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Foreword

Faculty of Medicine Osijek for the tenth time celebrates the Days of PhD candidates - Dies doctorandorum 2024., an annual and traditional event, and an important part of the Postgraduate Doctoral Study Of Biomedicine And Health. This public event is open to all members of the scientific community, with a focus on Ph.D. students presenting their research for discussion and debate.

All members of the scientific community are invited to share their experiences and mentor new colleagues at the beginning of their professional careers. As always, we hope to initiate social connections and increase collaboration between mentors and Ph.D. students, benefiting the scientific community and the postdoctoral study program itself.

The Faculty of Medicine Osijek aims to train its research postgraduates to develop sound research skills, acquire the latest theoretical knowledge, become critical thinkers in their chosen fields of study, and conduct inquiries in their areas of specialization professionally and ethically. This annual conference is designed to guide our students in communicating original ideas and offer new perspectives clearly and within coherent structures.

Furthermore, Ph.D. students' progress will be evaluated based on poster and oral presentations. As a result, the best poster presentations will be chosen which is an important factor when choosing the most successful doctoral student.

Dies doctorandorum reflects our relentless and constant efforts towards excellence in academic training and research, both for our students and our academics. I believe that through our commitment to excellence, and investment in continuous pursuits of knowledge and research, we can provide a meaningful and substantial contribution to the scientific community which is the ultimate purpose of doctoral postgraduate programs.

Professor Ivica Mihaljević, M.D., Ph.D.

Dean, Faculty of Medicine Osijek

Abstracts of annual seminars

Dissertation Proposal Title: PERSONAL, PROFESSIONAL, AND SITUATIONAL FACTORS AS PREDICTORS OF NURSING STUDENTS' DISHONEST AND UNPROFESSIONAL BEHAVIOR IN CLASSROOM AND CLINICAL SETTINGS

PhD candidate: Renata Apatić, MSN, RN, Health Center of Osijek-Baranja County, Croatia

Mentor: Assoc. Prof. Robert Lovrić, Ph.D., MSN, RN, Faculty of Dental Medicine and Health, Osijek, Croatia

Introduction: Academic dishonesty is a global problem in many higher education institutions and is increasing in nursing studies. Studies suggest a severe risk due to the association of nursing students' academic dishonesty (NSAD) in the classroom with dishonesty in clinical settings when it can directly jeopardize patient safety and healthcare quality. The contributions of various personal and situational factors (e.g., perception of punishment, peer influence, self-control, narcissism) to academic dishonesty have been studied. However, there are still many potential factors whose influences are not yet known.

Hypothesis: The NSAD incidence is related to personal, professional, and situational factors.

Aims: This study aims to provide a deeper insight into NSAD and attitudes and to determine the contribution of personal, professional, and situational factors to dishonest behavior in the classroom and clinical settings.

Materials/Participants and Methods: The study included first-year undergraduate and graduate nursing students (generation 2022/2023) at the Faculty of Dental Medicine and Health in Osijek.

An anonymous survey questionnaire consists of questions about the students' general characteristics and four validated Croatian versions of instruments: *Nursing Student Perceptions of Dishonesty Scale (NSPDS), Moral Competence Test (MCT), Emotional Competence Questionnaire (UEK-45),* and *Burns Perfectionism Scale*.

Research plan: The study will be conducted longitudinally during three academic years:

Phase 0 (Jan-Feb 2023):

- preparation of structured instruments for the study

Phase 1 (Mar 2023), **2 (Mar 2024)**, and **4 (Mar 2025)**:

- examination of attitudes and NSAD incidence in the classroom and clinical settings
- examination of students' moral competence, emotional competence and perfectionism traits

Phase 3 (Oct 2024):

- application of interventions to influence students' personal, professional, and situational factors (educating students and clinical mentors about the NSAD, potential risks and impacts on the patients' safety and the healthcare quality, the university code of ethics and student code of conduct, etc.)

Expected scientific contribution: The study will provide deeper insight into NSAD incidence and the possible contribution of personal, professional, and situational factors to dishonest behavior. The results will help higher education institutions design strategies to reduce NSAD. A higher academic honesty level will ensure higher healthcare quality and patient safety.

Keywords: academic dishonesty, nursing students, emotional competence, perfectionism, moral competence

Dissertation Proposal Title: "The role of miRNA-200 family members in chronic wound healing after local applications of platelet-rich plasma"

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Mentor: Assoc. Prof. Martina Mihalj, Department oh Physiology and Immunology, Faculty of Medicine Osijek, University Hospital Centre Osijek, Osijek, Croatia

Co-mentor: Assist. Prof. Mirjana Suver Stević, Biologist at Laboratory of molecular and HLA diagnostics University Hospital Center Osijek, Osijek, Croatia

Introduction: Chronic, non-healing, cutaneous wounds have become major medical and social burden worldwide. Reduced angiogenesis has been recognized as the major factor in the non-healing nature of the most types of chronic wounds. The wound healing process can generally be dissected into several interconnecting and overlapping stages, including immediate hemostasis, acute inflammation, proliferation and maturation. A key accompanying activity in the proliferation stage is the formation of new blood vessels, a process known as angiogenesis. The process is driven by synergistic activity of several factors, of which the vascular growth factor (VEGF) has a dominant role. In addition, beside genetic factors contributing to chronic wounds and underlying diseases, epigenetics has a prominent role in the formation and the maintenance of non-healing wounds. Epigenetic mechanisms include small non-coding RNA molecules (miRNA) with the capacity to bind and regulate expression of genes. Recent advantages of the chronic wound management include use of autologous platelet reach plasma (PRP) preparations, injected solei or in combination with hydrogels allowing prolonged release of the growth factors.

Hypothesis: The process of healing a chronic wound after the application of PRP includes regulation of E-cadherin and VEGF-mediated gene expression to members of the miRNA-200 family.

Aims:

- To make an objective assessment of the progress of the wound healing process after the application of PRP based on the size and quality of the tissue
- To determine the expression of certain miRNA-200 and their target genes in the marginal tissue of the wound before and after the application of PRP
- To determine whether there is a difference in the expression of studied miRNAs after PRP administration
- To determine whether PRP treatment affects gene expression for VEGF and e-cadherin

Materials/Participants and Methods: The examined group would consist of up to 40 patients with chronic wounds which will be randomly divided into two groups of subjects: (1) "PRP"

GROUP of patients who, in addition to routine treatment of chronic wounds, will also receive PRP treatment and (2) CONTROL GROUP of patients who will undergo only routine wound treatment.

Research plan: Patients will be included in a 6-week protocol during which the "PRP" group will receive PRP treatment on two occasions. For each patient, tissue samples would be taken from the wound for the purpose of miRNA and mRNA isolation, which would serve to determine the expression of certain miRNA-200 and their target genes (E-katherin, VEGF). In both groups of patients, an objective assessment of the wound size would be made using an automated 3D robotic camera.

Significance/Expected scientific contribution: The results of the proposed research will contribute to a better understanding of the role of miRNAs from the miRNA-200 family in the wound healing process. Positive results would make a significant contribution to the treatment of patients with chronic wounds. This kind of therapeutic procedure for the use of PRP in the healing of chronic wounds could be implemented in the standard treatment procedure at Clinical Hospital Center Osijek, which would enable better treatment of patients.

MeSH/Keywords:

- Chronic wound
- miRNA-200 family members
- Platelet-rich plasma

Dissertation Proposal Title: Sharing antibiotics among the general population as a modern public health challenge

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Mentor 1: Assoc. Prof. Ivan Miškulin, Ph.D., Faculty of Medicine Osijek, University of Osijek, Croatia

Co-mentor: Prof. Maja Miškulin, Ph.D., Faculty of Medicine Osijek, University of Osijek, Croatia

Introduction: Sharing antibiotics among adults (giving our antibiotics to someone else, or taking antibiotics from someone), without prior consultation with a doctor is a common phenomenon, and it is of important medical and public health significance, due to numerous possible harmful consequences. Antibiotic sharing affects the spread of antimicrobial resistance, which represents a global public health threat. Up until now, in Bosnia and Herzegovina, it hasn't been investigated why respondents share antibiotics, do they have sufficient knowledge about them, and whether they have a risk assessment strategy while deciding to share antibiotics.

Hypothesis: Sociodemographic and socioeconomic factors are connected to the behaviors and practices of respondents who share antibiotics and who resort to self-medication.

Aims:

1. Examine respondents' opinions and knowledge about antibiotics and their sharing without prior consultation with a doctor or pharmacist.
2. Examine the sociodemographic and socioeconomic factors of respondents who share antibiotics without a doctor’s prescription.
3. Identify sources of shared antibiotics.
4. Examine the frequency of antibiotic sharing.
5. Examine the reasons and circumstances under which the respondents share the antibiotics.
6. Examine potential negative and unwanted effects of antibiotic sharing.
7. Examine whether respondents have a risk assessment strategy when deciding about antibiotic sharing.

Materials/Participants and Methods: Participants will be the patients, recruited by the family doctor, who have been prescribed antibiotics at the level of primary health care at least once in their life, from the area of Bosnia and Herzegovina (Federation of Bosnia and Herzegovina, Republic of Serbs, and Brčko DC), age of 18-69 years. The quantitive part of the study will cover 500 participants, while the qualitative part of the study will consist of 45 participants, divided into 3 focus groups.

Research plan: The research will be conducted in 2 different directions. The quantitative part of the study will be conducted through the questionnaire. After questioning the sample of 500 participants, each participant will get a document, containing the risks and potential harm consequences of antibiotic sharing and consequences of antimicrobial resistance. A month after sharing interventional documents, the questioning of the participants will be conducted again. The qualitative part of the study will cover 45 participants, divided into 3 focus groups: patients who share antibiotics, people who do not resort to self medication, and pharmacists, who will be interviewed.

Significance/Expected scientific contribution: This research will define the factors that affect the behaviors of patients who borrow or lend antibiotics, or buy them without consulting the doctor. This research could also help to understand the reasons behind the sharing of antibiotics among patients. The results of the research will be useful in creating public health interventions to educate patients about the safe usage of medicaments, and it could help in designing different messages to reduce the risks associated with antibiotic sharing, as well as interventions for reducing antimicrobial resistance. The results could also help the regulatory authorities in developing plans to control the sale of antibiotics without medical prescription.

MeSH/Keywords: adults, behavior, population, cross-sectional studies, self-medication

Dissertation Proposal Title: Metabolic profiling of colon cancer, adjacent lymoh node and circulating tumor cells using the MALDI-TOF IMS

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Mentor: Assoc. Prof. Željko Debeljak, M.D., Ph.D., University hospital centre Osijek, Osijek, Croatia

Introduction: As colorectal cancer becomes the great burden of morbidity and mortality worldwide, often diagnosed at advanced stages, arises a need for the establisment of less invasive and more informative methods of early and metastatic dissease detection and personalised approach in treatment and follow-up. Circulating tumor cells (CTCs) are present in the blood of the colon cancer patients, independently of tumor stage. At the moment, just the number of CTCs in peripheral blood is used as a prognostic factor. Metabolic profile of CTCs has not been explored in great detail by informative techniques like MALDI TOF IMS.

Hypothesis: Metabolic profile of the tumor cells in the lymph node and CTCs differ from the metabolic profile of the primary tumor in colorectal cancer.

Aims: To establish an in-house protocol for detection of the circulating tumor cells from the whole blood. To examine the metabolic profile of the primary tumor, adjacent lymph node and CTCs. To determine the differences between those three samples for each patient.

Materials/Participants and Methods: Participants will be patients with metastatic colorectal cancer. After the surgical resection, samples of primary tumor, adjacent lymph node and CTCs from the blood sampled from the mesenteric vein during the operation will be prepared and analyzed by the pathologist and by MALDI-TOF IMS.

Research plan: 10 samples will be collected on the Abdominal surgery department in University clinical centre Osijek. Patients will be asked to sign informed consent form prior to the surgical resection. Samples of the tumor and lymph node will be analyzed in the standard fashion with a HE staining by the pathologist , after which will be analyzed by MALDI-TOF IMS. Blood samples will be processed in established protocol in clinical laboratory and analyzed by MALDI-TOF IMS. Data will be processed in Image reveal progam and statisticaly analyzed. Currently, we are optimizing the isolation of CTCs using the cell filtration kit, which will be presented on the poster.

Significance/Expected scientific contribution: Better understanding of tumor biology through metabolomic analysis, understanding of the pathways of tumor progression and

metastatic potential, establisment of the less invasive methods of the tumor progression follow-up, personalisation of the surgical and oncological therapy.

MeSH/Keywords: colorectal cancer, circulating tumor cell, single cell analysis, MALDI MS, liquid biopsy, EpCAM

Dissertation Proposal Title: Distribution of Ki-67 expression as surrogate tumour biology marker among molecular breast cancer subtypes

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Co-Mentor: Prof. Sven Kurbel, M.D., Ph.D., Faculty of Medicine Osijek, University of Osijek and Polyclinic Aviva, Zagreb, Croatia

Introduction: Breast cancer (BC) is an increasing epidemiological issue on global level characterized by high mortality rate.
BC is very heterogeneous disease with different gene profiling, histological, molecular, pathophysiological, clinical as well prognostic subcategories.
Immunohistological methods enable detection of ER, PR, HER2 oncogene and Ki-67 proliferative factor/mitotic index.
The therapeutic consequences of hormonal receptor status determination is of immense value as prognostic parameter for BC molecular subtypes.
The 12th St. Gallen Consensus proposed new BC classification considering these parameters – hormonal receptor expression and Ki-67 expression intensity, distinguishing following BC subtypes:

1) **Luminal A** (ER and/or PR positive, HER2 negative, Ki-67 <20%)
2) **Luminal B1 and B2** (HER2 negative, ER and/or PR positive, Ki-67 >20% vs. HER2 positive, ER and/or PR positive, any Ki-67),
3) **HER2 positive– nonluminal** (Erb-B2 expression/ HER2 positive, ER and PR negative);
4) **Basal-like or triple negative** (triple negative breast cancer: ER and PR negative, HER2 negative)

The clinicopathological and immunophenotype-based classification of BC and its nomenclature are widely used in standard diagnostic procedures with high implication on therapeutical strategies.

Hypothesis: Distribution of Ki-67 expression as surrogate tumour biology marker within and among molecular breast cancer subtypes predicts potentially different biological behaviour.

Aims:

The primary aim:

- to examine a relation between Ki-67 expression (as mitotic activity marker) with clinical, pathohistological and immunohistochemical characteristics of BC patients.

The secondary aims are:

- to identify differences among BC molecular subtypes and determine distribution of immunophenotypical features (hormonal receptors status (ER, PR), HER2 oncogene expression, Ki-67 expression)
- to examine presence of Ki-67 expression clusters among molecular BC subtypes
- to detect tumour variants within standard molecular BC subtypes with potentially different biological behaviour, dependent on still less known factors, based on Ki-67 clusters analysis

Materials/Participants and Methods:

a) Participants

2561 invasive breast cancer patients will be included in this comprehensive retrospective observational cohort study conducted in single-center, at the Pathology Department, Clinical Hospital Center Osijek.

b) Methods

Evaluation of archiv paraffin tumour blocks which are routinely processed with hematoxylin-eosin and immunochemical staining and will be complementary stained/analysed for missing data.
Accordingly to 12th St. Gallen Convention breast cancer subtypes will be classified.

c) Statistical methods:

Statistical analysis will be conducted on collected uniform dataset (Excell) to examine distribution of immunophenotypical features, similarities, and differences between molecular BC subtypes and primarily to identify Ki-67 clusters as known for Ki-67 to be a surrogate marker of tumour biological behaviour. Among BC molecular subtypes Ki-67 clusters of low and high mitotic activity will be identified using statistical method known as „expectation maximization“ (EM) clustering. Cluster detection will be automatically performed by statistical program StatSoft, Inc. (2011) STATISTICA using v-fold cross-validation algorithm.
Collected database will be analysed using program StatSoft, Inc. (2011) STATISTICA (Data Analysis Softwaresystem), version 10. www.statsoft.com.

Research plan: Conduction of the study, including collecting and analyzing data and publishing results will take approximately two years:

1) to form uniform dataset for 2561 BC patients

- demographical characteristics
- tumor analysis data from standard BC tumor block staining

2) to classify all molecular subtypes in Breast Cancer Patients according to St. Gallen Consensus

3) to conduct statistical analysis

- distribution of BC subtypes over considered demographical, clinico-pathological und histological parameters
- determine correlation of these variables with Ki-67 mitotic index/proliferation activity marker
- dentification of Ki-67 clusters among standard molecular subtypes

4) to publish of our results

Significance/Expected scientific contribution: Ki-67 is identified as a surrogate marker of tumour biology potentially linked to less identified factors, other than hormonal receptor status. The expanded knowledge on how predictive Ki-67 as proliferation marker in immunophenotype-based BC subtypes might be, could also elucidate future more differentiated (and more individualized) therapeutic strategies. The strength and comparative advantage of this study is the size of the investigated cohort and its uniformity, hoping to reveal representative results.

MeSH/Keywords: breast cancer, immunohistochemistry, molecular breast cancer subtypes, Ki-67 proliferation activity marker

Acknowledgement: Clinical Hospital Center Osijek, Department of Pathology

Dissertation Proposal Title: Human milk, a source of stem cells and exosomes, as a therapy for neonatal diseases.

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Co-mentor: Prof. Silvija Pušeljić, Ph.D., Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Introduction: Human milk has subsequently been shown to contain stem cells (hBSCs) with multilineage properties that exhibit variable expression of pluripotency genes normally found in human embryonic stem cells (hESCs). These genes included the transcription factors OCT4, SOX2, NANOG, which are known to form a fundamental circuit of self-renewal of human embryonic stem cells. In vivo, human milk stem cells differentiated into the cell lineages of all three germinal layers. It is believed that the above can continue postnatally through breastfeeding. Human milk leukocytes can pass into the digestive epithelium and enter the infant's systemic circulation. Stem cells from various sources are being investigated in many neonatal disorders, such as intraventricular hemorrhage (IVH), bronchopulmonary dysplasia (BPD), and necrotizing enterocolitis (NEC). In an observational study involving premature infants with severe IVH, early intranasal administration of human milk was shown to be effective in limiting brain damage. Bronchopulmonary dysplasia (BPD) is a chronic lung disease that occurs in premature infants, and is a major cause of neonatal morbidity and mortality worldwide. Inflammatory responses mediated by pro-inflammatory cytokines are crucial in the development of BPD. So far, no studies have been conducted that used human milk stem cells directly in BPD, and according to everything, they have potential applicability in this area. Necrotizing enterocolitis, which is an acquired, life-threatening disease characterized by inflammation, loss of villi and epithelial cells, and intestinal necrosis, is one of the main problems of premature infants. In the studies conducted so far, it has been established that human milk serves as a strong inhibitor of toll-like receptor 4 (TLR4), through the rich concentration of epidermal growth factor (EGF) secreted by the mammary gland into human milk, especially in the first weeks of life, which confirms that EGF protective factor of human milk. More recent studies have focused on exosomes, extracellular vesicles, of human milk that protect intestinal epithelial cells (IEC) from oxidative stress, stimulate intestinal stem cell activity, improve IEC proliferation and migration, and reduce the incidence and severity of experimental NEC. No human studies have yet been conducted using human milk in NEC, although the role of human milk in preventing the development of NEC is well known.

