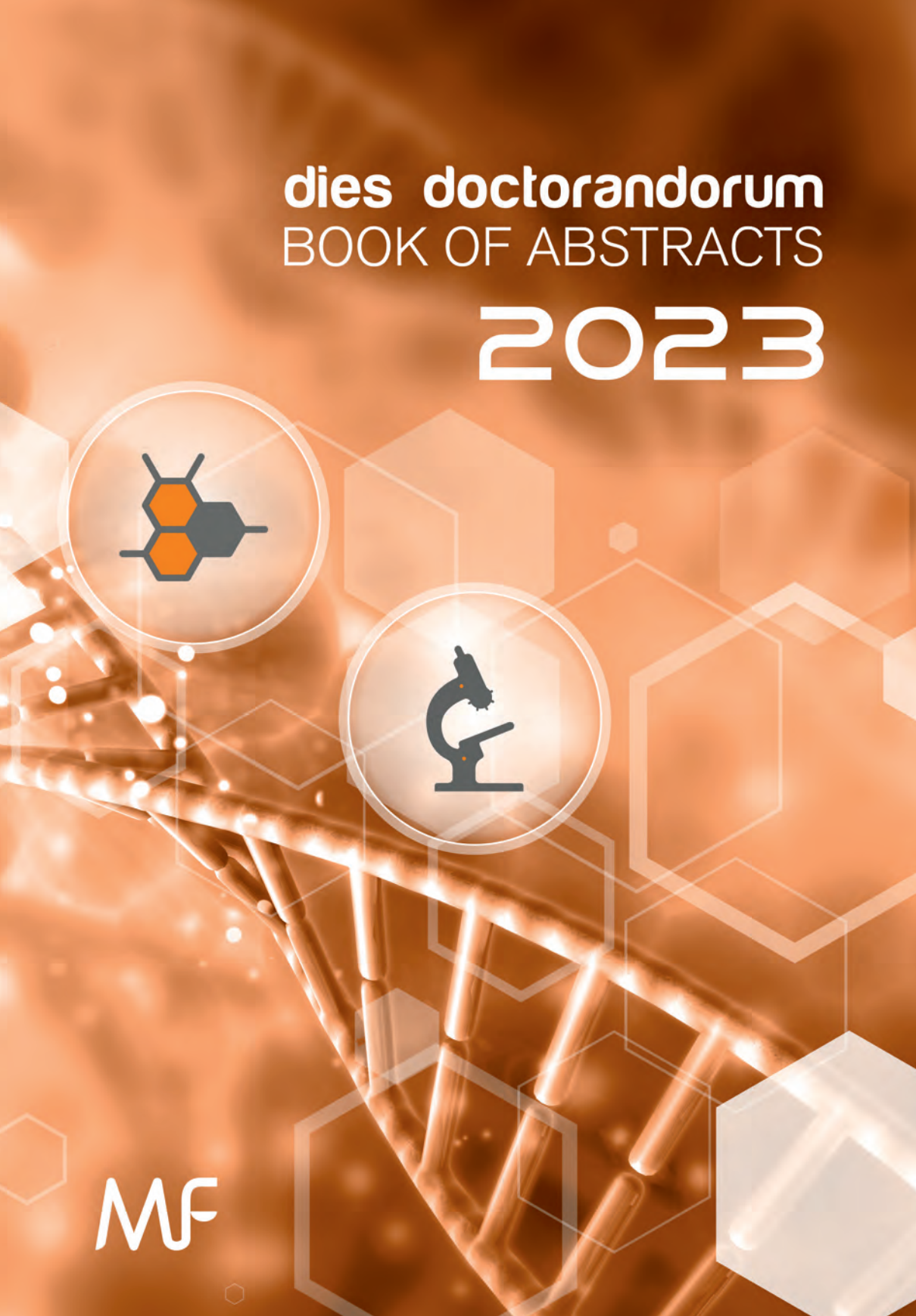
dies doctorandorum
BOOK OF ABSTRACTS
2023



UNIVERSITY OF OSIJEK, FACULTY OF MEDICINE OSIJEK

POSTGRADUATE DOCTORAL STUDY

OF BIOMEDICINE AND HEALTH

DIES DOCTORANDORUM 2023.

BOOK OF ABSTRACTS

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Foreword

Dies doctorandorum is an annual and traditional event that we will be hosting live again in 2023. This is an open and public event that welcomes all scientific community memebers, with a focus on Ph.D. students presenting their research work for discussion and debate.

The event is intended to present students' Ph.D. research projects, provide constructive feedback, and steer research in the appropriate direction enabling students to complete their postdoctoral studies on time. All members of the scientific community are invited to attend to exchange experiences and provide guidance to young colleagues at the beginning of their scientific careers. Moreover, our goal is to promote social interactions and strengthen collaboration between mentors and Ph.D. students, thus advancing the scientific community and the postdoctoral study' program itself.

Being a part of a community is also a crucial aspect of a scientific career. Interacting with fellow scholars allows scientists to stay updated and contribute to their field of research. It is often said that smaller conferences, such as ours, are where one learns more and has more rewarding relationships. It is also conceivable that senior scientist attending this event will be requested to provide letters for promotions and tenure decisions of younger collegues.

In addition, Ph.D. students' progress will be evaluated based on poster and oral presentations. As a result, the best poster presentations will be chosen and granted the Dean's Award by members of the Committee for Doctoral Studies.

To conclude, I would like to emphasize the importance of investigating relevant scientific problems, both exploiting and exploring research, and contributing 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: Association of treatment with sodium-glucose cotransporter 2 inhibitors and endothelial dysfunction in patients with diabetes mellitus type 2

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

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

Introduction: SGLT2 inhibitors are a novel therapeutic option for treating type 2 diabetes mellitus. This class of antidiabetic drugs has been shown to reduce the composite of cardiovascular mortality and heart failure hospitalizations in patients with heart failure, as demonstrated in multiple cardiovascular outcome trials. The exact mechanism behind their beneficial cardiovascular impact has not been fully elucidated. However, one of the proposed cardioprotective mechanisms is the positive effect of SGLT inhibitors on endothelial dysfunction in diabetic patients.

Hypothesis: SGLT2 inhibitors have been associated with a reduction in biomarkers of endothelial dysfunction in peripheral venous blood among diabetic patients.

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

Materials/Participants and Methods: This study includes 46 male and female patients who have started therapy with SGLT2 inhibitors at the Department of Diabetes, Endocrinology and Metabolism Disorders of Osijek University Hospital Centre in 2023 in accordance with current treatment guidelines for diabetic patients.

The exclusion criteria for this study include insulin therapy, GLP-1 agonists, macrovascular complications of diabetic disease such as acute myocardial infarction, stroke, and known significant stenosis of coronary or peripheral arteries, heart failure (NYHA class III and IV), malignancy, systemic inflammatory disease, kidney failure, and type 1 diabetes.

Serum markers of endothelial dysfunction and oxidative stress, including CRP, oxLDL, TNFα, PAI-1, vWF, IL-6, E-selectin, and VCAM-1, will be measured before and six months after the initiation of therapy.

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

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

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

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 of 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:

1. 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
2. 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
3. To determine whether there is a difference in the expression of studied miRNAs after PRP administration
4. 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 60 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: Effects of ketogenic diet on oxysterols synthesis in adult male and female C57black/6 mice

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Mentor: Assist. Prof. Božidar Muršić, M.D., Ph.D., Department of Neurosurgery, University Hospital Centre Osijek, Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Introduction: Ketogenic diet, based on a low carbohydrate and high fat intake, has been studied for its potential benefits in treatment of various diseases, among which are brain neoplasms. It is well known that this type of diet has its impact on cholesterol synthesis, while the influence on oxysterols synthesis is poorly understood.

Hypothesis: Ketogenic diet application in C57BL/6 mice will result in a significant change in oxysterol synthesis, tissue-specific lipid profile and expression of drug efflux pumps when compared to the control group of C57BL/6 mice on standard diet.

Aims:

1. To determine the differences in oxysterols synthesis in brain, kidney and hepatic tissue.
2. To measure and compare mitochondrial membrane potential, fusion and fission of targeted tissue under two dietary regimes using fluorescent dyes.
3. To perform a lipidomic analysis of targeted tissue samples using MALDI-TOF mass spectrometry.
4. To perform immunohistochemical analysis of targeted drug efflux pumps in brain, kidney and hepatic tissue.

Materials and Methods: Experiments will be carried out on 3 months old C57BL/6 mice divided into two groups, with equal sex distribution; the first group will be fed with ketogenic diet and the control group will receive standard diet. Behavioral tests and biochemical measurements of glucose and ketone bodies levels will be performed. After 3 months animals will be sacrificed and targeted tissues, which include brain, kidney and liver, will be isolated. Sterols will be extracted with PTAD (4-phenyl-1,2,4-triazoline-3,5-dione) derivatization procedure and further lipidomics analysis will be performed using mass spectrometry imaging MALDI-TOF. For measurement of mitochondrial membrane potential TMRM fluorescent dye will be used, and fusion/

fission will be determined using MitoTracker. Immunohistochemical analysis will be targeted toward multidrug resistance pumps.

Research plan: There will be 40 animals included in the study, 20 mice will be fed with ketogenic diet, while 20 mice will be on standard diet. The impact of diet will be tested using behavioral tests and blood sample analysis. Animals will be sacrificed after 3 months and further analysis will be performed on brain, kidney and hepatic tissue. Lipidomics and immunohistochemical analysis will be used for further investigation.

Expected scientific contribution: Given that the keto diet is proposed as an adjuvant therapy for brain neoplasms treatment, this research aims to determine sex specific diet-induced changes in brain, kidney and hepatic tissue which are important determinants of system adjustments before cytostatic application.

Keywords: oxysterols, mice, lipids, lipidomics, brain

Disertation Proposal Title: Metabolic characterization of follicular adenoma and follicular carcinoma of the thyroid gland by MALDI MSI

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Mentor: Prof. Andrijana Včeva M.D., Ph.D., University department of Otolaryngology, University Hospital Centre Osijek, Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Co Mentor: Assist. prof. Željko Zubčić, M.D., Ph.D., University department of Otolaryngology, University Hospital Centre Osijek, Osijek, Croatia

Introduction: Thyroid neoplasms are common in general population and appropriate preoperative workup is required to make adequate decision about further treatment options. In most of the cases partial (lobectomy, ishtmectomy) or total (total thyreoidectomy) surgical removal of thyroid parenchime is performed. Fine needle aspiration (FNA) combined with scintigraphy, ultrasound (US) imaging and hormone assay are most commonly used to define surgery extent. Aforementioned diagnostic methods are imperfect in detecting malignant cells and a number of patients undergo unnecessary surgery. One of the biggest problems in patohistologic diagnostic is to differentiate follicular thyroid adenoma (FTA) from follicular thyroid carcinoma (FTC). Previous studies used DESI MS- Desorption electrospray ionization mass spectrometry technology and did confim difference in metabolic characteristic between FTA and FTC. FTA showed prominent fatty acids (FA), phosphatidylserine (PS), phosphatidylglycerol (PG). FTC is metabolically described with phosphatidylinositol (PI), ceramide (CER), phosphati-dylethanolamine (PE) and several other metabolites, including succinate and malate. In this study we will perform metabolic characterization of FTA and FTC using matrix assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI) technology.

Hypothesis: Metabolic imprint of FTC differ from benign thyroid lesion (FTA), and MALDI MSI technology can provide useful informations in diagnostics of such pathologies on patohistologic samples with potential of becoming a standardized diagnostic tool.

Aims:

1. To describe metabolic differencies between FTA and FTC using MALDI MSI
2. To evaluate results of MALDI MSI in comparison to patohistologic findings

Materials/Participants and Methods: The study will include all patients within one year with suspect follicular lesions showed on preoperative FNA (standard indication for surgical treatment) and patients with patohistological findings of FTA and FTC after the surgery is performed. Metabolic analysis will be obtained using MALDI MSI imaging.

Research plan: A prospective study with no additional cost for patients. Estimated study duration is 12 months.

Expected scientific contribution: Better understanding of metabolic charateristics of follicular thyroid lesions, understanding the metabolic differencies between FTA and FTC which could lead to routine use of MALDI MSI in diagnosing of follicular thyroid lesions in comparisson to standard patohistological examination. Laying ground for further metabolic studies.

Keywords: thyroid, adenoma, carcinoma, MALDI MS, metabolic

Dissertation Proposal Title: Application of liquid biopsy as a diagnostic and prognostic factor in patients with colorectal cancer

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

Introduction: The incidence of colorectal cancer is on the rise, especially in young men, and ranks third for both sexes, with mortality on second. The stage of spread of the disease in time of diagnosis is the most important factor in survival, so the five-year survival of patients in stage I is 70-90%, and in stage IV 10-14%. In developed countries, the five-year survival rate is 65%. The diagnosis of colorectal cancer is made by radiological, endoscopic and laboratory methods, which are carried out in terms of prevention and screening. The gold standard for establishing the diagnosis is a colonoscopy with sampling, while the MSCT determines the extent of the disease. Among the biomarkers used are carcinoembryonic antigen (CEA) and CA 19-9, Ca 50 and CA 195. The most commonly used - CEA has a low sensitivity - 46% and a high specificity (89%) so it is used for monitoring and prognosis. Liquid biopsy is a minimally invasive method that isolates circulating tumor cells (CTC), circulating DNA, exosomes and microRNA (miRNA) from various body fluids (blood, urine, cerebrospinal fluid). MiRNAs are small, single-stranded non-coding RNAs consisting of 20-22 nucleotides that regulate the expression of oncogenes and tumor suppressor genes. Impaired miRNA expression affects different stages of colorectal cancer oncogenesis. MiRNAs are found in tissue fluids (plasma, serum, feces, urine) and are resistant to the action of RNAases because they are found in exosomes or are bound to lipoproteins and in that form they are stable. MiRNA clusters (a set of several miRNAs) can be used as diagnostic biomarkers (differentiating adenoma from CRC) but also as biomarkers in high-risk stratification. The research would include patients with newly diagnosed colorectal cancer, patients with verified adenomas with severe dysplasia and a control group. The control group will be patients from the National Colorectal Cancer Screening Program in whom we found no colorectal pathology. After endoscopic detection of colorectal carcinoma/polyps, patients would sign an informed consent form to participate in the study.

