure blood flow velocity through its lumen; also, a Biopac Student Lab device will be used for multichannel recording of different signals (infrared plethysmography and electrocardiography).

Research plan: Two groups of subjects, without any evident clinical data on circulatory or liver diseases, are formed. Two consecutive recordings are planned for each subject in the morning for two consecutive days. Every subject will, at the same time, have a blood flow velocity in the abdominal aorta, superior mesenteric artery, renal arteries and portal vein by Doppler ultrasound recorded, as well as plethysmography of capillary flow in little fingers of fists along with an ECG. After the first recording, the subject will receive two 500 mg L-arginine capsules (NO precursor) which they should take in the evening after a lighter meal not containing high amounts of protein, for the intention of periphery tissues to synthesize NO by tomorrow measurement.

Significance/Expected scientific contribution: Types of circulatory dysfunction according to age and cigarette smoking, Understanding regulation of blood flow through large regional arteries including liver arteries and through capillary network, Clinical peroral administration of L-arginine as a precursor of NO

MeSH/Keywords: L arginine, doppler, plethysmography, nitricoxide, flow, atherosclerosis

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

PhD candidate: Ivana Pavličević Tomas, Clinical Hospital Centre Osijek, Psychiatry Clinic, Osijek, Croatia

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

Introduction: Every person has the right to health care and the ability to achieve the highest possible level of health in accordance with legal requirements. Health care within the meaning of the Health Care Law includes a system of social, collective and individual measures, services and activities for the preservation and promotion of health, disease prevention, early detection of diseases, timely treatment, and health care and rehabilitation. According to the Health Care Law, every person is obliged to take care of their own health, to provide first aid in accordance with their knowledge, to participate in health-preventive activities and not to endanger the health of others. Mental health has been defined by the World Health Organization as a state of well-being in which an individual achieves his or her potential, can cope with normal life stress, work productively and fruitfully, and is able to contribute to the community. According to this approach, it is part of general health and an affirmative space for actualizing one's own life, not simply the absence of illness. People with mental health problems are known to be more difficult to function in their environment, and their stigmatization, which involves negative labeling, marginalization and avoidance because they have a mental illness, becomes a problem in itself. Therefore, it is concluded that persons with mental disorders do not adequately care for their own health in relation to the population without mental illness. Mental illness can have various powerful effects on a person's lifestyle, including social and financial status, which may put their physical health and overall functionality at risk.

Hypothesis: The level of care for one's own health, which includes care for physical, mental, sexual and spiritual health, is lower and more inadequate in persons with mental disorders compared to a healthy population.

Aims: The aim of this study is to determine whether there is a difference in the health care of people with schizophrenia and depression compared to the non-mentally ill

population, since we know that when it comes to mental health problems it is very difficult to focus on care about yourself. Lack of energy and loss of motivation with less insight into one's own condition can lead a person to neglect their physical health. To determine how lifestyle affects diseases in a healthy population and how it affects the course of illness in schizophrenic and depressed people. Compare quality of life and level of functioning with regard to lifestyle and health care in healthy, schizophrenic and depressed persons.

Materials/Participants and Methods: The study will be conducted in three separate groups, consisting of healthy people, people with schizophrenia and people with depressive disorder. These three groups of people will be examined and compared separately.
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 survey questionnaire and rating scales with the supervision of the examiner with access to primary care medical records and specialist findings. The survey questionnaire will be structured and designed for the purpose of the survey and will include data indicating the physical health of the study group with an emphasis on lifestyle (smoking, alcohol consumption, drug abuse, nutrition, exercise, personal hygiene) and physical illness (accompanying physical health care at the level of involvement and participation in primary, secondary and tertiary prevention measures; frequency and attendance at systematic examinations; dental health care). The assessment of mental health, as an important segment of general health, will be based on the completion of a survey questionnaire and rating scales, and an insight into the medical records at the level of primary, secondary and tertiary health care. Further research will be conducted through a survey questionnaire and access to medical records on sexual health care (contraceptive measures, prevention and treatment of sexually transmitted diseases, pregnancy monitoring) and spirituality (belonging to religion and community). The level of functioning will be assessed separately with regard to social and professional functionality (employment, life existence, working conditions, social interactions, leisure time).

Research plan: A cross-sectional study is used in this research.

Significance/Expected scientific contribution: The key findings of the dissertation will be reflected in a comprehensive and systematic account of caring for one's health between healthy, schizophrenic and depressed persons. The paper will use appropriate scientific methods to analyze and compare how and to what extent the examined population cares for physical, mental, sexual and spiritual health. How lifestyles, physical and mental illnesses reflect on the quality of life and the course of mental illness. Based on the results obtained, one can reflect on the manner and

development of primary, secondary and tertiary preventive measures to ensure the promotion of health in the Republic of Croatia.

MeSH/Keywords: healthy, schizophrenic, depressed, care, prevention

Dissertation proposal title: Injectable biphasic calcium phosphate vs. bovine xenograft in Guided Bone Regeneration - a prospective randomized clinical trial

Ph.D. candidate: Marija Pejakić, Faculty of Dental Medicine and Health Osijek, University of Osijek, Osijek, Croatia

Mentor1: Assist. Prof. Željka Perić Kačarević, Ph.D., Faculty of Dental Medicine and Health Osijek, University of Osijek, Osijek, Croatia
Mentor 2: Assist. Prof. Marko Matijević, Ph.D., Faculty of Dental Medicine and Health Osijek, University of Osijek, Osijek, Croatia

Introduction: Biomaterials for bone augmentation can be classified as bone substitute materials (allografts, xenografts, and alloplastic bone substitutes) and membranes for Guided Bone Regeneration (GBR), which is one of the most common methods used to promote bone regeneration in implant dentistry. Biphasic calcium phosphate is a mixture of hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP), of varying HA/β-TCP ratios, most commonly 60:40 and 70:30. Previous in vivo studies showed that injectable bone substitutes (I-BCP) based on β-TCP have osteoconductive properties. One clinical case on use of I-BCP in sinus-lift procedure was reported. However, currently, there are no clinical studies on the use I-BCP consisting of 60% HA and 40% β-TCP in GBR. In present study we want to asses histological properties of I-BCP in GBR.

Hypothesis: This study hypothesizes that there are no differences in potential for bone regeneration between I-BCP and xenograft of bovine origin.

Aims: to examine the bone regenerative potential of an I-BCP and xenograft by assessing descriptive histological results of bone biopsy harvested from the place of implantation, to determine histomorphometrical results (percentage of residual biomaterial, percentage of the soft tissue and percentage of the new bone) in bone biopsy harvested from the place of implantation, to register wound healing complications.

Participants and Methods: At the first stage of study 40 healthy patients with inidication for Guided Bone Regeneration (GBR) will be enrolled in study. Following the debridement, in 20 patients socket is filled with injectable biphasic calcium

phosphate (I-BCP) consisting of 60% hydroxyapatite (HA) and 40% beta-tricalcium phosphate (β-TCP), and in control group consisting of 20 patients socket will be filled with xenograft of bovine origin. In both groups a resorbable native collagen membrane is trimmed and adapted to cover the bone defect fully. The mucoperiosteal flap is readapted, and the wound is closed with single sutures. The sutures are removed after 10 days. Six months after the GBR, in the both groups the second stage of the study will start. In this stage, a re-entry procedure will be performed to harvest bone for histological analysis, and to place a dental implant. A trephine bur with an internal diameter of 2.5 mm will be used to harvest the bone from the central part of the pre-existing bone defect. Biopsies will go through the preparation process, cutting and coloring so the histological (qualitative and quantitative) analysis could be done.

Research plan: This prospective randomized clinical study will be performed in the period between October 2018 and March 2020. Patients will be recruited in a Community Health Center in Osijek. To enter the study, patients need to provide written consent. All surgeries will be performed by one experienced oral surgeon.

Significance/Expected scientific contribution: Sucessful bone augmentation and implant-supported prosthetic rehabilitation does not only depend on the clinical skills of the practitioner, but also on the appropriate choice and use of different biomaterials. The peculiarity of I-BCP is that their viscosity allows easy filling of the bone defect, which also reduces surgery time and the burden to the patient. The use of the I-BCP in augmentation procedures has been poorly investigated and currently there there are no published studies on the use I-BCP, so this study would be the first histological study on the usage of I-BCP in GBR.

MeSH/Keywords: biphasic calcium phosphate, xenograft, dental implantology, histology, histomorphometry

Dissertation Proposal Title: Effect of smoking and copd on immune response of lung adenocarcinoma

PhD candidate: Josip Pejić, Clinical hospital Dubrava, Zagreb, Croatia

Mentor: Assist. Prof. Igor Nikolić, M.D., Ph.D., Clinical hospital Dubrava, Zagreb, Croatia

Introduction: Lung cancer is a major public health problem worldwide, including Croatia. The leading cause of all cancer mortality. Considered extremely aggressive, has a very low five-year survival rate between of 15-20% patients. Approximately 80% -85% of lung cancer is treated by some method - surgical, chemotherapy, radiotherapy. Immune therapy has recently been started. The immune system plays an important role in lung cancer and chronic obstructive disease. Previous studies indicate that the immune response determines the course and prognosis of the disease.
For example PD-1 and PD-L1 check point inhibitors where clinical studies shows a significant response and improved survival. PD1 and PD-L1 cover only one aspect of the immune response in tumors, leaving more place for other interventions on immune defense of the tumor. Atezolizumab and Pembrolizumab are the most well-known representatives.

Hypothesis: The immune response in lung adenocarcinoma differs in chronic obstructive pulmonary disease (COPD), smokers and non-smokers. The immune response is an indicator of disease prognosis.

Aims: The main objective is to determine whether non-smokers, smokers, and COPD patients differ in their immune response to lung adenocarcinoma.
1. to determine markers of the immune response in the group of operated patients in Clinical hospital Dubrava
2. Colerate the presence of markers of the immune response with the stage of malignancy, accompanying lung pathology and smoking status.
3. Colerate biological markers of immune response with patient survival rates and local recurrence and / or distant metastases.