Hypothesis: Human milk is a complex biological fluid that can be compared to blood, possessing stem cells (hBSC) with multilineage properties and exosomes that are beneficial in

preventing the more severe consequences of neonatal diseases of intraventricular hemorrhage, bronchopulmonary dysplasia, and necrotizing enterocolitis.
Aims: To determine the presence and characteristics of milk pluripotent stem cells and exosomal vesicles in the milk of mothers of premature infants and the blood of premature infants and the milk of mothers of healthy children and the blood of healthy children. To determine the influence of milk pluripotent stem cells and exosomal vesicles on the outcome of treatment of breastfed and non-breastfed premature infants with associated neonatal diseases of intraventricular hemorrhage, bronchopulmonary dysplasia and necrotizing enterocolitis.

Participants and Methods: Newborns and premature infants exclusively fed for a period of one year will participate in the mentioned study, after the approval of the clinical trial. The composition of breast milk varies depending on the degree of lactation, therefore a sample of colostrum will be taken (in the first three days of the establishment of lactation), a sample of transitional milk (5th - 14th day after the establishment of lactation), and a sample of mature milk (after the 14th day of establishment of lactation). The respondents will be divided into four groups of breastfed and non-breastfed newborns and premature infants, with the sampling of breast milk and blood from breastfed newborns and premature infants at different time stages of the research.

Research plan: The study will be conducted at 50 breastfed newborns, 50 breastfed premature babies with IVH, BPD and NEC, 50 unbreastfed newborns, 50 unfed premature babies with IVH, BPD and NEC. Isolation of milk stem cells and exosomal vesicles will be performed with magnetic bead extraction kits. The clinical outcome of neonatal diseases depends primarily on antenatal and postnatal factors, as well as on the etiology and extent of the disease itself. In addition to the above, also about gestational age and birth weight.
IVH - need for relief lumbar punctures, anti-edematous therapy, ultrasound monitoring of bleeding resorption, leukomalacia.
BPD - duration of dependence on respiratory support, duration of dependence on oxygen supplementation, hypoxemic episodes (SpO2), need for surfactant, duration of antimicrobial therapy, need for inhalation therapy.
NEC - duration of antimicrobial therapy, speed of cessation of occult bleeding, speed of re-establishment of oral feeding, need for surgery, progress in body weight.

Significance/Expected scientific contribution: The expected contribution of the research would be the determination of the presence and characterization of milk stem cells and exosomal vesicles in the milk and blood of premature infants and their influence on the outcome of the treatment of this patient population.

MeSH/Keywords: Exosomal vesicle; Human milk; Stem cell

Dissertation Proposal Title: Molecular classification for targeted therapy and treatment of metastatic cancer of unknown primary using machine learning and the cBioPortal database

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Introduction: Cancer of Unknown Primary (CUP) presents a significant challenge in diagnosis and treatment due to the unknown primary site despite confirmed secondary tumors. This uncertainty complicates therapeutic decision-making, leading to poor treatment outcomes. However, advancements in tumor genomics offer a promising solution. Next-generation sequencing (NGS) not only ensures accurate diagnostics but also enables precise therapy in cancer treatment. Artificial intelligence and machine learning models, combined with large databases like cBioPortal for cancer genomics, provide a promising avenue for identifying the primary site of CUP tumors.

Hypothesis: We hypothesize that implementing big data analysis using machine learning techniques and molecular classification will accurately identify the primary tumor site in cases of CUP, thereby improving patient outcomes through targeted therapies.

Aims: This study aims to explore how molecular classification, utilizing machine learning methods and bioinformatics databases, can enhance the accuracy of identifying the primary tumor site.

Materials/Participants and Methods: This study will utilize data collected from over 10,000 patients stored in the cBioPortal database. With over 400 TB of raw data for 33 different tumor types, cBioPortal allows for the creation of virtual studies and analysis using machine learning

methods. We will conduct a machine learning-based analysis of secondary cancers with known primary sites using data from the publicly available cBioPortal database to create a statistical model predicting the primary site of tumors in cases of CUP.

Research Plan: Based on big data analysis, we will develop a statistical model to aid in identifying CUP's primary site, facilitating state-of-the-art precision therapy and treatment following the latest guidelines for tumors with specified primary sites.

Significance/Expected Scientific Contribution: Utilizing machine learning methods enables molecular classification, essential for precise primary tumor site detection and state-of-the-art targeted therapy. This approach represents breakthroughs in understanding the molecular basis of CUP's tumorigenesis and contributes to personalized treatment in oncology.

MeSH/Keywords: precision medicine, neoplasm metastasis, machine learning, computational biology, genomics

Dissertation Proposal Title: Association between gastrointestinal motility disorders and circulating markers in acute brain injury

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Mentor: Prof. Slavica Kvolik, Clinical Hospital Center Osijek, Department of Anesthesiology, Resuscitation and Intensive Care, Faculty of Medicine Osijek, University of Osijek, Croatia

Introduction: Acute brain injury is a major public health problem, because it is one of the leading causes of disability and death. It includes traumatic brain injury, hemorrhagic or ischemic insult.
Dysfunction of the gastrointestinal system is one of the most common consequences of acute brain injury, but often not the focus of attention in the treatment. A number of biomarkers of neurological damage such (S100b, NSE) are present in the circulation. The brain-gut axis itself is a complex network that includes numerous signaling pathways and any change leads to multifactorial effects that are reflected in numerous processes in the intestines and in the brain. Imaging methods are also important as they can indicate the intensity and localization of the injury.

Hypothesis: In acute brain injury, biomarkers (S100b, NSE) are disrupted, imaging tests are altered and the dysfunction of the gastrointestinal tract develops

Aims:

1. investigate correlation between biomarkers S100b, NSE and imaging tests after acute brain injury
2. examine the connection between imaging tests and gastrointestinal motility disorders
3. investigate correlation between biomarkers S100b, NSE after acute brain injury and gastrointestinal motility disorder
4. examine outcomes due to demographic indicators

Materials/Participants and Methods: The prospective observational study would include adult patients (>18 years) of both sexes who had to be operated because of acute brain injury (neurotrauma, spontaneous bleeding) and who required postoperative supervision in the Intensive care unit (ICU).
Patients under the age of 18, demented, immobile and those with a history of diagnosed neurological disease will be excluded from the study.

Research plan: In patients with acute brain injury hospitalized at the ICU outcomes would be compared in relation to demographic indicators, state of consciousness, biochemical and radiological indicators of neurological damage. Blood samples would be taken upon admission,

after 12 and 24 hours after addimison and circulating markers of neurological damage (S100b, NSE) would be analyzed. Imaging tests would be performed for the purpose of assessing neurological damage. Gastrointestinal motility disorder would be monitored by measuring content of the nasogastric tube.

Significance/Expected scientific contribution: With this study, we would establish the association of biomarkers (S100b, NSE) in acute brain injury with imaging findings and gastrointestinal motility disorder.

MeSH/Keywords: gastrointestinal motility; acute brain injury; biomarkers; S100b; gastric emptying

Dissertation Proposal Title: Gender-dependent predictive risk factors for the development of immunotherapy-mediated adverse events in non-small cell lung cancer patients treated with pembrolizumab

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Mentor: Assoc. Prof. Ines Bilić-Ćurčić, M.D., Ph.D., University Hospital Centre Osijek; Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

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

Introduction: Immunotherapy represents a milestone in the treatment of lung cancer was brought by immunotherapy. Immunotherapy medications are biologics, also called „checkpoint inhibitors“ and include the PD-1 (programmed cell death – 1) inhibitor pembrolizumab, which is used as monotherapy or in combination with chemotherapy in the first line of treatment for metastatic disease. Despite the revolutionary results in the treatment of cancer, immunotherapy can often cause various adverse events. Few studies have evaluated the difference between the incidence and severity of immunotherapy-mediated adverse events between men and women, and some data point out that the incidence might be higher in women. Also, certain molecules measured from the blood of the patients treated with immunotherapy have proved to be potential predictive biomarkers for the development of immunotherapy-mediated adverse events.

Hypothesis: Interleukin-6 and other molecules measured in the blood can be predictive biomarkers for the development of immunotherapy-mediated adverse events and these are more frequent and of a greater severity in women.

Aims:

1. To examine whether the difference in gender affects the occurrence and severity (grade) of recorded immunotherapy-mediated adverse events and other adverse events characteristic for the chemotherapy treatment protocol.

2. Test whether certain laboratory parameters (absolute lymphocyte count (ALC), absolute monocyte count (AMC), absolute eosinophil count (AEC), platelet values, absolute basophil count (ABC), neutrophil-lymphocyte ratio (NLR), platelet-lymphocytes ratio (PLR), monocyte-lymphocyte ratio (MLR), systemic immune-inflammation index (SII), lactate dehydrogenase and transaminases represent a predictive factor for the development of immunotherapy-mediated adverse events.

3. To investigate whether the elevated or decreased basal level of cytokine IL-6 can be considered a predictive factor for the development of immunotherapy-mediated adverse events.

4. To examine the influence of demographic and clinical parameters on the incidence and severity of side effects caused by immunotherapy.

Materials/Participants and Methods: The research will be conducted on 76 patients suffering from metastatic non-small cell lung cancer who will be divided into two groups by gender. Patients who start treatment with pembrolizumab at the Clinical Hospital Center Osijek will be included and will be monitored until disease progression or one year after the start of treatment.

Research plan: Demographic data (age, gender, body mass index, smoking status), data on comorbidities and chronic therapy, known drug allergies and data on existing malignant disease will be collected from patients before starting treatment with pembrolizumab. A blood sample will be taken from the patients to determine the basal values of the investigated parameters. Hematological parameters will be monitored before each application of a new cycle of treatment, and IL-6 will be measured again in the event of an immunotherapy-mediated adverse event. Severity of adverse events will be defined in 5 grades according to Common Terminology Criteria for Adverse Events (CTCAE) v5.0. Patients will also be contacted by phone between 3-week treatment cycles for information on possible adverse events. The type, grade and outcome of adverse events will be recorded. All data collected during the research will be analyzed using appropriate statistical methods.

Significance/Expected scientific contribution: Since adverse events of varying severity often occur with immunotherapy, some of which are life-threatening, it is important to identify predictive risk factors for their development. Identification of risk factors, early recognition and appropriate treatment of these adverse events are prerequisites for optimal patient treatment outcomes. Also, according to some research, there is a difference in the incidence and grade of immunotherapy-mediated adverse events depending on gender and it is important to determine whether this demographic parameter can direct doctors towards the expectation of a greater number of more severe adverse events in a particular gender.

MeSH/Keywords: Lung Neoplasms; Immunotherapy; Pembrolizumab; Interleukin-6; Adverse effects;

Dissertation Proposal Title: Assessment of myocardial viability in patients with chronic coronary syndrome using different diagnostic procedures

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Mentor: Assist. Prof. Darija Šnajder Mujkić, M.D., Ph.D., Clinical institute for nuclear medicine and radiation protection, University Center Hospital Osijek, Croatia. Faculty of Medicine Osijek, Croatia.

Co-mentor: Prof. Kristina Selthofer Relatić, M.D., Ph.D., Department for Heart and Vascular Diseases, University Center Hospital Osijek, Croatia. Faculty of Medicine Osijek, Croatia.

Introduction: Chronic total occlusion of a coronary vessel (CTO) represents a coronary artery without anterograde flow for a minimum of 3 months. According to estimates, every fourth patient with coronary heart disease has a CTO lesion. Myocardial revascularization of patients with CTO lesions has been proven to increase survival and improve quality of life. On the other hand, the risk of complications of percutaneous coronary intervention is higher in patients with a CTO lesion in contrast to patients without a CTO lesion. A great help in assessing the success and benefit of percutaneous coronary intervention is the analysis of myocardial viability. So far, no biomarkers have been found that would suggest the viability of the myocardium and thus help in the decision for revascularization.

Hypothesis: A combination of diagnostic methods including biomarkers, strain echocardiography and myocardial scintigraphy can predict viable myocardium in patients with chronic coronary syndrome.

Aims: To examine which factors are significant in the prediction of myocardial viability in patients with chronic coronary syndrome

Materials/Participants and Methods: Patients treated at the Department of Heart and Blood Vessel Diseases due to chronic coronary syndrome who manifest coronary disease as chronic coronary artery occlusion on coronary will be included. Minimal sample size is 58 patients.

Strain echocardiography will be performed at the Department of Heart and Blood Vessel Diseases on a GE Vivid e9 device (Chicago, USA). Scintigraphy of the myocardium at rest and under load will be performed according to the usual protocol at the Clinical Institute for Nuclear Medicine and Radiation Protection using Tc99m-MIBI on a GE Optima camera (Chicago, USA). Biomarker concentrations will be measured from serum at the Department of Physiology and Immunology of the Faculty of Medicine in Osijek.

Research plan: A cross sectional study will be performed. Estimated study duration is 12 months.

Significance/Expected scientific contribution: The research will provide new insight into the pathophysiological and biochemical mechanisms of myocardial viability assessment. The combination of diagnostic and biochemical procedures will contribute to better therapeutic options in the treatment of patients with CTO lesions.

MeSH/Keywords: Biomarkers; Chronic coronary syndrome; Myocardial scintigraphy; Myocardial viability; Strain echocardiography

Dissertation Proposal Title: Personality traits and shift work as predictors of circadian rhythm disorders and attention disorders in nurses in a hospital setting

PhD candidate: Željka Dujmić, MSN, General Hospital Dr. Josip Benčević, Slavonski Brod, Croatia

Mentor: Assist. Prof. Štefica Mikšić, Ph.D., MSN, RN, Faculty of Dental Medicine and Health Osijek, University of Osijek, Osijek, Croatia

Introduction: **S**hift work is one of the main features associated with healthcare workers, especially nurses/technicians. The definition of shift work includes that shift work is a type of work whose time schedule is permanently or frequently outside the classic daily working hours. The harmful effects of shift work can be classified into several categories, which include: biological functions, work efficiency and safety at work, social and family aspects, and health problems. Shift workers who work night shifts are especially susceptible to circadian rhythm disorders. In people who suffer from circadian rhythm disorders, increased secretion of cortisol has been recorded. The impact of shift work on many aspects of the lives of shift workers is widely recognized in the scientific literature. A series of studies specifically dealt with the impact of shift work on the cognitive performance of nurses. A high ability to perform all necessary tasks is expected from the medical staff at all times, because often even a small difference in the quality of the work performed can negatively affect the outcomes of the patient's treatment. In addition to variables related to shift work that affect attention deficit disorder and circadian rhythm disorder, it is reasonable to assume that personality traits are also related to this.

Hypothesis: Personality traits will affect circadian rhythm disorders and attention deficit disorder in shift work nurses.

Aims: The main goal of this research is to examine the impact of shift work on the presence of circadian rhythm disorders and attention disorders in nurses/technicians and their relationship with personality traits.

Materials/Participants and Methods: The research will be conducted in General hospital Dr. Josip Benčević in Slavonski Brod. The expected sample size is about 200 respondents. The respondents will wear Fitbit smart bracelets to measure sleep quality. The Morningness-

eveningness questionnaire, Mindful attention awareness scale, NEO five-factor inventory, Pittsburgh Sleep Quality Index will be used for the purposes of the research. The level of cortisol in the blood will also be controlled.

Research plan: This cross-sectional study will be conducted in the General Hospital Dr, Josip Benčević Slavonski Brod, during 12 months.

Significance/Expected scientific contribution: The proposed research will provide a deeper insight into the impact of shift work on attention disorders and disruption of the circadian rhythms of wakefulness and sleep of nurses in hospital institutions. The results of the research will present unique scientifically based evidence on the globally under-researched phenomenon of the association of shift work with attention deficit disorder and circadian rhythms of wakefulness and sleep with personality traits. The foundation will be laid for further professional and scientific research with the aim of improving the impact of shift work on the better organization of the work of nurses.

MeSH/Keywords: personality traits, shift work, atention deficit disorder, circadian rhythms disorder, nurse / technician

Dissertation Proposal Title: Changes of lysophosphatidylcholine concentrations in patients treated with aortic valve replacement

PhD candidate: Hrvoje Falak, M.D.

Mentor: Prof. Goran Krstačić, M.D., Ph.D, Institute for Prevention of Cardiovascular Diseases and Rehabilitation, Zagreb; Faculty of Medicine Osijek, Osijek, Croatia.

Introduction: Aortic valve stenosis (AVS) is the most prevalent valvular heart disease in the Western World with exponentially increased incidence with age. The yearly mortality rates increase up to 25% if left untreated. Currently, no effective pharmacological interventions have been established to treat or prevent AVS. The pathophysiology of aortic stenosis is very complex and begins with valvular endothelial injury and through lipid infiltration and oxidization, inflammatory cascade, ossification results with calcification. Two common options which are frequently employed include transcatheter aortic valve replacement (TAVR) and surgical aortic valve replacement (SAVR). Lysophosphatidylcholine (LPC) is considered as a metabolomic signature of calcified aortic valve and values correlate well with the severity of valvular stenosis. As LPC corelates well with severity of aortic stenosis, its levels should change with therapeutic. Therefore, LPC can be used as a biomarker to measure the outcomes following TAVR and SAVR and can be useful to compare their outcomes.

Hypothesis: Changes in LPC concentration in patients treated with TAVI or SAVR for aortic stenosis are associated with intervention outcomes.

Aims:

1. To investigate the association of serum lysophosphatidylcholine concentration and intervention type (TAVR or SAVR) three months after treatment.
2. To investigate the impact of changes in serum lysophosphatidylcholine concentration on echocardiographic variables in the cohort.
3. To investigate the impact of serum lysophosphatidylcholine concentration and biochemical variables in the cohort.

Materials/Participants and Methods: The study will include 50 consecutive patients with aortic stenosis undergoing treatment with no strict contraindications for any type of treatment. The indication for the type of treatment will be based on heart team consensus. As a control cohort, 15 patients without severe comorbidities will be included (HC). The diagnostic work-up of aortic stenosis will be performed according to the current guidelines of the European Society of Cardiology (ESC). Blood analyses and transthoracic echocardiography will be assessed in all patients prior and 12 weeks after treatment by a cardiologist who is accredited by ESC.

Research plan: This prospective, non-randomized observational cohort study will be conducted at the University Hospital Dubrava, Zagreb. Patients who meet the inclusion criteria and sign an informed consent form will be enrolled in the study. In the first year, data will be collected. The following year is planned for statistical processing, writing and publishing a scientific paper and writing of the doctoral thesis.

Significance/Expected scientific contribution: The correlation of serum LPC concentration with clinical outcome variables would contribute to a better understanding of metabolic processes in the pathophysiology of aortic stenosis, identifying possible biomarkers of the disease, monitoring the effects of therapy and intervention, finding potential targets for therapy, improving diagnostics and prognostic tools. This research can provide deeper insight into the pathophysiology of the disease, enabling a more personalized approach to treatment and improving healthcare.