Hypothesis: Combinations of certain miRNAs obtained by liquid biopsy can be used as biomarkers in the diagnosis of colorectal cancer

Aims:

1. Determination of the association between miRNA and pathohistological prognostic factors
2. To determine whether there is an association between miRNA and clinical staging of patients with CRC
3. Determine whether miRNA is present in pre-CRC, tubular adenomas with severe dysplasia
4. Determine if there is a change in miRNA after treatment, post-surgery or chemotherapy
5. Determine whether miRNA (certain clusters) can be used as an initial, independent diagnosis
6. Determine whether miRNA (specific clusters) can be used as an independent prognostic factor

Materials and Methods: The study was designed as a prospective clinical cohort study. The study will include 150 participants divided into three groups: patients with colorectal cancer, with adenomas and a control group. After endoscopic confirmation of colorectal carcinoma/polyp, the patient would sign an informed consent form to participate in the study. After endoscopic confirmation of CRC and awaiting pathohistological confirmation of the tumor, standard laboratory and radiology procedures would be performed for staging purposes and blood would be collected for miRNA analysis. After sample collection, the sample will be centrifuged in a test tube with EDTA (ethylene diamino tetraacetic acid) at 1900 g/10 min in a "swing-out" procedure within 1-2 hours, then the plasma will be separated and subjected to a second centrifugation at 3000 g/15 min. The plasma is then aliquoted and stored at - 80°C (-65 to -90°C). MiRNA is isolated in a test tube containing EDTA. MiRNA will be isolated using a commercial assay package (miRNeasy Serum/Plasma Advanced Kit) from the manufacturer Qiagen. MiRNA expression is determined using the quantitative chain reaction (qPCR) method - miRCury LNA miRNA PCR system manufactured by Qiagen. The presence of certain miRNAs is compared with the pathohistological and immunohistochemical features of the tumor and the clinical stage of the patient to determine the prognosis of the disease.

Research plan:

1. Screening of patients for pathology (CRC or adenoma)
2. Laboratory analysis of specimens
3. Statistical analysis
4. Publication of the results

Significance/Expected scientific contribution: The analysis of specific miRNAs and their clusters characteristic of colorectal cancer opens the field of targets for future drug development. If we could predict the course of the disease progression at the onset and tailor therapy to each patient, this would greatly improve treatment outcomes.

MESH Keywords: colon neoplasms, miRNAs, prognosis, diagnosis

Acknowledgements: I would like to thank my mentor Prof. Jasenka Wagner Kostadinović. Ph.D.

Association of galectin-3 and presence of significant atherosclerotic epicardial artery disease in patients with chronic coronary syndrome

Part of the Disertation Proposal: Use of cardiac biomarkers in the assessment of the significance of stable atherosclerotic coronary disease

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

Introduction: Cardiovascular diseases (CVD) remains a public health problem and one of the leading causes of death worldwide. Cardiac biomarkers, troponin (Tn), N-terminal pro-brain natriuretic peptide (NTproBNP), C-reactive proteine (CRP), and galectin-3 (Gal-3), are well-known tool in diagnostics of heart disease and in assessment of its outcome. The hypothesis is that these biomarkers have predictive value in evaluation the severity of stable coronary heart disease and correlate with its extensiveness. Gal-3 is one of numerous molecules involved as a mediator in process of atherosclerosis, triggering and enhancing this inflammatory process. Gal-3 assumes the promoter function of monocyte/macrophage chemoattraction, opsonization of apoptotic neutrophils and degranulation of mast cells. The question arises whether measured serum Gal-3 levels are related to the extent and severity of atherosclerotic disease or could be a possible predictor of future serious adverse events (MACE).

Aims: This study aimed to investigate association between serum Gal-3 levels and the presence of significant atherosclerotic epicardial artery disease in patients with stable coronary artery disease (CAD) and to assess whether serum Gal-3 levels can be used as a biomarker for major advance cardiac events (MACE) risk stratification in patients with stable coronary artery disease.

Materials/Participants and Methods: The study included 168 subjects with suspected CAD and indications for coronary angiography divided into three groups: PCI group (N=64), CABG group (N=57), and group with normal findings (control group, N=47). Gal-3 levels were measured by enzyme immunoassay (EIA).

Results: The mean value of Gal-3 in the study group was 19.98 ng/ml, while in the control group it was 9.51 ng/ml ($t=9.075$, $p < 0.001$). There was no statistically

significant difference in the levels of Gal-3 between the PCI and CABG groups, 18.84 and 21.27 ng/ml respectively (t=-1,402, p=0,164). Highest value of Gal-3 was found in the group of subjects with three-vessel disease (t=-3.652b, p<0.001).

Conclusion: Gal-3 has the potential to be a reliable marker for assessing the presence of significant CAD as well as a predictor of adverse cardiovascular events.

MeSH/Keywords: galectin-3, coronary artery disease, biomarker, chronic coronary syndrome, stable coronary artery disease

Abstract Title: Comparison of absorbed doses in the brain and other organs at risk in 116 single-fraction stereotactic radiosurgery treatment plans for high-definition multi-leaf and cone collimator on a Varian Edge linear accelerator.

Part of the Disertation Proposal: Comparison of absorbed dose to the brain in radiosurgery with multileaf collimator and conical collimator on linear eccelerator Varian EDGE

PhD candidate: Adlan Čehobašič, Special hospital Radiochirurgia Zagreb, Zagreb, Croatia

Mentor: Assist. Prof. Mladen Kasabašić, Department of physics and medical physics, Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Introduction: Brain metastases, traditionally seen as a terminal phase of cancer, generally affect 10-40% of patients with malignancies. Despite their poor prognosis, treatment modalities and technological advances have led to improved clinical outcomes for patients with brain metastases. Stereotactic radiosurgery (SRS) is a form of radiation therapy that involves delivering high doses of focused radiation to small, well-defined structures (lesions, target volumes) using tight beams of ionizing radiation such as X-rays, gamma rays, or protons. SRS aims to halt the proliferation of benign tumors or permanently destroy tumor tissue without an invasive approach while minimizing damage to surrounding healthy tissue. SRS can be administered in a single session or over a series of up to five fractions. SRS of brain metastases has been shown to achieve 85-95% local control at one year, with low mortality and average survival rates comparable to surgical treatment but at lower costs due to the shorter duration and fewer required hospitalization periods.

Radionecrosis can present itself with diverse symptoms, such as brain inflammation and swelling, seizures, alterations in cognition, and changes in motor function or coordination. Critical doses for developing radionecrosis are generally considered 10-12 Gy in a single fraction. To minimize the risk of side effects, it is important to achieve high-dose conformality, reducing the volume of healthy tissue receiving high doses of radiation.

Linear accelerator's photon beams can be focused using one or two sets of jaws and shaped using either a multileaf collimator (MLC) or conical collimator (CC). The MLC shapes the irradiation field using movable tungsten leaves, allowing it to conform

to the target volume (PTV) shape and reduce the dose to healthy tissue. Conversely, CC consists of a block with fixed conical shapes (cones) that are mechanically stable, generate less scatter compared to MLCs, and have a steeper dose gradient. Cones are preferred for irradiating small lesions due to three main reasons

1. The physical penumbra is the primary determinant of the steepness of the dose gradient in radiation therapy. It results from three independent sources: transmission, geometry, and scatter, which are influenced by factors such as the collimating system, scattering effects, and distance from the source to the target. These factors all contribute to the overall shape and intensity of the radiation beam. they provide a steeper penumbra and radiation dose fall off, thus better protecting OARs
2. they are mechanically more stable than MLCs, which can deviate from their desired position during leaf movements
3. small MLC fields may result in inaccurate dose calculations.

However, both MLC and CC have limitations. The mechanical stability of MLC is not the sole limitation of their use in radiation therapy. Additionally, modeling MLC and calculating doses can be challenging in treatment planning systems (TPS). For example, the minimum field size that can be calculated in Eclipse (Varian Medical Systems, Palo Alto, California) is 1 x 1 cm, which may not meet clinical standards for dose calculation. The smallest field size that can be imported into Varian Eclipse Beam Configuration is 2 x 2 cm for percent depth dose and profile curves. However, the output factor table can be populated for 1 x 1 cm field sizes. Modifying the effective target spot size could improve agreement for smaller field sizes.

The Eclipse Cone Dose Calculation (ECDC) algorithm utilizes three measurements: tissue-maximum ratio, off-axis ratio, and cone output factor. However, the ECDC algorithm has several limitations. It does not account for tissue heterogeneity, assumes that the beam axis is normal to the patient surface (without correction for oblique beam incidence), and does not consider backscatter. Additionally, the off-axis ratio is independent of depth, and arc beams are approximated as evenly distributed static fields. To calculate the average tissue-maximum ratio for the arc, the ECDC draws static beams at user-specified arc increments and then averages the tissue-maximum ratio from all of these beams to determine an average value for dosimetry calculations.

While the MLC is a standard component in linear accelerator systems, the CC is an expensive optional accessory purchased separately. The comparison of MLC and CC is a well-established topic in the literature, with numerous studies providing different perspectives on using CC in radiation therapy.

Despite the abundance of literature on the topic, a comprehensive understanding of the actual benefits of CC in clinical practice still needs to be discovered. To address this knowledge gap, we conducted a retrospective study of 116 metastases cases to compare CC to HD120 MLC (min. leaf width = 0.25 cm) in terms of treatment planning dose distributions outside of traget volumes and sparring organs at risk. The results of this study will provide valuable insights into the clinical advantages and disadvantages of CC and contribute to a more informed understanding of its value in radiotherapy practice.

Aims:

1. To compare radiation doses outside the target volumes in the treatment planning system for brain metastases. Specifically, to identify which collimator can provide fewer radiation doses around them for the same or similar treatment geometry
2. To compare the absorbed radiation doses in the brain for equal planning target volume coverage and prescription irradiation dose to determine if there is a statistically significant difference between the unnecessary radiation doses given to the brain as the main organ at risk
3. To determine whether there is a statistically significant difference in the absorbed radiation doses in other organs at risk for equal planning target volume coverage and prescription radiation dose

Materials/Participants and Methods: From the Aria© system (Varian Medical Systems, Palo Alto, California), 116 brain metastases were selected for patients who received SRS at Specijalna Bolnica Radiochirurgia Zagreb between 2017 and 2021. Brain metastases were contoured on 1 mm CT slice thickness as Clinical Target Volume (CTV), and a 1 mm isotropic margin was added for creating PTV. The selection criteria included PTV volumes between 0.1 and 6.5 cm3, a PTV shape as close to a sphere as possible, and no overlap with organs at risk (OARs) such as the optical pathway, chiasm, and brainstem. The PTVs were divided into four groups based on volume: group 1: 0.1-0.5 cm3, group 2: 0.6-0.9 cm3, group 3: 1.0-2.5 cm3, group 4: 2.6-6.5 cm3. The numbers of PTVs in groups were: 35 for group 1, 35 for group 2, 35 for group 3, and 11 for group 4.

The study employed the following planning parameters: a 6X FFF energy, RapidArc (VMAT) technique, a single fraction, and normalization of 25 Gy covering 99.5% of the planning target volume (PTV) for groups 1-2, and 25 Gy covering 95% of the PTV for groups 3-4.

Treatment plans utilizing a HD120 MLC were based on a single isocenter, while plans using CC employed one to ten isocenters. The single isocenter arcs for any collimator comprised one full arc with a couch angle of 0 and nine partial arcs with identical

start and stop angles and couch rotations. The multi-isocentric arcs, specific to the CC plans, were composed of four non-coplanar partial arcs individually selected for each isocenter.

The CC's planning module was set with arc resolution of 1 degree, a dose matrix resolution of 1 mm and a slice interval of 1 mm were used for optimization. Final dose calculations were performed using an arc angle resolution of 1 degree, dose matrix resolution of 0.6 mm, and dose slice interval of 0.6 mm.

The comparison of plans involved the evaluation of the following parameters for 5Gy and 10Gy brain dose volumes, and maximum dose for organs at risk (OARs).

Results: The advantage of CC in sparing brain tissue was slight for group 1, while HD120 MLC provided better results for the other groups. Statistical analysis showed no significant differences in datasets across groups 2-4, except for the 10 Gy volume in group 3. In HD120 MLC plans, the V_{5Gy} was lower by 0.92 cm^3 for group 2, 3.28 cm^3 for group 3, and 6.59 cm^3 for group 4. Similarly, the V_{10Gy} was lower by 0.04 cm^3, 1.27 cm^3, and 0.47 cm^3 for groups 2, 3, and 4, respectively. In group 1, statistical analysis revealed significant differences across the groups. The volume of brain tissue receiving 5 Gy ranged from 1.81 to 7.44 cm^3 with a mean value of 4.54 Gy for HD120 MLC, while for CC plans, the absorbed dose for the same radiation doses ranged from 1.13 to 6.78 cm^3 with a mean value of 3.2 cm^3. The volume of brain tissue receiving 10 Gy ranged from 0.62 to 2.72 cm^3 with a mean value of 1.63 cm^3 for HD120 MLC and 0.38 to 2.29 cm^3 with a mean value of 1.09 cm^3 for CC plans. The results indicate that the difference could be even higher for smaller volumes. However, these findings should be interpreted in light of previous research indicating that radiation doses of 10 Gy and 12 Gy are critical for potential radionecrosis, with no more than 10 cm^3 of brain tissue receiving these doses. Although the numerical differences between the collimators are significant, a 0.5 cm^3 higher dose absorbed in the brain in HD120 MLC plans should be an acceptable trade-off when considering the CC's imaging issues mentioned earlier.

Absorbed dose OARs differ from the trend of absorbed dose in the brain. OARs are located further away from the PTV and receive lower radiation doses. While this is favorable for sparing these organs, it poses a challenge for CC planning. The optimizer in the HD120 MLC plans can include numerous dose constraints to shape the dose fluence in the plan. However, optimizing the low radiation doses is difficult when the OARs are positioned far from the PTV. Very high-priority dose constraints must be placed in the optimizing software, which can violate the achieved radiation dose distributions inside and outside the PTV.

The results for doses absorbed by OARs varied between plans. While the statistical analysis showed statistically significant differences for both eyes, lens, and optic nerve on the right side, but it is hard to notice significant differences across organs except for a few outliers that influenced the statistics. Results are not following any trend, so interpreting the results solely on the numbers from Table 5 is difficult. For example, the brainstem received a lower dose in HD120 MLC plans, the right eye in CC plans, and the optic nerve in HD120 MLC plans. Based on the results obtained, it can be inferred that the steeper dose fall-off observed in CC is not a significant factor in sparing the OARs.

Conclusion: The conical collimator has been thought to allow steeper dose fall-off outside the PTV, potentially leading to better sparing of OARs. However, the results of this study indicate that this may not necessarily be the case for the Varian Edge linear accelerator. After planning 116 pairs of treatment plans for single-fraction heterogeneous doses, it was found that cones could produce results almost as good as HD120 MLC, and the true benefit of steeper dose fall-off is questionable. Regarding steeper dose fall-off, CC only shows slightly better results in dose distributions outside the PTV for PTV volumes below 0.5 cm^3. In these cases, cones do tend to spare the brain a little more than HD120 MLC, but other OARs do not show the same benefit. However, the question arises as to whether the differences of 0.3 cm^3 and 0.7 cm^3 for V10Gy and V5Gy are clinically significant for patient treatments. The incidence of radiation necrosis and swelling, among other side effects, increases with the size of the PTV. Therefore, large metastases are typically treated with 3-5 fractions instead of a single fraction. Given CC offers no advantage over HD120 MLC for larger lesions, CC will not be used. In cases with smaller lesions, where all dose constraints are well below critical values, the differences in the absorbed doses in the brain have no clinical benefit. The results of this study are showing that steeper dose fall-off plays small, almost insignificant role in brain metastases therapy for voumes below 0,5 cm^3, and that cones can generally give results almost as good as HD120 MLC.