Materials/ Participiants and Methods: Patients with lung adenocarcinoma operated in Clinical hospital Dubrava in 2012 and 2013 (100 patients per year) were classified into groups based on COPD and without additional lung pathology.

Groups of patients with adenocarcinoma of the lung: a) COPD-smokers, b) COPD ex-smokers, c) non-COPD-smokers d) non-COPD-ex-smokers, e) non-COPD-smokers

MATERIALS - 1. paraffin cubes stored at the Department of Pathology, Clinical hospital Dubrava
2. The necessary antibodies for immunohistochemistry and PCR primers will be obtained for immunoassay purposes.

1. Patient screening will be performed on the criteria defined by the study design
2. Paraffin blocks will be analyzed by classical immunohistochemical methods set for specific markers (such as CD4, CD8, CD20, and antigen presenting cells for MDSCs from TAM (tumor assosiated macrophages, etc.) - the number of positive cells infiltrating the tumor will be determined and the number of positive cells in the near and far region of the tumor

The results will be presented in tables and graphs. Normality of data distribution will be analyzed (Smirnov-Kolmogorov test) and corresponding parametric and / or non-parametric statistical analyzes and methods of data presentation will be applied according to the obtained results.

Quantitative variables will be presented through the median and associated interquartile ranges, while categorical variables will be presented through frequencies and corresponding proportions. Any P value less than 0.05 will be considered statistically significant. The analysis will use STATISTICA 10.0 software (www.statsoft.com)

Significance/Expected scientific contribution: A significant contribution to a better understanding role of the immune response against lung adenocarcinoma and the impact of active smoking and COPD on the survival.

Dissertation Proposal Title: Individual measurement of intima and media thickness in correlation with arterial hypertension, diabetes mellitus and smoking in patients with and without ischemic stroke.

PhD candidate: Romana Perković, M.D., Department of Neurology, University Hospital Osijek, Osijek, Croatia

Mentor: Prof. Silva Butković Soldo, M.D., Ph.D., Department of Neurology, Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Introduction: In order to identify asymptomatic patients with latent vascular risk, we need to upgrade and extend the methods for identifying such patients. There is a shared opinion that simple, inexpensive and non-invasive methods for recognizing subclinical atherosclerosis should be used, such as measurment of intima and media thickness in carotid arteries.

Hypothesis: Determine the thickness of individual layers of the carotid artery wall depending on specific risk factors in patients with and without stroke in a given age group.

Aims: The main objective of the non-invasive diagnostic procedure - ultrasound examination of the carotid arteries – is to determine the thickness of individual layers of the carotid artery wall as the basis of subclinical arteriosclerosis and one of the main risk factors for cerebrovascular disease, depending on other common cerebrovascular disease risk factors such as arterial hypertension, diabetes mellitus and smoking. The secondary objectives of the study are: to determine the correlation of wall thickness in patients with risk factors and to determine possible sex differences; to determine the significance of the duration of the presence of risk factors necessary for the detection of pathological ultrasound changes of the carotid arteries.

Participants and Methods The research will include subjects, male and female, from eastern Croatia between 50 and 70 years of age which have been hospitalized for ischemic stroke at Clinic for Neurology at Clinical Hospital Centre Osijek. Control group will be subjects who come to the Clinic for Neurology for regular outpatient ultrasound imaging. Ultrasound scanning will be performed by a single expert sonographer using a 7 MHz linear transducer. Maximal height (thickness) will be

measured in common carotid artery and internal carotid artery, measuring intima and media thickness individually.

Research plan: Duration of the research will be aproximately one year, starting with comprehensive literature review, following by ultrasound imaging of hospitalized patients and outpatient volunteers. Third part of the research plan will be statistical analysis of gathered dana, following by data publishing.

Significance/Expected scientific contribution: Intima and media thickness in correlation with specific risk factors can identify asymptomatic people who will benefit from aggressive preventive measures which will consequently lower number of atherosclerotic cerebrovascular incidents.

MeSH/Keywords: carotid artery, atherosclerosis, intima media thickness, risk factors, stroke

Abstract Title: Association of alexithymia with laboratory values, sleep quality and datime sleepiness among hemodialysis patients

Part of the Disertation Proposal: Depression, sleep disorders, defense mechanisms and alexithymia among patients on chronic hemodialysis program

PhD candidate: Đorđe Pojatić, Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia, General County Hospital Vinkovci, Vinkovci, Croatia

Mentor 1: Assoc. Prof. Dunja Degmečić, M.D., Ph.D., Faculty of Medicine Osijek, University of Osijek, Croatia, Department for Psychiatry, Clinical Hospital Centre Osijek, Osijek, Croatia
Mentor 2: Assist. Prof. Davorin Pezerović, M.D., Ph.D., Faculty of Dental Medicine and Health Osijek, University of Osijek, Croatia, General County Hospital Vinkovci, Vinkovci, Croatia

Introduction: Alexithymia is a construct, stable personality trait that implies difficulty distinguishing between emotions and the body sensations, difficulty in describing feelings to other people, constricted imaginal processes and a cognitive style of thinking that is oriented towards external stimuli rather than feelings (1). Moreover, alexithymia is also associated with lower sleep quality, depression, lower survival and quality of life among hemodialysis patients (HD patients) (2). Yet, it is unknown if there is any correlation between alexithymia and biochemical parameters of HD patients or duration of hemodialysis program. It is also unknown whether there is any corelation between alexithymia and excessive daytime sleepiness in HD patients.

Aims: Aims of this study was to determine alexithymia association with excessive daytime sleepiness, laboratory values of potassium, phosphorus, leukocytes and duration of HD patients hemodialysis.

Participants and Methods: The sample consisted of 83 HD patients (47 males and 36 females) on chronic hemoadialysis programe, mean age 63,95 years. Participants answered questions from the sociodemographic questionnaire,Toronto Alexithymia Scale 26 (TAS 26) and Epworth Sleepiness Scale (ESS), both validated in Croatian (3, 4). On the same day, participants gave blood samples for analysis of biochemical values.

Results: Based on alexithymia levels there is significant difference in serum phosphorus levels among HD patients (p=0.027, Kruskal Wallis H test). Alexithymic patients had lower levels of serum phosphorus compared to HD patients without alexithymia (p=0.022). HD patients had significantly different level of alexithymia based on duration of hemodialysis (p=0.034, Kruskal Wallis H test), respectively HD patients with mild levels of alexithymia had longer duration of hemodialysis compared to patients without alexithymia (p=0.035). Alexithymic HD patients had significantly higer levels of daytime sleepiness compared to patients without alexithymia (p<0.000, Kruskal Wallis H test). Alexithymia levels were positively correlated with leukocyte levels (p=0.004, Pearson correlation coefficient). Subscale of TAS 26, externaly oriented thinking correlated positively with potassium levels (p=0.032, Spearman correlation coefficients).

Conclusion: Alexithymia is an important personality trait in HD which is strongly associated with low phosphorus levels and high leukocyte levels. Alexithymic HD patients had excessive daytime sleepiness.Duration of hemodialysis may increase the rise levels of alexithymia in HD patients.

MeSH/Keywords: alexithymia, hemodialysis patients, excessive daytime sleepiness, phosphorus, potassium

Dissertation Proposal title: Comparison of serum concentrations of folic acid in a patient with therapeutic resistant and non- resistant depression

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Mentor 1: Assist. Prof. Bjanka Vukan – Ćusa, M.D., Ph.D., Clinic for Psychiatry University Hospital Center Zagreb, Zagreb, Croatia
Mentor 2: Prof. Ivan Požgain, M.D., Ph.D., Clinic for Psychiatry University Hospital Center Osijek, Osijek, Croatia

Introduction: Major depressive disorder is the second leading cause of disability worldwide and affects an estimated 350 million people. Although big progress in treatment of depression has been made , an estimated 15% od depressed patients da not respound adequate to pharmacotherapy or psychotherapy. An antidepressant trial lasting at least 4 weeks at an optimal dose of the prescribed antidepressant (at least as high as the lowest dose defined as effective in the product datasheet) was considered as an adequate treatment. Treatment resistance was defined as not reaching a HAM-D-17 score≤17 after at least 2 adequate consecutive antidepressant trials administered during the last episode. Non-resistance was defined as a HAM-D-17 score≤17 after a single antidepressant treatment or at the second trial after 1 failure. Depression increases the risk of severe physical disorders such as cancer, diabetes, stroke, inflammation and acute coronary syndrome through multiple molecular mechanisms. depression is often connected with stress and a live style, which implies smoking, low physical exercise, sleep disturbances, less social activities and unhealthy nutrition. Several biomarkers relevant for different facets of depressive disorders are differentially regulated by antidepressant treatment and connected again to metabolic, inflammatory, cardiovascular and apoptotic components of the pathophysiology, i.e. adiponectin, apolipoprotein-B, B-type natriuretic peptide, cortisol, C-reactive protein, cysteine, homocysteine, fibrinogen, growth-differentiation factor-15, glycated hemoglobin A1c, leptin, high-density lipoprotein, interleukin-6, insulin-like growth factor-1, low-density lipoprotein, myeloperoxidase, osteoprotegerin, tumor necrosis factor-α, troponins, triglycerides. Folic acid, also called vitamin B9 is actually a pteroylglutamic acid consisting of three components: 2-amino-4-hydroxypteridine nucleus, p-aminobenzoic acid and glutamic acid. It is important for DNA synthesis and conversion of some amino acids. Folate (vitamin B_9) plays a crucial role in fundamental