MeSH/Keywords: Aortic stenosis, Lysophosphatidylcholine, Biomarkers, Transcatheter aortic valve replacement (TAVR), SAVR

Dissertation Proposal Title: Impact of SARS-CoV-2 virus infection on acute and long-term disruption of glycemic control

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

Co-mentor: Assoc. Prof. Marija Santini, M.D.,Ph.D., University Hospital for Infectious Diseases, Zagreb, Croatia

Introduction: The COVID-19 pandemic has underscored a notable link between SARS-CoV-2 infection and increased susceptibility to severe illness and elevated mortality rates in individuals with diabetes. This association is likely influenced by intensified immune responses and hyperglycemia in diabetic patients, predisposing them to a pro-inflammatory and procoagulant condition and impairing immune functions via various biological mechanisms. Recent evidence points to a bidirectional relationship between COVID-19 and diabetes, where the virus may not only precipitate the emergence of new-onset diabetes but also exacerbate existing metabolic dysfunctions. The interaction of the virus with angiotensin-converting enzyme 2 (ACE2) receptors, which are prevalent in critical metabolic organs, could interfere with glucometabolic pathways, inducing hyperglycemia and potentially triggering novel pathophysiological processes. There has been an increase in observed cases of hyperglycemia in adults with no prior history of diabetes linked to COVID-19, associated with considerable morbidity and mortality. While inflammation and cytokine activation from the infection may lead to insulin resistance and consequent stress hyperglycemia, the extent to which direct viral attack on pancreatic islet cells reduces insulin production remains uncertain. Moreover, COVID-19 has been associated with the onset of diabetes, as seen in the abrupt hyperglycemia in individuals without diabetes, diabetic ketoacidosis in existing diabetic patients with the virus, and new diabetes diagnoses in COVID-19 patients. Although the underlying mechanisms between COVID-19 and diabetes are still being investigated, it is obvious that both conditions share stress-induced pathways that interact in a two-way direction. The long-term effects of COVID-19 on glucose metabolism are yet to be fully understood, highlighting the importance of continued research to ascertain potential enduring alterations or the emergence of a new diabetes form.

Hypothesis: SARS-CoV-2 infection interferes with glucose control and increases the risk of developing diabetes. COVID-19 can trigger diabetes onset through direct harm to the pancreatic

beta cells, heightened insulin resistance due to widespread inflammation, or hormonal changes that affect glucose metabolism. Individuals who are diagnosed with new-onset diabetes during COVID-19 may face a higher risk of adverse outcomes compared to those without diabetes or with pre-existing diabetes.

Aims: This research is designed to investigate the complex molecular mechanisms responsible for the diabetogenic impact of COVID-19. This study aims to explore the correlation between glucose regulation anomalies and SARS-CoV-2 infection in subjects without prior diabetes diagnoses. Additionally, the study seeks to delineate the mechanisms through which hyperglycemia develops associated with COVID-19. Patient outcomes will be monitored and documented over at least three months following COVID-19 infection. This monitoring aims to assess whether individuals with newly identified diabetes experience worse disease outcomes or increased complications and to ascertain whether the diabetes resolves or persists as a chronic condition

Materials/participants and Methods: The research will enroll a cohort comprising patients admitted to the Infectious Diseases Clinic who are confirmed to have SARS-CoV-2 infection, categorizing them into those with insulin resistance and newly diagnosed diabetes, and those without diabetes. Each group will consist of a minimum of 64 participants, totaling at least 128 individuals. Upon admission, detailed patient histories will be documented, and serum biomarkers will be examined. The research will systematically assess various parameters such as CBC, CRP, PCT, blood glucose, urea, creatinine, electrolytes, liver enzymes, inflammatory markers, and coagulation factors. Additional assays will include HbA1c, fasting plasma glucose, insulin, C-peptide, HOMA IR, HOMA B, leptin, adiponectin, and the Human Cytokine 17-plex immunoassay. Outcomes related to the disease, including recovery, complications, mortality, and the resolution or diagnosis of diabetes mellitus, will be tracked up to three months post-COVID-19 infection.

Research plan: This prospective cohort study, spanning 1-2 years, will be undertaken at the Clinic of Infectious Diseases, University Hospital Centre Osijek. Patient observations and glycemia levels will be tracked for a minimum duration of three months post-recovery from COVID-19. Additionally, the incidence of diabetes newly diagnosed subsequent to coronavirus disease-2019 infection will be systematically documented.

Significance/Expected scientific contribution: The contribution of this study lies in elucidating the causal relationship between COVID-19 and diabetes, along with the early laboratory detection of individuals at risk of developing diabetes. This knowledge will facilitate prompt and appropriate medical interventions, and offer a deeper understanding of the distinctive characteristics of diabetes

induced by COVID-19. This study emphasizes the importance of early detection and management of diabetes, especially in the context of the ongoing pandemic, to mitigate the risk of severe COVID-19 outcomes.

MeSH/Keywords: COVID-19, SARS-CoV-2, Diabetes mellitus, Hyperglycaemia, COVID-19 induced diabetes

Dissertation Proposal Title: Diagnostic accuracy of the biological indicators leucine-rich alpha 2-glycoprotein and calprotectin in patients with suspected acute appendicitis

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Mentor: Assoc. Prof. Višnja Adam Nesek, Clinical Hospital "Sveti Duh", Faculty of Dental Medicine and Health Osijek, Osijek, Croatia

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

Introduction: Acute abdominal pain represents 7-10 % of all Emergency Department visits. While appendicitis is one of the main causes of acute abdominal surgery, its precise diagnosis remains challenging. Negative appendectomy rates can be as high as 40%, while the rate of perforation indicative of delayed surgery can reach up to 30%. Therefore, more effective preoperative screening is needed for patients with suspected appendicitis. Several studies have investigated the role of new biomarkers in improving the diagnosis of appendicitis. Leucine-rich alpha 2-glycoprotein (LRG1) and calprotectin (CP) are two biomarkers that have demonstrated promising outcomes. Unlike C-reactive protein, which is synthesized in the liver, LRG1 and CP are produced at the site of inflammation. As a result, their elevated levels may be detectable in the early stages of a disease, potentially before other typical indicators of inflammation increase. This study aims to further assess the diagnostic accuracy of LRG1 and CP in patients with suspected acute appendicitis.

Hypothesis: Serum values of leucine-rich alpha-2-glycoprotein 1 and calprotectin are more reliable biological indicators of acute appendicitis in adults compared to standard laboratory indicators.

Aims: To determine the diagnostic accuracy of leucine-rich alpha-2-glycoprotein 1 and calprotectin as new biological indicators in the diagnosis of acute appendicitis.

Materials/Participants and Methods: The study will include patients with a clinical suspicion of acute appendicitis in the Emergency Department of General Hospital Našice and Clinical Hospital "Sveti Duh", along with their controls. Minimum sample size is 125 patients. The serum levels of LRG1 and CP will be measured from blood samples using commercially available ELISA kits at

the Department of Physiology and Immunology of the Faculty of Medicine in Osijek. The study is approved by the Ethics Committee of the Faculty of Medicine Osijek.

Research plan: A prospective cohort study will be conducted. **Significance/Expected scientific contribution:** The study could assess the sensitivity, specificity, and predictive value of the investigated biomarkers, allowing for the determination of optimal combinations of markers or thresholds for diagnosing and differentiating acute appendicitis from other conditions with similar symptoms in emergency medical care. These advancements may improve treatment outcomes and reduce complications from delayed diagnoses, contributing significantly to the medical field.

MeSH/Keywords: Abdominal pain; Appendicitis; Biological Indicators; Calprotectin; LRTG1 protein

Dissertation Proposal Title: The role of socioeconomic factors and LDL-levels on suicidal ideation of patients with multiple sclerosis

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Co-Mentor: Prof. Dunja Degmecic, M.D., Ph.D., University Hospital Osijek; Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Introduction: The incidence of depression and suicide is greater in patients with multiple sclerosis compared to healthy populations and is proportional to the extension of neurological deficits and reduction of the quality of life. Serum LDL- levels are associated with higher depression incidence.

Hypothesis: Socioeconomic factors and lower LDL are associated with the depression and suicidal ideation of patients with multiple sclerosis

Aims:

- to evaluate the role of socioeconomic factors and life stress events on depression and suicidal ideation of patients with multiple sclerosis
- to investigate the role of serum LDL-levels on depression and suicidal ideation of patients with multiple sclerosis

Materials/ Participants and Methods: This cross-sectional study will include patients with multiple sclerosis of the State Hospital Villach over a period of six months. The expected sample size is 130 subjects. The subject’s disability level will be determined using the Expanded Disability Status Scale (EDSS) based on neurological examination. German versions of Patient Health Questionnaire-9 (PHQ-9), World Health Organisation Quality of Life and Columbia-Suicide-Severity-Rating-Scale (C-SSRS) will be used to evaluate quality of life, depression and suicidal intention. Socioeconomic status will be obtained separately through unvalidated questionnaire.

After filling out of the above mentioned questionnaires, blood will be taken from every subject to determine LDL-serum levels.

Research plan: Participants in this study will be voluntary and recruited from the State Hospital Villach in Villach, Austria

Significance/ Expected scientific contribution: This study will provide insight into possible connection of serum LDL levels on depression and suicidal ideation of patients with multiple sclerosis, thus strengthening the hypothesis of an underlying inflammation as a common cause of both depression and hyperlipidemia.

MeSH/Keywords: multiple sclerosis, LDL, quality of life, suicide, depression

Dissertation Proposal Title: Influence of genotype variants HER2/neu gene on treatment outcomes in early breast cancer with monoclonal antibody trastuzumab

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Co-mentor: Assist. Prof. Kristijan Dinjar M.D., Ph.D., Osijek University Hospital Centre, Department of Maxillofacial and Oral Surgery, Osijek, Croatia

Introduction: According to the latest available statistical data from the Cancer Registry of the Republic of Croatia, breast cancer is the most common malignancy among women, accounting for 25% of cases. Statistical data for Europe regarding the incidence of breast cancer in women in 2021 are consistent with data for Croatia. The disease is most commonly detected as either localized to the breast or spread to the ipsilateral locoregional lymph nodes in approximately 80% of patients. Breast cancer represents a heterogeneous disease based on its molecular profile. Depending on the immunohistochemical staining of receptors (estrogen receptors - ER, progesterone receptors - PR, and human epidermal growth factor receptor 2 without a ligand - HER2), it is classified into several molecular subtypes. Specific oncological treatments are defined based on the biological characteristics of the tumor. HER2 overexpression is present in approximately 20% of breast cancers and is associated with clinically more aggressive disease biology. ER positivity and HER2 gene overexpression are recognized as dominant drivers of breast cancer cell proliferation, and blocking their signaling pathways achieves the most effective therapeutic response. The HER2 receptor is a member of the EGFR receptor family, and its inhibition reduces intracellular tyrosine kinase phosphorylation, decreases the activation of mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) signaling pathways, ultimately leading to reduced cell proliferation and promotion of apoptosis. A review article by Daniyal and colleagues highlighted that genetic polymorphism is a cause of breast cancer resistance to specific oncological treatments. Several studies have explored the impact of HER2/neu gene polymorphism at codon 655 [Ile655Val] on the outcome of trastuzumab treatment. Similarly, research describing the association between polymorphism of this gene and the frequency of trastuzumab-induced cardiotoxicity is available.

Hypothesis: Genotypic variants of the HER2/neu gene influence the outcomes of early HER2-positive breast cancer treatment with the monoclonal antibody trastuzumab.

Aims: This study aims to clarify if specific genotypic variants of the HER2/neu gene may be associated with variations in treatment efficacy, disease progression, and overall survival rates. Understanding the genetic determinants of response to trastuzumab therapy could potentially lead to the identification of predictive biomarkers for treatment outcomes and facilitate personalized therapeutic strategies in the management of HER2-positive breast cancer. Additionally, we would investigate whether the occurrence of cardiotoxicity, recognized as one of the most significant side effects of specific oncological treatment, affects specific treatment outcomes.

Materials/Participants and Methods: The study population comprises patients who underwent adjuvant trastuzumab treatment for early-stage HER2-positive breast cancer between 2007 and 2016 at the Department of Oncology and Nuclear Medicine, University Hospital Center "Sestre milosrdnice," Zagreb. Retrospective data collection from medical records obtained during treatment and subsequent follow-up will be conducted. Descriptive statistics will be performed initially, followed by statistical analysis using SPSS version 20.0 software.

Research plan: The participants will be categorized into two groups: Group A, where trastuzumab treatment did not result in cardiotoxic side effects, and Group B, where trastuzumab treatment-induced cardiotoxicity was one of the most significant side effects. Research Method: Retrospective data collection from available medical records obtained during specific oncological treatment and subsequent follow-up.

Significance/Expected scientific contribution: The overexpression of the HER-2/neu gene and receptor glycoprotein is a significant clinical feature of aggressiveness in this breast cancer subtype, making it an ideal therapeutic target. The planned study aims to investigate whether genotypic variants of the HER2/neu gene influence the outcomes of early breast cancer treatment with the monoclonal antibody trastuzumab. If a correlation between the genotypic variants of the gene and treatment outcomes with targeted anti-HER2 therapy is established, such findings would harbor clinical significance in the era of personalized oncological treatment and could potentially be utilized as predictive biomarkers in oncological treatment planning.

MeSH/Keywords: HER2/neu gene polymorphism, trastuzumab, early breast cancer, treatment outcomes

Dissertation Proposal Title: The connection between personality traits, anxiety and styles of coping with stress in nurses and nursing students and their influence on professional self-concept and conflict resolution methods.

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

Introduction: Personality traits are defined as tendencies of individuals to act in certain ways. It is known that individuals with different personality traits have competence in different areas. A higher level of one's "professional self" can influence more effective communication with patients. There are different approaches to conflict management: accommodation, compromise, avoidance, competition and collaboration. Personality traits and professional factors can influence on the development of anxiety. Coping strategies have been extensively studied and they include adaptive cognitive, adaptive behavioral, emotion-focused, and occupation-focused coping.

Hypothesis: Neuroticism, extraversion and agreeableness as personality traits, anxiety and stress coping styles in nurses and nursing students will influence professional self-concept and conflict resolution methods.

Aims:

- Examine the connection between professional self-evaluation and ways of resolving conflicts with personality traits, ways of dealing with stress and anxiety in nursing students and nurses.

- Examine the differences in ways of resolving conflicts and professional self-evaluation according to demographic variables and variables related to studying and working status.

- Examine which predictors from the researched variables are significant in the prediction of professional self-evaluation.

Materials/Participants and Methods: Research will include nurses and technicians from the University Hospital Centre Osijek and students of Nursing from Faculty of Dental Medicine and

Health Osijek. Participants will be assessed through questionnaires: The BFI-44, NSCQ, PCS, Brief-COPE and BAI along with demographic data. The expected number of participants is 500.

Research plan: Collecting data. Conducting research. Analysis of data and results. Publishing the results.

Significance/Expected scientific contribution: The results will raise awareness of the need for specific conflict management education into the curriculum of nursing students. At the hospital level it will contribute to the additional education of nurses/technicians.

MeSH/Keywords: personality traits; conflict; anxiety; coping strategies; self concept.

Abstract Title: The Association between Quality of Life and Body Mass Index with Patient Anxiety on Hemodialysis

Part of the Disertation Proposal: Association of quality of life and risk of malnutrition with incidence of depression, anxiety and stress in patients treated with hemodialysis and peritoneal dialysis.

PhD candidate: Tihomir Jovanović, MSN, General Hospital of Pakrac, Pakrac, Croatia

Mentor: Prof. Štefica Mikšić, Ph.D., MSN, RN, Faculty of Dental Medicine and Health Osijek, University of Osijek, Osijek, Croatia

Introduction: Renal replacement therapy through dialysis affects the quality of life of patients, recognized as a reliable predictor of long-term survival in dialysis patients. Apart from quality of life, the significance of malnutrition has been acknowledged in increasing morbidity and mortality rates, as well as in hindering recovery from various infections, injuries, and surgical procedures. Dialysis patients also face issues such as stress and anxiety, with mental health problems potentially progressing to depression and more severe conditions.

Aims: To examine the association between quality of life and body mass index with patient anxiety on hemodialysis.

Materials/Participants and Methods: A pilot study conducted in March 2024 involved 25 dialysis patients from the General Hospital Pakrac and the Croatian Veterans Hospital, comprising 17 (68%) males and 8 (32%) females. The average age of patients was M = 67.48 (SD = 7.83; range 54 to 79 years), with an average duration of dialysis treatment of M = 6.04 years (SD = 5.12; range 1 to 16 years). Sociodemographic questionnaire, SF-36 health survey, and DASS questionnaire were utilized. Descriptive statistical methods were employed to describe the distribution of frequency of variables. Mean values of numerical variables were expressed as arithmetic mean, range, and standard deviation. Spearman correlations were used to examine associations.

Results: Anxiety was moderately negatively correlated with health as an aspect of quality of life (rho = -0.525; P = 0.007), while it was highly negatively correlated with physical health limitations (rho = -0.556; P = 0.004), emotional well-being (rho = -0.506; P = 0.010), and social functioning

(rho = -0.666; P < 0.001), and highly positively correlated with fatigue (rho = 0.700; P < 0.001), indicating that higher anxiety was associated with greater fatigue and lower physical health limitations, emotional well-being, social functioning, and health as an aspect of quality of life. The body mass index of dialysis patients was highly negatively correlated with anxiety (rho = -0.804; P = 0.001), indicating that higher anxiety was associated with lower body mass index, and vice versa.

Conclusion: Understanding the relationship between quality of life, body mass index, and anxiety states is crucial in recognizing key factors affecting the well-being of these patients. Information on improving quality of life can promote the development of interventions aimed at enhancing aspects of life such as nutrition, physical activity, emotional support, and social connectedness.

MeSH/Keywords: Anxiety; Health-Related Quality Of Life; Malnutrition; Renal Dialysis

Dissertation Proposal Title: The association of Sodium-Glucose Cotransporter 2 Inhibitors therapy with elastographic and molecular markers of liver injury in patients with Type 2 Diabetes Mellitus

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Introduction: The most common liver disease worldwide, Metabolic-associated fatty liver disease (MAFLD), with only one drug approved so far (Resmetirom), has practically no approved pharmacotherapy in routine practice. It is characterized by deposition of at least 5% lipids within hepatocytes caused by the interplay between genetic, immunological, environmental and metabolic factors. Histologically, MAFLD can be divided into two distinct conditions: Metabolic-associated fatty liver (MAFL) and Metabolic-associated steatohepatitis (MASH) which may lead to severe conditions such as cirrhosis, liver failure and hepatocellular carcinoma. Progression of MAFLD is usually slow and asymptomatic, and therefore is often diagnosed at a very late stage. The most important factor for the prognosis of mortality in MAFLD patients is the stage of hepatic fibrosis. MAFLD occurs in almost all patients with a metabolic disorder such as metabolic syndrome and type 2 diabetes mellitus, and conversely, patients with MAFLD will typically develop some kind of metabolic disorder. Hence, MAFLD is a new accepted terminology for the former disease name Non-alcoholic fatty liver disease (NAFLD), which does not accurately reflect the disease pathology and treatment strategy. Sodium-glucose cotransporter 2 (SGLT2) inhibitors are antidiabetic drugs that already got approval beyond type 2 diabetes mellitus, namely chronic kidney disease and heart failure. SGLT2 inhibitors show significant beneficial effects on many processes such as autophagy, apoptosis, endoplasmic reticulum stress, oxidative stress and low-grade inflammation in patients with MAFLD. The effects of SGLT2 inhibitors on the development and progression of MAFLD are not yet fully understood and need to be further investigated in clinical settings.

Hypothesis: Therapy with SGLT2 inhibitors leads to changes in the expression of lipogenesis biomarkers and in liver tissue stiffness in patients with type 2 diabetes mellitus.