MeSH/Keywords: stereotactic radiosurgery, treatment planning, radiation dose, organs at risk, linear accelerator

Adenosine A1 and A2a receptor protein expression in cerebral blood vessels of Sprague-Dawley rats exposed to hyperbaric oxygen

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Introduction: Hyperbaric oxygenation (HBO_2) affects gene and protein expression, modulate signaling pathways and influence vascular structure and function. This changes vasomotor responses that is of great importance in physiopathological processes. Adenosine receptors (AR) may play an important role in the mechanism of action of oxygen as a signaling molecule and vasoactive substance, and their expression may be affected by HBO_2.

Aim: Present study aimed to determine the protein expression of adenosine A1 and A2a receptors in cerebral blood vessels of Sprague-Dawley rats after exposure to acute and intermittent HBO_2.

Participants and Methods: Male and femal healthy Sprague-Dawley rats aged 8-10 weeks were divided into 3 groups: CTRL (control non HBO_2), A-HBO_2 (single animals exposed to HBO_2 for 2 hours) and 4D-HBO_2 (animals exposed to HBO_2 at a pressure od 2 bars for 2 hours daily for 4 days, with saple collection on the fifth day). Superficial brain blood vessels were collected to determine the protein expression of adenosine

A1 and A2a receptors using the Western blot method. All experimental procedures conformed to the European Guidelines for the Care and Use of Laboratory Animals (directive 86/609) and were approved by the local and national Ethical Committee (#2158-61-07-21-88; EP-348/2021).

Results: A1 receptor protein expression was significantly reduced in A-HBO_2 rats compared to 4D-HBO_2 (relative expression in relation to ß-actin 0,5865 +/- 0,1259 vs 0,9316 +/- 0,1152, p=0.0191). A-HBO_2 group had significantly reduced protein expression of A2a receptors compared to CTRL (0,2380 +/- 0,09028 vs 0,6833 +/- 0,1054, p<0.001) and 4D-HBO_2 (0,2380 +/- 0,09028 vs 0,7204 +/- 0,08244, p<0.001). 4D-HBO_2 group had a similar protein expression of both adenosine receptors as the CTRL group.

Conclusion: Acute HBO_2 significantly reduced protein expression of adenosine A1 and A2a receptors, whereas exposure to intermittent HBO2 render similar protein expression as in control. These results suggest that changes in adenosine receptor protein expression might be involved in the mechanisms through which HBO modifies vascular reactivity.

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Keywords: hyperbaric oxygenation, adenosine receptors, cerebral blood vessels, protein expression, Sprague-Dawley rats

COVID-19 / changes in methods and quality of life related to health

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

Introduction: The COVID-19 pandemic and the measures to prevent the spread of the virus have not only had a significant impact on health systems around the world but also on the lifestyle of the population, especially on eating habits and physical activity. The issue of vaccination amidst the COVID-19 pandemic has caused a rift among scientists, leading to a loss of trust from the general population and strengthening the influence of anti-vaxxers.

Aim:

1. to investigate the way of life and quality of life-related to health in subjects who had CVD and those who were not ill;
2. evaluate the factors that can influence the relationship between CVD and its consequences in the observed population

Materials and methods: Patients who tested positive for PCR in Virovitica-Podravina County were categorized into three groups: those who received treatment for COVID-19 in a hospital setting, those who received treatment at home, and those who tested negative for COVID-19. A questionnaire was used to collect sociodemographic, socioeconomic, and anamnestic information from all respondents.

Results: The study surveyed a total of 356 participants, consisting of 139 hospital-treated COVID-19 patients, 97 home-treated COVID-19 patients, and 120 respondents who tested negative for COVID-19. On average, hospitalized patients were 68 years old, weighed 93.8 kg, and comprised 70% of women, while home-treated patients were 42 years old, weighed 91 kg, and included 59% of women. COVID-19-negative respondents had an average age of 57 years, weighed 85 kg, and were 66% women. Of the participants, 51.1% of hospitalized patients, 60.8% of patients treated at home and 74.2% of COVID-19 negative subjects were vaccinated against COVID-19. Those who

declined vaccination accounted for 18.7% of hospitalized patients, 22.6% of home-treated patients, and 19% of COVID-19-negative respondents. Of those hospitalized, 64% showed a decrease in body weight, compared to 32% of home-treated patients, and 70% of COVID-19-negative respondents, who had no change in body weight. 73,7 % of hospitalized and 68.5% of home-treated patients, and 63.4% of COVID-19-negative respondents agreed or completely agreed with their religious beliefs.

Conclusion: COVID-19 infection notably impacts the quality and manner of one's life. Females are more numerous in both groups, and patients with higher body weight are more represented in the group treated in the hospital. Unvaccinated patients with COVID-19 exhibited a more severe manifestation of the disease and required hospitalization. Patients who received treatment in the hospital experienced more frequent weight loss than those treated at home or those who tested negative for COVID-19. The strength of religious belief was more pronounced among COVID-19 patients compared to COVID-19-negative respondents.

MeSH keywords: COVID-19 Virus Disease; Lifestyle Factors; Quality of Life; Religious Beliefs; Mental Health

Dissertation Proposal Title: Role of the Wnt signaling pathway in fatty acids induced fibrogenesis in a co-cultured model of hepatic fibrosis.

PhD candidate: Dominik Gjoni, M.D.

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

Introduction: Liver fibrosis represents a significant global health problem. Due to the fact that fibrosis progression leads to the development of cirrhosis (last stage of fibrosis) and liver cancer. Therefore, liver fibrosis represents a great challenge for clinicians and scientist and a great economic burden for the healthcare systems worldwide, with numerous etiologies and pathophysiological mechanisms, high rates of progression into more severe stages of the disease eventually leading to cirrhosis (with numerous complications) and liver cancer development. Common pathophysiological mechanisms involved in fibrosis development, regardless of the etiology, are: cytokine release and chronic inflammation, hepatocyte death, hepatic stellate cells (HSC) activation, disruption of the endothelial or epithelial barrier. Liver fibrogenesis represents a complex process which requires extracellular and cellular signaling. Among several intracellular signaling pathways involved in the pathophysiology of liver fibrosis Wnt pathway has a growing role. Based on the involvement of the ß-catenin, Wnt signaling pathway is principally divided into two classes: canonical and non-canonical Wnt signaling pathway. In the canonical Wnt signaling pathway, ß-catenin is the key signaling molecule, a protein with dual functions, acting as a transcription factor and an adhesion molecule. The transcription factor function is mostly regulated by Wnt proteins, which are mainly secreted to the extracellular space, and by bounding they initiate signaling processes leading to ß-catenin's cascade reaction. In the absence of Wnt signaling, ß-catenin is located in the cytoplasm in the low regime, where its stability is controlled by a destruction complex composed of protein Axin, adenomatous polyposis coli (APC), glycogen kinase 3β (GSK-3β) and casein kinase 1 (CK1). ß-catenin is phosphorylated by CK1 and GSK-3β and subsequently ubiquitinated by ß-transducin repeat containing protein (ß-TrCP) and finally degraded by the proteasome. The canonical pathway is activated when Wnt proteins bind to the Frizzled transmembrane receptor (Fz) and the Fz-coreceptors low-density LRP 5/6. Relocation of Axin to LRP 5/6 due to Fz/Dishevelled (Dvl) complex leads to phosphorylation of LRP 5/6. Activated Dvl then dissociates the destruction complex, resulting in the inactivation of GSK-3ß,

without resulting phosphorylation of the ß-catenin. Subsequently, the proportion of unphosphorylated ß-catenin increases, followed by its translocation to the nucleus. Although the mechanism of ß-catenin's translocation to nucleus is yet unnknown, the main point is to be bined to lymphoid enhancer binding factor (LEF)/T-cell specific transcription factor (TCF) in order to initiate targeted genes transcription. On molecular level to generate a transcriptionally active complex, ß-catenin must recruit either one of the two transcriptional coactivators cAMP response element binding protein (CBP) or p300. Non-canonical signaling pathways are ß-catenin independent. They encompass non-canonical Wnt/Ca2+ pathway and planar cell polarity (PCP) pathway. In Wnt/Ca2+ signaling pathway, Wnt proteins (such as Wnt5a) trigger a signaling cascade that results in activation of the cytoplasmic protein Dvl, which up-regulates the concentration of cytoplasmatic Ca2+ and subsequently activates the protein kinase C (PKC) and calcium sensitive enzymes calmodulin kinase II (CamKII). Another non-canonical Wnt signaling pathway is the PCP, also known as Wnt/c-Jun N-terminal kinase (JNK) pathway, important in cytoskeletal organization. The Wnt/PCP ligands (Wnt5a, Wnt7, Wnt 11) bind to Fz receptor encompassing Dvl-mediated stimulation of the small GTPases Rho and Rac. Subsequently, activation of kinase (ROK and JNK) is stimulated, which, in the end, are comprehensively involved in cell growth and differentiation (including HSC in the proces of hapatal fibrosis). Wnt signaling has showed to be activated in liver fibrosis with some elements of the Wnt signaling pathway up-regulated in the fibrogenic process such as Wnt5a and its receptor frizzled 2 included in differentiation of quiescent HSCs into myofibroblasts and ECM production. Significant upregulation in the expression of LRP co-receptor and β-catenin has also been documented. When β-catenin, an important regulator of cellular proliferation and differentiation, is inhibited, synthesis of types I and III collagen in HSCs is reduced. Inhibition of the Wnt/β-catenin signaling pathway using selective β-catenin/CBP inhibitor ICG-001 significantly inhibits HSC activation, collagen deposition, HSC contractility and migration in vitro and strongly attenuates fibrogenesis, inflammation and angiogenesis in vivo.

Hypothesis: Activation of Wnt signaling pathway is related to activation of human hepatic stellate cells and induces fibrogenesis in a cell culture model of MAFLD induced fibrosis.

Aims:

1. To measure the fat accumulation in the MAFLD induced fibrosis model on co-cultured human hepatic stellate cells (HSC) and human Huh7 hepatocytes by microscopic visualization and Oil-Red-O staining and by measuring triglyceride levels

2. To measure mRNA expression of fibrogenic markers (ACTA2, TIMP metallopeptidase inhibitor 1, TIMP metallopeptidase inhibitor 2, Collagen, type 1, alpha 1, Heat shock protein 47, Matrix metallopeptidase 2) involved in the process of fibrogenesis in the MAFLD induced fibrosis in simultaneous co-culture model and monoculture by RT-PCR method
3. To measure the expression of Wnt5a and Wnt3a in the process of fibrogenesis in the MAFLD induced fibrosis co-culture cell model by immunofluorescence in simultaneous co-culture of Huh7 and HSC cells and in monoculture of Huh7 and HSC cells
4. To investigate the correlation of Wnt5a and Wnt3a protein expression with the expression of genes involved in the process of fibrogenesis, cell survival parameters and the amount of triglycerides in the MAFLD induced fibrosis cell culture model

Materials/Participants and Methods: In this study it will be used: Human Huh7 hepatocytes; LX-2 Human Hepatic Stellate (HSC) Cell Line; DMEM (Dulbbecco's modified eagle medium), Capricorn Scientific, Ebsdorfergrund Germany; Fetal bovine serum- FBS; Penycilin/streptomycin solution; Colorimetric MTT Cell Growth Kit; Oil-Red-O, ChemCruz; Glutathione Colorimetric Detection Kit; Tri Reagent ; High Capacity cDNA Reverse Transcription Kit; Taq PCR Core Kit, Qiagen, Hilden, Germany; Trigliceryde TG GPO-PAP (Glycerol 3 phosphate oxidase - 4-Amino-antipyrine).

First step is the establishment of the MAFLD induced fibrosis on co-cultured human hepatic stellate cells (HSC) LX2 and human Huh7 hepatocytes. After that it will follow measurement of cell survival upon exposure to the fatty acids and evaluation of fatty acids effects by testing cell viability by MTT method, TC20, TC50. After that we will Measure the effects of fatty acids on the formation of fat droplets by visualizing the fat accumulation in Huh7 and LX2 HSC cells. It will continue with evaluation of the effects of fat accumulation in co-cultured Huh7 and LX2 HSC cells by measuring triglyceride levels and determination of the level of expression of genes involved in the processes of fibrogenesis and determination of the expression of Wnt5a and Wnt3a proteins in the process of fibrogenesis in the MAFLD induced fibrosis cell co-culture model by Immunofluorescence.

Research plan: In the first year, samples will be determined in the laboratory. The following year is planned for statistical processing, writing and publishing a scientific papers and writing of the doctoral thesis

Significance/Expected scientific contribution: Liver fibrosis remains an interesting and still under-explored research area. There are many mechanisms involved in its development, which further complicates its etiology. Establishing a cell culture model

of MAFLD induced fibrosis on co-cultured human hepatic stellate cells (HSC) and human Huh7 hepatocytes would provide a new means of assessing the occurrence and consequences of fatty acids induced fibrogenesis. Studying the effects of fatty acids on co-cultured cell model of fibrosis, would further elucidate the involvement of Wnt signaling pathway in fibgrogenesis.

MeSH/Keywords: Wnt signal pathway;Metabolic dysfunction-associated fatty liver disease;Fibrogenesis;Human Huh-7 cell line;Human Myofibroblastic Hepatic Stellate Cells

Dissertation Proposal Title: Importance of early risk assessment of vertebral compressive fractures in patients with inflammatory rheumatic diseases. Difference in clinical manifestations and other risk factors by gender.

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Mentor: Prof. Marija Glasnović, Department of Rheumatology, Clinical Immunology and Allergology, Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Introduction: Inflammatory rheumatic autoimmune diseases are characterized by a distinct variety of immunological and clinical manifestations conditioned by the biological course of the disease, but also by the use of immunosuppressive or immunomodulating therapeutic procedures. Vertebral compression fractures are a widespread disease and one of the most common complications of inflammatory rheumatic diseases. Prevalence data from population studies indicate significant differences in overall prevalence in women and men. Although the prevalence of osteoporosis is higher in women, men have a higher risk of mortality after fracture. Despite the high mortality and morbidity in men, unfortunately, most randomized controlled trials for the treatment of osteoporosis include only postmenopausal women, resulting in a challenge in the management of osteoporosis in men. When they are symptomatic, they cause significant pain and impair the overall quality of life, and represent a major public health problem. The most important component of prevention is early screening of people with a high risk of developing osteoporosis. According to clinical research, it is possible to prevent the onset of osteoporosis and reduce its progression exclusively through a multidisciplinary approach. Primary level health workers are in constant contact with patients and, according to their knowledge and skills, they should identify people at risk of osteoporosis, properly advise them and refer them to specialist health profiles in order to start the treatment of the underlying disease as early as possible.

Hypothesis: Data on the prevalence of vertebral fractures were not presented in any major study due to methodological problems associated with their verification. Early recognition and good management of inflammatory rheumatic diseases can prevent osteoporotic fractures, and in already registered patients there is a possibility of newer interventional therapeutic options.