cellular processes, including nucleic acid biosynthesis, methyl group biogenesis and amino acid metabolism. The detection and correction of folate deficiency prevents megaloblastic anaemia and reduces the risk of neural tube defects. Coexisting deficiencies of folate and vitamin B_{12} are associated with cognitive decline, depression and neuropathy. 5-Methyltetrahydrofolate, the main form of folate found in blood, is essential for the vitamin B_{12}-dependent methionine synthase mediated remethylation of homocysteine to methionine. As such, homocysteine measurement reflects cellular folate and vitamin B_{12} use. Vitamin B_{12}, also called cobalamin is a water-soluble vitamin that is involved in the metabolism of every cell of the human body: it is a cofactor in DNA synthesis, and in both fatty acid and amino acid metabolism methylation and mitochondrial metabolism,.It is particularly important in the normal functioning of the nervous system via its role in the synthesis of myelin, and in the maturation of developing red blood cells in the bone marrow. Subclinical deficiency affects between 2.5% and 26% of the general population. Amino acid homocysteine is formed in the metabolism of methionine that is introduced into the body food as a product of numerous S-adenosyl-methionine-dependent transmitting reactions. An important role homocysteine-methionine metabolism is the creation of a donor of S-adenosyl-methionine (SAM) methyl groups in a number of biological reactions in the human body. Homocysteine can be recycled into methionine or converted into cysteine with the aid of certain vitamins B. It is proven that brain neurons create homocysteine and circulate over the blood-brain barrier in both direction. Plasma homocysteine concentrations are 20 to 10 times higher than in cerebrospinal fluid, but there is a positive correlation between these two concentrations. In conditions of experimentally induced deficiency of folate neurons produce more homocysteine than neurons in folate rich media. The role of folate in the metabolism of homocysteine in the brain is confirmed and in vivo. Namely, in the cerebrospinal fluid of patients receiving antifolate therapy / methotrexate / lower folate and SAM values were found and elevated homocysteine concentrations ulation. Hyperhomocysteinemia has been associated with various adverse health conditions, including cardiovascular disease, fractures, dementia, decreased physical functioning and mortality In addition, observational studies suggest a link between elevated homocysteine concentrations and depressive symptoms .A meta-analysis of nine observational studies showed that persons with Hcy ≥12.5 μmol/L had a 70% higher risk of prevalent depression than persons with Hcy concentrations <12.5 μmol/L . Supplementation with vitamin B_{12} and folic acid decreases Hcy concentrations and may thereby reduce depressive symptoms and improve quality of life.

We review the findings in major depression of a low plasma and particularly red cell folate, but also of low vitamin B12 status. Both low folate and low vitamin B12 status have been found in studies of depressive patients, and an association between depression and low levels of the two vitamins is found in studies of the general

population. Low plasma or serum folate has also been found in patients with recurrent mood disorders treated by lithium. A link between depression and low folate has similarly been found in patients with alcoholism. The role of vitamin B_{12} and folate in depression is due to their role in transmethylation reactions, which are crucial for the formation of neurotransmitters (e.g. serotonin, epinephrine, nicotinamides, purines, phospholipids). The proposed mechanism, is that low levels of folate or vitamin B_{12} can disrupt transmethylation reaction, leading to an accumulation of homocysteine (hyperhomocysteinemia) and to impaired metabolism of neurotransmitters (especially the hydroxylation of dopamine and serotonin from tyrosine and tryptophan), phospholipids, myelin, and receptors. High homocysteine levels in the blood can lead to vascular injuries by oxidative mechanisms which can contribute to cerebral dysfunction. All of these can lead to the development of various disorders, including depression. Lowering elevated plasma homocysteine (Hcy) concentrations by supplementing vitamin B_{12} and folic acid may reduce depressive symptoms and improve health-related quality of life.

Hypothesis: serum folic acid concentration is lower in patients with resistant depression than those with good therapeutic response, serum vitamin B 12 concentration is lower in patients with resistant depression than those with good therapeutic response, Serum homocysteine concentration is higher in patients with resistant depression than those with good therapeutic response

Aims: Our primary aim is to demonstrate the difference in serum concentrations of folic acid among patients with resistant and non-resistant depression. We want to prove that serum concentrations of folic acid is lower in patients with resistant depression .Our secondary aim is to demonstrate the difference in serum concentrations of vitamin B 12 and homocysteine among patients with resistant and non-resistant depression. We want to prove that serum concentrations of vitamin B 12 is lower in patients with resistant depression and that the homocystin concentration is higher.

Materials/Participants and Methods: In this cross-sectional study, we will include patients suffering from depressive disorder who have achieved symptomatic improvement in the therapy of antidepressants and patients who have shown resistance to multiple antidepressants in sufficient time and sufficient doses in the treatment so far. Respondents will continue to take antidepressant therapy. The examiner will conduct a psychiatric interview, validate the diagnosis in accordance with the existing classification systems (MKB-10 and DSM-5) and take sociodemographic data. Then we will apply the two-grade depression scales (Hamilton's Scenario for Depression, HAMD-17 and Montgomery-Åsberg's Depression Scale, MADRS) . Then blood will be taken to measure folic acid, vitamin B 12, and homocysteine concentration in both

groups of patients. We are planning to include 116 patients. The required size of the sample is calculated for the purpose of verifying the three hypotheses on the difference in concentrations of folate, vitamin B12 and homocysteine in patients with major depression disorder (VDP) and nonresistant resistant to therapy. Expected values in the group of patients with VDP non-resistant to therapy were estimated based on Assies et al. 2015: arithmetic mean (standard deviation) of folic acid 3,12 (0,15), vitamin B12 314 (53,0), homocysteine 2,23 (0,15). (1) The lowest difference we consider to be theoretically and clinically relevant is +/- 10%. Targeted statistical strength is determined at 0.80, and the level of statistical significance at $p < 0.05$. We will check the hypothesis using the three univariate Mann-Whitney-Wilcoxon two-way test of the statistical significance of the difference. Under these preconditions, $n = 17$ for folic acid, $n = 49$ for vitamin B12 and $n = 10$ for homocysteine is required for each group. Since the largest sample is needed to check the hypothesis of difference in the homocysteine level, this is the number we planned. Expecting ≤15% of incorrectly collected data, the initial sample size was estimated at $n = 58$ in each group, ie $n = 116$ in total. The required sample size was calculated using 2000 Monte Carlo samples in the program: PASS 15 Power Analysis and Sample Size Software (2017). NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/pass. The investigation will take place at the Psychiatric Clinic.

Research plan: In this cross-sectional study, we will include patients suffering from depressive disorder who have achieved symptomatic improvement in the therapy of antidepressants and patients who have shown resistance to multiple antidepressants in sufficient time and sufficient doses in the treatment so far. The number of patients per group will be 58, a total of 116 patients.

Patients who make an antidepressant-resistant group will be hospitalized at the psychiatric clinic University Hospital Center in Zagreb, while other non-resistant antidepressant patients will be treated out of the hospital. Before engaging in the research each patient will be thoroughly described in the research process and will be asked to sign informed consent for participation. Research will only be available to women aged 18-65 who have no antipsychotics in therapy. The subjects will continue to take antidepressant therapy. The examiner will conduct a psychiatric interview, confirm the diagnosis in accordance with the existing classification systems (MKB-10 and DSM-5) and take sociodemographic data (expected 40 minutes). Then we will apply the two-grade depression scales (Hamilton's Scenario for Depression, HAMD-17 and Montgomery-Åsberg's Depression Scale, MADRS). Blood (5 to 10 mL) will then be taken to measure folic acid, vitamin B 12 and homocysteine concentrations in both patient groups. Within the aforementioned research, we will ask patients to complete the questionnaire on religion (about 15 minutes) and the cigarette consignment questionnaire (about 10 minutes).

Significance/Expected scientific contribution: Low folate levels are linked to a poor response to antidepressants, and treatment with folic acid is shown to improve response to antidepressants. A recent study also suggests that high vitamin B12 status may be associated with better treatment outcome. Folate and vitamin B12 are major determinants of one-carbon metabolism, in which S-adenosylmethionine (SAM) is formed. SAM donates methyl groups that are crucial for neurological function. Increased plasma homocysteine is a functional marker of both folate and vitamin B12 deficiency. Increased homocysteine levels are found in depressive patients. In a large population study from Norway increased plasma homocysteine was associated with increased risk of depression but not anxiety. There is now substantial evidence of a common decrease in serum/red blood cell folate, serum vitamin B12 and an increase in plasma homocysteine in depression. Given the current knowledge of the level of folic acid, vitamin b 12 and homocysteine levels and their impact on depression treatment, the results of our research would contribute to better quality patient treatment, given that folic acid and vitamin b 12 are easily replenished by oral intakes in the body. Also depression is conducive to the many somatic illnesses it is important to be effective in treating resistant depression. Studies suggest that insufficient folate and vitamin B_{12} status may contribute to major depressive disorder and that supplementation might be useful in this condition. For further research, the question remains to what extent a certain way of nutrition contributes to the increase in folic acid and vitamin B12 concentrations.

MeSH/Keywords: Non – resistant depression: Resistant depression, Folic acid, Vitamin B12, Homocystein

Dissertation Proposal Title: Quality of Myocardial Protection Using Calafiore Cardioplegic Solution Compared to Other Solutions Used in Aortic Valve Replacement

PhD Candidate: Marko Rimac, M.D., Health Centre Osijek, Osijek, Croatia

Mentor: Assist. Prof. Igor Lekšan, M.D., Ph.D., Health Centre Osijek, Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Introduction: Inadequate myocardial protection in the cross-clamping period is an issue of concern in cardiac surgery. Cardioplegic solutions improve tolerance to ischemia and reperfusion by preserving myocardial energy reserves and preventing electrolyte imbalances and buffering acidosis. Aortic stenosis causes changes in the structure of the heart in the form of left ventricular hypertrophy. Accordingly, the metabolic needs of the heart increase substantially. Therefore, the application of cardioplegic solution in patients suffering from such conditions is extremely important for the purpose of preventing irreversible cardiac changes following aortic cross-clamping.

Hypothesis: Application of warm blood cardioplegic solution produces an adequate level of myocardial protection, which results in unaffected cardiac contractility.

Aims: To evaluate the efficacy and safety of Calafiore cardioplegic solution in comparison with other cardioplegic solutions.