Aims: To measure liver tissue stiffness with elastography, biochemical analysis to determine glucose, HbA1c, total blood count, standard markers of liver damage, lipogenesis biomarkers SREBP-1 (Sterol Regulatory Element-Binding Protein-1), PPAR (Peroxisome Proliferator-

Activated Receptor) alpha, PPAR gamma, MTTP (Microsomal Triglyceride Transfer Protein) involved in the process of mitochondrial dysfunction and lipogenesis before initiation of therapy with SGLT2 inhibitors and 6 months after initiation of therapy. To study the association between serum indicators of lipogenesis and mitochondrial dysfunction and liver tissue stiffness of patients before initiation of SGLT2 inhibitors therapy and 6 months after initiation of therapy.

Materials/Participants and Methods: This will be a prospective cohort observational study with at least 57 participants (i.e 63 with 10% calculated for attrition) who were prescribed SGLT2 inhibitors for the first time in general medical practices in Osijek. Inclusion criteria: 1.Signed informed consent, information for the participants and questionnaire, 2. Patients that are 18 years of age or older, 3.Diagnosis of type 2 diabetes mellitus, 4.Patients being treated with an SGLT2 inhibitor for the first time, 5.Patients who have been on stable antihyperglycemic therapy for 90 days (3 months) before enrollment in the study. Exclusion criteria: 1. Patients taking drugs that are extremely hepatotoxic, i.e., require additional monitoring of liver function during therapy (e.g., chemotherapeutic agents, biological therapy), 2. Patients taking drugs which cause drug-induced fatty liver disease (DIFLD): amiodarone, tamoxifen, methotrexate, 5-Fluorouracil, irinotecan, l-asparaginase, valproate, tetracycline, nucleoside reverse transcriptase inhibitors (NRTIs such as lamivudine, tenofovir, zidovudine etc.), 3. Patients with liver cancer, hemochromatosis, primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), hepatitis C virus (HCV), hepatitis B virus (HBV), liver cirrhosis or autoimmune hepatitis, 4. Patients who are alcohol addicted, i.e., consume more than two alcoholic beverages per day (for women) or more than three alcoholic beverages per day (for men), 5. Mentally ill patients who are incapable of making their own independent decisions and have a legal custodian, 6. Pregnant women and nursing mothers, 7. Patients who are on insulin therapy. All patients will complete a questionnaire on gender, age, lifestyle and nutrition habits to investigate the association with improvement of liver status with SGLT2 inhibitor therapy. Outcomes will be assessed by elastography before initiation of therapy and 6 months after initiation of SGLT2 inhibitor therapy. Enzyme-linked immunosorbent assay (ELISA) will be used to assess serum levels of lipogenesis biomarkers before initiation of SGLT2 inhibitor therapy and 6 months after.

Research plan: Following recruitment, elastography will be performed to determine liver tissue stiffness and blood samples will be taken to determine levels of glucose, HbA1c, total blood count, ALT, AST, ALP, GGT, albumin, proteins, coagulation levels, bilirubin total and conjugated, lipid profile, SREBP-1, PPAR alpha, PPAR gamma, MTTP before the initiation of SGLT2 inhibitor therapy and 6 months after initiation of therapy at the Mursa Medical Centar L.L.C. or Health Center Osijek-Baranja County in Osijek.

Significance/Expected scientific contribution: To the best of our knowledge, this study will be the first to investigate the association between the use of SGLT2 inhibitors and elastographic and molecular markers of liver injury in patients with type 2 diabetes mellitus. This study will evaluate whether SGLT2 inhibitor therapy leads to elastographic changes in the liver of patients with type 2 diabetes as well as changes in the expression of markers of lipogenesis and mitochondrial

dysfunction and whether they have a correlation with each other that may ultimately contribute to the design of new studies that will evaluate the potential use of these markers in the diagnosis and monitoring of MAFLD, as well as the possible expansion of the indication for the use of SGLT2 inhibitors.

MeSH/Keywords: biomarkers, elastography, sodium and glucose cotransporter 2 inhibitors, liver injury, metabolic-associated fatty liver disease

Dissertation Proposal Title: Effect of treatment of non-metastatic colorectal cancer on differences in glycosylation of Immunoglobulin G

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Mentor: Prof. Robert Smolić, Ph.D., Medical Centre Mursa, Faculty of Dental Medicine and Health Osijek, University of Osijek, Osijek, Croatia

Co-mentor: Asst. Prof. Ilijan Tomaš, Ph.D., Clinic of Oncology, University Hospital Centre Osijek; Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Introduction: Glycans, complex oligosaccharides linked to membrane and secretory proteins, undergo glycosylation, a process involving multiple enzymes and metabolites, occurring in the endoplasmic reticulum and Golgi complex. Although protein glycosylation, especially N-glycosylation, is well-studied, other types like O-glycosylation and C-glycosylation also exist. Immunoglobulin G (IgG), a vital component of immune defense, undergoes glycosylation affecting its function; decreased galactosylation is associated with various cancers, including colorectal cancer. IgG glycans hold promise as biomarkers for cancer progression. Colorectal cancer incidence is rising in Croatia, emphasizing the need for improved prevention and treatment strategies. However, research on the impact of neoadjuvant chemoradiotherapy and surgical treatment on IgG glycosylation remains limited.

Hypothesis: The application of neoadjuvant chemoradiotherapy and surgical treatment of colorectal carcinoma leads to changes in the composition of glycans in patients with colorectal carcinoma.

Aims:

1. To determine the glycan profile of IgG molecules in patients with non-metastatic colorectal carcinoma before neoadjuvant chemoradiotherapy and surgical treatment.
2. Determine the glycan profile of IgG molecules in patients with non-metastatic colorectal carcinoma during their first visit, immediately after neoadjuvant chemotherapy, immediately after surgical treatment.

3. Investigate whether there is a difference in the glycan profile of IgG molecules in patients with non-metastatic colorectal carcinoma before the initiation of neoadjuvant therapy and surgical treatment and after the implementation of neoadjuvant chemoradiotherapy and surgical treatment.

Participants and Methods: This study will enroll newly diagnosed colorectal cancer patients who will be candidates for neoadjuvant chemoradiotherapy and after for surgical management and will be treated at the Oncology Clinic, Clinical Hospital Center Osijek. Exclusion criteria are reluctance to participate, acute infections, additional malignancies, autoimmune disorders, injuries and smoking. A minimum of 36 participants is needed to identify meaningful variations in numerical variables over three assessments, with a significance level of 0.05 and a power of 0.95 (G*Power ver 3.1.2).

Research plan: Upon obtaining Ethical Approval, participants will provide informed consent by signing a consent form. Data collection prior to commencing neoadjuvant chemoradiotherapy and will encompass medical history, familial medical background, details of chronic therapies, ECOG status, TNM disease stage evaluation, and the collection of a 4ml blood sample at the Clinic of Oncology, Clinical Hospital Center Osijek, from 8 to 10 am, after an overnight fast. Blood samples will be preserved at -20°C until IgG glycosylation analysis at Genos's glycobiology laboratory. Samples will be procured three times: pre-therapy, after neoadjuvant chemoradiotherapy and after surgical therapy.

Significance/Expected scientific contribution: The study aims to determine if changes in IgG glycan profiles correlate with neoadjuvant and surgical treatments in colorectal carcinoma patients. It will prospectively monitor glycan profile changes in non-metastatic colorectal carcinoma patients undergoing these treatments. The findings may introduce IgG glycosylation determination as new biomarkers for monitoring treatment effectiveness.

MeSH/Keywords: chemotherapy, colorectal neoplasms, immunoglobulin G, glycosylation, neodjuvant therapy, radiotherapy, surgical oncology

The influence of the coronavirus disease severity on the sex life of sexually active adults in Croatia

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

Co-mentor: Assist. Prof. Tomislav Franić, M.D., Ph.D., Department of Psychiatry, University Hospital of Split, Split, Croatia, School of Medicine, University of Split, Split, Croatia

Introduction: Sexual dysfunctions are a growing public health problem whose prevalence varies between 25.8% and up to 91.0%, with a higher frequency in women compared to men. There are more and more studies that talk about the impact of the coronavirus disease on sexual life, i.e. the incidence of sexual dysfunction.

Hypothesis:

1) The coronavirus disease affected the occurrence of sexual disorders in men and women in the Republic of Croatia.

2) The severity of the clinical picture of the coronavirus disease is proportional to the quantity and quality of sexual disorders.

Aims:

1. To examine whether the coronavirus disease has affected the sexual life of sexually active adults in the Republic of Croatia.

2. To examine whether the coronavirus disease had an equal effect on the occurrence of sexual disorders in men and women.

3. To investigate the impact of the recovered coronavirus disease on the sexual life by age group.

4. To examine whether the more severe clinical picture of the coronavirus disease had a significant impact on sexual life.

5. To examine the incidence of sexual disorders due to the coronavirus disease.

Participants and Methods: We will include 800 adults (ages 30 to 65) sexually active men and women who have recovered from the coronavirus disease, at least 6 months ago, didn't have a verified sexual disorder before the coronavirus disease, don't have a serious somatic illness, and are willing to participate in the research. The study will be conducted by filling in a questionnaire through which basic sociodemographic data on the subject and basic data on overcoming the infection of COVID-19, quality of life, the impact of the pandemic of COVID-19 and sexual dysfunctions will be collected.

Research plan: The first part of the research plan consists of collecting data from respondents, which we intend to carry out by the end of this year. The following year is planned for statistical processing, writing and publishing a scientific paper, and writing of the doctoral thesis.

Expected scientific contribution: The proposed research will actualize the problem of sexual dysfunctions after the coronavirus disease and pandemic. It will be examined to what extent the traumatic experience of the pandemic can be connected with the prevalence of sexual dysfunctions. The quality of life of people with sexual dysfunction will also be examined. We believe that it is important to destigmatize and stimulate people with sexual dysfunctions to seek help. The impact of the pandemic and the coronavirus disease itself has been poorly researched, and that is why we believe that the contribution of our research is important.

Keywords: COVID-19, coronavirus, sex life, sexual dysfunction, quality of life

Abstract Title: Prescribing new cardioprotective antidiabetics in Croatia from a family medicine perspective

Part of the Dissertation Proposal: A practice of prescribing new antidiabetic medicines with protective cardiovascular effects in family medicine and possibilities for improvement

PhD candidate: Tomislav Kurevija, M.D., Department of Family Medicine, Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia, Health Center of Osijek-Baranja County

Mentor: Assoc. Prof. Silvija Canecki-Varžić, M.D., Ph.D., Head of Clinic for Internal Medicine and Department of Endocrinology in University Hospital Center Osijek, Department of Pathophysiology, Faculty of Medicine, University of Osijek, Osijek, Croatia

Co-mentor: Prof. Ljiljana Trtica Majnarić, M.D., Ph.D., Department of Family Medicine, Faculty of Medicine, University of Osijek, Osijek, Croatia

Introduction: Managing patients with type 2 diabetes (T2D) is often challenging, and achieving target therapeutic goals can be difficult. With the emergence of new groups of antidiabetic drugs, glucagon-like peptide 1 receptor agonists (GLP-1ra) and sodium-glucose cotransporter-2 inhibitors (SGLT2-i), for which the beneficial cardiovascular (CV) effects were shown in CV outcome trials (CVOTs), expectations have been raised for the improvement management of T2D patients. However, despite their proven CV efficacy, their prescription rates remain low.

Aims: To determine current prescription rates and confidence in prescribing new antidiabetic drugs among family doctors (FDs) in Croatia, and modalities for optimizing their prescribing.

Participants and Methods: As part of doctoral research, a qualitative study was conducted on a sample of 86 examinees out of the planned 180. Two self-developed and thematically related questionnaires were sent to FDs across Croatia via email. The questions were in the form of a Likert scale. To obtain accurate data about the number of T2D patients and prescription rates, respondents checked the e-health records.

Results: The respondents were mostly female, with a ratio of 67.4% to 32.6%. A majority of them lived in urban areas (62%) rather than rural areas (38%). The respondents included specialists in family medicine (45.3%), family medicine residents (29.1%), non-specialists (24%), and those with

other specializations (2%). Together, they treated over 12.000 patients with T2D. The prescription rates for SGLT2i and GLP1-ra were 16.7% and 13.1%, respectively. A significant number of respondents (61.6%) indicated that they prescribed SGLT2i independently of the endocrinologists' recommendations, and 74.4% of them expressed high confidence in doing so. If given the option, 82.6% of respondents would like to prescribe GLP-1ra on their own initiative, but only 57% of them felt highly confident enough to do so. The top suggestions for increasing the prescription rates of GLP1-ra and SGLT2i included improving e-health records by adding panels and algorithms to support CV risk assessment, adopting structured data collection methods for all patients, and providing continuing education for FDs about CVOTs.

Conclusion: Despite the proven CV efficacy of novel antidiabetic drugs, their prescription rates are low. Improvements could include modifying e-health records to better manage T2D patients and providing continuous education to FDs.

Keywords: SGLT2-i, GLP1-ra, type 2 diabetes, cardioprotective agents, drug prescription

Abstract title: Optimizing choice of skin surrogates for surface guided stereotactic body radiotherapy of lower lung lesions using four-dimensional computed tomography

Part of the dissertation proposal: There are differences between respiratory skin magnitudes and skin-to-lesion correlations between skin segments on different thoracic regions that can be detected using 4DCT

PhD candidate: Vanda Leipold, Specialty hospital Radiochirurgia Zagreb, Zagreb, Croatia

Mentor: Assoc. Prof. Hrvoje Brkić, Department of Biophysics and Medical Physics, Faculty of Medicine, University of Osijek, Osijek, Croatia

Introduction: Surface guided radiotherapy allows for precise patient setup and motion management while reducing the need for kV imaging.
Optical cameras monitor a region of interest (ROI) on the patient's skin and use it as a surrogate for lung lesion motion. The position of the lesion can be inferred since a correlation exists between skin surrogate and lesion motion.
However, not all ROIs move with the same magnitudes, nor are they equally correlated to lesion respiratory motion - both of which is important when choosing an ROI surrogate.

Aims: In this study, respiratory magnitudes and correlations of nine different ROIs to a target in the lower lung lobe are analyzed.

Materials/Participants and Methods: 58 four-dimensional computed tomography (4DCT) images of different patients acquired for treatment planning in our institution were analyzed.
Respiratory ROI and a target structure (TS) motion have been conducted using an in-house developed computer program.
Respiratory excursions of ROIs and TS have been measured, and Pearson’s correlations between ROI and TS respiratory motion have been computed.

Results: Differences in respiratory magnitudes and ROI-to-TS correlations have been detected between ROIs.
ROIs without rib cage support had larger respiratory magnitudes and higher correlations to TS motion.
TS had larger respiratory magnitudes than ROIs in all areas.

Conclusion: In free breathing technique, ROIs in umbilical, lumbar and epigastric regions may be a better choice for tracking lower lung lesion motion.

These conclusions may not be applicable to patients treated in breath hold technique.

Possible effects of sex, age and adipose tissue should be analyzed.

Significance/Expected scientific contribution: This research can help establish differences in respiratory skin magnitudes and ROI-to-TS correlations between skin regions, further improving application of surface guidance for lung treatment.

10. MeSH/Keywords: Patient Positioning; Radiotherapy, Image-Guided; Tomography, X-Ray Computed; Four-Dimensional Computed Tomography; Body Surface Area

Abstract Title: Cognitive behavioral interventions in managing anxiety and depression in adolescents with type 1 diabetes mellitus

PhD candidate: Maja Lovrić, clinical psychologist

Mentor: Assoc. Prof. Silvija Pušeljić, M.D., Ph.D.

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

Introduction: Type 1 diabetes mellitus (T1DM), along with asthma, is the most common chronic disease affecting children. According to data from the Pediatric Clinic of UHC Osijek, in Osijek-Baranja County, 350 children and adolescents are currently diagnosed with T1DM. Glycated hemoglobin (HbA1c) values are used to assess long-term glycemic control. Mental disorders affect approximately 33%-42% of adolescents with T1DM, which is 2-3 times higher than in a healthy peer population. The research results indicate the use of cognitive-behavioral interventions for better disease management and mental health preservation.

Hypothesis: Anxiety and depression are associated with the regulation of type 1 diabetes mellitus in adolescents.
The cognitive behavioral interventions have been associated with reducing anxiety and depression and improving disease management in adolescents with T1DM.

Aims: The study aimed to assess the effect of cognitive behavioral interventions on disease regulation (HbA1c), anxiety and depression in adolescents with T1DM.

Participants and Methods: The study includes 200 adolescents with T1DM who will be psychologically evaluated at the Pediatric Clinic of the University Hospital Centre Osijek. The intervention will involve a structured program focused on various cognitive-behavioral strategies that have been proven to be effective in the treatment of anxiety and depression. These interventions will take place at the Pediatric Clinic during six weekly group meetings lasting 90 minutes each. Data will be collected before and immediately after the intervention period. The psychological assessment will include a structured clinical interview and the use of psychological measuring instruments (WISC-IV, SKAD, SDD PedsQL). Values of glycosylated hemoglobin (HbA1c) will also be collected.

The exclusion criteria comprise adolescents with an IQ < 70, adolescents whose disease has lasted for less than one year and adolescents with other somatic disorders.

The written consent of the respondents and one of their parents/guardians will be required.

Research plan: This prospective observational study will be conducted at the Pediatric Clinic of University Hospital Centre Osijek. The research will take two years.

Expected scientific contribution: Reducing anxiety and depression with cognitive behavioral interventions leads to better disease regulation, improved quality of life for adolescents and their families, as well as lower treatment costs.

MeSH/Key words: Anxiety, depression, cognitive behavioral interventions, adolescence, type 1 diabetes mellitus

Abstract Title: Preliminary research results on predictors of patient and nurse satisfaction as indicators of the process in healthcare quality management system

Part of the Dissertation Proposal: Predictors of patient and nurse satisfaction as indicators of the process in healthcare quality management system

PhD candidate: Marin Mamić, mag. med. techn., General County Hospital of Požega, Croatia

Mentor: Assist. Prof. Štefica Mikšić, MsN, PhD., The Faculty of Dental Medicine and Health, University of Osijek, Croatia

Co-mentor: Tihana Mendeš, M.D., Ph.D., Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Introduction: Patient satisfaction with the quality of healthcare reflects the relationship between perceived care received and the level of care patients expected to receive upon arrival at the healthcare facility, representing a balance between expectations and perceptions. Therefore, patient satisfaction can be considered as the subjective assessment of a patient's cognitive and emotional response resulting from the interaction between their expectations and perceptions of actual care. As patient satisfaction is a crucial indicator of overall healthcare quality, it is important to identify factors that may influence it.

Aims:

1. To examine the contribution of variables related to demographic characteristics, job, and personality traits of nurses and technicians on the level of patient satisfaction as an indicator of the process in the healthcare quality management system.

2. To investigate the contribution of variables related to demographic characteristics, health status, and personality traits of patients on the level of patient satisfaction as an indicator of the process in the healthcare quality management system.

Materials/Participants and Methods: The preliminary study was conducted at the General Hospital Požega during the year 2023. The study involved 240 patients and 65 nurses and technicians. The research utilized the following questionnaires: Demographic questionnaires for

patients and medical staff, Patient Satisfaction with Nursing Care Quality Questionnaire (PSNCQQ), Organizational Justice Questionnaire, and the NEO Five-Factor Inventory (NEO FFI).