Aims:

1. To determine the role of risk factors in the occurrence of vertebral compression fractures
2. To show the prevalence of the occurrence in men and women
3. To point out the importance of differences in the expression of the clinical picture and the harmful effect of uncontrolled rheumatic diseases on the loss of bone density
4. To show the importance of prevention, early detection and the earliest possible initiation of adequate treatment in order to maintain working ability and preserve quality of life.

Materials/Participants and Methods: A selected group of patients treated at the Department of Rheumatology, Clinical Immunology and Allergology of the Clinical Hospital Center Osijek will be included in the trial. The database of patients treated at the Department of Rheumatology, Clinical Immunology and Allergology of the Clinical Hospital Center Osijek in a twelve-year period (2008-2020) will serve as a research instrument that will offer answers to the aforementioned questions in the interpretation of the obtained and evaluated numerous results, retrospectively and prospectively.

Research plan: In the first year, subjects from database of patients treated at the Department of Rheumatology, Clinical Immunology and Allergology of Clinical Hospital Center Osijek will be included in the study. Datas will be collected, and clinical parameters determined. The following year is planned for statistical processing, writing and publishing a scientific papers and writing of the doctoral thesis. It is necessary to compare the analyzed results obtained from the medical documentation with the results of already conducted studies and determine whether the obtained results match the statistical data.

Significance/Expected scientific contribution: Compression fractures of the vertebrae impair the quality of life, cause psychological consequences, loss of self-esteem and isolation from social events, and greater family burden. All the above lead to an increase in the number of visits to family medicine doctors and specialists, a greater number of hospitalizations, and ultimately, with long-term treatment, a significant increase in treatment costs. Therefore, quick recognition and adequate treatment is the challenge and goal of every healthcare system.

MeSH/Keywords: Vertebral Compression Fractures; Inflammatory Rheumatic Diseases; Osteoporosis; Risk Factors; Prevalence

Dissertation Proposal Title: Clinical significance of determining the volume of the implantation endometrium using three-dimensional ultrasound VOCAL softwares.

PhD candidate: Stefan Gjoni, Department of Obstetrics and Gynaecology, General Hospital Pula, Pula, Croatia

Mentor: Prof. Siniša Šijanović, M.D. PhD., Department of Obstetrics and Gynaecology at Clinical Hospital Osijek, Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Introduction: Three-dimensional ultrasound in gynecology have great diagnostic, scientific and educational potential, but unfortunately poor applications on all three mentioned levels. Implantation, or the implantation window, is the biggest unknown in reproductive medicine. Mother nature solved the problem in such a way that after ovulation, under the influence of progesterone, it changed or transformed the proliferating endometrium into a mucous membrane rich in glycogen (polymerized glucose). Modern reproductive medicine approaches implantation as a potential biological problem with two-dimensional ultrasound. Advanced, three-dimensional technologies for the objective determination of the volume of secretory-changed endometrium still do not have protocol status in the diagnostic treatment of infertile women. In the volume of secretory changed endometrium there is a real energy potential for blastocyst implantation, i.e. the beginning of a new life. VOCAL (Virtual Organ Computer-aided AnaLysis) is a semi-automatic 3D software technology for volume measurement of ovoid organs and volume estimation of blood flow.

Hypothesis: The volume of the implantation endometrium is a great unknown in reproductive medicine. So far, no one has standardized its size in that phase of the menstrual cycle in any group of women within the fertile age. The volume of the endometrium measured in that phase of the menstrual cycle by an objective ultrasound method (VOCAL) is a true indicator of implantation capacity, that is, a woman's biological ability to undergo medically assisted fertilization procedures.

Aims: Determine the standard values of the volume of secretory changed endometrium, in an objective ultrasound way by three-dimensional ultrasound VOCAL (Virtual Organ Computer-aided AnaLysis) technology.

Materials/Participants and Methods: The research will be conducted in three different groups of women of reproductive age. In each group there will be at least

50 women or a total of at least 150 women of fertile age: 1. women who have given birth to eutrophic children, 2. women who have not yet given birth and have not been pregnant, 3. women with sterility problems.

Research plan: In the first year, subjects will be included in the study, 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: Upon completion of the proposed study, the standard values of secretory altered endometrium will be known, as well as the optimal endometrial volume when it is most receptive, i.e. its implantation window, and thereby improve the success of the results of medically assisted fertilization.

MeSH/Keywords: The volume of secretory changed endometrium, Three-dimensional ultrasound, VOCAL (Virtual Organ Computer-aided AnaLysis), Ultrasound standards in reproductive medicine, Sterility.

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

Ph.D. Candidate: Matea Hudolin, M.D., County Hospital Villach, Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

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

Co-Mentor: Assist. Prof. Hrvoje Budincevic, M.D., Ph.D., University Hospital „Sveti Duh", Zagreb, Croatia; Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Introduction: Incidence of depression and suicidal ideation is greater in patients with multiple sclerosis compared to healthy population. The incidence 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 on quality of life, 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 County Hospital Villach during the period of one year. The expected sample size is 200 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-Severiity-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 County Hospital Villach in Villach, Austria

Significance/ Expected scientific contribution: This study will provide insight into possible connection of serum LDL-levels and socioeconomic factors on depression and suicidal ideation of patients with multiple sclerosis enabling the further development of better suicide programmes for this patient population.

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

Dissertation Proposal Title: Risk of falls in the elderly: comparison of monotherapy with anxiolytics versus the combination of anxiolytics with other psychopharmaceuticals

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

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

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

The START STOP criteria represent frequently used explicit criteria for potentially inappropriate drugs in the elderly-benzodiazepines, psychofarmaceuticals, hypnotics.

Anxiolytics must be administered in the lowest possible doses in elderly people, because the same concentrations they have a stronger pharmacodynamic effect in elderly people.

Hypothesis: An elevated concentration of anxiolytics in the blood of the elderly is associated with a lower risk of falls compared to a simultaneously elevated concentration of anxiolytics and other concurrently administered psychofarmaceuticals.

Aims:

Main goals:

1. To investigate wheter the concentration of anxiolytics in the blood of elderly people affects the frequency of falls less than the combination of concentrations of anxiolitycs with other psychofarmaceuticals
2. 2) To assess the risk of falling to the MORSE FALL SCALE in institutionalized and independent elderly people

Secondary goals:

1. To examine whether there are significant differences in the quality of life of respodents who live in a institution and those who live alone
2. To examine whether there are significant differences in the quality of life with regard to gender, level of education and marital status
3. To examine whether there is connection between the frequency off falls and the quality of life
4. To examine witch factors influence the greater frequency of falls

Materials/Participants and Methods: 128 respondents will be included in the research who take psychofarmaceutics als as permanent therapy for a period of at least 3 months.

The estimated sample size is 60 respondents from institutions - Family Home for the Elderly and the Infirm "St. Anthony" Šag and the remaining 68 respondents will be recruited from the specialist family medicine office, Assistant Professor Mario Ćurković, PhD, Health Center of Osijek-Baranja County and family medical office Posavski Podgajci - Rajevo Selo, Health Center Županja, team leader Anja Jakovčević, MD, family medicine specialist.

All survey participants will be asked to complete a questionnaire containing a total of 40 questions. The survey consists of 8 questions on SOCIODEMOGFRAFIC data (gender, age, place of residence, marital status, education, medications used, previous falls, chronic illnesses-arterial hypertension, COPD, diabetes mellitus, osteoarthritis, obesity, Parkinson's disease), 26 questions from the standardized questionnaire of the World Health Organization on quality of life - WHOQOL-BREF, 6 questions from the standardized questionnaire for assessing the risk of falls in the elderly - MORSE FALL SCALE. In addition to filling out questionnaires, all subjects will be tested for blood levels of psychotropic drugs in the blood in the clinical biochemical laboratory of KBC Osijek and KBC Zagreb.

Research plan: We started the research in two specialist family medicine surgeries. Subjects using anxiolytics and anxiolytics in combination with other psychotropic drugs were given questionnaires with 40 questions and instructions were issued for determining the concentration of psychotropic drugs in the blood. Since it takes several weeks for the medical-biochemical laboratory to process the samples, we are still waiting for the findings. The findings will be sent to us via the CEZIH portal and we will have an accurate insight into the concentrations of the required drugs in the blood of the subjects.

We will do the same with the respondents from the Home for the Elderly and the Infirm.

We plan to conduct the research for 12 months because we believe that we need this time frame to collect relevant data.

Expected scientific contribution: The results of this research will provide important data on whether the concentration of anxiolytics affects the frequency of falls in the elderly less than the combination of concentrations of anxiolytics with other psychofarmaceuticals.

Avoiding these drugs and their combinations, better managing these drugs, using these drugs for a shorter period of time, and regularly reviewing prescribed medications by your family doctor could significantly reduce the incidence of falls and / or fractures in the elderly.

Reducing falls and consequent injuries, especially fractures, can significantly affect the quality of life of the elderly, reduce mortality, reduce the cost of health care facilities, which allocate them to patients during their hospitalizations and rehabilitation.

Keywords: psyhotropic drugs, anxiolytics, falls, fractures, elderly people

Dissertation Proposal Title: Hemodynamic characteristics of ruptured intracranial aneurysms, severity of subarachnoid hemorrhage and development of vasospasm

PhD candidate: Dragan Jankovic, M.D., Department of Neurosurgery, University Medical Center of Mainz, Mainz, Germany

Mentor: Prof. Krešimir Rotim, M.D., Ph.D., Department of Neurosurgery, Sisters of Mercy University Hospital Center, Zagreb,

Co-mentor: Assis. Prof. Sanja Tomasovic, M.D., Ph.D., Department of Neurology, University Hospital "Holy Spirit", Zagreb, Croatia

Introduction: The spontaneous rupture of an intracranial aneurysm results in aneurysmal subarachnoid hemorrhage (aSAH), a potentially life-threatening neurovascular condition. Early diagnosis and occlusion of the aneurysm are of crucial importance in the treatment of aneurysms. It is important to avoid aneurysm rebleeding and secondary brain damage, mostly caused by vasospasm and delayed cerebral ischemia.

Considering that the integrity of the blood-brain barrier after aneurysmal subarachnoid haemorrhage is disrupted, the question arises of whether hemodynamic parameters measured using computational fluid dynamics affect the development and severity of vasospasm.

Hypothesis: The occurrence and severity of vasospasm after aneurysmal subarachnoid hemorrhage will be affected by the degree of subarachnoid hemorrhage and the patient's neurological condition upon admission to the hospital and in dependence on hemodynamic parameters of ruptured intracranial aneurysm.

Aims:

1. to examine how the hemodynamic characteristics of a ruptured intracranial aneurysm and the severity of subarachnoid hemorrhage affect the development and severity of vasospasm
2. to analyze the hemodynamic characteristics of ruptured intracranial aneurysms and parent arteries using computational fluid dynamics
3. to examine the severity of subarachnoid hemorrhage and vasospasm
4. to examine which factors are significant in predicting the development and severity of vasospasm

Participants and Methods: This research will be conducted as a cross-sectional study with historical data, which will include patients treated at the Department of Neurosurgery, Clinical Hospital Center Sisters of Mercy, with a diagnosis of ruptured intracranial aneurysm.

Patients will be divided into two groups - patients with radiologically confirmed vasospasm and patients without radiologically diagnosed vasospasm. The following variables will be examined: age, sex, location of the aneurysm, clinical condition at admission, the severity of subarachnoid hemorrhage and vasospasm, and presence of intracerebral and intraventricular hemorrhage.

The vascular geometry of the aneurysm and parent artery will be obtained from CT angiography data and reconstructed using a medical image processing package Ziostation2.

Hemodynamic analysis of intracranial aneurysms and parent arteries will be performed using the Hemoscope software.

The following hemodynamic parameters will be studied: maximum pressure, wall shear stress, wall shear stress magnitude, wall shear stress direction, velocity and streamline.

Research plan:

1. Screening of patients according to inclusion and exclusion criteria
2. Determining the severity of subarachnoid hemorrhage and vasospasm
3. Analysis of hemodynamic characteristics of ruptured aneurysms using computational fluid dynamics
4. Statistical analysis
5. Publication

Keywords: computational fluid dynamics; intracranial aneurysm; hemodynamics; subarachnoid hemorrhage; vasospasm, intracranial

Expected scientific contribution: Given that early diagnosis of vasospasm is important to prevent secondary brain injury, the information from this study could be useful in predicting the risk of developing vasospasm after aneurysmal subarachnoid hemorrhage. Our results would help to better understand the behavior of the blood vessel wall during vasospasm.

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:

1. 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.
2. Examine the differences in ways of resolving conflicts and professional self-evaluation according to demographic variables and variables related to studying and working status.
3. 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 throught

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 for the need of 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.

Dissertation Proposal Title: Assessment of the social support and psychosomatic symptoms of patients treated with renal function replacement

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

Introduction: Social support is an important protective factor in everyday life and in dealing with various stressors occurring in a daily or weekly routine. Those who have friends, partners, and family members who support them are better in health than those with fewer contacts. Psychosomatic symptoms are described as physical symptoms occurring as one or more symptoms at the same time and maybe weaker to very high intensity that usually interferes with daily functioning. In developing psychosomatic symptoms, the individual's defenses, health, inherited traits, and social impact, including social support and culture, play an important role. Patients treated with renal replacement experience various psychosomatic symptoms before, during, and after hemodialysis.

Hypothesis: A lower level of social support is associated with a poorer self-assessment of the psychosomatic symptoms of patients treated with renal function replacement.

Aims: To examine the perception of social support and psychosomatic symptoms of patients treated with renal function replacement.

Materials/Participants and methods: The study will include patients treated with renal replacement in dialysis centers. 300 subjects are planned to be tested (n = 300). A socio-demographic questionnaire, Social support scale, and Psychosomatic symptoms scale will be used for the research.

Research Plan: A cross-section study will be conducted in the period from October 2023 to March 2024.

Significance/expected Scientific contribution: This study will provide data on the level of social support and self-assessment of the health of patients treated with renal replacement. Understanding the relationship between social support and self-

assessment of health in patients undergoing hemodialysis can guide healthcare providers, family members, and social services on the importance of social support to this patient group.

Mesh/Keywords: renal Insufficiency, chronic; renal dialysis units, hospital; support, Social; self assurance (Psychology); Nutritional deficiency;

Thermographic analysis of the metabolic activity of the breast in healthy women

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

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Introduction: The risk of breast cancer (BC) laterality emergence can be associated with breast size, vascularity and metabolic activity.

Thermography is an indicator of physiological function, unlike MMG, magnetic resonance imaging (MRI) and ultrasound, which reveal anatomical characteristics.

The thermography measures breasts' thermal print based on the metabolic activity of cells.

Digital infrared thermal imaging (DITI) can register temperature distribution patterns to a sensitivity of 0.08 °C or less, and deviations from the symmetry pattern can be identified.

The thermal symmetry of the healthy human body has a definition to be a maximum value of 0.570 °C for two bilateral regions of interest.

Paired organs in a human body may have anatomical and physiological asymmetry, which could influence the laterality of cancer occurrence.

The analysis focuses on characterising women's basal steady temperature profiles in specifically defined regions of interest and determining the thermal symmetry values for a healthy woman and standard patterns of temperature distribution in the whole breast.