Materials/Participants and Methods: Prospective study based on a statistically significant sample of patients with isolated aortic defects is planned. The study will be carried out at the Department of Cardiac Surgery of the Clinical Hospital Centre Osijek. EF and LVD will be determined preoperatively. Duration of aortic clamping and need for defibrillation following clamp release will be monitored intraoperatively. Postoperatively, the values of cardioselective enzymes and laboratory indicators of cardiac insufficiency will be monitored in equal intervals, whereas the frequency of new onset AF, application of inotropic therapy and EF will be checked in the early postoperative period and after one year.

Research plan: Upon signing informed consent, the study will be conducted on a total of 100 patients, both male and female, suffering from isolated aortic defects.

The study will not include patients with cardiac comorbidity. The study will also not include patients with kidney failure and patients undergoing chronic antipsychotic treatment due to the fact that such conditions can affect the values of cardioselective enzymes. Preoperative evaluation will include heart ultrasound performed within the previous 6 months. Postoperatively, the patients will undergo laboratory ultrasound and heart rhythm screening. Based on those indicators, the quality of myocardial protection using warm blood cardioplegic solution will be assessed in comparison with the standard cardioplegic solution on the basis of data from large-scale multi-centre studies.

Significance/Expected scientific contribution: Application of Calafiore cardioplegic solution effectively protects the myocardium in aortic valve replacement.

MeSH/Keywords: Calafiore cardioplegic solution, myocardial protection, cross-clamping, AF, EF

Dissertation Proposal Title: Diagnostic accuracy of serum calprotectin for early diagnosis of sepsis in patients presenting to the emergency department with suspected sepsis.

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Mentor: Assist. Prof. Višnja Nesek Adam, M.D., Ph.D., University Hospital "Sveti Duh", Zagreb, Croatia

Introduction: Sepsis is a potentially life-threatening complication of an infection. It occurs when chemicals released into the bloodstream to fight the infection trigger inflammatory responses throughout the body. This inflammation can trigger a cascade of changes that can damage multiple organ systems, causing them to fail. Sepsis still has high mortality despite available diagnostic tests and therapeutic methods. Numerous studies have shown that administration of antibiotic therapy within the first hour of documented hypotension is associated with a survival rate of 79.9%. However, the early diagnosis of patients with sepsis remains a challenge for clinicians in the emergency department. Biomarkers that have been assessed clinically such as C-reactive protein (CRP) and procalcitonin (PTC) do not have 100% specificity for sepsis. Mentioned above indicates the need to find and introduce new diagnostic and laboratory procedures at the very early stages of the disease. Acute phase protein calprotectin is a new biomarker that has been proposed for the diagnosis of many inflammatory conditions. Calprotectin is an antimicrobial peptide that protects cells against invasive microorganisms and regulates adhesion of leukocytes to the endothelium and extracellular matrix during the inflammatory process. It is released by innate immunity cells immediately after hostpathogen interaction and is detectable in body fluids by means of a simple ELISA technique. The aim of this study would be to investigate the diagnostic accuracy of serum calprotectin for early diagnosis of sepsis in patients presenting to the emergency department with suspected sepsis.

Hypothesis: Serum calprotectin is a more reliable biochemical marker for early diagnosis of sepsis compared to the previous laboratory parameters (CRP, PTC).

Aims: The main aim would be to determine the diagnostic accuracy of serum calprotectin for early diagnosis of sepsis in patients presenting to the emergency department with suspected sepsis.

Specific objectives would be:

- to identify demographic characteristics (age and gender) and associated diseases (cardiovascular diseases, chronic renal insufficiency, liver lesions, diabetes, cerebrovascular disease).
- to determine clinical status, vital signs, multiple organ dysfunction.
- calculate the initial Sequential Organ Failure Assessment (SOFA) score and determine its correlation with the serum calprotectin levels.
- to determine measures of diagnostic accuracy for serum calprotectin: sensitivity, specificity, predictive values, likelihood ratios and the area under the curve (AUC).
- to determine relationship between calprotectin levels and clinical stages of sepsis (uncomplicated sepsis, severe sepsis, septic shock).
- to determine differences in diagnostic accuracy of serum calprotectin compared with diagnostic accuracy of previous laboratory parameters (CRP, PTC).
- to determine prognostic value of serum calprotectin in predicting an outcome (mortality, duration of hospitalization) compared with current indicators (albumin, lactate and sodium concentration, SOFA score).

Materials/Participants and Methods: We would preforme a cross-sectional study of patients presenting to the Emergency Department of Clinical Hospital Sveti Duh with suspected sepsis. The group, 'with suspected sepsis', would consists of patients who have suspected infection + two or more criteria for systemic inflammatory response syndrome (SIRS): body temperature > 38°C or <36°C; heart rate > 90/min; respiratory rate > 20/min or SpO2 <90% in room air or <94% with O2; leukocytes > 12 x10 9 or <4 x 10 9. We would take informed consent to conduct the study. Excluding criteria would be age <18 years, pregnant women, patients who do not want to participate in the study.

Research plan: In all patients with suspected sepsis during stay in the Emergency Department we would monitor the clinical status and vital signs (noninvasive blood pressure, body temperature, heart rate, respiratory rate, oxygen saturation levels), level of consciousness, acute phase proteins (CRP, PTC), multiple organ dysfunction (partial pressure of oxygen in arterial blood (pO2), creatinine level, platelet count, bilirubin level) and serum calprotectin. A blood samples would be taken by properly qualified and authorized persons immediately upon arrival to the Emergency Department. We would calculate the initial SOFA score by using clinical findings and laboratory test results (pO2, creatinine level, bilirubin level and abnormally low blood pressure). The final diagnosis would be based on bacteriological analysis (hemoculture, urine culture, sputum and stool culture, cerebrospinal fluid culture and other microbiological cultures depending on the clinical findings). Patients would be

divided into two groups according to final diagnosis : patients without sepsis and those with diagnosed sepsis. Group of patients with sepsis would be divided into three groups: those with uncomplicated sepsis, severe sepsis and septic shock.

Significance/Expected scientific contribution: The results obtained in this study would provide us with new information on diagnostic accuracy of serum calprotectin for early diagnosis of sepsis in patients presenting to the emergency department. Considering the mentioned difficulties in diagnosing sepsis, the availability of accurate sepsis biomarker to facilitate diagnosis could be of use to enable timely appropriate treatment to be started, thus optimizing a patient's chances of survival.

MeSH/Keywords: serum calprotectin, sepsis, emergency department, sepsis biomarkers, early diagnosis

Dissertation Proposal Title: Periprocedural myocardial injury after elective percutaneous coronary intervention – comparison of high sensitivity and conventional troponin

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Mentor: Assist. Prof. Tomislav Letilović, M.D., Ph.D., CH „Merkur", Zagreb, Croatia; University of Zagreb, Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Introduction: Percutaneous coronary intervention (PCI) is the most widely used method of treating symptomatic patients with stable coronary artery disease. Periprocedural myocardial injury (PMI), as a result of PCI, correlates with the early and long term survival. Most of the data regarding association of PMI and adverse cardiac events come from studies using conventional troponins. In modern clinical practice conventional troponin is being replaced by high sensitivity troponin (HSTN). There is paucity of data regarding the role of HSTN in definition and diagnosis of PMI.

Hypothesis: Using high sensitivity troponin leads to increased incidence and intensity of PMI after elective PCI when compared to conventional troponin.

Aims: To investigate the incidence and intensity of PMI, using various available definitions, after elective PCI by measuring HSTN. To compare the data obtained from the control group in whom PMI was detected using conventional troponin – "historical controls".
To correlate PMI with various clinical characteristics.

Materials/Participants and Methods: Study included two groups of consecutive patients with stable coronary artery disease. In the first group, treated during 2017 and 2018 with PCI at the Department of Cardiology, CH "Merkur", were patients in whom presence of PMI was defined using HSTN. The control group were patients, treated from 2012 to 2015, in whom PMI was analysed using conventional troponin. Incidence and intensity of PMI were evaluated using measurements of basal levels of troponin and values 16 hours after PCI. Analyses were done using different available definitions of PMI and further correlated with relevant clinical characteristics.

Research plan: As all the clinical data are already collected future work will be focused on statistical analyses.

Significance/Expected scientific contribution: With this research we will hopefully further clarify the role of HSTN in evaluating PMI, a phenomenon shown to be correlated with adverse clinical events.

MeSH/Keywords: periprocedural myocardial injury, high-sensitivity troponin

Dissertation Proposal Title: The influence of the immune and microbiological status of a woman in the presence of precancerous changes of the cervical mucosa

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Introduction: It has been proven that many sexually transmitted diseases (STDs) cause strong immune inflammatory responses by a host, and that certain microbial flora favours their development. Limited data are available about the effect of immune and microbiological status on the presence of precancerous changes of the cervix. It should be considered that chronic human papillomavirus (HPV) infection is one of the leading causes of premalignant and malignant changes of the cervix.

Hypothesis: Immune and microbiological status, with the known existence of chronic HPV infection, determines the formation of precancerous changes of the cervix**.**

Aim: To show the influence of the vagina and cervix microbial flora and more immunity indicators on the presence of precancerous changes of the cervix.

Participants and Methods: The research will cover 100 women divided into two groups. The first group consists of 50 healthy women who are HPV negative and whose last two Pap smears within 5 years were negative. The second group consists of 50 women who are, due to pathohistologically proven precancerous lesions oft he cervix, planned to be treated by LLETZ or cold knife conisation and are HPV positive.

Research plan: After signing informed consent and taking medical history from HPV-positive women with pathohistologically proven precancerous changes of the cervix, molecular diagnostics will determine the microbiological status, and immunoassays of NK cell activity and the CH50 complement test will determine the immune status of a patient. The same tests will be performed during regular gynaecological check-ups in the group of healthy women. In a comprehensive statistical analysis, we will show whether there is the influence of the immune and microbiological status in the existence of precancerous changes of the cervix.

Significance/Expected scientific contribution: This study could indicate us the potential correlation of the presence of precancerous changes of the cervix with the microbiological status of the vagina, and the parameters of the woman's immune status, which would be of high importance for the success of the treatment of the cervix precancerous changes.