Results: Linear regression analysis revealed that demographic and personal variables significantly explain 29.2% of the variance in patient satisfaction with nursing care quality (AR2=0.292; p<0.001). Significant predictors of patient satisfaction with nursing care quality from the patient's perspective were place of residence (p=0.044), health status (p=0.003), agreeableness personality trait (p=0.003), and conscientiousness (p=0.010). From the perspective of nurses and technicians, linear regression analysis showed that demographic, personal, and job-related variables significantly explain 36.2% of the variance in patient satisfaction with nursing care quality (AR2=0.362; p<0.001). Significant predictors from the perspective of nurses and technicians were procedural justice (p=0.003), interactional justice (p=0.005), and the personality trait of extraversion (p=0.043).

Conclusion: Preliminary findings suggest that patient satisfaction with healthcare quality may be influenced by both patient personal variables and those of nurses/technicians.

MeSH/Keywords: Job satisfaction, Justice, Nursing care, Organization, Personality traits, Quality.

Dissertation Proposal Title: Influence of *SLCO1B1* gene polymorphism and vitamin D concentration on the required dose of statins to achieve the target value of LDL-cholesterol and on side effects of statins in postmenopausal women

PhD candidate: Romana Marušić M.D., National Memorial Hospital „Dr. Juraj Njavro" Vukovar, Vukovar, Croatia

Mentor: Assist. Prof. Saška Marczi, Clinical Institute for Transfusion Medicine, Clinical Hospital Center Osijek, Osijek, Croatia

Introduction: The organic anion transport polypeptide 1B1 (OATP1B1), encoded by the *SLCO1B1* gene, significantly transfers and eliminates certain statins. Reduced activity of the OTP1B1 transporter results in reduced efficacy of statins and a greater likelihood of side effects. The most decisive influence on the unwanted side effects of statin use is the polymorphism rs4149056 (c.521T > C, p.V174A) in the *SLCO1B1* gene; three genotypes were identified and classified about their influence on statin metabolism in the liver. The implementation of pharmacogenomics knowledge is lagging in its application in clinical practice because there still need to be clear guidelines and recommendations on adapting specific knowledge of genetic tests to treatment.

Hypothesis: Postmenopausal women with *SLCO1B1* genotypes T/C and C/C (rs4149056) who are on atorvastatin therapy have a higher incidence of side effects and need higher drug doses to achieve LDL-cholesterol target values than postmenopausal women of the mentioned *SLCO1B1* genotypes on rosuvastatin therapy.
The statin therapy administered, atorvastatin or rosuvastatin, does not affect the incidence of side effects in postmenopausal women with low vitamin D concentrations.

Aims:
Primary objective:
1. To examine the influence of *SLCO1B*1 gene polymorphism and vitamin D concentration on the dose of statin needed to achieve the target value of LDL-cholesterol and the side effects of statin in postmenopausal women.

Participants and Methods: The research will include females between the ages of 45 and 70 who should use a statin divided into two groups: atorvastatin and rosuvastatin as statin therapy.

Research plan: At the beginning of the research, the *SLCO1B1* gene polymorphism, vitamin D concentration, lipid profile, creatine kinase (CK), alanine aminotransferase (ALT), aspartate - aminotransferase (AST), gamma-glutamyltransferase (yGT) will determine. In all subjects, the treatment will begin with the dose of statin therapy that recommends considering the initial values of LDL cholesterol at the time of inclusion in the study. Further measurements will be performed every four weeks, up to a total of 4 months. The side effects of statin therapy will be monitored based on clinical manifestations and by monitoring the concentration of AST, ALT, yGT, and CK in the serum.

Expected scientific contribution: This study can help set clear guidelines and recommendations based on genetic tests to help choose the type of statin and prevent side effects when reaching target values.

Keywords: adverse effects, hydroxymethylglutaryl-CoA reductase inhibitors, low density lipoprotein, menopause, pharmacogenetics, *SLCO1B1*, vitamin D

Dissertation Proposal Title: Alterations of plasma proteins' N-glycosylation under LDL-cholesterol optimization with atorvastatin therapy after percutaneous coronary intervention in acute coronary syndrome

PhD candidate: Domagoj Mišković, M.D., General Hospital Slavonski Brod, Croatia

Mentor: Prof**.** Irzal Hadžibegović, M.D., Ph.D., University Hospital Dubrava, Zagreb, Croatia

Introduction: Considering the complexity of glycosylation, the potential of glycans as biomarkers in the prevention, detection, and monitoring of the therapy of various diseases is known. Despite the known positive effects of low LDL-cholesterol values, less than 50% of patients after acute coronary syndrome achieve target values. The goal is to investigate possible connection between N-glycans in plasma and a weaker response to atorvastatin therapy

Hypothesis: The composition of IgG N-glycans and total plasma proteins is significantly different between patients who achieve target LDL-cholesterol values to patients who do not achieve

Aims: The aim of the research is to determine whether there is a connection between the composition of N-glycome IgG and total plasma proteins with the response to atorvastatin therapy

Materials/Participants and Methods: the test group are statin naive patients with acute coronary syndrome who underwent percutaneous coronary intervention and who have no contraindication to taking the maximum dose of atorvastatin. It is planned to include 78 patients with acute coronary syndrome. The control group of subjects would consist of 20 subjects with similar demographic and clinical characteristics who are being introduced to atorvastatin for the first time in therapy as part of primary prevention. A venous blood sample will be taken from the subjects for plasma N-glycan analysis, which will be centrifuged and stored at -20 C.

Research plan: The test group will have a lipid control on the first day, 60th, 90th and 365th day after acute coronary syndrome. N-glycans of total plasma proteins will be controlled on day 1, day 5-7 (at discharge) and day 60 after acute coronary syndrome

Significance/Expected scientific contribution: The association of IgG N-glycans and total plasma proteins with LDL-cholesterol optimization will be analyzed prospectively. N-glycan analysis could predict which patients are likely to have difficult LDL-cholesterol control and an increased risk of an adverse cardiovascular event. Such a group could be the target group for the early introduction of additional forms of treatment. N-glycan analysis could become a useful parameter in the treatment of patients with acute coronary syndrome.

MeSH/Keywords: Acute coronary syndrome, Polysaccharides (Glycans), Atorvastatin, LDL-cholesterol, Percutaneus Coronary Intervention

Abstract Title: Clinical Results of Knee Osteoarthritis Treatment with Autologous Microfragmented Adipose Tissue Containing Mesenchymal Stem Cells Compared to Hyaluronic Acid

Part of the Dissertation Proposal: Effects of Knee Osteoarthritis Treatment with Autologous Microfragmented Adipose Tissue Containing Mesenchymal Stem Cells Compared to Hyaluronic Acid

PhD candidate: Vilim Molnar, M.D., Clinic for Orthopaedic Surgery, St. Catherine Specialty Hospital, Zagreb, Croatia

Mentor: Prof. Dragan Primorac, M.D., Ph.D., St. Catherine Specialty Hospital, Zagreb, Croatia; School of Medicine, Josip Juraj Strossmayer University of Osijek; Medical School, University of Split; Department of Biochemistry & Molecular Biology, The Pennsylvania State University, State College, PA, USA; The Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven, West Haven, CT, USA; Medical School REGIOMED, Coburg, Germany; Medical School, University of Rijeka; Faculty of Dental Medicine and Health, Josip Juraj Strossmayer University of Osijek; Medical School, University of Mostar, Bosnia and Herzegovina; National Forensic Sciences University, Gujarat, India

Introduction: Osteoarthritis (OA) is the most common progressive musculoskeletal condition affecting joints, particularly the hips and knees. It is estimated that 250 million people globally suffer from OA, with the prevalence of knee OA having significantly increased over recent decades. The dominant symptoms are pain and mobility loss, with joint replacement surgery being the only option for end-stage knee OA. However, secondary prevention therapeutic measures, including injections of hyaluronic acid (HA) or autologous micro-fragmented adipose tissue (MFAT) containing mesenchymal stem cells (MSCs), can slow disease progression. Recent studies at St. Catherine Specialty Hospital have shown significant symptom relief after MFAT application, proving its efficacy even 2 years post-treatment, while HA injections, though more accessible and cost-effective, offer temporary relief typically lasting 6 to 9 months.

Aims: This study aimed to evaluate the effects of MFAT and HA on knee symptoms, pain, and function using questionnaires such as the Visual Analog Scale (VAS), Western Ontario and McMaster Universities OA Index (WOMAC), and Knee Injury and OA Outcome Score (KOOS) before therapy, and at 1- and 6-months post-application in patients with knee OA.

Materials/Participants and Methods: Conducted as part of the European research project IRI2 (KK.01.2.1.02.0173), the study included 53 patients aged 30-75 with mild to moderate knee OA. Detailed inclusion and exclusion criteria (available at isrctn.com/ISRCTN88966184) were implemented to form a cohort from the same phenotypic group corresponding to the inflammatory phenotype of knee OA. Patients were randomized into two groups and blinded to the therapy they would receive. 35 patients were treated with MFAT (7 mL, Lipogems®), 18 with HA (Hyalubrix 60®), and followed for 6 months.

Results: Statistical analysis showed significant clinical improvement in both groups with changes in questionnaire scores (KOOS, WOMAC and VAS, $p<0.05$). Particularly, the MFAT group showed a statistically significant improvement in the KOOS Symptoms score after 6 months compared to the HA, indicating a superior effect of MFAT in terms of mobility, effusion, and stiffness ($p=0.008$). Furthermore, patients treated with MFAT exhibited continued symptom improvement (observed in all questionnaires) at 6 months compared to the 1-month post-treatment point, a trend not observed with HA, which showed its peak effect at 1 month ($p<0.05$).

Conclusion: In conclusion, both MFAT and HA treatments demonstrated significant clinical improvements in knee OA patients over 6 months. However, MFAT treatment, with its potent anti-inflammatory properties, showed a superior effect in improving symptoms such as mobility, effusion, and stiffness, particularly in the KOOS Symptoms score. Additionally, MFAT patients experienced continued symptom improvement over the 6 months, unlike those treated with HA, who displayed peak effects at 1 month. These findings suggest that MFAT may offer a more durable and progressive therapeutic benefit for knee OA compared to HA.

MeSH/Keywords: adipose tissue, mesenchymal stem cells, hyaluronic acid, knee osteoarthritis, phenotype

Abstract Title: Effect of consumption of chicken meat enriched with omega-3 polyunsaturated fatty acids on microvascular function

Part of the Dissertation Proposal: „Effect of consumption of food enriched with omega-3 polyunsaturated fatty acids on vascular function and oxidative stress levels in healthy young subjects"

PhD candidate: Tihana Nađ, M.D., Clinic of Pediatrics, University Hospital Centre Osijek, Department of Pediatrics, Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Mentor: Assoc. Prof. Ivana Jukić, M.D., Ph.D., Department of Physiology and Immunology, Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Co-Mentor: Darjan Kardum, M.D., Ph.D., Department of Pediatrics, Zadar General Hospital, Department of Pediatrics, Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

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

Aim: Present study aimed to evaluate the effects of n-3 PUFAs enriched chicken meat consumption on microvascular reactivity in healthy young volunteers.

Participants and Methods: 39 healthy young participants were included in this double-blind, placebo-controlled, randomized, interventional study. All participants consumed approximately 500 g of quick-roasted chicken meat for 21 days, and were divided in two groups: Control group (N=20) – regular chicken meat and n-3 PUFAs group (N=19) – chicken meat enriched with n-3 PUFAs.
Anthropometric, hemodynamic and biochemical measurements were performed to all participants. Laser Doppler flowmetry (LDF) was used to assess the forearm skin microvascular endothelium - dependent/independent vasodilation by iontophoresis of acetylcholine (ACh) and sodium nitroprusside (SNP), respectively. All measurements were performed before and after each dietary protocol.

The study protocol and procedures met the standards of the latest revision of the Declaration of Helsinki and were approved by the Ethics Committee of the Faculty of Medicine, University of Osijek, Osijek, Croatia (Cl: 602-04/23-08/03; No: 2158-61-46-23-125). This study is part of a clinical research study investigating the effects of functionally enriched chicken eggs on cardiovascular function registered at ClinicalTrials.gov (accessed on 11 September 2023) (NCT04564690).

Results: After a 3-week dietary protocol, the microvascular response to ACh was significantly increased in the n-3 PUFAs group, while there was no difference in the Control group. In both groups, microvascular response to SNP was not changed compared to the basal values.

Conclusion: Results of the present study indicate a beneficial effect of n-3 PUFAs consumed in enriched chicken meat on endothelium-dependent vasodilation in healthy young people.

MeSH/Keywords: functional food, omega-3 polyunsaturated fatty acid, vascular function, endothelium

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Dissertation Proposal Title: Correlation of open-angle glaucoma of different degrees of damage with the occurrence of anxiety and depression

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Introduction: The second most common cause of blindness in the world is glaucoma. It affects 2% of the average population over 40 years of age and impaired quality of life of sick patients is caused by impaired vision quality of the patient. Glaucoma includes several diseases of different aetiologies and their common characteristic is that they result in a progressive and irreversible deterioration of the visual nerve and retinal nerve fibers, with corresponding outbursts in the visual field. It is necessary to identify it as early as possible in order to begin the treatment of the patient before it causes irreversible structural changes**.**

Hypothesis: Open-angle glaucoma of different degrees of damage is statistically significantly associated with the incidence of anxiety and depression.

Aims:

1) To examine the incidence characteristics of anxiety, depression and open-angle glaucoma in patients with glaucoma treated during the period from October 2023 to June 2024
2) Examine the characteristics of anxious and depressed patients with open-angle glaucoma by age, gender and type of anti-glaucoma therapy
3) Examine the characteristics of non-anxious and non-depressed patients with open-angle glaucoma age, gender and type of anti-glaucoma therapy

Materials/Participants and Methods: Participants will be patients in the glaucoma infirmary at the Clinic for Eye Diseases (CED) of Clinical Hospital Centre Osijek (CHCOS), due to the need for a follow-up examination and patients monitored due to previously known elevated intraocular pressure (IOP) values or glaucoma. Persons under the age of 18 and over the age of 70 and ones

who are not being treated for glaucoma or are not being controlled for elevated IOP values will be excluded from the survey.
The research will be conducted by the *Crown-Crisp Experience Index* (CCEI) which is designed to identify and measure common symptoms and personality traits within conventional categories of psychoneurotic diseases and personality disorders. The *General Anxiety Disorder-7* (GAD-7) questionnaire which is a validated instrument for identifying a generalized anxiety disorder and *Patient Health Questionnaire-9* (PHQ-9) characterized by good sensitivity and specificity for the detection of depressive disorders will also be used. Additional needed data will also be examined and recorded in a complementary survey sheet.

Research plan: Participants will be patients in the glaucoma infirmary at the CED of CHCOS who will be submitted to standard follow-up examination, as well as to CCEI, GAD-7, PHQ-9 and an additional survey sheet. The planned duration of the research is from October 2023 to June 2024.

Expected scientific contribution: The proposed research will try to more clearly determine the connection of open-angle glaucoma of different degrees of damage with the occurrence of anxiety and depression, and whether the occurrence of anxiety and depression affects the progression of open-angle glaucoma. If anxiety and depression are identified as risk factors for this type of glaucoma, a multidisciplinary approach to glaucoma treatment is required because adequate treatment of anxiety and depression is a possible way to slow down the progression of open-angle glaucoma.

Keywords: anxiety; depression; glaucoma; intraocular pressure

Dissertation Proposal Title: Comparison of the Clinical Effects of Leukocyte-Poor and Leukocyte-Rich Platelet-Rich Plasma in the Treatment of Knee Osteoarthritis.

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

Introduction: Knee osteoarthritis is a common musculoskeletal disease with over 620 million patients worldwide. The insidious disease progression is such that the treatment for the end stage of the disease is a total knee arthroplasty. However, the dawn of orthobiologics has enabled the prolongation of knee osteoarthritis progression so that patients can live with their native knees much longer than previously projected.

Hypothesis: Intra-articular administration of standardized leukocyte-rich platelet-rich plasma (LR-PRP) shows no difference in clinical effects, including symptom resolution, pain reduction, and improvement of function, compared with leukocyte poor platelet-rich plasma (LP-PRP) after 12 months in patients with knee osteoarthritis.

Aims: The aim of the research is to provide more precise answers about the effectiveness of platelet-rich plasma therapy, including LR-PRP and LP-PRP preparations in patients with knee osteoarthritis, such as:

● Objectivization of the effect of therapy by analyzing clinical data based on WOMAC, KOOS and VAS scores after 1, 3, 6 and 12 months

● Comparison of orthopedic scores (WOMAC, KOOS, VAS) of LR-PRP therapy and LP-PRP intra-articular injection therapy

● Correlate the effectiveness of LR-PRP and LP-PRP therapy on knee osteoarthritis with the number of platelets and leukocytes, by observing clinical scores and examinations.

Materials/Participants and Methods: Patients are selected based on clinical presentation of knee pain lasting for longer than 6 months. Patients are between 18-75 years old. Criteria for admitting patients to the study were no prior history of traumatic osteoarthritis, mechanical axis deviation less

than 5°, intact ligaments of the knee, and menisci that had no clinically impactful tears. Patients are assessed using X-ray and magnetic resonance imaging, as well as VAS, KOOS, and WOMAC scores. Blood samples are processed by using a proprietary protocol to produce LP-PRP or LR-PRP. The concentration of leukocytes and platelets present in the whole blood and PRP are measured on a differential hematology analyzer. To provide high-quality products for our patients, we are aiming for a minimal thrombocyte PRP concentration of 7×10^9. Patients will be randomly assigned to the LP and LR groups and will be blinded as well as the administering physician. Finally, the PRP will be injected intraarticularly into the knee via ultrasound guidance. Patients will then be assessed at 0, 1, 3, 6 and 12 months by the VAS, KOOS, and WOMAC scores.

Research plan: The prospective, interventional, randomized, double-blind study will be conducted at St. Catherine Specialty Hospital.

Expected scientific contribution: Standardization of the production process of LR-PRP, and LP-PRP, with confirmation of its clinical effectiveness, will contribute to the inclusion of PRP as a therapy in the conservative treatment of knee osteoarthritis.

MeSH/Keywords: platelet-rich-plasma, knee osteoarthritis, leukocyte-rich platelet-rich plasma, leukocyte-poor platelet-rich plasma, orthobiologics

Dissertation Proposal Title: Treatment of neurogenic bladder in children using non-invasive neuromodulation

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Introduction: Neurogenic bladder (NB) is defined as any change in the physiological function of the bladder due to central or peripheral neurological lesions. Congenital neural tube defects (CDNT) are common abnormalities that lead to neurogenic bladder in children.

Spinal dysraphism (SD) is responsible for up to 93% of cases (open myelodysplasia 85%, closed/occult dysraphism: 8%) of NB cases.

Patients with SD have problems with storage and/or emptying of urine because the spinal

cord lesion disrupts nervous system control of urinary voiding mechanisms. The results in urinary incontinence, increased risk of urinary tract infection, and risk of renal injury. The abnormalities of lower urinary tract dynamics include:

●Flaccid bladder

●High pressure bladder

●Hyperreflexic bladder

●Open bladder neck and/or overactive external/internal sphincter

●Lack of coordination between the bladder and the external sphincter during voiding and/or bladder filling

The goals for the management of neurogenic bladder in patients with SB are to preserve kidney function and for the patient to have independent continence of bowel and bladder at a developmentally appropriate age.

The procedures used in the treatment are clean intermittent catheterization (CIC), anticholinergic agents (AchA), in some cases prophylactic antibiotics. A part of the patient remains symptomatic despite the applied therapy. Treatment is often difficult due to off-label branding and patient age.