The more significant the temperature difference in a smaller area of breast interest is, the more suspicious area will be.

The reason of that are pathophysiological and morphological changes of the breast. Considering that the tumour is making the whole breast warmer, its presence in a specific quadrant will cause even higher temperature release in the same quadrant.

These thermal variations can be measured using thermography.

Thermography provides a significant advantage as an early indicator of breast disorder, owing to the first emergence of thermal signs indicating errant function.

Infrared thermography is a method, which detects infrared energy emitted from objects, converts it into temperatures and can display an image of temperature distribution.

Breasts have an average temperature difference of less than 0.5 °.

The real challenge in natural science is to understand, recognise and classified these temperature changes as a higher metabolic activity due to hormonal changes and consequently a higher demand for the blood inflow, inflammation or pre-cancerogenesis stage.

The perception that breast cancer appears more commonly in the left than the right side breast has been of interest to the scientific community for many years.

However, the reason for the increased appearance of breast cancer in the left breast is still unclear.

The presence of an abnormal, asymmetric infrared heat pattern of the breast increases a women's risk of getting breast cancer at least 10-fold (Gautherie and Gros).

Aims: Establishment of the laterality of breast temperature changes in healthy women and its association with breast's size, vascularity, and metabolic activity.

Calculating of the degree of thermal asymmetry and vascular characteristics for the risk of developing breast cancer.

Making the National Thermographic Protocol (NTP) for women with higher temperature asymmetry who should have frequent follow-ups and monitoring because standardised protocol will enable us to observe temperature dynamics, record them and later analyse and compare them for a better assessment and evaluation of the cancer risk prediction.

Materials and Methods: 340 healthy women between 19-66 years of age underwent non-contact thermographic imaging during preventive screening.

In a thermally controlled room without air circulation, where the room temperature was maintained between 20-21 °C with a relative humidity of 50-65%.

During the thermographic imaging, the participants were asked to stand and keep their hands on their neck one meter away from the camera.

The recording was taken at the moment of the maximal inhalation with the arms above the head.

An infrared (IR) thermographic camera (FLIR T335; FLIR Systems Pty Ltd, Australia) with a detection range of -20 °C to +650 °C, with thermal sensitivity less than 0.05 °C, IR resolution of 320 x 240 focal plane array detector (76,800 pixels) and with the image frequency of 9Hz was used

Analysis of the data was performed by using the FLIR Tools software (FLIR Systems, Inc., North Billerica, MA, USA).

Method of analysis (thermogram): Ville Marie Breast Thermography Grading Scale methodology as adopted by the Thermography Service of Integrative Life Solutions (Clemmons, NC).

Thermograms were classified into 1 of 5 thermobiological (TH) groups based on vascularity and breast temperature, where TH-1 represents (Normal) absence of any vascular pattern to mild vascular symmetry and TH-2 (Normal Vascular) represents significant but asymmetrical vascular pattern to moderate vascular asymmetry, if stable. The last three groups of this classification are TH-3 (Equivocal), one abnormal sign, TH-4 (Abnormal), two abnormal signs and TH-5 (Severely Abnormal), three abnormal signs.

A unilateral pathology or any other condition that created an abnormal circulation were recorded as an asymmetrical thermographic pattern if the temperature difference was above 0.570 °C (Fig.2).

Unfortunately, though, there is no screening tool currently available that provides 100% predictability of a cancerous tumour's presence.

The only definitive diagnostic tool is a biopsy.

A unilateral pathology or any other condition that created an abnormal circulation were recorded as an asymmetrical thermographic pattern if the temperature difference was above 0.570 °C.

Breast glandular tissue density was classified based on the MMG (BIRADS) classification system.

Vascularity was determined after visualising the thermograms and inspecting the pattern of blood vessel distribution using the grey-scale visualisation method (Fig.1) on the FLIR operative system.

Descriptive statistical analysis was used to evaluate data.

The Student's dependent paired t-test was used to test the statistical significance of the collected data between the mean values obtained for the left and right breast.

Results: There were no significant size differences between the left and the right breast ($p=0.209$).

Dependent on the differences in the sizes of the left and the right breast, three groups were determined (the left and the right breast were of equal size; the left breast was larger than the right; the right breast was larger than the left one (Fig.3)

Maximum, minimum and average temperatures of the left and the right breast are shown in Table 2.

There was a significant difference for the maximum temperature between the left and the right breast ($t= 3.196$, $p<0.02$), as well as for the average temperature ($t= 3.558$, $p<0.01$).

There was no significant difference between the left and the right breast considering the minimum temperature ($t= 1.534$, $p>0.05$).

Data analysis has been done per age group, and there was no temperature difference dependent on the age group.

Vascularity features of the breast were observed as well and its influence on heat production.

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

Conclusions: Thermography is currently the only method that can detect physiological- pathophysiological changes of metabolic activity in the breast, specifically in high-risk regions for cancer occurrence.

Left breasts were found to have significantly higher maximum and average temperature than right breast.

The left breast was larger in 39% and more vascularised in 38% of cases.

Heart heat signature did not influence the heat distribution pattern as much as it was expected.

It can also raise suspicions of pre-cancerous stages based on temperature distribution differences, so radiotherapy could be avoided.

The standardised National Thermographic Protocol (NTP) will enable us a better assessment and evaluation of the cancer risk prediction.

When combined with other examinations, thermography may contribute to the best possible evaluation of breast health or pathology.

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

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Dissertation Proposal Title: The association of sodium and glucose cotransporter 2 inhibitors use with elastographic and molecular markers of liver injury in patients with type 2 diabetes

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Introduction: Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease with no approved pharmacotherapy. NAFLD is characterised by deposition of at least 5% lipids within hepatocytes caused by genetic, immunological, environmental, metabolic factors and not by alcohol consumption. Histologically, NAFLD can be divided into two distinct conditions: non-alcoholic fatty liver (NAFL or steatosis) and non-alcoholic steatohepatitis (NASH) which may lead to severe conditions such as cirrhosis, liver failure and hepatocellular carcinoma. Progression of NAFLD is usually slow and asymptomatic, and therefore is often diagnosed at a very late stage. NAFLD occurs in almost all patients with metabolic syndrome and/or type 2 diabetes mellitus. SGLT2 (sodium-glucose cotransporter 2) inhibitors are antidiabetic drugs that show significant beneficial effects on many processes such as autophagy, apoptosis, endoplasmic reticulum stress, oxidative stress and low-grade inflammation in patients with NAFLD. The effect of SGLT2 inhibitors on the development and progression of NAFLD are not fully understood.

Hypothesis: Administration of SGLT-2 inhibitors reduces the expression of lipogenesis markers in patients with type 2 diabetes.

Aims: To measure liver tissue density with elastography, determine standard markers of liver damage, lipogenesis markers and genes responsible for the expression of SREBP-1 (Sterol Regulatory Element-Binding Protein-1), PPAR alpha (Peroxisome Proliferator-Activated Receptor alpha), PPAR gamma, TNF-alpha (Tumor Necrosis Factor-alpha), TNF-beta, MTTP (Microsomal Triglyceride Transfer Protein) and IL-8 (Interleukin-8) 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 expression of genes involved in the process of lipogenesis and mitochondrial dysfunction with liver tissue density 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 who were prescribed SGLT2 inhibitors in general medical practices in Osijek. Inclusion criteria: patients who started therapy with SGLT2 inhibitors for the first time. Exclusion criteria: patients with confirmed fatty liver disease and with other liver diseases, patients taking drugs that affect the liver (glucocorticoids, metamizole, methotrexate, antiepileptic drugs, chemotherapeutic agents, alcohol etc.). 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. ELISA and RT-PCR will be used to assess standard biomarkers of liver injury, serum levels and gene expression before initiation of SGLT2 inhibitor therapy and 6 months after.

Research plan: Following recruitment, elastography will be performed to determine the density of liver tissue and blood samples will be taken to determine serum levels of ALT, AST, ALP, GGT, albumin, proteins, coagulation levels, bilirubin total and conjugated, lipid profile, SREBP-1, PPAR alpha, PPAR gamma, MTTP, TNF-alpha, TNF-beta and IL-8 as well as for RNA isolation to determine gene expression before the initiation of SGLT2 inhibitor therapy and 6 months after initiation of therapy at the Medical Centar Mursa in Osijek.

Significance/Expected scientific contribution: To the best of our knowledge, this study will for the first time investigate the association between SGLT2 inhibitors administration and elastographic and molecular markers of liver injury in patients with type 2 diabetes mellitus. This study will evaluate whether SGLT2 inhibitor therapy improves liver status in patients with type 2 diabetes mellitus and may help to establish new methods for NAFLD diagnosis at early stage of disease in the future.

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

Abstract title: Prognostic role of miRNA 21 and association with HPV status in carcinoma of the oral cavity and oropharynx

Part of Disertation Proposal: Current knowledge of HNSCC miRNA profiles is still incomplete, especially in the context of HPV positive cancer. A prospective cohort study.

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Introduction: Head and neck cancer (HNC) is the sixth most common malignancy. They are associated with tobacco and alcohol use and human papillomavirus (HPV). Over 90% of all HNC are squamous cell carcinomas (HNSCC). 550,000 cases per year, while 896 new cases were estimated in Croatia in 2015.

HNSCC is often diagnosed at a late stage, when it is difficult to treat, and the five-year survival rate is only 40-50%.

The basic principle of treatment of such patients is still mutilating surgery with possible postoperative radiotherapy.

Studies also show that HNSCC appears to be an epigenetic disease rather than a genetic one. Studies on epigenetic changes in HNSCC, such as miRNA profiling, hold promise for finding specific biomarkers for tumor patients

Aims:

1. To investigate the association of miRNA 21 with the stage of oral and/or oropharyngeal cancer.
2. To determine whether there is a connection between miRNA 21 and HPV expression, i.e. HPV genotype.
3. To determine the impact of miRNA 21 on the survival and prognosis of patients with oral and/or oropharyngeal cancer.
4. To investigate the relationship between miRNA 21 and pathohistological

characteristics of tumors (perineural, vascular invasion, tumor differentiation).
5. To determine whether there is a difference in the expression level of miRNA 21 in oral and oropharyngeal cancer.
6. To determine whether there is a difference in prognosis depending on the expression of miRNA 21 in oral and oropharyngeal cancer.
7. To determine whether there is a connection between miRNA 21 expression and alcohol consumption and smoking in patients with oral/oropharyngeal cancer.

Materials and methods: A power analysis was performed to determine the required number of patients in the study, and it is 64 for a test power of 80% and the level of statistical significance is p=0.05.

The research will include patients with squamous cell carcinoma with a primary lesion in the oral cavity and oropharynx who were primarily treated surgically at the Clinic for Maxillofacial Surgery of the Clinical Hospital Dubrava and the Clinic for ENT and Maxillofacial Surgery University Clinical Hospital Mostar from January 1, 2017 to January 1, 2020 . Only patients who had squamous cell carcinoma of the oral cavity or oropharynx were included .

The study did not include patients whose tumor pathohistologically was not squamous cell carcinoma of the oral cavity/oropharynx, who were primarily irradiated or underwent chemotherapy, or who previously had surgery for oral cavity or oropharynx cancer.

The surgically removed tumor was subjected to pathohistological analysis, which is a standard procedure.

During the operation, a piece of the tumor was taken, which was further processed at the Institute for Molecular Medicine, "Ruđer Bošković" Institute (IRB) in terms of miRNA epigenetic analysis as well as HPV isolation using the PCR method.

The clinical status of the patient after surgical treatment will be monitored for at least one year after the operation, which includes the presence or absence of the primary tumor, time of recurrence, time of regional metastasis, time of distant metastasis, survival in months. Likewise, the existence of perineural and perivaxular infiltration and the degree of tumor differentiation will be distinguished from the pathohistological findings.

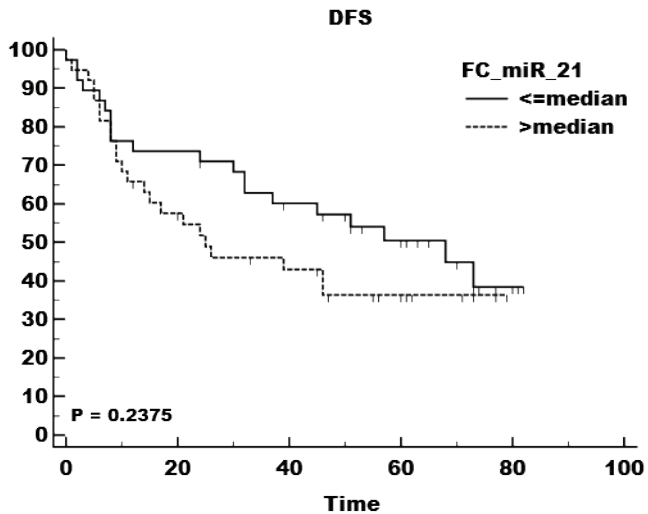
Results: Men prevailed in the study (73.7%, 56/76) in comparison with women (26.3%, 20/76). The mean age of all patients was 61.7±11.6 (range 31-87, median 61). Approximately one third of patients were never smokers and never drinkers (34.2%, 26/76), while more than 60% of patients reported tobacco and alcohol

use. Most tumours were in the oral region (78.9%, 60/76), while others were in the oropharyngeal region (21.1%, 16/76). According to clinical TNM (cTNM) most patients presented at stage IV cancer (47.4%). Samples were classified by the HPV status into two groups: 18 (23.7%) samples with HPV-positive and 58 samples with HPV-negative HNSCC (76.3%). Among HPV-positive tumours HPV16 was found in most cases (77.7%, 14/18). Consistent upregulation of miR-21 was seen across the study population and all subgroups.

Relative expression (median and interquartile range (IQR), fold change (FC) versus normal control sample) of miRNA 21 in the study population and subgroups.

	Total		HPV negative		HPV positive		O		OP		O-		O+		OP-		OP+	
RQ miR-21	76	0.4 (0.3-0.5)	58	0.4 (0.3-0.5)	18	0.3 (0.2-0.8)	60	0.4 (0.3-0.6)	16	0.4 (0.2-0.5)	48	0.4 (0.3-0.5)	12	0.4 (0.2-0.8)	10	0.4 (0.3-0.8)	6	0.2 (0.1-0.4)

In survival curves overexpression of mir-21 was associated with worse prognosis although it wasn't statistically significant.



However in Cox multivariate regression analysis for overall survival (OS) gender, presence of perineural and angioinvasion, tumour resection margin, lymph node yield and the expression of miR-21 were independent prognostic factors. MiR-21 was also found to be significant in disease-free survival model.

Conclusion: According to the results of this study we can conclude that miRNA 21 is generally overexpressed in most cancers of the oral cavity and oropharynx and it was proven as negative prognostic factor.