MeSH/Keywords: HPV, PAP smear, microbiome, CH50, NK cells

Dissertation Proposal Title: Correlation of left ventricular diastolic dysfunction with endothelial dysfunction biomarkers for people with low and moderate cardiovascular risk

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Mentor 2: Prof. Josip Vincelj, M.D., Ph.D., FESC, Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Introduction: Cardiovascular Disease (CVD) is the leading cause of mortality worldwide. In the 10-year risk assessment of a fatal cardiovascular incident we use a SCORE based on which patients are divided into 4 categories: 1. Low risk is defined by SCORE <1%, 2. Moderate risk by SCORE 1 - 5%, 3. High risk by SCORE 5 - 10%., and 4. Very high risk by SCORE ≥ 10%. In high-risk patients we include also those with proven CVD, diabetes mellitus (DM), renal failure (RF), cholesterol > 8 mmol/L and blood pressure ≥ 180/110 mmHg.
Endothelial dysfunction is an early manifestation of vascular disease and occurs in the early, asymptomatic stage of atherosclerosis. Asymmetric dimethylarginine (ADMA), the endogenous competitive endothelial nitric oxide (eNOS) inhibitor, is a newly-recognized endothelial dysfunction biomarker whose plasma concentration increases in patients with proven CVD, arterial hypertension (AH), hypercholesterolemia, hyperhomocysteinemia, DM RF and chronic liver disese.
Left ventricular diastolic dysfunction (LVDD) is a reflection of reduced relaxation and increased left ventricular wall stiffness. LVDD rate increases with age, and asymptomatic LVDD occurs in an adult population older than 45 years. Valvular disease, aging, hypertrophic cardiomyopathy, ischemic heart disease, obesity, diabetes and hypertension are known risk factors for earlier LVDD development. LVDD is estimated by two-dimensional transthoracic echocardiography (2D TTE) and biomarker brain natriuretic peptide (BNP).
While recent studies confirm the association between ADMA and LVDD in patients with known CVD, little is known about their association in asymptomatic people with low and moderate cardiovascular risk.

Hypothesis: There is a positive correlation between the plasma concentration of ADMA and the LVDD in people with low and moderate cardiovascular risk**.**

Aims: To evaluate LVDD by 2D TTE and N-terminal pro BNP (NT pro BNP) from venous blood sample by ECLIA method, To evaluate the endothelial function of examinees by measuring the plasma concentration of ADMA by ELISA method, To assess the impact of SCORE on the occurrence of LVDD, To assess the impact of SCORE on the plasma concentration of ADMA, To examine the differences in the appearance of LVDD and plasma concentrations of ADMA depending on age and sex.

Participants and Methods: The study will include about 120 examinees of both sexes, 40 to 65 years old, with SCORE <5% and a negative ergometry. Examinees will be divided into two groups: 1.SCORE <1% and 2. SCORE 1-5%. For estimation of LVDD we will use pulse and tissue doppler on a Philips Affinity 30 ultrasound device. NT pro BNP and ADMA will be determined from the venous blood of examinees by ECLIA and ELISA method.

Research Plan: It is a cross-sectional study. The research will be conducted at the Health Center Osijek and Department of Clinical Laboratory Diagnostic of Clinical Hospital Center Osijek. The planned study time is 6 months.

Significance/Expected scientific contribution: Confirmation of the hypothesis would contribute to a better understanding of asymptomatic LVDD in people with low and moderate cardiovascular risk.

MeSH/Keywords: cardiovascular diseases, endothelium, ventricular function, diastole, adults

Dissertation Proposal Title: Impact of arterial stiffness and endocan concentration on survival in patients on chronic hemodialysis

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Introduction: Cardiovascular disease (CVD) is the main cause of morbidity and mortality in chronic kidney disease (CKD) patients. Arterial stiffness results in increased central pulse pressure, cardiac workload, and left ventricular hypertrophy and has been shown to be a strong predictor for cardiovascular morbidity and mortality in dialysis population. Endocan is a soluble proteoglycan of 50 kDa expressed by the vascular endothelium and recent study reported that endocan is overexpressed in cancer, sepsis, obesity, CKD and inflammatory conditions. Serum endocan levels were applied to a novel prediction model of all-cause mortality and cardiovascular events in CKD patients.

Hypothesis: Patients on chronic hemodialysis with increased pulse wave velocity (PWV) as a measure of arterial stiffness, should have increased concentration of plasma endocan level as a marker of endothelial dysfunction and vascular damage and poor cardiovascular outcome.

Aims: To investigate relationship between PWV and endocan concentration in patients on chronic hemodialysis and theirs impact on cardiovascular outcome. To investigate the relationship between PWV and endocan with other biochemical parameters. To test the ability of PWV and endocan as a predictors of cardiovascular events

Participants and Methods: One hundred stable chronic hemodialysis patients will be enrolled in the prospective study. Also we will include patients with CKD who are not on hemodialysis and healthy controls. We will exclude patients with malignant diseases, acute inflammatory processes, tachyarrhythmias, hystory of stroke or myocardial infarction last 3 months and dialysis vintage less than three months. All included patients will be followed-up for time-to-event analysis until occurrence of fatal or nonfatal CVE. Information on fatal and nonfatal CVE including death, stroke, and myocardial infarction.

Research plan: Measurement of PWV will be performed with Mobil-O-Graph Agedio B 900 device before the midweek dialysis session and right after. All blood samples will be obtained and analyzed in Central laboratory of University Hospital Osijek.

Expected scientific contribution: There was no study that compares arterial stiffness with endocan concentration and occurance of cardiovascular events in patients on chronic hemodialysis. The main contribution of this study will be to predict the population of patients on chronic hemodialysis which has particularly increase risk of poore cardiovascular outcome on the basis of relatively simple PWV measurement and biochemical analysis.

Keywords: Hemodialysis, CVD, arterial stiffness, PWV, endocan

Abstract Title: Alexithymia and anxiety sensitivity in panic disorder

Part of the Disertation Proposal: Alexithymia and anxiety sensitivity in panic disorder

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Introduction: Panic disorder differs among other anxiety disorders mainly presenting with physical symptoms such as palpitations, chest pain, dizziness, trembling, nausea, sweating. The current prevalence of panic disorder in the general population is about 2%. Panic disorder has high rates of relapse (25% -50% of patients within six months of therapy discontinuation), a significant negative impact on the quality of life of patients, and is associated with high total treatment costs.
Many studies have found significant associations between anxiety sensitivity and panic disorder. A small number of empirical studies highlighted the association between alexithymia and panic disorder. Alexithymia and anxiety sensitivity are dimensional personality constructs, although they share common features that potentially act as diathesis for translating emotional distress into a somatic experience.

Aims: The aim of the current study is to examine the influence of both of these personality traits on panic disorder, as well as to investigate independent contributions of alexithymia and anxiety sensitivity to the overall association of both concepts with the severity of panic disorder, and to investigate the association between alexithymia and anxiety sensitivity on panic disorder after adjustment for confounding variables.

Participants and Methods: A cross-sectional study was carried out at Psychiatric Hospital "Sveti Ivan". Targeted population was outpatients of both sexes with diagnosed panic disorder ages 18 to 50 years without psychiatric or somatic comorbidities. A consecutive sample of all patients was chosen according to the order of arrival for review. We included 92 patients diagnosed with panic disorder. The arithmetic mean (standard deviation) of the age of the respondents was 27 (9.59) years and 59/92 (64.1%) of the sample were women.

Results: Anxiety sensitivity as well as all three subscales of the Toronto alexithymia scale were statistically significantly associated with the severity of the panic disorder. However, in the multivariate analysis and after adjusting for all other variables and age and gender, only anxiety sensitivity was statistically significantly associated with panic disorder severity.

Conclusion: In our sample, preliminary results showed that anxiety sensitivity and alexithymia are associated with the panic disorder. Therefore, focusing therapeutic interventions on alexithymia and anxiety sensitivity could improve treatment and prognosis of panic disorder.

MeSH/Keywords: panic disorder, alexithymia, anxiety sensitivity, panic disorder severity scale, Toronto alexithymia scale

Dissertation Proposal Title: Spirituality and religiosity in schizophrenia

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Mentor: Assist. Prof. Branka Aukst Margetić, M.D., Ph.D., Department of Psychiatry, University Hospital Sestre milosrdnice, Zagreb, Croatia

Introduction: Schizophrenia is one of the most severe forms of psychiatric disorders. Religiosity and spirituality are highly expressed in patients with schizophrenia. The link between religiosity and psychopathology has been examined in a series of studies, however, prospective studies examining the connection between religiosity/spirituality and recovery are very rare and the results are not unambiguous.

Hypothesis: The degree of religiosity and spirituality before treatment is associated with the degree of recovery of schizophrenic patients
Different components of spirituality and religiosity have different contributions to recovery in schizophrenic patients
Pharmacotherapeutic and socio-therapeutic psychiatric treatment with spiritual and religious therapeutic content leads to a greater degree of recovery than pharmacotherapeutic and socio-therapeutic treatment without spiritual and religious content.

Aims:

1. To examine whether different patterns of change in religiosity/spirituality during treatment are associated with different degrees of recovery.
2. To examine the factor structure of the questionnaire "Short multidimensional measurement of religiosity/spirituality" on a group of schizophrenic patients in treatment.
3. To examine the association between degree of religiosity/spirituality and degree of recovery as measured by differences in scores on the Positive and Negative Symptoms Scale (PANSS) at the beginning of and one month after the treatment.
4. To examine the relationship between the degree of religiosity/spirituality and the degree of recovery of negative symptoms of schizophrenia
5. To examine the relationship between the degree of religiosity/spirituality and the degree of recovery of positive symptoms of schizophrenia.

6. To examine how various components of spirituality/religiosity obtained by factor analysis of the questionnaire "Short multidimensional measurement of religiosity/ spirituality" on a group of schizophrenic patients in treatment contribute to recovery.
7. To examine the time fluctuations in religiosity/spirituality between the start of the treatment and one month after the treatment.
8. To examine whether there is a difference in the efficiency of different types of treatment, pharmacotherapeutic and socio-therapeutic psychiatric treatment with spiritual and religious therapeutic content compared to pharmacotherapeutic and socio-therapeutic treatment without spiritual and religious content, given the degree of recovery.