Transcutaneous tibial nerve stimulation (TTNS) is a non-invasive neuromodulation method used in children and adults with overactive bladder, non-obstructive retention, chronic constipation, fecal incontinence and chronic pelvic pain syndrome. Despite the potential positive effect on urinary symptoms, the experiences of children and their parents with this long-term treatment and the effect on QOL are not well known, primarily due to the lack of good clinical studies.

Hypothesis: Transcutaneous tibial nerve stimulation (TTNS) is a suitable non-invasive neuromodulation treatment for neurogenic bladder in children with spina bifida

Aims:

1. to use non-invasive stimulation of the tibial nerve with the intention to achieve desired therapeutic effects.
2. to assess whether there is an objective acute effect of TTNS on urodynamic parameters.
3. to determine the following parameters before and after therapy: incontinence day/night, number of UTI, sensation of bowel and bladder fullness, number of stools, Bristol Stole Chart assessment, presence of encopresis, urine stream strength

Materials/Participants and Methods: The study includes 30 patients with neurogenic bladder resulting from open/closed spinal dysraphism, as well as some other less common and acquired neurological conditions. The age range will be from 4 to 18 years. The research will not include children who are undergoing some other form of treatment that could affect bladder function.

Before the start of neuromodulation treatment for TTNS, all subjects will undergo an electromyoneurographic measurement (EMNG) and urodynamic evaluation as well as after 12 weeks of therapy.

TTNS neuromodulation therapy will be carried out for 12 weeks, every day at home for 30 minutes.

Research plan: This prospective observational study will be conducted at the Clinic for Pediatric Medicine Helena, Zagreb and Children's Hospital Zagreb. The research will last 12 months.

Expected scientific contribution: Neuromodulation allows us a new approach to patients with neurogenic bladder dysfunction and can help in recovery functions or alleviating symptoms that have a neurological basis. The results of the proposed research will contribute to a better understanding of the role of TTNS in the treatment of neurogenic bladder. The results will enable a better assessment of TTNS in children with NB.

MeSH/Keywords: neurogenic bladder, transcutaneous tibial nerve stimulation, urodynamic, neuromodulation, n.tibialis posterior

Dissertation Proposal Title: Association of glycosylation changes with interpatient variability in the frequency and intensity of adverse effects in breast cancer patients treated with aromatase inhibitors or selective estrogen receptor modulators

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Introduction: Glycosylation, an enzymatic process in eukaryotic cells, involves attaching carbohydrates to proteins or lipids. These glycans, including monosaccharides, oligosaccharides, and polysaccharides, form glycoconjugates, playing vital roles in cellular functions such as stability, solubility, and immunity. Disruptions in N-glycosylation can lead to severe diseases. Changes in IgG antibody glycosylation are linked to infections, cancers, and autoimmune disorders. Carcinoma development is associated with glycosylation alterations, particularly in tumor cells displaying unique glycan patterns. These changes, including those in O-glycans, N-glycans, and glycosaminoglycans, have implications for breast cancer progression, serving as biomarkers for tumor aggressiveness, potential side effects, and therapy response. Elevated levels of sialylated IgG in breast cancer tissues correlate with poor prognosis and metastasis, suggesting potential therapeutic targets. In summary, glycosylation changes influence metastasis and carcinogenesis, offering diagnostic and therapeutic insights in oncology. Endocrine therapy is a crucial therapy option for breast cancer patients with positive hormone receptors. Despite its importance, its impact on glycosylation changes is not well understood. This study aims to assess how introducing endocrine therapy affects N-glycosylation of IgG molecules in breast cancer patients.

Hypothesis: Glycosylation changes before and after endocrine therapy in hormone-sensitive breast cancers are associated with individual differences in the frequency and severity of side effects of treatment.

Aims: This study aims to identify IgG glycan composition and profile in breast cancer patients with positive hormone receptors before and after 3 and 6 months of starting endocrine therapy. It also investigates differences in IgG glycan profiles pre- and post-endocrine therapy and their association with the frequency and severity of side effects.

Participants and Methods: This study will enroll newly diagnosed breast cancer patients with positive hormone receptors who qualify for endocrine therapy and will be treated at the Oncology Clinic, Clinical Hospital Center Osijek. Exclusion criteria are reluctance to participate, acute infections, additional malignancies, autoimmune disorders, injuries, previous hormone therapy, and smoking. A minimum of 44 participants is needed to identify meaningful variations in numerical variables over three assessments, with a significance level of 0.05 and a power of 0.95 (G*Power ver 3.1.2).

Research plan: After obtaining Ethical Approval, consenting participants will sign an informed consent form. Data collection before starting endocrine therapy includes past illnesses, family history, chronic therapy details, ECOG status, TNM disease stage assessment, and a 4ml blood sample collection at the Department of Oncology, Clinical Hospital Center Osijek, between 8 and 10 am following an overnight fast. Blood samples will be stored at -20°C until IgG glycosylation analysis at Genos's glycobiology laboratory. Samples will be taken three times: before therapy, at 3 months, and at 6 months.

Significance/Expected scientific contribution: This study aims to assess the associations between IgG glycan profile changes after starting endocrine therapy in breast cancer patients with positive hormone receptors. Additionally, it will prospectively track glycan profile modifications during therapy and examine their potential association with side effect occurrence.

MeSH/Keywords: Breast Cancer; Glycans; Endocrine Therapy; Glycosylation; Hormone Receptors

Dissertation Proposal Title: Homocysteine levels as early indicators of cobalamin deficiency in infancy

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Introduction: Vitamin B12, a crucial nutrient found in animal-derived foods, plays a vital role in metabolism and organism development, including DNA formation, red blood cell production, and myelin sheath formation. Vitamin B12 deficiency in infants can have long-term consequences on cognitive development. Although B12 deficiency is not routinely measured in many countries, some newborn screening tests can indicate potential deficiency. B12 supplementation in infants with deficiency can quickly improve motor development. However, previous studies have mainly focused on the biochemical aspects of B12 deficiency, while simultaneous assessment of clinical and developmental status of the child is often lacking. This study aims to investigate B12 and homocysteine levels in newborns and their neurological status.

Hypothesis: Elevated homocysteine levels coupled with reduced total circulating cobalamin are a more sensitive indicator of cobalamin deficiency than circulating cobalamin levels alone.

Aims: Determine homocysteine and cobalamin levels in delivered newborns immediately after birth. Assess the neurological status of delivered newborns and identify those at neurodevelopmental risk. Establish correlations between clinical symptoms and signs of cobalamin deficiency with homocysteine and cobalamin levels, and establish reference values.

Participants and Methods: The research will be structured as a cross-sectional study. It will involve infants of both genders who were examined as outpatients at the Department of Pediatrics, Clinical Hospital Center Osijek, over a period of one year. The study aims to include approximately 300 infants of both genders aged 2 to 12 months without previous perinatal risk factors (prematurity, developmental anomalies, mechanical ventilation, etc.).

Research plan: Clinical examination of the infant according to routine practice and depending on the clinical condition will be done by a pediatrician-neuropediatrist, and neurodevelopment will be assessed with the Denver Developmental Screening Test. Before the clinical examination at the hospital, the parents will fill out a questionnaire that includes questions with presumed risk factors for vitamin B12 deficiency, including maternal nutrition, maternal diseases, pregnancy and infant nutrition. Taking a venous blood sample to determine the concentration of total homocysteine, cobalamin, methylmalonic acid and complete blood count.
Based on the results, two groups of respondents were formed:
1. Infants who have positive criteria for cobalamin deficiency - positive hematological/biochemical parameters, clinical criteria
2. infants without deviations in biochemical and hematological parameters

Significance/Expected scientific contribution: The scientific contribution of the future doctoral work is that this work would also be shown to be scientific procedures proving that the limit value of homocysteine and cobalamin is significantly different from the currently applied values of the same and that the current values are not adequate for the infant age. This can change and improve good clinical practice by being, and based on, medicine evidence-based, using new criteria to single out infants with a real deficit
cobalamin in the early stage of development in which the application of simple and cheap therapy would be faster and more efficient, which would avoid the serious consequences of an unrecognized deficit of the same.

MeSH/Keywords: Cobalamin deficiency; Infant; Methylmalonic Acid; Neurodevelopment

Dissertation Proposal Title: Knowledge about inflammatory bowel diseases and therapy is an important predictive factor of therapeutic adherence

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Introduction: Inflammatory bowel disease (IBD) consists of autoimmune diseases with a chronic course characterized by inflammation of the alimentary tract. The course of IBD is unpredictable and is manifested by remissions and relapses, which reduce the quality of life of patients and often lead to disability in the long term. In patients with IBD, therapeutic non-adherence leads to a loss of response to therapy, more frequent hospitalizations, and increased treatment costs. The most common, simplest, and fastest measurement of therapeutic adherence is through a questionnaire. There is no validated questionnaire for measuring adherence to subcutaneous biological therapy. Knowledge about IBD and therapy contributes to better therapeutic adherence and contributes to reducing the need to apply more advanced forms of treatment, which indicates a clinically stable disease. The actual impact of knowledge about IBD and therapy on the clinical course of the illness, as well as the adherence to subcutaneous biological therapy among IBD patients in Croatia, is currently unknown. This research aims to identify these factors and their effects on the disease to improve treatment outcomes for those affected by IBD.

Hypothesis: Knowledge about the disease and therapy affects the clinical course of the disease and adherence to medication in patients with IBD treated with subcutaneously applied biological therapy.

Aims: To determine the impact of knowledge about IBD and therapy on the clinical course of the disease and adherence to subcutaneous biological therapy in patients with IBD. Validate a questionnaire for measuring adherence to subcutaneous biological therapy in patients with IBD.

Participants and Methods: The research will include 300 subjects of both sexes between the ages of 18 and 70 who meet the endoscopic, radiological, and histological criteria of IBD and who are treated with subcutaneous forms of biological therapy. From the prepared questionnaire, data will be collected on the demographic and socioeconomic characteristics of the respondents, data on lifestyle habits, the peculiarities of the disease, and on the clinical course of the disease. Knowledge about the disease and therapy will be measured by the IBD-KNOW questionnaire. Adherence to subcutaneous biological therapy will be measured by a questionnaire that will be formed based on the available literature, evaluated, and modified by experts in the field, and validated by research.

Research plan: A six-month cross-sectional, multicenter study will include IBD patients undergoing subcutaneous biological therapy at hospitals in Koprivnica, Varaždin and Zagreb.

Expected scientific contribution: We will assess how knowledge about IBD, and subcutaneous biological therapy affects the disease's progression. We will validate a questionnaire to measure adherence to this therapy, providing a standardized tool for future research. This will be the first measurement of adherence to subcutaneous biological therapy among Croatian patients with IBD.

Keywords: IBD, biologic therapy, IBD knowledge, adherence, clinical outcome

Doctoral thesis proposal: "Connection of oral rimegepant use with middle cerebral arteries vasoreactivity in patients with episodic migraine"

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Introduction: Migraine is a common neurological disorder that belongs to primary headaches and is characterized by moderate to severe headaches. It belongs to debilitating primary headaches which affect more than a billion people worldwide. The word migraine originates from the Greek word „hemicrania " later changed in Latin to „hemigranae" and in French to migraine. Migraine is a significant public health problem worldwide because it is one of the most common neurological disorders as well as one of the most common causes of disability among neurological disorders. Epidemiological data show that migraine is a common disease with a prevalence of around 14% in the general population. Female to male occurrence ratio is 3:1. According to literature, in the USA migraine affects 36 million Americans, from which there are 8,7 million women and 2,6 million men. By the frequency, 3,4 million women and 1,1 million men have one or more migraine attacks per month. The female population between 40 and 49 years of age from low-income households has a particularly high risk for migraine, which leads to more often use of emergency room services in comparison with the rest of the population. Research conducted in the USA showed that patients with chronic migraine lose 4,6 hours on average during the week and have 19% less salary than the rest of the population, in comparison with patients with episodic migraine who lose 1,1 hours on average during the week (1). Complex and mostly vague mechanisms of migraine development, bring to the conclusion that it is a result of different social and biological risk factors such as hormone disbalance, genetic and epigenetic factors, and cardiovascular, neurological and autoimmune diseases. Migraine has a strong genetic component, but at the moment there is no clear pattern of inheritance.

According to research, around 76% of patients with migraine expressed that there are provoking factors that cause migraine attacks. Most common are: stress in 80%, hormonal changes during the menstrual cycle in 65%, skipped meals in 57%, climate changes, too much or not enough sleep in 50%, intensive odors in 40%, pain as part of a cervical syndrome in 38%, alcohol ingestion in 38%, smoking in 36%, food in 27% (full-fat cheese, chocolate, etc.), exercise in 22%, sexual activity in 5%.

Pain usually affecting one side of the head is the reason why it is also called hemicrania, but it can affect any part of the head. Headaches are often associated with vegetative symptoms like nausea and vomiting as well as photophobia and phonophobia. The duration of untreated migraine headaches is variable, usually lasting between 4 and 72 hours.

In migraine, vascular changes do not always correlate with clinical observations. While changes in cerebral flow correlate with aura, the headache starts in a phase of oligemia, and after hypoperfusion. Today it is speculated that migraine is a state of the brain due to deranged neuronal excitability (10). Migraine occurrence is most commonly a result of homeostasis impairment which happens with activation of hypothalamus, brainstem structures and limbic cortical areas (10). Aura in migraine usually manifests with visual disturbances, but symptoms of sensory, motor, speech and brainstem dysfunction can also occur. New animal studies revealed a potential connection between cortical spreading depression and headache, showing that cortical spreading depression can activate meningeal trigeminovascular afferent branches and evoke a series of changes in meninges and brainstem, which can be associated with activation of trigeminal nociceptive pathways and development of headache in migraine. There is no clear evidence that aura and cortical spreading depression are the primary trigger of migraine attacks in people who have migraine with aura (2). Studies have shown that the most common triggers of migraine are stress, severe sensory stimulus, fatigue, meal skipping and menstrual cycle (2). Key event can be neuron hyperexcitability, which induces branches of the trigeminal nerve in meningeal arteries, which cause neurogenic inflammation and pain through the trigeminal nerve. Among important neuropeptides which are released and which take part in meningeal inflammation is calcitonin gene-related peptide (CGRP), P substance (SP), neurotoxin A (NKA), and vasoactive intestinal peptide (VIP). On the other hand, from nucleus salivarius superior (SSN), with reversible parasympathetic reflex, vasodilatation of meningeal arteries occurs, which is epiphenomenon in migraine attack, and not the main mechanism of headache occurence (2). There are two hypotheses about neuron hyperexcitability which lead to a migraine attack. According to the first hypothesis, cortical spreading depression caused by neuron hyperexcitability in the cortex causes pain and aura. These neurons inject neuronal inflammation into the trigeminovascular system which sends painful signals through the brainstem in the thalamus and sensory cortex (2). According to a second hypothesis, three groups of nuclei in the brainstem (locus ceuruleus, raphe nucleus and periaqueductal grey matter) due to abnormal activity do not inhibit incoming signals from trigeminocervical complex, but facilitate painful stimuli. In the same way, they can induce cortical spreading depression in the cortex which leads to pain aggravation and accompanying symptoms (sensitivity to light, smell and sound) (2). The trigeminovascular system starts in a dense tangle of nociceptors that innervate cranial meningeal vasculature, the main projection of which is trigeminal ganglion (TG), and which includes second-line neuron synapse in the brainstem and dorsal horn of upper spinal cord segments, called trigeminocervical complex (TCC). The aforementioned has a direct ascending connection in brainstem areas and periaqueductal gray matter, thalamus and hypothalamus, and through trigeminothalamical and trigeminohypothalamical projections connects to cortical neurons. Apart from ascending connections, there is a returning connection from TCC to the parasympathetic system through the superior salivatory nucleus (SNN) and sphenopalatine ganglion (SPG) (2). Activation of this system leads to stimulation of nociceptive neurons which innervate dura mater and release vasoactive neuropeptides like calcitonin gene-related peptide (CGRP), pituitary adenylate cyclase 38–activating polypeptide (PACAP-38), P substance,

neurokinin A, causing neurogenic inflammation. This leads to vasodilation, plasma extravasation, monocyte degranulation, thrombocyte activation and release of serotonin, bradykinins, histamines and prostaglandins. After being activated by endogenic inflammatory mediators following the neuropeptides effect, peripheral trigeminovascular neurons are becoming more sensitive to repetitive dural stimuli which leads to peripheral sensitization of trigeminal ganglion and which clinically manifests itself with pulsatile pain aggravated by coughing and bending over (2). Sensory input for pain is transmitted through the second neuron in trigeminocervical complex to higher subcortical and cortical structures. If a second migraine attack is not ceased during strengthened sensory inputs, or if the frequency of shorter migraine attacks is increased, central sensitization in TCC and/or thalamic nuclei can occur (2).

CGRP molecule has its effect on different receptors, but binding to CGRP receptor complex is significant for migraine pathophysiology. Triptans decrease the level of CGRP which leads to alleviation of migraine pain. Selective antagonists of CGRP receptors can effectively reduce migraine pain (2). CGRP is present in neurons of trigeminal ganglion and is released from peripheral nerves which start a cascade of events including increased nitrogen oxide synthesis and trigeminal nerve hypersensitivity. Neurons in trigeminal ganglia have receptors for CGRP where the excessive release of CGRP in migraine attack stimulates neurons in maintaining of pain leading to peripheral sensitization, while in the trigeminal nucleus, nucleus caudalis and nucleus spinalis it also leads to central sensitization (2).

Sudden fall of serotonin in trigeminovascular system contributes to neurogenic inflammation. Serotonin function on neurons 5HT1 B/D is to inhibit the release of CGRP in presynaptic neurons. As there is a fall of serotonin levels in migraine, CGRP increases with consequential stimulation of neurogenic inflammation and pain. On peripheral branches of meningeal arteries, serotonin has strong pro-nociceptive activity and increases CGRP levels with subsequent activation of 5HT-3 neurons (2). Pituitary adenylate cyclase-activating polypeptide (PACAP) is similar to vasoactive intestinal peptide (VIP). PACAP is located in the peripheral and central nervous system.

According to the recommendation of the International Headache Society, migraine can be classified as migraine without aura, migraine with aura and chronic migraine. Migraine without aura is the most common and it occurs in 75% of cases.

Migraine can clinically present in two forms: migraine with or without aura, and by frequency of attacks it can present as episodic or chronic migraine. By definition, episodic migraine is characterized by headaches that occur in less than 15 days during a month. Migraine therapy can be divided into therapy for acute migraine attacks, preventive therapy and non-pharmacological therapy. Regarding the aforementioned, preventive therapy is the least used (2-14%).

Therapy for acute migraine attacks can be classified as first, second and third line of treatment and as additional preventive therapy. The first line of therapy includes: acetylsalicylic acid, ibuprofen, diclofenac, paracetamol, antiemetics domperidone and metoclopramide. Second-line therapy for acute migraine attacks includes: zolmitriptan, almotriptan, eletriptan, frovatriptan, naratriptan, rizatriptan. Around one-third of patients do not respond adequately on triptans and $\geq 20\%$ of patients have contraindications for therapy with triptans due to cardiovascular disease. The third line of therapy includes gepants and ditans. Gepants that can be used are ubrogepant, rimegepant 75 mg which has been approved for acute and preventive migraine therapy. Lasimiditan is used from the group of ditans.