MeSH / Keywords: microRNA, miR-21, HPV, HNC, Mouth neoplasms, Survival, Prognosis

Changes in the proteome of exosomes shed by rat liver after subtoxic exposure to acetaminophen

Part of Disertation Proposal: Changes in the proteome od exosomes shed by rat liver after subtoxic exposure to acetaminophen

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Introduction: Liver damage after abuse of several agents, like alcohol, energy drinks, different bacterial and fungal toxins, as well as pre- and post-market drug complications are frequent causes of damage and as a final consequence also failure of this vital organ . Several drugs like painkillers are responsible for so-called drug-induced liver injury (DILI). DILI represents a severe clinical and economical challenge . Acetaminophen (APAP) is the painkiller and antipyretic drug, when used in therapeutic doses, APAP overdose is a leading case of DILI, especially in the presence of alcohol consume.

Most frequently used models od liver injury are poisoning by galactosamine and lipopolysaccharides from cell envelopes of different bacteria, like *Escherichia coli*. These and similar toxic agents promote acute liver inflammation following by damage of this organ. DILI, alcohol abuse , over consume of alcohol and painkillers and their combination as well as bacterial toxins and mycotoxins can cause severe liver disease and finally failure of this vital organ. There are several models for both DILI and alcoholic liver disease, mostly by use of rat or mice as experimental animals.

Analysis of body fluids, especially plasma, serum and urine has a long history and change of concentration of proteins that are synthesized in the liver, as well as changes in their posttranslational modifications is an important diagnostic and prognostic tool . Omics investigations of body fluid samples can provide additional information on the way of diagnosis and treatment . In diagnostics of liver diseases liquid biopsy can supplement or even completely replace the invasive and painful liver biopsy.

Extracellular vesicles (EVs) are released by liver, and they can be also detected in body fluids. Changes in MVs composition after liver injury and under other pathological conditions is became an important indication on the way for diagnosis of liver

diseases. These nanoparticles are a heterogeneous population with a size between 30 and 1000nm (and up to 2000nm in the case of apoptotic bodies). They are selectively enriched by proteins, lipids, and ribonucleic acids and released from EVs producing cells. The smallest ones, the exosomes, play a crucial role in both physiological and various pathological processes . They also have an emerging role in biomarker discovery, and recently also in therapeutic applications for treatment of diseases.

Liver-derived EVs can be also secreted into the bloodstream or excreted by urine. They are carriers for specific mRNAs and micro RNAs, as well as intracellular proteins and proteins coming from different cellular membranes. Microvesicular RNAs are early indicators of organ injury , and/or other diseases like viral hepatitis or malignancy . EVs contain several proteins, and some of them are specifically enriched in these membrane-enveloped nanoparticles. Specific changes of proteome of MVs are consequence of organ injury, malignant modification, or other pathological conditions, e.g. viral infection or inflammation. Consequently, EVs are important source of disease biomarkers . Their use as important tools for disease treatment, especially for delivery of anti-cancer drugs is recently topic of intensive studies .

Composition of liver EVs depends of particular cell state. The presence of some liver-specific proteins indirectly supported the assumption for presence of liver-derived EVs in the bloodstream and other body fluids, and few years later it was also experimentally confirmed by detecting some liver-derived proteins in exosome-like vesicles purified from mouse and/or rat urine or serum samples . Human liver biopsy is a very invasive and painful process, and the identification of biomarkers for detection of pathological changes in this organ, preferably in body fluids, should be a method of choice. For identification of possible biomarker candidates, the investigation of EVs isolated from livers or shed into body fluids of model animals was performed. In EVs released by cultured primary hepatocytes, several members of liver-specific cytochromes P450, uridinediphosphate-glucuranosyl-transferase (UGT) and glutathione S-transferase (GST) protein families were identified . Rodríguez-Suárez et al. detected some heat-shock proteins [HSP90 and HSP70] as potential biomarker candidates for experimental galactosamine induced hepatitis in rats. Higher concentration of these two proteins was also detected in EVs released by primary hepatocytes as well as in serum of treated rats.

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

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

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

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

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

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

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

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

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

MS/MS spectra were searched against the Uniprot rat protein using the Mascot algorithm v.2.3.2 provided by Matrix Science. The exact procedures were previously given [39]. Shortly: Mascot searches were performed with the following parameters:

trypsin enzyme specificity, 2 possible missed cleavages, 20ppm mass tolerance. Search parameters specified a differential modification of oxidation on methionine and a static modification of carbamidomethylation (+57.0215 Da) on cysteine. Protein quantification was performed using ProteoIQ software v. 2.3.05 (BioInquire, Bogart, GA, USA) with spectra count data. To provide high confidence on peptide sequence assignment and protein identification, data were filtered with following stringent criteria: Mowse score > 28 for all charge states, at least 2 peptides per protein, 1% peptide false discovery rate (FDR) and 1% protein FDR.

Results: Isolated perfused liver offers a means to collect MV shed under conditions that approach those *in situ*. This approach provides large quantities of highly enriched MVs, making further fractionation based on size or expression of cell-type specific markers a feasible undertaking. Based on TEM analysis MVs shed by normal liver have different size, starting with a diameter larger 250 nm, 100-250 nm, and smaller than 100 nm. Acetaminophen intoxication enhances shedding of a population of very small MVs, suggesting injury increases shedding of MV from a particular cell type e.g. endothelial cells or causes a change in the process by which MV are formed or released . Major differences (arrows) were apparent in the protein composition shown by SDS-PAGE (see arrows) that will be further analyzed by LC-MS/MS.

The problem of detection of "real-vesicular proteins" was recently addressed by Choi et al.. We early recognized this problem when working with plasma membrane proteins, and different sample preparation strategies, in-gel tryptic digestion, as well glycoprotein deglycosylation before trypsin digestion were applied . This strategy yielded in detection of hydrophobic proteins with one or more membrane-spanning domains in both preparations of liver MVs. Cytosolic intravesicular proteins actin as well as tubulin that according to these Choi et al. were "protected from the action of trypsin" were also detected in both MVs fractions after use of presented strategies for sample preparation and proteolytic digestion.

Out of over 200 proteins, only 46 (26%) of them are detected in both MV preparations. The protein pattern in MVs fraction of non-treated liver differs significantly from this one in the fraction of MVs isolated from the liver of acetaminophen treated rats, where more different proteins were detected. This result was validated by multiple MS analyses of both fractions as well as by presented analysis by SDS-PAGE that also shows large difference in protein patterns between two samples . To exclude difference in protein compositions that is caused by liver damage during perfusion, presence of some proteins that are markers for liver cells' injury was controlled. Aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) are markers for hepatocellular injury. In both preparations AST was not detected at all, and

concentrate of LDH in MVs is at the threshold of detection limit. Purine nucleoside phosphorylase, an enzyme that is localized in non-parenchymal cells such as vascular endothelium and Kupffer cells was detected only in acetaminophen-treated cells . However, this enzyme is also marker of oxidative injury that can also be consequence of toxicity of applied high drug concentration.

Cytochromes P450 (CDP450) are a superfamily of liver enzymes that oxidize steroids and fatty acids. These enzymes are also responsible for oxidation and clearance of xenobiotics like. Together with uridinediphoshate-glucuronosyl-transferase (UGT), glutathione S-transferase (GST) and carbamoyl phosphate synthetase these proteins are discussed as biomarkers for hepatocyte-derived biomarkers. Above listed proteins were detected only in MVs isolated from normal liver, and not in the MVs from treated liver. At the other hand, proteins from the annexin family, namely annexin A1, annexin A3, and annexin A5 were detected only in MVs shed by liver after injury . Annexin A6 was detected in both MV fractions. However, the concentration of this plasma membrane associated protein was much higher in MVs of acetaminophen-treated liver. Stepwise disappearance of these proteins, especially of the members of the CD P450 protein superfamily and of alpha-1-macroglobulin in liver derived MVs, as well as the appearance or increasing concentration of "typical MVs proteins" like members of the annexin family can be additional tool for early detection of liver injury .

Conclusions: Healthy liver cells shad MVs into blood stream and other body fluid. Similar process occurs in liver cells after both the injury caused by different toxic agents and after malignant modification, that also secrete MVs by use of identical or similar mechanisms. However, already by electron microscopy, differences in size and form of these MVs can be observed . Liver MVs are identified as important source of potential disease biomarkers. They can be detected by use of so-called liquid biopsy and can be used for simple and fast diagnosis of liver injuries and/or malignant changes of this organ.

Change in the proteome liver MVs was mostly studied on the analysis of microvesicles secreted by primary hepatocytes that were grown in cell culture, and important biomarker candidates for liver injury were identified. The liver is a complex organ, and hepatocytes constitute about 80% of the cell population of the liver. Residual 20% are occupied by other cell populations like Kupffer cells, hepatic stellate cells, endothelial cells and mesothelial cells that also play very important role in liver function. These cells also shed MVs, and they can be detected in body fluids. The liver perfusion animal model presented here can be applied as useful addition to already used methods for identification of biomarker candidates for both liver injury and malignancy. It also offers a useful alternative for very aggressive and stressful liver biopsy. The absence or low concentration of biomarkers for liver injury caused by the

perfusion demonstrates that the artifacts in MVs proteome caused by the perfusion procedure are minimal, and that this protocol can be used for their isolation and further analysis.

Keywords: Liver injury, Microvesicles, Perfusion, Proteome, Biomarker Candidates

Abstract Title: 2-Week Low-Salt Diet Ameliorates Cutaneous Inflammation and Improves Endothelium-Dependent Vasodilation in Psoriasis Patients

Part of the Dissertation Proposal: Effects of Low Salt Dietary Intake on Th17-Mediated Inflammation and Vascular Reactivity in Patients with Psoriasis

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Mentor: Assoc. Prof. Martina Mihalj, M.D., Ph.D., Department of Dermatology and Venereology, University Hospital Osijek; Department of Physiology and Immunology, Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Co-mentor: Assoc. Prof. Ana Stupin, M.D., Ph.D., Department of Physiology and Immunology, Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Introduction: Psoriasis presents an independent cardiovascular risk factor characterized by chronic low-grade systemic inflammation and oxidative stress which altogether might lead to endothelial dysfunction. It has been reported that increased oxidative stress has a pivotal role in high dietary sodium-induced endothelial dysfunction. Previous studies on sodium accumulation in psoriatic skin lesions and the sodium-induced augmentation in Th17 immune response, raise the question on the complex interplay between sodium and psoriasis, especially in the context of cardiovascular morbidity.

Aims: We aimed to investigate the effect of a 2-week low-salt diet on endothelium-dependent and endothelium-independent cutaneous microvascular vasodilation in patients with psoriasis vulgaris.

Materials/Participants and Methods: The study was designed as an interventional study in which patients with psoriasis were assigned to same study protocol. Severity of psoriasis was determined using the Psoriasis Area and Severity Index (PASI). The participants were instructed to maintain a low-salt diet (LS diet) according to DASH eating plan, with sodium intake of 1500 mg (3.75 g of salt), within the period of 14 days. Cutaneous microvascular reactivity in response to vascular occlusion (post-occlusive reactive hyperemia, PORH), iontophoresis of acetylcholine (ACh induced dilation, AChID) and sodium nitroprusside (SNP induces dilation, SNPID), and local thermal heating (local thermal hyperemia, LTH) were assessed using laser Doppler flowmetry (LDF).

Results: Twenty psoriasis patients completed the study protocol. 24-hour natriuresis confirmed that participant conformed to the LS diet. PASI, serum creatinine, systolic and diastolic blood pressure significantly decreased following 2-week LS diet compared to baseline measurements. Furthermore, both PORH and AChID (considered endothelium-dependent vasodilation) significantly increased after the 2-week LS diet compared with baseline measurements (PORH: baseline 100.1±2.1 vs. LS diet 120.9±34.1; p=0.048; AChID: baseline 12.6 (7.1 15.4) vs. 12.1 (9.1 20.9), p=0.048). SNPID and LTH did not significantly change following LS diet compared to baseline.

Conclusion: A 2-week LS diet led to a decrease in clinical presentation and cutaneous inflammation in subjects with psoriasis, and to an increase in endothelium-dependent vascular reactivity in psoriasis patients, as seen in significantly increased PORH and AChID. According to LTH results, it seems that LS diet affects vascular endothelium potentially via modulating endothelium derived vasoactive mediators other than nitric oxide.

MeSH/Keywords: (5) Diet, Sodium-Restricted; psoriasis; Laser-Doppler Flowmetry; microcirculation; endothelium

Abstract title: Using 4-dimensional Computed Tomography to detect effects of adipose tissue on skin surrogates for lung lesion respiratory motion

Part of the dissertation proposal: There is a connection between respiratory induced skin and lung lesion motion that can be detected using 4DCT, and it is affected by the amount of the patient's adipose tissue.

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

Introduction: Optical Surface Guidance (SG) systems track skin respiratory motion and use it as a surrogate for lesion respiratory motion during Stereotactic Body Radiation Therapy (SBRT). This enables intrafraction motion management without additional ionizing irradiation imaging. It has been shown that the patient's Adipose Tissue Amount (ATA) can affect the success of intrafraction motion management.

The goal of this study is to determine the relationship between skin and lung lesion respiratory motion using 4-dimensional Computed Tomography (4DCT), and how it is affected by the ATA.

Aims: To generate patient specific skin respiratory motion maps, and correlation maps between skin and lung lesion respiratory motions using 4DCT. To compare these maps between patients with different ATA.

Materials/Participants and Methods: 4DCT images of 28 patients of the same gender were acquired in our institution for the purpose of SBRT planning. For each of the 10 4DCT phases, a body structure (skin), and a bifurcation of a blood vessel in the lower right lung lobe (tracking structure), were delineated, and their displacements measured. Patient's skin was divided into 9 segments for purposes of comparison.

Results: Pearson correlation coefficient (R) was calculated for displacements of skin segments and tracking structure. Correlation maps and motion magnitude maps of the patient's respiratory skin motion were generated. Maps for patients of different ATAs were compared, and significant differences were detected.

Conclusion: Differences in ATA between patients affect respiratory skin motion of regions typically used for skin surface tracking.

Significance/Expected scientific contribution: This research can help to establish how different ATAs affect respiratory skin amplitudes and correlations to respiratory induced motion of lower lung lesion respiratory motion. Clinically, this can lead to better understanding of skin dynamics and to more precise application of surface guidance for lung treatment.

MeSH/Keywords: Patient Positioning; Radiotherapy, Image-Guided; Tomography, X-Ray Computed, Adipose Tissue, Body Surface Area;

Dissertation Proposal Title: Predictors of nurse care quality by nurses/technicians and hospitalized patients

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

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

Introduction: Nurses and technicians spend most of their time with patients, from the moment they arrive at the ward until they are discharged from the institution, It can be expected that satisfaction with health care is an important factor in assessing the quality of overall health care and will affect satisfaction with overall care during treatment. However, the assessment of the quality of health care is a subjective assessment of the one who receives the healthcare service and depends on various factors, both for the one who receives the service and for the one who provides the service. In our research, we will examine how much the personal variables of nurses / technicians and patients influence the assessment of the quality of health care from the patient's perspective.

Hypothesis:

1. Predictors of the quality of health care from the perspective of nurses / technicians are job satisfaction and perceived organizational justice.
2. Predictors of the quality of health care from the patient's perspective are neuroticism, extraversion and agreeableness as personality traits, as well as the length of hospitalization, level of education and perceived state of health.