Participants and Method: The study will include 100 schizophrenia patients at Special Hospital Sveti Rafael and 100 schizophrenia patients at Neuropsychiatric Hospital "Dr. Ivan Barbot" Popovača. Respondents will be interviewed with questionnaires in the field of spirituality and religiosity after answering socio-demographic questionnaires. Multidimensional measurement of religiousness/ spirituality in health research will be used to examine religiosity and spirituality.
For the purposes of our research, we will conduct factor analysis on the data collected with this questionnaire to determine the factors/components that characterize the religiosity/spirituality of schizophrenic persons. We will also check the metric characteristics of this questionnaire based on the data obtained in our study. We will look at and compare the factors/components obtained separately to accomplish some of our research goals with respect to their relationship to recovery.
The intensity of schizophrenic symptoms will be measured by the Positive and Negative Symptoms Scale (PANSS).

Research plan: In both hospitals, questionnaires will be distributed at the beginning of treatment and one month after the treatment. At Saint Raphael Special Hospital, in addition to the usual pharmacotherapy and socio-therapeutic treatment, a religious and spiritual treatment program will be implemented. This includes individual and group spiritual conversations with the priest, group animation through prayer, attending a communal Mass and receiving sacraments.
The aforementioned questionnaires will be used on the control group of subjects at the Neuropsychiatric Hospital "Dr. Ivan Barbot", however, without spiritual and religious therapeutic content being applied during hospitalization.
The intensity of schizophrenic symptoms will be measured by the Positive and Negative Symptoms Scale. The assessment will be based on a structured clinical 30-45 minutes interview conducted by a psychiatrist. Short multidimensional measurement of religiosity/spirituality will be distributed in a form of a questionnaire to patients at

the beginning of the treatment and one month after the treatment to be filled out with the support of the examiner.

Significance/Expected scientific contribution: The research is expected to contribute to the understanding of the relationship between spirituality and mental health and examine its possible wider application in psychiatry.

MeSH/Keywords: spirituality, religiosity, schizophrenia, recovery

Dissertation proposal title: Estimation of prognostic value of RDW in metastatic colorectal cancer depending on the location of the primary process

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Introduction: Colorectal cancer is a leading malignant in the gastrointestinal tract and occurs in both sexes almost equally. According to the Croatian Cancer Registry (2017) among men it shares second and third place together with prostate cancer, behind the lung cancer, while among women it is in second place right behind breast cancer. In terms of mortality by malignancies, it ranks high in both sexes. At the time of diagnosis, 75% of patients have operable disease and 50-60% of patients will develop metastatic disease. The most common locations of metastases are the liver and lungs. Studies have confirmed that there is a difference in overall survival (OS) in metastatic colorectal cancer in patients whose primary process develops on the left colon area (33.3 months) unlike patients whose primary process develops on the right colon area (19.4 months). RDW (Red blood cell distribution width) is a parameter of standard full blood count tests that reflects the size variability of erythrocytes. In recent studies, RDW levels have been associated with ischemic heart disease, hypertension, and inflammatory bowel disease. More recent studies have shown that raised RDWvalues may be a significant prognostic factor for various types of cancers. The same studies have shown its association with low overall survival rate and time for disease to progress.

Hypothesis: Metastatic right-sided colorectal cancer has higher values of RDW than left-sided colorectal cancer, which is associated with worse treatment outcome, shorter overall survival rate, and lower therapy response.

Aims: Investigate the prognostic and predictive effect of RDW on metastatic CRC according to localization of primary tumor, its association with worse treatment outcome inspite of identical chemotherapy and biologic therapy and to determine whether there is a difference in the values of RDW in left-sided and right-sided metastatic colorectal cancers.

Materials/Participants and Methods: A minimum of 90 newly diagnosed metatastic colorectal patients, of both sexes, older than 18 years. We will collect laboratory data on complete blood count from medical records of reference laboratories

Research plan: We will analyze about 90 patients with newly metatstatic colorectal cancer, collect their blood test results including RDW value, tumor pathological features, locations of metastasis and primary tumor then we›ll group them. After 2 and 3 years of follow-up, the survival status will be defined, and the above data will be statistically analyzed.

Significance/Expected scientific contribution: Confirmation of the prognostic significance of raised RDW values in patients with mCRC and its impact on PFS and OS depending on the location of primary colorectal cancer with unambiguous meaning in clinical usage.

MeSH/Keywords: metastatic colorectal cancer, RDW, left sided tumor, right sided tumor

Dissertation Proposal Title: Influence of vestibular rehabilitation on the cupular and otolith senses in patients with unilateral vestibular hypofunction

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Introduction: The benefit of vestibular rehabilitation (VR) in patients with unilateral vestibular hypofunction (UVH) has been confirmed with many studies. However, all of them so far have shown the influence of VR only on a cupular sense of horizontal canal. In recent years, there has been a growing awareness of the existence of various forms of UVH, depending on which of the cupular or otolith senses are affected. With the help of ocular and cervical vestibular evoked myogenic potentials (o and c VEMPs), Video Head Impulse Test (vHIT) and Caloric Test (CT), we are now able to detect different forms, and combinations of vestibular sensory lesions.

Hypothesis: Patients with impaired cupular and otolith senses are less likely to recover compared to patients with isolated sensory impairment, Vestibular rehabilitation affects the subjective and objective improvement of patients with isolated impairment of the otolith senses.

Aims: Compare the condition of the damaged cupular and otolith senses before and after VR according to relevant parameters: unilateral weakness and directional preponderance in CT, interaural amplitude ratio in o and c VEMP, vestibulo-ocular reflex (VOR) gain in vHIT, Determine the incidence of abnormal VOR gain of the horizontal semicircular canal in vHIT with pathological CT in acute / subacute and chronic UVH, Determine the incidence of abnormal o and / or c VEMP with abnormal CT, Examine the correlation between the time period from the onset of symptoms to the beginning of VR and the degree of recovery of the vestibular senses, Compare the subjective assessment of handicap and quality of life before and after VR in patients with UVH based on the DHI and SF-36 questionnaire results, Determine the number of patients who did not recover after performing VR for 6 weeks considering the involvement of cupular and / or otolith senses and the stage of UVH.

Materials/Participants and Methods: The study will include 60 patients with UVH. Prior to joining the study, all patients will be offered to sign an informed consent document for participation. To determine UVH, CT, vHIT, o and c VEMP will be used. Handicap and quality of life of patients will be examined with DHI and SF-36 questionnaires. All patients will perform VR for 6 weeks.

Research plan: At first visit patients will be diagnosed with UVH based on otoneurological examination and results of CT, vHIT, o and cVEMP. Also, they will fill out DHI and SF-36 questionnaire. All patients with UVH will perform VR excercises with a physiotherapist. After 3 weeks of VR, patients will fill out DHI and SF-36, for estimation of subjective recovery. Six weeks after the initial examination, CT, o and cVEMP, vHIT and filling out the questionnaires will be repeated. The planned duration of the study is 14 months / until the planned number of patients is collected.

Significance/Expected scientific contribution: Confirmation of the hypothesis that VR has a positive effect on the recovery of otolith sense and that patients with combined impairment of the cupular and otolith senses recover less than patients who have isolated impairment of cupular or otolith senses. The study highlights the need for using a wider set of tests in UVH diagnostics, which help in establishing a precise diagnosis and planning further treatment.

MeSH/Keywords: vertigo; vestibulopathy, unilateral; vestibular evoked myogenic potentials; caloric test; video Head Impulse Test

Dissertation Proposal Title: Regional arterial and capillary blood flow characteristics in extremities of patients without circulatory difficulties, depending on age, nicotine use and oral intake of L-arginine

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Mentor: Assoc. Prof. Lada Zibar, M.D., Ph.D., Faculty of Medicine Osijek, University of Osijek; CH Merkur, Zagreb, Croatia

Introduction: In the studies of atherosclerosis clinical medicine mostly uses radiological methods (standard x-ray images, ultrasound examination, CT), with Doppler ultrasound being one of the most commonly used. In this case, respondents' blood flow is measured and the resistance in certain parts of the blood vessels is calculated (most commonly in carotid arteries, basilar and vertebral arteries, etc.) This procedure can only be done up to a certain level of branching of the blood vessels. For research purposes, and still not as a part of the standard clinical practice, various devices for measuring blood flow through the capillaries are used. Infrared plethysmography is the most common, where, based on the change in the infrared reflection of erythrocytes in the capillaries, i.e. depending on the amount of haemoglobin, the curve of blood flow through the capillaries can be seen. The aforementioned researches are very important for a more accurate understanding of the bloodstream, especially in the extremities (toes, arms) and parts of the epicranium, especially in the outer ear (*otitis externa*). There are relatively few studies (*Bilateral photoplethysmography studies of the leg arterial stenosis*) using two methods of measuring blood flow simultaneously, i.e. blood flow at regional artery levels and blood flow at the capillary level of the same body part. It is important to emphasize that in addition to measuring blood flow velocity, the pulse wave propagation rate can also be measured. Thus, there are measuring systems which show slower pulse wave propagation in elastic arterial tree in comparison to a rigid arterial tree in patients with generalized atherosclerosis

Hypothesis: It is hypothesized that older age and nicotine use reinforce vascular dysfunction and that oral administration of L-arginine alters the flow in the capillary system of such individuals.

Aims: The aim of the study is to show the behaviour of the circulatory system at rest in healthy respondents by measuring the blood flow through the regional arteries using the common Doppler apparatus, and by measuring blood flow through the capillary system of the same part of the body by infrared plethysmography after the usual diet and less than 24 hours after taking an additional amount of L-arginine, which increases the availability of NO in tissues. The study will observe the effect of sex, nicotine use and age on the blood flow through regional arteries and at the level of capillary junctions, as well as to what extent they modulate the effect of L-arginine.