Preventive therapy of migraine consists of continuous treatment in patients in whom migraine significantly impairs life quality. There is an indication for preventive therapy if migraine occurs more than two times during a month if attacks have a more severe clinical presentation or last longer, and in case medications for acute migraine attacks are overused. Preventive migraine therapy is divided in three lines. First-line therapy includes beta-blockers (atenolol, bisoprolol, metoprolol, propranolol), antiepileptic topiramate, and angiotensin 2 antagonist candesartan. Second-line therapy includes amitriptyline, flunarizine, and sodium valproate. Third line therapy includes botulinum toxin, erenumab, fremanezumab, galcanezumab, eptinezumab.

Rimegepant is a second-generation gepant for oral use. It has been approved for acute treatment of migraine with or without aura in adults and for preventive treatment of episodic migraine in adults in several countries including the USA and EU. Rimegepant is an antagonist of CGRP receptor with high binding affinity which blocks the activity of CGRP with yet unclear mechanism.

In oral use, rimegepant is absorbed with maximal concentration after 1,5 hours. Median time to Cmax (Tmax) was 1,48 hours with ODT (orally disintegrating tablet) formulation versus 1,92 with conventional tablet formulation ($p = 0,0021$), a difference of 26 minutes (3). After a supratherapeutic dose of 300 mg, absolute oral bioavailability of rimegepant was approximately 64%. Approximately 96% of rimegepant is bound to plasma proteins (3). Rimegepant is primarily metabolized with CYP3A4 and, to a lesser extent, with CYP2C9. It is mostly eliminated (≈ 77% of dose) as an unmodified drug, whereby 78% of the drug is found in feces (42% as an unmodified drug) and 24% in urine (51% as an unmodified drug).

Rimegepant has half-life of ≈ 11 h (3).

There was no impact of sex, age, race, ethnicity, body mass, migraine status, CYP2C9 genotype, renal insufficiency (mild, moderate or severe) or liver damage (mild or severe) on the pharmacokinetics of rimegepant (3). Rimegepant has not been tested in patients with end-stage renal disease (creatinine clearance < 15 ml/min) or in dialysis patients. Exposure to rimegepant was ≈ 2 higher in patients with severe liver damage than in matching controls with normal liver function, therefore rimegepant use should be avoided in patients with severe liver damage (3).

The efficacy of rimegepant for the acute treatment of migraine has been estimated in four randomized, double-blind, multicentric, placebo-controlled trials of phase III (3).

Trials included patients between 18 and 65 years of age with a positive history of 2 to 8 migraine attacks of moderate to severe intensity per month, < 15 days per month with headache (with or without migraine) during the past 3 months and the onset of migraine before 50 years of age. ODT formulation used in one dose of 75 mg of rimegepant, provided better efficacy than placebo for acute migraine treatment. Two hours after rimegepant use, a significantly higher number of patients in the group who received ODT rimegepant compared to the placebo group didn't have headaches.

The efficacy of rimegepant for preventive migraine treatment has been estimated in randomized, double-blind, multicentric, placebo-controlled trials of phase II/III. Rimegepant, used every other day, has been more efficient than placebo for preventive treatment of migraine (3). Rimegepant is the first approved drug that has indications for acute and preventive migraine treatment.

Hypothesis: Oral use of rimegepant causes vasoreactivity of middle cerebral arteries in patients with episodic migraine.

Aims: The aim of this research is to provide evidence that rimegepant causes vasoreactivity in middle cerebral arteries. Such activity could be demonstrated by measuring mean blood flow velocity (MBFV) in middle cerebral arteries using transcranial Doppler (TCD). It will be important to determine time span in which significant vasoreactivity of middle cerebral arteries occurs after oral intake of rimegepant, as well as period in which vasoreactivity is smallest.

Materials and participants: In this research, 50 patients, between 18 and 60 years of age will be enrolled. Patients will be detected in policlinic for headaches or general neurological policlinic in Sveti Duh University Hospital. The inclusion criterion will be episodic migraine diagnosed by history and examination after secondary causes have been excluded by earlier neuroimaging (brain MSCT or MRI). Excluding criteria will be age (<18 or >60 years), positive neuroimaging finding for secondary headache, migraine headaches that do not fulfill International Headache Society ICHD-3 criteria for episodic migraine, severe cerebrovascular disorder, severe liver or renal impairment and uncontrolled arterial hypertension.

Methods and research plan: Research will be conducted in the Department for Neurology of Sveti Duh University Hospital.

In prospective research, 50 patients will be enrolled between 18 and 60 years of age after appropriate screening in the headache clinic and general neurological clinic of Sveti Duh University Hospital. Patients will be admitted to an outpatient unit of the Department for Neurology of University Hospital Sveti Duh. The beginning of research is planned in September 2024. while the expected duration of research is 12 months.

At screening, history of age, sex, frequency and intensity of migraine headache, frequency of specific and non-specific analgesic therapy use, previous specific or non-specific prophylactic migraine therapy, liver and renal diseases, presence of peripheral arterial disease or previous stroke/transient ischemic attack, uncontrolled arterial hypertension, consumption of tobacco, coffee, tea or other caffeine-containing products in 12 hours previous to testing, eating habits and stress will be taken.
In selected patients, which would fulfill the criteria for episodic migraine, will be performed blood pressure measurement and laboratory tests: urea, creatinine, sodium (Na), potassium (K), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT) and total bilirubin. Transcranial Doppler (TCD) breath-holding test for assessment of cerebrovascular reactivity will be performed in a standardized manner. Breath holding index will be calculated for the middle cerebral artery in the following manner: mean blood flow velocity (MBFV) in the middle cerebral artery will be measured for 5 minutes for baseline value and after that patient will be asked to hold their breath during normal breathing for 20 to 30 seconds (to avoid Valsalva maneuver) and maximal value at the end of that cycle will be considered. The difference between the maximal value and baseline value divided by time will be calculated to get breath-holding index (BHI).

If blood tests are satisfactory, TCD will be performed at three time points (before rimegepant treatment, two hours after the first rimegepant intake and 48 hours after the last rimegepant intake). Mean blood flow velocity (MBFV) in middle cerebral arteries will be measured and the result will be expressed in centimeters per second (cm/sec). Results will be noted in appropriate tables.
Each participant will be provided with an Informed consent form approved by the Ethical committee of 'Sveti Duh' University Hospital. All patient test results will be noted in research exclusively under codes and all patient-related data will be anonymous during analysis. After gathering the expected number of patients, statistical analysis will be performed. P values of <0.05 will be considered statistically significant.

Expected scientific contribution: Migraine is among leading neurological disorders with a significant effect on the working ability and functioning of the sick. Because of it there is a great significance in reducing migraine headaches as well as preventing them. Considering that rimegepant has approval for use in acute and episodic migraine headaches, it is important to determine the connection between rimegepant with cerebral vessel vasoreactivity. If the hypothesis is confirmed, results will help us better understand connection between rimegepant and middle cerebral arteries vasoreactivity. The theoretical contribution will be a better understanding of pathophysiological processes in episodic migraine.

Dissertation Proposal Title: Influence of virtual reality vestibular rehabilitation on cognitive functions and vestibular recovery in patients with unilateral vestibular impairment

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Introduction: Vestibular rehabilitation based on virtual reality exposure therapy is a novel therapeutic option for the treatment of unilateral vestibular hypofunction. The exact mechanism behind vestibular recovery after virtual reality exposure therapy is not fully elucidated. One suggested explanation is a positive impact on cervical and ocular vestibular evoked myogenic potentials. Furthermore, few studies suggested that virtual reality therapy can improve poor cognitive functions which is also something to have in mind as poor cognitive functions are commonly found in patients with unilateral vestibular impairment.

Hypothesis: Vestibular rehabilitation based on virtual reality exposure therapy positively affects cognitive functions and vestibular recovery in patients with unilateral vestibular impairment.

Aims:

1. Compare the subjective scope of vestibular sense recovery based on the results of DHI and SF-36 questionnaire before and after therapy.

2. Compare the objective scope of vestibular sense recovery based on covert and overt saccades and on result of rotational tests before and after therapy.

3. Compare cognitive functions before and after therapy among patients with unilateral vestibular impairment based on results of the Montreal Cognitive Assessment score (MoCA).

Materials/Participants and Methods: The study will include at least 60 patients divided into two groups. All patients must be diagnosed with unilateral vestibular impairment. One group will implement standard vestibular rehabilitation procedures while the other group will implement

vestibular rehabilitation based on virtual reality exposure therapy. Subjective progress will be measured by DHI and SF-36 questionnaires in both groups while cognitive functions will be measured in all patients by Montreal Cognitive Assessment (MoCA).

Research plan: A prospective study will be conducted at the Department for Otorhinolaryngology and Head and Neck Surgery of University Hospital Center Osijek. The estimated duration of study is 12 months.

Significance/Expected scientific contribution: The emphasis of the study is on a comprehensive and multidisciplinary approach to the patients so we can diagnose this impairment more precisely and implement new, relatively unknown and technologically more advanced vestibular rehabilitation in clinical practice.

MeSH/Keywords: virtual reality, vertigo, rehabilitation, vestibular disease, dizziness

Abstract Title: BPC 157 treatment attenuates occlusion-like syndrome in rat glaucoma model with three cauterized episcleral veins

Part of the Dissertation Proposal: Vascular and multiorgan dysfunction syndrome (occlusion syndrome), peripheral and central in rat glaucoma and BPC 157 therapy

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Introduction: Open-angle glaucoma is a chronic, progressive, and irreversible optic neuropathy with multifactorial origins. The interplay among cerebrospinal fluid, intravascular and intraocular pressures has long since been the theory behind the pathogenesis of glaucoma. Recent investigations demonstrated that therapy utilizing stable gastric pentadecapeptide BPC 157 promptly normalizes intraocular pressure. Furthermore, the therapeutic potential of BPC 157 in mitigating vascular and multiorgan failure in various occlusive and "occlusion-like" syndromes has been elucidated.

Aims: The primary objective of this study was to validate the advantageous effects of BPC 157 on systemic manifestations induced by glaucoma in rats.

Materials/Participants and Methods: Acute open-angle glaucoma was induced in deeply anesthetized Sprague-Dawley rats by cauterizing three episcleral veins in both eyes. BPC 157 (and saline in the control group) in two doses (10µg/kg and 10 ng/kg) was applied as intragastrical, subcutaneous and local (two drop/eye) treatment 15 minutes, 1 hour and 24 hours following glaucoma induction. Volumes of major blood vessels and organs were assessed using a USB microscope camera. Pressures within the vasculature and intraocular pressure were measured. Blood clots were removed and weighed after sacrifice.

Results: Rats in the control group exhibited intracranial, portal and caval hypertension and aortal hypotension, accompanied by brain swelling, vein congestion and blood clots. Treatment with all regimens of BPC 157 attenuated those pathological changes.

Conclusion: Acute glaucoma induces immediate (15 minutes) and lasting (24 hours) systemic changes centrally (intracranial hypertension, brain swelling) and peripherally (vascular congestion, clots, pressure changes) all of which are ameliorated by the application of BPC 157 regimens.

MeSH/Keywords: Pentadecapeptide BPC 157; Glaucoma; Rat; Therapy; Multiple Organ Failure; Occlusion/Occlusion-like syndrome

Dissertation Proposal Title: Are HER2 gene single nucleotide polymorphisms associated with trastuzumab-induced cardiotoxicity?

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Introduction: A humanized monoclonal antibody trastuzumab, that binds to epidermal growth factor receptor 2 (HER2) is the standard of treatment for early and metastatic HER2-positive breast cancer. Overexpression of human epidermal growth factor 2 (HER2) occurs in approximately 15-25% of all breast cancers, and HER2 positivity is associated with a more aggressive phenotype, higher recurrence rates and worse prognosis. Targeted anti-HER2 treatment with trastuzumab significantly improved disease outcomes, and although well tolerated, it is associated with potential serious side effects of cardiotoxicity. The underlying mechanism of cardiotoxicity development is still unclear, however, it is mainly associated with HER2 receptor blockade in cardiac muscle, and unlike anthracycline-related cardiotoxicity, mostly reversible. Trastuzumab-induced cardiotoxicity ranges from asymptomatic and reversible left ventricular systolic dysfunction to irreversible dilated cardiomyopathy with symptoms of heart failure. The incidence of asymptomatic left ventricular ejection fraction (LVEF) decrease (defined as a decrease of > 10% from baseline value or below 50% in total) is estimated to 10% in adjuvant treatment setting, whereas more serious forms of cardiotoxicity along with symptomatic left ventricular failure occurred in up to 4% of cases. It is known that older patients, ones who had prior anthracycline therapy, as well as patients who suffer from obesity or diabetes had a higher risk of developing trastuzumab-induced cardiotoxicity. But what about individual genetic risk? Single-nucleotide polymorphisms (SNPs), which are the most common type of genetic variation among people, are defined as variations at a single position (nucleotide) in DNA sequence, which can occur in protein-encoding genes (multiple allele genes) and noncoding regions of DNA. SNPs are associated with variations in the efficacy of systemic treatment, and also, treatment toxicities. By now, the most evaluated HER2 gene polymorphism is Ile655Val, linked to variable expression of HER2 transmembrane protein, which could have an impact on treatment outcomes, overall survival and cardiotoxicity incidence. At present, there is limited data in this regard, with trials conducted on small population size. Another known single nucleotide polymorphism Pro1170Ala (RS 1058808) could be linked to trastuzumab-induced cardiotoxicity, but data is conflicting, and further investigation is needed. By this time, small number of studies that evaluated the impact of HER2 genotypes (Pro/Pro; Pro/Ala; Ala/Ala) on cardiotoxicity risk, indicated that Pro/Pro genotype is associated with higher cardiotoxicity risk in comparison to the other two variants. Few studies presented statistically significant differences in HER2 protein expression on the tumor cell surface associated with Pro1170Ala polymorphism, but

further investigation is required, especially in Caucasian population. Two more single nucleotide polymorphisms are known to be associated with different treatment outcomes, as well as trastuzumab-related cardiotoxicity. Polymorphisms of the genes that code FCγ receptors, FCGR2A-H131R (RS 1801274) and FCGR3A – V158F (RS 396991), which are known to be included into immunological response to antibody-dependent cytotoxicity (which is one of the proposed mechanisms of trastuzumab action), could be associated with cardiotoxicity development, with so far conflicting results published.

Hypothesis: The number of breast cancer patients treated with anti-HER2 antibody trastuzumab in an adjuvant setting is increasing. Could single nucleotide polymorphisms of HER2 and other genes, along with other biomarkers, be used for stratification of cardiotoxicity risk? In addition, could it potentially be used as a predictive biomarker for earlier implementation of preventive medications, especially considering long-term outcomes and better overall survival of those patients?

Aims: To investigate Pro1170Ala single nucleotide polymorphism of HER2 gene and determine genotype distribution (Pro/Pro; Pro/Ala; Ala/Ala) among patients treated with anti-HER2 monoclonal antibody trastuzumab in the adjuvant setting of early HER2 positive breast cancer. Also, the aim is to explore the potential association between specific genotypes and increased cardiotoxicity risk, between specific genotypes and HER2 receptor expression on tumor cell surface and as well, the impact of HER2 genotype on biological characteristics of the tumor, presence of risk factors, grade of heart failure, cardiotoxicity reversibility, recovery time and late cardiotoxicity rates.

Materials/Participants and Methods: 177 patients would be included in the research, with HER2 positive early breast cancer who have been treated with trastuzumab in an adjuvant setting from year 2007 to year 2016, at University Hospital Center Sestre Milosrdnice (University Hospital for Tumors and University Department of Oncology and Nuclear Medicine). They are divided in two subgroups, group A (99 participants) who didn't develop cardiotoxicity, and group B (78 participants) who developed trastuzumab-induced cardiotoxicity.

Research plan: Patients` follow-up would be done at present time, by medical history data gathering and analysis, performing echocardiography exam and biochemical measurement of amino-terminal pro-B type natriuretic peptide (NT-proBNP) levels. NT-proBNP is a biomarker used in heart failure assessment, and its plasma levels are in direct correlation with the level of heart failure and further clinical outcomes. Also, the pathology report would be reevaluated in search for HER2 receptor expression on the tumor cell surface. In addition, information about the biological characteristics of the tumor would be obtained (tumor nuclear grade, hormone receptor status, Ki-67 proliferation index) as well as evaluation of the presence of risk factors (diabetes, hypertension, hyperlipidemia, family history), determination of the grade of heart failure and cardiotoxicity reversibility evaluation along with cardiotoxicity recovery time. An echocardiography exam and

biochemical measurement of NT-proBNP levels would be focused on late cardiotoxicity assessment. Pro1170Ala polymorphism would be determined using primers provided by Medical Intertrade company, and research would be carried out at Department of Clinical Chemistry Sestre milosrdnice (University Hospital Center Sestre milosrdnice).

Significance/Expected scientific contribution: The number of breast cancer patients treated with anti-HER2 monoclonal antibody trastuzumab in the adjuvant setting is rising and long-term outcomes of these patients, including overall survival, are improving. Taking into consideration that cardiotoxicity is the most serious acute and chronic side effect of trastuzumab treatment and one of the main reasons for trastuzumab interruption and discontinuation, further investigation is required in the search for potential predictive biomarkers that would help clinicians to identify patients with higher risk of cardiotoxicity development, who would, therefore, have the benefit of earlier implementation of preventive measures. So far, research has been done to determine the connection between SNP and risk of cancer, with a focus on different levels of risk in various ethnic subgroups. Also, there is data about single nucleotide polymorphisms association with variable systemic treatment efficacy as well as treatment toxicities in various tumor sites. Additional research on HER2 gene single nucleotide polymorphisms, as well as other genes connected to cardiotoxicity, could explain a possible genetic susceptibility to cardiotoxicity development. Shortly, with the individualized approach as the standard of care in early breast cancer, cardiotoxicity genetic risk assessment could become widely used in everyday clinical practice, along with other available biomarkers. One step ahead, the identification of SNPs connected with cardiotoxicity could lead to further analysis and whole genome sequencing in specific populations and ethnic groups, in order to detect clear genetic susceptibility to certain conditions connected to oncology treatment and toxicities.

MeSH/Keywords: single nucleotide polymorphism, cardiotoxicity, HER2 gene, trastuzumab

Abstract Title: Prognostic significance of GDF-15 levels and other laboratory findings in hospitalized COVID-19 patients

Part of the Dissertation Proposal: Early biomarkers of the risk of developing a severe and complicated course of the disease in hospitalized patients with SARS-CoV-2 infection

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Introduction: Growth differentiation factor 15 (GDF-15) is a cytokine induced by tissue stress and associated with inflammatory states. Recent research suggests increased GDF-15 levels in response to SARS-CoV-2, but results remain inconclusive.

Aims: The aim of this prospective observational study was to investigate the prognostic and diagnostic roles of GDF-15 and routine clinical laboratory parameters in hospitalized COVID-19 patients.

Materials/Participants and Methods: The study was conducted on 95 adult hospitalized COVID-19 patients in Croatia. Upon admission, blood analysis was performed, and medical data were collected. Routine hematological, biochemical, and coagulation laboratory parameters were analyzed as well as GDF-15 levels. The data collected from medical history included age, sex,

vaccination information, information about earlier SARS-CoV-2 infection, comorbidities (diabetes mellitus, cardiac disease, lung disease, chronic kidney disease), clinical signs and symptoms of disease at admission, systolic and diastolic blood pressure, peripheral blood oxygen saturation, heart rate, respiratory rate, Glasgow Coma Scale (GCS) score, length of hospitalization, the need for ICU admission, and clinical outcomes. Additionally, it was documented whether the patient required a high-flow nasal cannula (HFNC) and/or invasive mechanical ventilation (IMV) during hospitalization. CURB-65 and qSOFA scores were calculated. The patients were categorized based on survival, ICU admission, and hospitalization duration. Statistical analysis was done using logistic regression and ROC curve methods.