Aims: To examine the predictors of the quality of health care from the perspective of nurses / technicians and hospitalized patients.

Materials/Participants and Methods: The research will include nurses /technicians of all levels of education and patients hospitalized in the wards. It was planned to examine 150 employees and 500 patients. The Job Satisfaction Index and Perceived Organizational Support Scale will be used on the sample of nurses / technicians, while the Neo five-factor inventory and the PSNCQQ quality of care questionnaire will be used on the sample of patients.

Research plan: This cross-sectional study will be conducted in the General County Hospital of Požega, in all departments during 12 months.

Significance/Expected scientific contribution: The result of this research would be scientific proof of the influence of psychological and organizational factors on the assessment of the quality of health care. By detecting predictors of the quality of health care, it will be possible to act in the prevention and elimination of factors in the organization that reduce the quality related to nurses /technicians. Also, we will try detecting the factors, which are related to patients, that contribute to the change in the perception of the quality of health care.

MeSH/Keywords: Quality of nursing care, Job satisfaction, Personality traits, Organizational justice

Abstract Title: Preanalytical aspects of isolation and sample preparation protocols for the single-cell imaging mass spectrometry analysis of human B-lymphocytes

Part of the Disertation Proposal: Metabolic characterization of B-lymphocytes in the chronic lymphocytic leukemia using imaging mass spectrometry

PhD candidate: Ivana Marković, Clinical Isntitute for Laboratory Diagnostics, University Hospital Centre Osijek, Osijek, Croatia

Mentor: Prof. Željko Debeljak, M.D., Ph.D., Clinical Institute for Laboratory Diagnostics, Osijek University Hospital, Croatia

Introduction: Single-cell MALDI-TOF imaging mass spectrometry (IMS) is a technology that enables the spatial metabolic characterization of individual cells and their environment. Single-cell IMS of human B-lymphocytes is expected to be useful in clinical conditions where their number or metabolism is altered, eg in chronic lymphocytic leukemia and other hematological neoplasmas.

Aims: to develop isolation and sample preparation protocols for the IMS of individual human B-lymphocytes.

Materials/Participants and Methods: Residual K3-EDTA whole blood samples (N=22) were used for the evaluation of 41 different B-lymphocyte isolation and sample preparation protocol. 150 µL of whole blood was mixed and incubated for 20 minutes with 20 µL of CD 19-FITC antibody solution (Beckton Dickinson, Franklin Lakes, New Jersey, USA). 2 mL of BD FACS Lysing Solution for red blood cell lysis was added and incubated for 5 or 10 minutes. The solution was then centrifuged for 5 minutes at 3500, 2000, 900, 600 or 400 g and washed with PBS 2 or 3 times. 150 µL of isolated leukocytes in PBS were transferred to an indium tin oxide (ITO) glass slide (Merck, Darmstadt, Germany) using a cytocentrifuge at 700 or 600 g for 8, 6, 4 or 2 minutes. After 1 second fixation with 100% methanol, the slide was placed in a volatile buffer solution (150 mM ammonium acetate or 100 mM N-ethylmorpholine formate) for 10 or 20 seconds. Matrix α-Cyano-4-hydroxycinnamic acid (CHCA) (Merck, Darmstadt, Germany) was sublimated on ITO slides for 2.5, 3, 3.5 or 4 minutes. The matrix on the ITO slide was recrystallized using 400 µL of 5% methanol in water solution in a sealed container heated to 64.7°C for 70, 40 or 30 seconds. The isolation and sample preparation procedure was evaluated and analyzed using an iMScope TRIO MALDI-TOF analyzer (Shimadzu, Kyoto, Japan). An integrated light and fluorescence microscope was used

for gross assessment of cell integrity and identification of CD19+ B-lymphocytes. A detailed assessment of cell integrity was obtained by MS analysis by evaluating the spatial distribution of membrane phospholipid signals on the cell surface.

Results: Increased cellular decay was observed under prolonged exposure to Lysing Solution, centrifugation speed higher than 900g and more than 2 washing steps. Decay was observed with fluorescent mycroscope where bare nuclei fluoresce due to the CHCA binding to DNA. Furthermore, increased decay was also observed with rough cytocentrifuge conditions (more than 600g and 4min) during the cell transfer on the ITO slide. In sample preparation protocol, longer exposure to volatile buffer induces cell bursting (especially with N-ethylmorpholine formate). CHCA sublimation and recrystallization longer than 2.5 min and 30 sec, respectively, reduces transparency and cell visibility under the light microscope, and disable B-lymphocyte identification. MS analysis of membrane phospholipids in inadequate sample preparations revealed cell debris that was invisible to light microscopy.

Conclusion: Adequate and dainty B-lymphocyte isolation procedure from human blood and consecutive sample preparation procedure for IMS are essential for obtaining reliable and reproducibile results.

MeSH/Keywords: B-lymphocyte, MALDI-MS, sample preparation methods, CD19

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, 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, and yGT in the serum, and in the case of myalgia, by determining CK.

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

Pharmacogenomic Testing in Croatia: Allele Frequencies of Genes Encoding Enzymes, Receptors, Transporters and Major Histocompatibility Complex (MHC) Antigen.

Part of the Dissertation Proposal: Potential health and economic benefits of proactive pharmacogenomic testing in the population of the Republic of Croatia

PhD candidate: Vid Matišić, M.D., St. Catherine Specialty Hospital, Zagreb, Croatia

Mentor: Prof. Dragan Primorac, M.D., Ph.D., St. Catherine Specialty Hospital, Zagreb, Croatia and Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Introduction: Personalized medicine can add considerable value to healthcare, where pharmacogenomics (PGx) is a critical component of personalized medicine. The field of pharmacogenomics is actively developing in the present day, with stakeholders from both the public and industry sectors implementing new solutions to improve the safety and efficacy of medications. In our institution, panel-based testing for single nucleotide polymorphisms (SNPs) was implemented for 27 genes (91 polymorphisms) known to influence the pharmacologic properties of over 300 drugs.

Aim: The aim of this retrospective cross-sectional study was to investigate the allele frequencies of genes encoding enzymes and receptors in a Croatian population. The population investigated are patients who had undergone pharmacogenetic testing using the RightMed panel in the period from 2018 until 2023.

Participants and Methods: Retrospective analysis of patient health records was performed, which included 522 patients who underwent pharmacogenomic testing by the RightMed panel using a TaqMan quantitative real-time PCR method and CNV analysis to determine the SNPs in the 27 targeted genes. Allele frequency were determined for the 26 reported genes.

Results: The analysis demonstrated wild-type alleles to be the most frequent for CYP2B6 (61.8%), CYP2C9 (76.4%), CYP2C19 (60%), CYP2D6 (37,3%), CYP3A4 (93.9%), CYP4F2 (69.4%), DPYD (99%), DRD2 (93.5%), NUDT15 (99.6%), TPMT (97.5%), UGT1A1 (61%), HTR2C (80.8%), IFNL4 (68.6%), OPRM1 (87.5%), SLC6A4 (61.4%), VKORC1 (56.5%). COMT locus rs4680 was found to have nucleotide G in 48% and nucleotide A in 52% of patients. GRIK 4 locus rs1954787 had the nucleotide T in 45.5% and C in 54.5%. HTR2A locus rs7997012 nucleotide A was present in 46.5% and G in 53.5%. CYP1A2 had the increased inducibility allele *1F present in 62.2%, while the wild-type

allele was present in 30.9%. CYP3A5 had a reduced function *3 allele frequency of 93.9%. F II and F V wild-type alleles were present in 98.5% and 98.4%, respectively. MTHFR rs1801133 C allele was present in 65.7% and rs1801131 A allele in 67.7%. CYP2C cluster rs12777823 G allele was present in 85.1%. HLA-A *31:01 allele was found only in 1.8%. HLA-B *57:01 and HLA-B *58:01 were found in 1.9% and 1.6%, respectively.

Conclusion: This study contributes to the growing body of literature on the prevalence of genetic variations that influence drug response in different populations. The allele frequencies observed in our study can serve as a valuable reference for future studies investigating drug efficacy and safety in the Croatian population. Further research efforts should be made to investigate the potential of proactive pharmacogenomic testing to improve therapeutic outcomes for patients.

MeSH/Keywords: pharmacogenomics, gene-drug interactions, allele frequency, personalized medicine, SNPs

Acknowledgment: None.

Prognostic value of CDC20 and Securin in patients with Diffuse large B-cell lymphoma

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Introduction: Diffuce large B-cell lymphoma (DLBCL) is the most common lymphoma and belongs to aggressive lymphomas. The pathophysiological mechanisms of tumorigenesis in DLBCL are still not well understood. DLBCL tumor cells are cells with a high proliferation index, so studying the regulation of cell entry into mitosis is of particular interest. The anaphase-promoting complex/cyclosome with co-activator CDC20, cell division cycle protein 20, plays a key role in the transition from metaphase by causing the degradation of securin, a separase inhibitor. Studies have shown that CDC20 acts as an oncogene during oncogenesis. Given the importance of the regulatory protein securin, there is an indication that high expression of securin along with CDC20 on DLBCL cells, measured at diagnosis, is an independent prognostic factor for disease progression.

Hypothesis: In DLBCL patients, high expression of CDC20 and securin measured at diagnosis is an independent prognostic factor for disease progression, overall survival, and event-free survival.

Aims: To investigate the expression level of CDC20 and securin in tumor tissues from DLBCL patients, to investigate whether the expression of securin and CDC20 in DLBCL tumor cells at the time of diagnosis is an independent prognostic factor for survival, and to evaluate their joint prognostic activity and their relationship at the time of diagnosis with laboratory and clinical characteristics.

Material/Participants and Methods: Review of the archives of the Department of Hematology identified patients who met the criteria for participation in the study. Paraffin blocks of formalin-fixed, histologically verified DLBCL tissue samples from the archival material of the Department of Pathology of KBC Osijek. Immunohistochemical analysis of these blocks with Securin and Cdc20 antibodies.

Research plan: Identification of patients who met criteria for study participation. Analysis of patient demographic and clinical data. Immunohistochemical treatment of tissue samples with Securin and Cdc20 antibodies. Investigation of the expression level of CDC20 and Securin in the tumor tissues.

Expected Scientific Contribution: The discovery of new immunohistochemical prognostic markers is an important contribution to the establishment of therapeutic guidelines.

MeSH/Keywords: diffuse large B cell lymphoma, cell division cycle protein 20, securin, prognostic value, survival

Dissertation Proposal Title: The association of anxiety with open-angle glaucoma of different degrees

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Mentor: Assoc. Prof. Katarina Dodig-Ćurković, M.D., Ph.D., Department of Child and Adolescent Psychiatry, Clinical Hospital Centre Osijek, Osijek, Croatia

Co-mentor: Assoc. Prof. Dubravka Biuk, M.D., Ph.D., Clinic for Eye Diseases, Clinical Hospital Centre Osijek, Osijek, Croatia

Introduction: The second most common cause of blindness in the world is glaucoma. It affects 2% of 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 etiologies and their common characteristic is that they result in a progressive and irreversible deterioration of the visual nerve and retinal nerve fibres, with corresponding outbursts in the visual field. It is necessary to identify it as early as possible in order to begin with the treatment of the patient, before it causes irreversible structural changes

Hypothesis: Anxiety is statistically significantly associated with different degrees of open-angle glaucoma.

Aims:

1. To examine whether there is an association between anxiety and open-angle glaucoma
2. Examine the proportion of anxious patients suffering from open-angle glaucoma
3. Examine the proportion of patients without signs of anxiety suffering from open-angle
4. To examine whether there is a statistically significant difference in the number of anxious patients suffering from open-angle glaucoma from patients suffering from open-angle glaucoma without signs of anxiety during the period from March 2023 to December 2023

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 that are not being treated for glaucoma or are not being controlled for elevated IOP values will be excluded from the survey.

Prior to the implementation of the Crown-Crisp Experience Index (CCEI), respondents will personally sign the informed consent and consent for its implementation and data collection. Examined and recorded will be sex and age of the subject, degree and duration of an open-angle glaucoma, length of treatment and number of used antiglaucoma medications, number of antiglaucoma drips drugs in the patient and whether the target IOP was achieved in the patient with this treatment.

The research will be conducted by the CCEI which is designed to identify and measure common symptoms and personality traits within conventional categories of psychoneurotic diseases and personality disorders. The overall score gives a measure of general emotional instability or neuroticism with a profile of six scores per subscale.

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. Planned duration of the research is ten months, from March 2023 to December 2023.

Expected scientific contribution: The proposed research will more clearly determine whether anxiety is connected with open-angle glaucoma of various degrees. In addition, the significant professional contribution of this research will be reflected in the improvement of the understanding that, by recognizing anxiety in patients, there is a possibility of preventing the development of open-angle glaucoma.

Keywords: anxiety; glaucoma; intraocular pressure

Abstract Title: Serum concentrations of Hepcidin-25 in colorectal cancer, a pilot study

Part of the Disertation Proposal: Serum Hepcidin-25 in colorectal cancer

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Introduction: Hepcidin-25 (Hep-25) as a key regulator in iron (Fe) metabolism is a potential new marker in colorectal cancer (CRC) diagnosis. Survival and proliferation of CRC cells depend on Fe concentration resulting in chemotherapy resistance if Fe cell concentration is high. Moreover, Fe deficit is correlated with poorer disease prognosis. Hep-25 as a prognostic marker can be correlated with poor therapeutic response.

Aim: The aim was to investigate Fe metabolism by measuring Fe metabolism parameters and Hep-25 in CRC patients.

Materials/Participants and Methods: Venipuncture was performed according to the guidelines at the Osijek University Hospital Centre, Osijek, Croatia and at the Clinical Hospital Centre "Sestre milosrdnice", Institute for tumors, Zagreb, Croatia in August 2020. All participants were male adults 18 years and older; a signed informed consent was provided. CRC diagnosis was confirmed by the clinician after colonoscopy and patients were divided into two groups: CRC I: newly discovered (N=15), and CRC II: diagnosed with CRC for more than one year (N=33). Fe metabolism parameters measured: Fe, transferrin (Trf), and ferritin (Fer) using the Beckman Coulter AU480 analyzer (Beckman Coulter, Inc., Brea, USA) by the spectrophotometric (Fe) and immunoturbidimetric method (Trf, Fer). Hep-25 was measured by the automatic ELISA method using DRG Hybrid XL (DRG Instruments, Marburg, Germany). All data were calculated using the MedCalc program (version 12.4.0.0. MedCalc Software, Marakerke, Belgium).

Results: Hep-25 serum concentrations in the CRC I group is statistically significantly lower compared to the CRC II group. Fe and Trf did not change between these two groups and Fer concentrations were significantly higher in the CRC II group.

Conclusion: Newly discovered CRC patients have altered Fe metabolism and lower Hep-25 serum concentration in comparison to patients living with CRC for more than one year. Hep-25 has the potential to augment clinical diagnostic and therapeutic practice. More research is needed to strengthen these findings.