Research plan: There will be 4 groups of respondents with no clinical or anamnestic data on the existence of atherosclerotic disease. We will measure their blood flow through the regional arteries of the arms, legs and carotid arteries with a Doppler ultrasound device, and we will simultaneously measure the capillary blood flow in the outer ear and little fingers of the hands and feet (*digitus minimus*) via the infrared plethysmography. The ECG will simultaneously be recorded as well.

Participants and Methods: Each group will consist of at least 30 respondents. The first two groups are young, i.e. under the age of 30, divided into smokers and non-smokers. Smokers are defined as persons who have smoked at least 10 cigarettes per day in the past year. The other two groups are over the age of 40, also divided into smokers and non-smokers.
A modern Doppler device with Alok color display will be used to measure blood flow through the regional blood vessels as well as the Biopac Student Lab apparatus, which allows for multi-channel recording of different signals. This study will record infrared plethysmography and ECG in resting respondents. It is estimated for each respondent to undergo 2 consecutive tests, one during the morning and the other one after 24 h. After the first test, each respondent will consummate a light meal containing no significant amounts of protein, followed by 2 capsules of 500 mg L-arginine. The aim is that in the second test, i.e. after 24 h, peripheral tissues are able to synthesize NO without any difficulties in the absence of adequate oxygenation.

Significance/Expected scientific contribution: The expected scientific input is the advance in understanding blood flow at capillary levels in healthy subjects depending on age and nicotine use

MeSH/Keywords: Atherosclerosis, Color Doppler, Blood flow, Capillaries, Plethysmography

Dissertation Proposal Title: Diagnostic value of CA 19-9 and CEA concentrations in pancreatic juice and the predictive value of tumor markers for the survival of patients with pancreatic cancer

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Introduction: Malignancies are the second cause of death in the population, with pancreatic cancer in both sexes accounting for 3% of all malignancies. According to the Cancer Registry, 600 new cases are detected annually in the Republic of Croatia. Although pancreatic cancer ranks ninth in frequency with other cancers, it ranks fourth in mortality with a five-year patient survival rate of less than-. Given such a small five-year survival, efforts should be made to discover methods for the early detection of pancreatic cancer. Also, methods need to be developed to solidify the characteristics of each individual cancer and adjust the oncological treatment accordingly. Tumor markers, CA 19-9, and CEA can be used as indicators of cancer, and may also be predictors of metastatic potential and cancer recurrence. Because pancreatic juice is a rich source of tumor-specific proteins and relatively readily available during surgical resection, the potential of CA 19-9 and CEA from pancreatic juice to be prognostic biomarkers needs to be established.

Hypothesis: Concentrations of CA 19-9 and CEA tumor markers in pancreatic juice of patients with pancreatic cancer are also elevated when serum concentrations are within normal limits and have a high predictive value in assessing patient survival and treatment.

Aims: To determine the concentration of tumor markers CA 19-9 and CEA in pancreatic juice in patients with operable, inoperable pancreatic cancer and patients with benign disease (choledocholythiasis), Compare the values of serum concentrations of tumor markers and their values in pancreatic juice in all three groups of patients, To determine whether there is an association between the concentration of tumor markers in pancreatic juice and type of pancreatic cancer and survival of patients with pancreatic cancer

Materials/Participants and Methods: Patients diagnosed with operable and inoperable pancreatic cancer will be included in the study with third group of patient with benign disease as control group. Surgery for resection of the pancreatic head will be performed in group of patients with operable pancreatic head carcinomas. Sample size calculation will be performed after the results of the pilot study have been obtained. For the purpose of the prospective study, the sample to be taken is pancreatic juice. During the surgical procedure for resection of the pancreatic head, after the resection is performed, the main pancreatic duct remains open. The pancreatic juice flows freely from it. We will take 1 ml of juice and apply it to the cuvette. The sample is then directly transferred to the laboratory of the Clinical Hospital Center Osijek, where it is treated with the ECLIA immunohistochemical electroluminescence method on a Roche Elecsys 1010/2010 immunochemical analyzer. In second and third group of patients which include patients with inoperable pancreatic carcinomas and patients with benign diseases like choledocholythiasis pancreatic juice will be taken during endoscopic retrograde cholangiopancreatography which will be diagnostic and therapeutic method for those patients who needs entering main pancreatic duct. It that moment 1 ml of pancreatic juice will be taken and will be transported and analised in same fashion as mentioned above. It is important to emphasize that when sampling at no point does the patient suffer pain, affect the length and course of surgery and treatment, the outcome of surgery and further treatment, and before each sampling, the patient will be able to independently decide whether to participate in the study with a clear an explanation of which will be confirmed by the signed consent.

Research plan: Once the research hypothesis has been defined, a further research work plan will consist of forming informed consent for patients who will be invited to participate in the research work, and the research work together with the informed consent will be presented to the Ethics Committee of the Osijek Clinical Hospital and the Osijek School of Medicine. This is followed by the next phase, which includes sample collection in all three of these patient groups. When sufficient samples are collected per group (15), a pilot research study will be formed on which to base future doctoral dissertation.

Significance/Expected scientific contribution: The study will greatly contribute to clarifying the potential of CA 19-9 and CEA as prognostic biomarkers and the individual approach to planning adjuvant and neoadjuvant chemotherapy before and after surgery and understanding the metastatic potential and recurrence of each cancer. Intraoperative sampling will allow differentiation between pancreatic inflammation and tumor change, which is often difficult to clarify. It will allow further

processing of pancreatic juice and help to find new prognostic tumor biomarkers that will ultimately allow early detection of pancreatic cancer and better patient survival.

MeSH/Keywords: pancreas, pancreatic cancer, biochemical tumor markers, pancreatic juice, survival rate, carbohydrate antigen 19-9, carcinoembrionic antigen

Abstract title: DNA mismatch repair gene mutation and loss of CDX2 expression in colorectal cancers – Preliminary results

Dissertation proposal title: Frequency of loss of CDX2 protein expression in patients with colorectal cancer depends on the type of DNA mismatch repair gene mutation

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Introduction: In modern healthcare, there is a galloping trend of personalized medicine that is increasingly being introduced as standard in clinical practice. The basis of personalized medicine is the genetic background of the patient and the disease. This is especially important in oncology. Based on the genetic background of the tumor patients are treated with targeted chemotherapy. Colon cancer is a perfect example: treatment with a monoclonal antibody (Pembrolizumab) depends on whether the tumor has a mutation of DNA mismatch repair genes (MMR genes: MLH 1, MSH2, MSH6, PMS2). On the other hand, studies are suggesting that the loss of CDX2 protein expression leads to tumor resistance to various chemotherapeutics. This is the first study in the Republic of Croatia to link MMR protein mutation (MMR deficiency) and loss of CDX2 protein expression in colorectal tumors.

Aims: Determine the frequency of mutations of DNA mismatch repair genes in patients with colorectal cancer and determine whether there is a correlation between a specific mutation and age and gender of patients with colorectal cancer, Determine the frequency of loss of CDX2 protein expression in patients with colorectal cancer and its correlation with patients age and gender, Investigate whether there is a link between increased or decreased frequency of loss of expression of CDX2 protein with one or more mutation of DNA mismatch repair genes.

Materials and Methods: The materials are paraffin-embedded tumor tissue samples taken from the surgically removed tumor specimen. Diagnosis of colorectal cancer is confirmed histologically on tissue samples stained with standard hematoxylin and eosin method. The representative paraffin-embedded tumor tissue samples

are then immunohistochemically stained on CDX2, MLH1, MSH2, MSH6, and PMS2. Immunohistochemical expression of these proteins is classified as 0 (the negative nucleus of tumor cells) and + (the positive nucleus of tumor cells.). Data such as age, gender, localization, pathohistological diagnosis, tumor diameter, mucinous component are gathered in for every patient.

Results: Preliminary results include patients operated for colorectal cancer in the Vinkovci County Hospital in the period 2016-2018. There were 192 patients and 192 tumors (123 men (64%) and 69 women (36%)). In 20 tumors (10%), there is a mutation of one of the DNA mismatch repair genes (MMR deficiency), and in 11 tumors (6%) there is a loss CDX2 expression. In 3 tumors (1.5%), there was a combination of MMR deficiency and decreased expression of CDX2 protein. The average age of patients in the whole sample is 68 years, in MMR deficient tumors patients are on average 70 years old, and in tumors with loss of CDX2 expression 65 years old. MMR-deficient tumors are more often located in the right colon (R 70% - L 30%), tumors in the entire sample are more often located in the left colon (R 27% - L 73%), while tumors with reduced CDX2 protein expression are equally located in the right and left colon (D 56% - L46%). MMR-deficient tumors have a mucinous component in 50% of tumors, as opposed to the other two groups (whole sample 24%, tumors with loss of CDX2 expression 27%). The diameter of tumors that are MMR deficient is 5.24 centimeters, while the other two groups of tumors are 4.45 centimeters in diameter.

Conclusion: The results show that the characteristics of tumors with loss of CDX2 expression are similar to the characteristics of the tumors in the whole sample. In contrast, MMR-deficient tumors more frequently have a mucinous component within the tumor, are larger in diameter and more frequently occur in the right colon in comparison with the other two groups. These preliminary results are comparable with the results published in the literature. However, to complete this research, it is necessary to expand the sample and make a statistical analysis so that it can confirm or reject the hypothesis and reach a definitive conclusion.

Keywords: mismatch repair genes, CDX2, colorectal cancer, immunohistochemistry

Abstract Title: The Development of Dental Medicine in Osijek in the 18th century

Part of the Disertation Proposal: The Development of Dental Medicine in Osijek

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Mentor: Prof. Aleksandar Včev, M.D., Ph.D., Faculty of Dental Medicine and Health Osijek; Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Introduction: In order to investigate the development of dentistry in Osijek, it was necessary to observe the development of the city itself from the time of the Mursa to the modern city of Osijek. Given the lack of information on tools used to treat the teeth from the Mursa period, we focused on the period when military facilities were built in Osijek, which is considered to be the beginning of the modern city of Osijek. Considering the presence of army during this period, it is logical to conclude that they had to have developed health care in the form of wound care, treatment of some diseases as well as dental diseases. During the research we came across interesting materials, laws, and the mention of the first names of "medicus" and "chyrurgus", as well as instruments for dental treatment of that time, which will be briefly explained in the Results section.