Results: Logistic regression showed two independent predictors of negative outcome: CURB-65 score (OR = 2.55) and LDH (OR = 1.005); one predictor of ICU admission: LDH (OR = 1.004); and one predictor of prolonged hospitalization: the need for high-flow nasal cannula (HFNC) upon admission (OR = 4.75). The ROC curve revealed diagnostic indicators of negative outcome: age, CURB-65 score, LDH, and GDF-15. The largest area under the curve (AUC) is represented by GDF-15 (AUC = 0.767, specificity 65.6, sensitivity 83.9) with a cutoff value of 3528 pg/mL. For ICU admission, significant diagnostic indicators are LDH, CRP, and IL-6; and significant diagnostic indicators of prolonged hospitalization are CK, GGT, and oxygenation with HFNC upon admission.

Conclusion: This study strengthens the significance of usually used laboratory parameters and clinical scores in evaluating COVID-19. Additionally, it shows the emerging potential for a new prognostic approach using GDF-15 levels upon hospitalization of affected individuals.

MeSH/Keywords: COVID-19; Growth Differentiation Factor 15; Mortality; Prognosis; SARS-CoV-2

Abstract Title: Effect of consumption of chicken meat enriched with carnosine on microvascular function in patients with chronic coronary artery disease

Part of the Disertation Proposal: Effect of consumption of chicken meat enriched with carnosine on microvascular function and immune system in patients with chronic coronary artery disease

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Introduction: Cardiovascular disease remains the leading cause of death globally. Evidence from animal studies showed that carnosine supplementation may reduce body weight, blood pressure level, serum lipid levels and atherosclerotic plaque instability. Due to its anti-inflammatory, antioxidant and anti-ishemic roles in the body, food enrichment with carnosine could be useful in improving and enhancing cardiovascular health.

Aims: The aim of this study is to investigate the effects of the consumption of enriched chicken meat with carnosine on blood pressure levels, biochemical parameters and microvascular reactivity in patients with chronic coronary artery disease.

Materials/Participants and Methods: This was a double-blind, placebo-controlled clinical study that included 19 participants with existing chronic coronary artery disease randomized into the control group (N = 9), who ate ordinary chicken meat and carnosine group (N = 10), who ate enriched chicken meat for 21 days. Microvascular endothelium-dependent vasodilation (post-occlusive reactive hyperemia, PORH; and acetylcholine-induced dilation, AChID) and endothelium-independent vasodilation (sodium-nitroprusside induced dilation, SNPID) were assessed by laser Doppler flowmetry. Anthropometric parameters, blood pressure and biochemical parameters were measured before and after the dietary protocol. The study was approved by the Ethical Committees of Faculty of Medicine Osijek and University Hospital Centre Osijek, and all participants gave written informed consent.

Results: Following dietary protocol, body mass index (BMI), systolic (SBP), diastolic (DBP) and mean (MAP) arterial blood pressure were significantly decreased in carnosine group. Biochemical parameters did not change in both control and carnosine group following dietary protocol. PORH and SNPID were significantly increased in the carnosine group while they remained unchanged in the control group. AChID did not significantly change in both control and carnosine group.

Conclusion: Consumption of functionally enriched chicken meat for 21 days has a favorable effect on blood pressure levels and a positive effect on microvascular reactivity in patients with chronic coronary artery disease.

MeSH/Keywords: carnosine; endothelium; functional food; microvascular function; vasodilation

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Dissertation Proposal Title: Association of donor-related factors with aggregate occurrence in apheresis-derived platelet concentrates

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

Introduction: Aggregates in apheresis-derived platelet concentrates (APCs) are a well-known phenomenon. A tendency for aggregate formation has been described in some donors ("clumpy donors"), but the exact cause has not yet been discovered.

Hypothesis: Donors with repetitive aggregate occurrence compared to donors with no history of aggregates have:

1. more expressed platelet activation markers
2. different coagulation status (significant difference in coagulation markers)
3. different characteristics.

Aims: Examine donor-related factors (donors' platelet activation, coagulation status, donor characteristics – age, sex, body mass index, smoking, medicines, dietary supplements) in donors with repetitive aggregates in APCs and compare them with the control group.

Materials/Participants and Methods: Participants will be allocated in two groups:

- aggregate group – plateletpheresis donors at Croatian Institute of Transfusion Medicine (CITM), who had ≥2 aggregate occurrences in APCs, with no permanent contraindication for blood donation. The expected number of donors: 100.
- control group – plateletpheresis donors at CITM who did not have aggregate occurrence in APCs during the observed period, with no permanent contraindication for donating blood. The expected number of donors: 100.

Harmonization of the groups: beside the main condition of aggregate absence in APCs, control group will be formed from the donors whose donations were equally distributed during the year and during the day. The observed period from which donors will be allocated in the two groups will be 2018-2022.

Research plan: Available data on donors, donations and procedures will be collected from the IT system. Additional data on donor habits will be obtained by a short survey. For the main purpose of this research, additional tests will be performed on donors' blood samples: platelet aggregation with ADP and collagen, prothrombin time, activated partial thromboplastin time, fibrinogen, FVIII, FIX, von Willebrandt factor. Flow cytometry will be used for platelet activation analysis with CD62P and PAC-1 markers.

Significance/Expected scientific contribution: This research is a contribution in resolving molecular and pathophysiological cause of aggregates in platelet concentrates in the group of donors with repetitive aggregates. This research would be the first one dealing with platelet activation markers in the donor bloodstream, coagulation factors and donor characteristics among the donors with repetitive aggregates.

MeSH/Keywords: blood donors, platelet activation, platelet membrane glycoproteins, platelet aggregation, blood coagulation factors

Abstract title: Sex and age of patients with functional dyspepsia and associated comorbidities

Dissertation Proposal Title: Differences between male and female in psychosocial factors associated with functional dyspepsia

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

Introduction: Functional dyspepsia (FD) is a common gastrointestinal (GI) disorder with persistent and recurring symptoms like abdominal pain, bloating, early satiety, and epigastric burning. To make a diagnosis of FD, doctors must exclude any pathological changes in the upper GI system mucosa. FD is more common in women, affecting around 20-40% of the Western world's population, and is less prevalent in older people. FD is closely linked to psychological factors like anxiety, depression, and chronic stress, due to connections between the brain and GI system known as the Gut-Brain Axis. The gut microbiome plays a mediating role in this network, which explains the association of FD with psychological disorders and other chronic conditions.

Aims: To determine the age-dependent distribution of males and females and their relationship in a sample of patients with FD examined so far. The study aims to identify the main GI and non-GI comorbidity associated with FD.

Participants and Methods: The study included adults (18+) referred by their family doctors for initial endoscopic examination due to dyspeptic disorders. The preliminary analysis was conducted on the sample of 59 examinees out of the planned 200. Persons with negative endoscopy results are considered to have FD, and further information is obtained through questioning.

Results: The sample includes 32 females and 27 males in three age groups. Most participants are aged 18-40, with equal sex distribution. As for mental disorders, 27% of the participants have anxiety and 6% have depression. There are more females than males with FD who have anxiety (ratio 1.3). Furthermore, 14 (22.4%) participants have other GI diseases, with Irritable Bowel Syndrome (IBS) being the most common (7 or 12.1%). In addition, a significant part of participants

(37 or 59.3%) has non-GI comorbidities, including cardio-metabolic diseases and hypertension, then allergies, and other immunologically mediated diseases.

Conclusion: The survey on FD has revealed some interesting results. It showed more females than males with the condition, along with a significant presence of young people and individuals with non-GI comorbidities and mental disorders. Females with FD are more susceptible to anxiety disorders. These insights can assist in targeted treatment and management of FD.

MeSH/Keywords: Dyspepsia; Functional dyspepsia; Gastrointestinal endoscopy; Stress, Physiological;
Comorbidity

Dissertation proposal tittle: Electrical activity of the upper leg musculature in three-dimensional space during physical exercise

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Introduction: Local muscle fatigue is a complex phenomenon, representing a combination of metabolic, structural and energetic changes in the working muscle itself. Continuous monitoring of local muscle fatigue can be easily demonstrated by using superficial electrography since biochemical and physiological changes during muscle fatigue are reflected and can be recorded as changes in electric signal above the skin surface of the observed working muscles.

Hypothesis: The hypothesis of the proposed research is that a method of detecting muscle fatigue in real-time can be developed by recording the exercise-induced electrical activity of the upper leg musculature in high resolution. The exercise is expected to induce electrically measurable changes in the coordinated work of individual muscles as well as changes in their activity.

Aims: The main goal of the research is to measure the electrical field that develops and changes during the contraction of the thigh muscles to determine the characteristics of changes in electrical activity during exercise, depending on age, gender and training.

Materials/ participants and methods: The research will be conducted on a group of thirty healthy active rowers, male and female. Exposure to 200 Won rowing erg will last 300 seconds per subject.

Research plan: In the first year, subjects will be included in the study, and data will be determined. The following year is planned for statistical processing, writing and publishing a scientific paper and writing of the doctoral thesis.

Significance / expected scientific contribution: We hope that upon the completion of the investigation of the electrical activity of the muscles during exertion data obtained by the surface electrographic measurements will be used to gain new insights into the occurrence of skeletal muscle fatigue.

MeSH / Keywords: effort, thigh muscles, electrophysiology, rowing

Abstract Title: Antimicrobial and antibiofilm properties of melittin and nisin

Part of the Dissertation Proposal: "*In vitro* evaluation of effect of surfactin and antimicrobial peptides on biofilm-forming ability"

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Introduction: Biofilms are complex communities of microorganisms that adhere to various surfaces and produce a protective matrix of polysaccharides, proteins, and DNA. One of the major challenges of biofilm infections is their increased resistance to antibiotics and they also pose a serious threat to public health. Therefore, there is an urgent need for novel strategies to prevent and treat biofilm infections, such as antimicrobial peptides.

Aims: To evaluate minimum inhibitory concentrations and antibiofilm effects of melittin and nisin on different ATCC strains of bacteria and yeasts.

Materials and Methods: The minimum inhibitory concentration (MIC) of melittin and nisin was determined by the two-fold serial dilution method in Mueller Hinton broth and microtiter plates with 96 wells. Stock solutions of melittin and nisin were prepared, two-fold serial dilution was made in microtiter plates, and bacterial inoculum was added. The microtiter plates were incubated overnight and after incubation the MIC was observed. MIC represents the lowest concentration of the tested substance that inhibited the visible growth of bacteria.
The effect of melittin and nisin on biofilm formation was tested using the co-incubation method. Two-fold serial dilutions of the tested substance were prepared again, and bacterial inoculum was added. After overnight incubation, the rest of the procedure was carried out by modifying the method described by Stepanović et al. The plates were washed with phosphate buffer saline (PBS), then 0.2% crystal violet was added, and after 15 minutes they were washed again with PBS. Solubilization was performed with 96% ethanol. The absorption of the solution was measured with a spectrophotometer at 570 nm.

Results: MIC of nisin was detected for five Gram-positive strains (1 – 16 µg/ml) as well as for *A. baumannii* (32 µg/ml). Biofilm reduction was observed for Gram-positive strains, *A. baumannii*, *K. pneumoniae* and *P. aeruginosa*. Surprisingly, *C. albicans* showed slight biofilm induction.
MIC of melittin was 4 – 8 µg/ml for Gram-positive strains and 16 – 64 µg/ml for Gram-negative strains, except for *P. aeruginosa* (>256 µg/ml). *C. albicans* was also susceptible to melittin (MIC 32 µg/ml). Biofilm reduction was observed for all tested strains.

Conclusion: This study shows that both melittin and nisin have antimicrobial and antibiofilm properties. The antimicrobial spectrum of nisin was oriented against Gram-positive bacteria while melittin was effective against Gram-positive and Gram-negative bacteria, as well as *C. albicans*. The antibiofilm spectrum of both molecules was slightly wider. A combination of these two molecules could be even more potent and grant further investigation.

MeSH / Keywords: antimicrobial peptides, biofilms, melittin, microbial sensitivity tests, nisin

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

Željka Dujmić, MSN	Assist. Prof. Štefica Mikšić, Ph.D., MSN, RN	Personality traits and shift work as predictors of circadian rhythm disorders and attention disorders in nurses in a hospital setting	
Hrvoje Falak, M.D.	Prof. Goran Krstačić, M.D., Ph.D.	Changes of lysophosphatidylcholine concentrations in patients treated with aortic valve replacement	
Barbara Grubišić, M.D.	Assoc. Prof. Ines Bilić Ćurčić, M.D., Ph.D.	Impact of SARS-CoV-2 virus infection on acute and long-term disruption of glycemic control	
Lea Gvozdanović	Assoc. Prof. Višnja Adam Nesek	Diagnostic accuracy of the biological indicators leucine-rich alpha 2-glycoprotein and calprotectin in patients with suspected acute appendicitis	
Matea Hudolin, M.D.	Assist. Prof. Hrvoje Budincevic, M.D., Ph.D.	The role of socioeconomic factors and LDL-levels on suicidal ideation of patients with multiple sclerosis	
Petra Jakšić M.D.	Assist. Prof. Robert Šeparović M.D., Ph.D.	Influence of genotype variants HER2/neu gene on treatment outcomes in early breast cancer with monoclonal antibody trastuzumab	
Ivana Jelinčić, MsN	Prof. Dunja Degmečić, M.D., Ph.D.	The connection between personality traits, anxiety and styles of coping with stress in nurses and nursing students and their influence on professional self-concept and conflict resolution methods	
Tihomir Jovanović, MSN	Prof. Štefica Mikšić, Ph.D., MSN, RN	The Association between Quality of Life and Body Mass Index with Patient Anxiety on Hemodialysis	
Farah Khaznadar, M.Pharm.	Prof. Martina Smolić, M.D., Ph.D.	The association of Sodium-Glucose Cotransporter 2 Inhibitors therapy with elastographic and molecular markers of liver injury in patients with Type 2 Diabetes Mellitus	
Darko Kotromanović	Prof. Robert Smolić, Ph.D.	Effect of treatment of non-metastatic colorectal cancer on differences in glycosylation of Immunoglobulin G	
Marinela Krištić, M.D.	Assist. Prof. Ivan Miškulin, M.D., Ph.D.	The influence of the coronavirus disease severity on the sex life of sexually active adults in Croatia	
Tomislav Kurevija, M.D.	Assoc. Prof. Silvija Canecki-Varžić, M.D., Ph.D.	Prescribing new cardioprotective antidiabetics in Croatia from a family medicine perspective	

Vanda Leipold	Assoc. Prof. Hrvoje Brkić	Optimizing choice of skin surrogates for surface guided stereotactic body radiotherapy of lower lung lesions using four-dimensional computed tomography	
Maja Lovrić	Assoc. Prof. Silvija Pušeljić, M.D., Ph.D.	Cognitive behavioral interventions in managing anxiety and depression in adolescents with type 1 diabetes mellitus	
Marin Mamić	Assist. Prof. Štefica Mikšić, MsN, PhD.	Preliminary research results on predictors of patient and nurse satisfaction as indicators of the process in healthcare quality management system	
Romana Marušić M.D.	Assist. Prof. Saška Marczi	Influence of *SLCO1B1* gene polymorphism and vitamin D concentration on the required dose of statins to achieve the target value of LDL-cholesterol and on side effects of statins in postmenopausal women	
Domagoj Mišković, M.D.	Prof. Irzal Hadžibegović, M.D., Ph.D.	Alterations of plasma proteins' N-glycosylation under LDL-cholesterol optimization with atorvastatin therapy after percutaneous coronary intervention in acute coronary syndrome	
Vilim Molnar, M.D.	Prof. Dragan Primorac, M.D., Ph.D.	Clinical Results of Knee Osteoarthritis Treatment with Autologous Microfragmented Adipose Tissue Containing Mesenchymal Stem Cells Compared to Hyaluronic Acid	
Tihana Nađ, M.D.	Assoc. Prof. Ivana Jukić, M.D., Ph.D.	Effect of consumption of chicken meat enriched with omega-3 polyunsaturated fatty acids on microvascular function	
Marija Olujić, M.D.	Prof. Katarina Dodig-Ćurković, M.D., Ph.D.	Correlation of open-angle glaucoma of different degrees of damage with the occurrence of anxiety and depression	
Eduard Stjepan Pavelić	Prof. Dragan Primorac, M.D., Ph.D.	Comparison of the Clinical Effects of Leukocyte-Poor and Leukocyte-Rich Platelet-Rich Plasma in the Treatment of Knee Osteoarthritis	
Sanja Pejić Roško	Prof. Andrea Cvitković Roić, M.D., Ph.D.	Treatment of neurogenic bladder in children using non-invasive neuromodulation	
Luka Perić	Prof. Martina Smolić, M.D., Ph.D.	Association of glycosylation changes with interpatient variability in the frequency and intensity of adverse effects in breast cancer patients treated with aromatase inhibitors or selective estrogen receptor modulators	

Nora Pušeljić	Prof. Marija Heffer, Ph.D.	Homocysteine levels as early indicators of cobalamin deficiency in infancy	
Petra Puž Britvić	Assoc. Prof. Ivan Miškulin, Ph.D.	Knowledge about inflammatory bowel diseases and therapy is an important predictive factor of therapeutic adherence	
Zurap Raifi	Asst. Prof. Sanja Tomasović, M.D.	Connection of oral rimegepant use with middle cerebral arteries vasoreactivity in patients with episodic migraine	
Matej Rezo, M.D.	Prof. Andrijana Včeva, M.D., Ph.D.	Influence of virtual reality vestibular rehabilitation on cognitive functions and vestibular recovery in patients with unilateral vestibular impairment	
Marko Sablić, M.D.	Assoc. Prof. Antonio Kokot, M.D., Ph.D.	BPC 157 treatment attenuates occlusion-like syndrome in rat glaucoma model with three cauterized episcleral veins	
Petra Sertić	Robert Šeparović, M.D., Ph.D.	Are HER2 gene single nucleotide polymorphisms associated with trastuzumab-induced cardiotoxicity?	
Luka Švitek	Asst. Prof. Sanja Mandić, Ph.D.	Prognostic significance of GDF-15 levels and other laboratory findings in hospitalized COVID-19 patients	
Dora Uršić, M.D.	Assoc. Prof. Aleksandar Kibel, M.D., Ph.D.	Effect of consumption of chicken meat enriched with carnosine on microvascular function in patients with chronic coronary artery disease	
Matea Vinković, M.D.	Assist. Prof. Irena Jukić, M.D., Ph.D.	Association of donor-related factors with aggregate occurrence in apheresis-derived platelet concentrates	
Mile Volarić, M.D.	Prof. Ljiljana Trtica Majnarić, M.D. Ph.D.	Sex and age of patients with functional dyspepsia and associated comorbidities	
Marko Zelenić	Prof. Sven Kurbel M.D., Ph.D.	Electrical activity of the upper leg musculature in three-dimensional space during physical exercise	
Marko Živkov, M.D.	Assist. prof. Maja Bogdan, M.D., Ph.D.	Antimicrobial and antibiofilm properties of melittin and nisin	