Aims: To study the development of dentistry in Osijek during the 18th century, the role of the first „medicus" and the instruments they used in the beginning of dentistry in this area.

Materials/Participants and Methods:
In this research we have used the State Archives in Osijek:
Clinical Hospital Osijek, HR-DAOS-208, 580 books, 6 boxes of archives, 1874-1955.
Medical Center of National Health Osijek, HR-DAOS-204, 1 volume, 9 boxes of archival material, 1925-1956.
Cotar District Unutarnji Grad, HR-DAOS-0002, 8 volumes, 1 box of archives, 1697-1786.
Cotar District Donji Grad, HR-DAOS-0003,7 book, 1 box of archives, 1722-1786.
Cotar District Gornji Grad, HR-DAOS-0004, 7 volumes, 1 box of archives, 1774-1786.
United Cotar District Osijek, HR-DAOS-0005, 13 volumes, 3 boxes of archives, 1786-1809.

Municipality of the Free and Royal City of Osijek, HR-DAOS-0006, 410 books, 820 boxes of archives, 1809-1848.
Royal Cotar District, HR-DAOS-0008, 45 volumes, 1889-1918.
City Hall Osijek, HR-DAOS-0010, 1420 books, 3166 boxes, 1848-1918.
During the research, the methods of deduction, description, analysis, synthesis and comparison were used.

Results: The first information about the health service in Osijek dates from 1697, which is understandable considering the fact that a large number of soldiers were present in the city at that time, especially after the construction of the new fort from 1712-1719. Also in reference to the rest of the city's population, as early as 1700, the first known city chyrurgus, or feldsher healer, Gaspar Aman, who had previously served in the army, is mentioned. The military authorities in Osijek have several feldshers, trained doctors and a hospital, but unfortunately, the health service among citizens and civilians is mainly performed by barber surgeons who, in addition to simple surgical procedures and treatment of some diseases, also deal with the treatment and extraction of teeth. In the records of the cotar municipalities examined so far, we have found information about the first references to Medicus in Osijek in the early 18th century. Their activity was related to the army stationed in Osijek, but they also carried out certain activities for the civilian population. "Medicus" could be the actual initiators of dentistry in Osijek, since they dealt with the treatment of various diseases and, among them, dental diseases, with adequate education and required work permits.

Conclusion: In the cotar district records we have found information about the first references to Medicus in Osijek, in the beginning of the 16th century. At that time, their activities were closely related to the army stationed in Osijek. Through the review of archival funds, we also found that they performed certain health care activities for the civilian population, including the treatment of dental disease.

MeSH/Keywords: Dentistry, History, 18th Century, Military

Abstract Title: Correlation of folate receptor alpha expression with clinicopathological parameters in triple negative breast cancer

Part of the Disertation Proposal: Folate receptor alpha expression in triple negative breast cancer

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Mentor: Assoc. Prof. Branka Lončar, M.D., Ph.D., Clinical Hospital Center Osijek, Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Introduction: Triple negative breast cancer (TNBC) is defined by the lack of estrogen and progesterone receptor as well as human epidermal growth factor receptor 2 expression. It has more aggressive course and lower survival rates compared to other breast cancer subtypes, and is, at present, without guided therapy. Over recent years folate receptor alpha (FRA) emerged as a striking target for directed therapies, with reported benefits in some epithelial tumors, thus suggesting the potential benefit in breast cancer.

Aims: To determine the correlation of FRA expression with clinicopathological parameters in TNBC.

Participants and Methods: Our preliminary study included 100 women diagnosed with TNBC in Clinical Hospital Center Osijek, between January 1, 2008 and December 31, 2017. Factors of inclusion were TNBC molecular subtype and positive FRA membranous staining, whereas other molecular subtypes and negative FRA staining were excluding factors. Clinicopathological parameters included age of patient at the time of diagnose, tumor size and grade, Ki-67 score and morphologic type of tumor. Parameters were correlated with FRA expression (scored with *H-score*) using Spearman's rank correlation coefficient and *Mann–Whitney U test*.

Results: Study involved 100 women between ages 31-89 (mean 59). Tumors were almost equally left and right sided and ranged in size from 0.5 to 10.5 cm (mean 2.2). Ki-67 score ranged 0 to 90 (mean 70). These parameters showed weak correlation with H-score, without statistical significance. Morphologic types of observed TNBC included

2 cases of lobular invasive cancer and 98 ductal invasive cancers. 70 women were diagnosed as Bloom-Richardson grade III, 21 as grade II and 1 as grade I carcinoma. Using Mann–Whitney U test we showed that there is correlation of H-score with these two parameters. Significantly higher H-score is found in patients with lobular invasive carcinoma as well as in patients with lower histological grade, with p values of 0.02 and 0.04 respectively.

Conclusion: This study reports that lower grade TNBC as well as certain morphologic types, express high levels of FRA, thus making them a potential target for anti-FRA therapy.

MeSH/Keywords: TNBC, FRA, breast cancer, H-score, histological grading

Abstract Title: Effects of ganglioside synthesis inhibition on neurite length and insulin signaling pathway in differentiated SH-SY5Y neuroblastoma cell line.

Part of the Dissertation Proposal: Effects of changes in glycolipid cell membrane composition on the composition of lipid rafts in SH-SY5Y human neuroblastoma cell line

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

Introduction: The insulin signaling pathway has been extensively researched in recent two decades with an emphasis on type two diabetes and the development of insulin resistance. With the recognition of Alzheimer's disease as one of the forms of insulin resistance focus was shifted on central nervous system and a better understanding of underlying mechanisms of the disease. A large portion of cell to cell communication and neuroplasticity depends on small cholesterol-rich cell membrane microdomains called lipid rafts (LR). The composition of LR is tightly regulated with respect to function in signal transduction. Flotillin 1 (Flot 1) is an integral LR protein important in endocytosis and vesicular trafficking. Brain and neural cells contain large amounts of complex gangliosides such as GM1, GD1a, GD1b, and GT1b, and their roles are various from regulating normal myelination to cell migration. Gangliosides are also an integral part of LR involved in receptor-ligand interaction. One of the complex gangliosides - GM1 is observed to affect insulin receptor signal transduction *in vitro* and as consequence levels activated protein kinase B (pAKT). Concentration and positioning of gangliosides are also important for the spread of action potential what may imply their role in the morphology of neurites and may affect the positioning of immunoglobulin synaptic molecules involved in synaptogenesis like neuroplastin Np65.

Hypothesis: Disturbing ganglioside synthesis affect the expression of Np65, levels of pAKT and neurite length.

Aims: To determine the potency of ganglioside synthesis inhibitors P4 and CDP71 in SH-SY5Ycells; to determine the expression of lipid raft marker flotillin and promotor of neurite outgrowth Np65 under ganglioside synthesis down-regulation; to determine activation of insulin downstream signaling molecule AKT under the condition of ganglioside deprivation.

Materials/Participants and Methods: SH-SY5Y neuroblastoma cell line was differentiated with 10uM retinoic acid and separately treated with ganglioside synthesis inhibitors P4 and CDP 71 during 48 hours. All experiments were done in growth medium unsupplemented with fetal bovine serum (FBS). Solvent controls (ethanol/DMSO) were used for each inhibitor. Total neurite length was measured in 4% PFA fixed cells, immunostained against GM1 and imaged alongside a darkfield condenser lens. Neurites of 100 cells per group were measured in ImageJ program - data are presented as the total length of neurite (μm). The inhibition effect was assessed by GD1b/GT1b/flotillin-1 immunostaining. All cells were imaged on the confocal microscope (Fluoview 1000) and staining was quantified as intensity per individual cell in the ImageJ program. The activity of the insulin signal transduction pathway was measured in various conditions (P4 & CDP 71 treatment) after insulin challenge (2μg/ml during 1 hour). After treatment, cells were homogenized in RIPA buffer and prepared for western blotting. Western blot membranes were probed against Np65 and insulin receptor downstream signaling molecule AKT and its activated form pAKT. Normalization of signal intensity was performed by probing the membranes against housekeeping protein glyceraldehyde 3 phosphate dehydrogenase. Quantification was done in the ImageJ program.

Results: Treatment with P4 significantly inhibits the biosynthesis of GM1 ($p<0.001$) GD1b ($p<0.001$) and GT1b ($p<0.05$) gangliosides without effect on length of the neurite. At the same time flotillin 1 expression in a membrane ($p<0.001$) and levels of AKT ($p<0.05$) compared to the control group decreased. P4 treated cells expressed less Np65 if compared to control cells ($p<0.05$). Second inhibitor CDP 71 effects on a synthesis of gangliosides are observed just for two out of three GM1 ($p<0.01$) and GT1b ($p<0.05$), GD1b inhibition effect is omitted. DMSO has a significant lowering effect on levels of GM1 ($p<0.001$) and GT1b ($p<0.01$). Significantly shorter neurites are observed compared both to control ($p<0.001$) and solvent ($p<0.001$) group as well as levels of flotillin 1 ($p<0.01$). A similar effect to the first inhibitor is observed with lower levels of total Np65 ($p<0.05$). The addition of inhibitors changes base levels of pAKT compared to control and overall response to insulin stimulus that can be observed in lower pAKT levels ($p<0.001$) compared to the control group.

Conclusion: The inhibition of ganglioside synthesis changes the activity of insulin signaling pathway in inhibitors treated cells because stimulation with insulin does not increase but rather decreases overall levels of pAKT and what can be interpreted as insulin resistance. Change in the composition of lipid rafts under the effect of inhibitors is also observed in the lower expression of Flotillin1 and Np65. Inhibition of neuritogenesis is more prominent in CDP 71 treated cells than in P4 treated cells and thus we can conclude different mechanisms of action of each inhibitor. These findings must be additionally confirmed by immuno co-precipitation of insulin receptor, Np65, and major gangliosides.

MeSH/Keywords: Ganglioside, Np65, pAKT, Neurite length, SH-SY5Y

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