MeSH/Keywords: enzyme-linked immunosorbent assay, colorectal neoplasms, hepcidins, iron, prognosis

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Abstract Title: The effect of BPC 157 on the systemic sequelae of induced open angle glaucoma in rat model

Part of the Disertation Proposal: Vascular and multiorgan failure (occlusion syndrome) peripheral and central in rat glaucoma. BPC 157 therapy.

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Introduction: Open-angle glaucoma is a chronic, progressive, and irreversible multifactorial optic neuropathy. The relationship between cerebrospinal fluid pressure, blood pressure and intraocular pressure have been understood as an important mechanism in the pathogenesis of glaucomatous changes. In recent study, it has been shown that therapy with stable gastric pentadecapeptide BPC 157 regimens immediately normalized intraocular pressure, preserved ganglion cells with normal fundus, retinal and choroidal blood vessel, and optic nerve presentation in open angle rat glaucoma model. Additionally, we revealed the therapy effect of BPC 157 on vascular failure and multiorgan failure in various models of occlusion and "occlusion-like" syndromes.

Aims: The aim of this study was to prove the beneficial effects of BPC 157 on the systemic effects induced by glaucoma in rats.

Materials/Participants and Methods: In deeply anesthetized female Albino Wistar rats, we cauterized 3 episcleral veins in both eyes to induce open angle glaucoma. BPC 157 was applied intragastric 15 minutes after cauterisation. Brain volume in craniotomized rats and the volumes of major blood vessels were measured by USB microscope camera. The pressures in the superior sagittal sinus as well as major blood vessels were measured.

Results: Rats in control group exhibited intracranial hypertension and aortal hypotension. Gross brain swelling and vein congestion was observed. BPC 157 therapy attenuated all pathological changes.

Conclusion: Acute glaucoma causes systemic blood changes which were reversed by BPC 157 application.

MeSH/Keywords: (5) pentadekapeptid BPC 157, glaucoma, rat, therapy

Dissertation Proposal Title: Use of lipidomics and changes in lipidomic status in patients with depressive disorder treated with antidepressants

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Introduction: From the available literature, we are familiar with the fact that lipid peroxidation is increased in depressive disorders, which results from increased oxidative stress of the organism and causes changes in lipid metabolism. Published studies show that depression is associated with low cholesterol levels and modulation of neurotransmitter signalling (5-HT1a, 5-HT2, and D1) by cholesterol in lipid rafts. By analysing the obtained mass spectrometry data, with the help of bioinformatics tools for statistical analysis, a complete representation of the lipidome and its adaptation to depressive disorder is obtained. It has been proven that several psychotropic drugs change the levels of individual sterol derivatives, so changes in sterol concentrations can be related to symptoms of depression and the clinical presentation of the disease.

Hypothesis: Psychopharmaceuticals significantly alter the lipid profile in patients with depressive disorder treated with antidepressants treated at the Psychiatric Clinic

Aims:

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

Materials/Participants and methods: The study will include patients with depressive disorders who are treated as outpatients and inpatients at the Psychiatry Clinic. A detailed clinical and psychiatric examination of the patient is carried out and diagnostic criteria are established according to the ICD-10 classification of depressive disorder. After that, an extended questionnaire on socio-demographic data, constructed for the needs of this research, is carried out in the selected population. Blood samples will be collected in serum tubes. In all serum samples, the types and concentrations of antidepressants will be determined by serum extraction followed by spectrometric analysis.

Research plan: Collect samples and conduct research. Sample analysis. Analysis of results. Publication of a pilot study based on the obtained results. Publication of final work results.

Significance/expected scientific contribution: The application of lipidomics in clinical studies would provide new insights into lipid profiling and the pathophysiological mechanisms underlying depressive disorders. The main goal of this research is to determine the detailed lipid status of patients with depressive disorder who are treated with different groups of antidepressants. Also, the goal is to gain insight into the concentration of antidepressants in the patient's blood, whether it is monotherapy or polytherapy, and to correlate the concentration of antidepressants in the blood with different segments of lipid status.

MeSH/Keywords: depressive disorder, antidepressants, lipidomics, mental disorders, ICD-10

Dissertation Proposal Title: Diagnostic criteria of early-onset neonatal sepsis (EOS) in preterm infants on multiple level

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Introduction: A life-threatening condition in premature infants caused by a systemic inflammatory response to an invasive microbiological agent is called neonatal sepsis. The term "neonatal sepsis" is used when the onset of the infection occurs within the first 28 days of life, and it can be early-onset (EOS), which occurs within the first seven days of life, or late-onset, which occurs in the rest of neonatal period. The diagnosis of early-onset sepsis is usually not confirmed by biomarkers, because they are non-specific at that age, but it establishes clinically and proves by blood culture (current gold standard), which takes up to several days for results. For this reason, it is necessary to find new and more precise diagnostic biomarkers for neonatal sepsis.

Hypothesis: It is necessary to introduce new biochemical markers on a multilevel approach in the diagnosis of neonatal early-onset sepsis, in order to detect it before the appearance of its clinical indicators.

Aims:

1. To determine the diagnostic value of serum concentrations of hs-CRP, IL6, presepsin, pro-adrenomedullin, and procalcitonin, in EOS, in preterm infants, with gestational age <37 weeks and a birth weight <2500 grams.
2. To determine the levels and interrelationships of previously mentioned biochemical markers, haematological indicators and their temporally resolved dynamics in early neonatal sepsis.
3. To analyze the significance of the pathology of pregnancy, childbirth, and the placenta on the development of EOS

Materials/Participants and Methods: The study was organized as a prospective cohort. The research will be conducted at the Department of Neonatology (NICU level III), University Hospital Center (UHC) Osijek. It was planned to include 166 premature infants of low birth weight, of both sexes, born in 2022 and 2023. The umbilical cord blood will be sampled immediately after birth, and processed in the Medical-

biochemical laboratory of UHC Osijek. Data on the pathology of pregnancy, childbirth, and placenta will be included.

Inclusive criteria:

- gestational age less than 37 weeks

- birth weight less than 2500 grams

Research plan: The initial pediatric examination will be performed immediately after delivery. Blood will be sampled from the umbilical cord for biochemical and microbiological analysis, and newborns will be referred to the Department of Neonatology, where venous blood will be routinely sampled for analysis of hematological indicators. The diagnosis of early-onset sepsis will be made clinically, based on the physical status of the newborn and predisposing factors (pathology of pregnancy, birth and placenta). Clinical and laboratory monitoring of patients will be carried out until the moment of their healing, that is, discharge from the hospital.

Significance/expected scientific contribution: We would like to point out the possibility of non-invasive cord blood sampling as a new and adequate approach for the analysis of biochemical markers of EOS. To introduce new and potentially more useful biomarkers on a multi-level, with the aim of increasing the precision of EOS.

MeSH/Keywords: Preterm infants, Early-onset neonatal sepsis (EOS), Presepsin (P-SEP), Pro-adrenomedullin (MR-proADM), Procalcitonin (Pct), Interleukin-6 (IL6), high sensitivity CRP (hs-CRP), Placental pathology

Dissertation Proposal Title: Effects of sodium-glucose cotransporter-2 inhibitors on the vascular endothelial function in patients with type 2 diabetes and developed peripheral artery disease

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Introduction: Several randomized controlled trials have demonstrated the advantages of sodium-glucose cotransporter-2 inhibitors (SGLT2i) for people with diabetes, heart failure, and kidney disease since they were first approved. Unlike well-known conditions like ischemic heart disease and stroke, patients with lower limb peripheral arterial disease (PAD) were underrepresented in those trials. Furthermore, an increased chance of amputations associated with individual SGLT2i has been the subject of some concern, although there was a lack of precise information on baseline PAD status. Laser Doppler flowmetry (LDF) is an established method for evaluating cutaneous microcirculation, including in patients with PAD. In our research, we will determine the effect of SGLT2i therapy on microcirculation in patients with type 2 diabetes (T2D) and PAD.

Hypothesis: SGLT2i therapy in patients with T2D and PAD has a beneficial effect on vascular function and inflammation markers.

Aims:

1. To examine the effect of SGLT2i therapy on the skin's microcirculation of the foot.
2. To examine the effect of SGLT2i therapy on glycemic parameters, lipid profile, inflammation biomarkers, pro- and anti-inflammatory cytokines.

Materials/Participants and Methods: The research will be conducted on subjects with T2D and established PAD who have been referred to the Department of Endocrinology at the Clinical Hospital Centre Osijek. To observe a medium effect in the difference of numerical variables, with a significance level of 0.05 and a power of 0.8, the minimum required sample size is 42 subjects. The subjects' anamnestic data will be gathered and body composition will be analyzed by bioimpedance. A venous

blood sample will be taken to assess glycemic status, lipid levels, inflammatory parameters, and other biochemical safety parameters. Microcirculatory blood flow will be evaluated by LDF.

Research plan: Participants will have two visits during this study at which aforementioned data will be collected. After the first visit participants will start SGLT2i therapy, while the second visit will be after a 12-week treatment period.

Significance/Expected scientific contribution: The proposed research will result in new knowledge about the effects of SGLT2i therapy on microcirculation in T2D patients with developed PAD, which could help in the selection of an adequate pharmacological treatment in the aforementioned patient population. The results of the proposed study would potentially represent an additional incentive for more comprehensive and extensive research of this kind.

Keywords: type 2 diabetes; peripheral artery disease; sodium-glucose cotransporter-2 inhibitors; vascular function; laser Doppler flowmetry

Dissertation Proposal Title: Characteristics of Cytokine Response in Patients with Temporomandibular disorders treated with Occlusal Splint Therapy

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

Introduction: Temporomandibular disorder (TMD) is a multifactorial disease with complex etiopathogenesis. The main symptoms are persistent pain in the masticatory muscles and preauricular region, limitations in jaw function, and sounds in the temporomandibular joints. The most commonly used therapy is the occlusal splint (OS). The therapeutic effect of OS is attributed to various factors, however the exact mechanism is still unknown. One of the potential diagnostic methods is the use of molecular biomarkers. Studies have demonstrated a correlation between inflammatory cytokines and pain in TMD patients compared to healthy controls. Molecular biomarker levels can be determined from tissue samples and body fluids. Gingival crevicular fluid (GCF) is a serum exudate that enters the gingival sulcus from the gingival plexus of blood vessels and contains proteins, various cell types, electrolytes, cytokines, etc. Considering its composition, wide availability, non-invasive method, and speed of collection, GCF could be a new source of TMD biomarkers with potential diagnostic, prognostic, and therapeutic purposes.

Hypothesis: Proinflammatory cytokines level in GCF and serum are related to pain intensity before and after occlusal splint therapy in patients with painful temporomandibular disorders.

Aims:

1. to determine cytokines level in GCF and serum before and after OS therapy
2. to determine the degree of psychosocial dysfunction and oral health-related quality of life before and after OS therapy
3. investigate the correlation between cytokines level in GCF and serum
4. to determine the effect of OS on treatment outcomes, pain intensity, dysfunction, and psychosocial status of patients with painful TMD

Materials/Participants and Methods: 28 participants diagnosed with myalgia, arthralgia, headache attributed to TMP and/or painful disk displacement (with and without reduction) according to DC /TMD will be included in the study. Participants will be asked to complete self-assessment questionnaires: Graded Chronic Pain Scale (v2), Jaw Functional Limitation Scale-20, Patient Health Questionnaire-9, Genaral Anxiety Disorder-7, Patient Health Questionnaire-15, Oral Behaviors Checklist, Oral health Impact Profile, and Perceived Stress Scale-10. The occlusal splints will be fabricated from hard acrylic resin by the same dental technician in the dental laboratory. The GCF sample will be collected from the sulcus using sterile tweezers and sterile paper sticks and placed in an Eppendorf tube containing 250 µl of 0.9% NaCl solution. Blood samples will be collected in Vacutainers and stored at +4°C until centrifugation. For multicomplex quantitative analysis of proinflammatory cytokines (interleukin 1 beta (IL -1β), interleukin 6 (IL - 6), interleukin 7 (IL -7), interleukin 8 (IL -8), interleukin 13 (IL -13), tumor necrosis factor alpha (TNF-α)), adapted ProcartaPlex multiplex assays will be used.

Research plan: Patients who meet the inclusion criteria and sign an informed consent form will be enrolled in the study. GCF and blood samples will be collected and questionnaires will be completed before beginning of the therapy and at follow-up examinations one month and two months after. Participants and GCF samples will be collected at the Dental Prosthodontics Office at the Health Center Osijek-Baranja County while blood samples will be collected at the Mursa Medical Center. The biochemical analyzes will be performed in the Laboratory of Translational Medicine at the Department of Integrative Medicine, Faculty of Dental Medicine and Health Osijek.

Expected scientific contribution: This research will attempt to determine the effect of occlusal splint therapy on the concentration of inflammatory cytokines. In addition, this research will examine the role of GCF as a valuable source of molecular biomarkers that will be important in the future for the diagnosis, prognosis, and treatment of painful TMJ disorders.

MeSH/Keywords: biomarkers, cytokines, gingival crevicular fluid, occlusal splint, temporomandibular joint disorders

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

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

Hypothesis:

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

Aims:

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

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

Materials/Participants and Methods: The research included eighth grade students from selected primary schools. Prior to joining the study, all parents were offered to sign an informed consent document for their children's participation. Also, all parents were asked to complete a short questionnaire that included sociodemographic data and The Carolina HPV Immunization Attitudes and Beliefs Scale – CHIAS (McRee, Brewer, Reiter, Gottlieb i Smith, 2010.; Delač, Korajlija, 2019.). In addition to sociodemographic data, the student questionnaire contained several scales that assess knowledge about HPV and the vaccine, as well as an assessment of one's own knowledge (Caskey, 2009.; Delač, Korajlija, 2019.), and a scale of health beliefs and attitudes about HPV (MacArthur, 2017.; Delač, Korajlija, 2019.). Students, who participated in the research, were divided into 2 groups: a group of respondents who had health education, which is why they solved the questionnaire 4 times, and a control group that filled out the questionnaire 2 times - at the beginning and end of the research. During first visit to the primary school, all students involved in the research solved a questionnaire. Then, a group of respondents had a short lecture on health education, after which they again solved the survey questionnaire, as well as 4 weeks and 8 weeks after the health education. The control group of students also re-solved the survey questionnaire after 8 weeks. The duration of the research is one school year.

Results: According to preliminary results, the vaccination rate of this year's generation of eighth grade students in the selected schools is 61 %. The total vaccination rate of the participants in the observed generation is 64%. In all observed schools, this year's the vaccination rate is over 50% (53 % - 69 %), while last year's vaccination rate of the same observed school were ranged from 35 % to 66 %. The vaccination rate of the control group is the same as the vaccination rate of a group of respondents which is 64 %. However, students can decide to be vaccinated until the end of the school year, which is why there is a possibility that the percentage of vaccinated students will increase. Other data are still being statistically processed.

Conclusion: In the observed generation, there was an increase in vaccination rate against HPV by 11 %. The obtained results suggest the positive impact of this research and health education for the vaccination rate against HPV of the target population.

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

Effect of perioperative blood adiponectin levels on acute infflammatory response in pastients after masjor asbdominasl surgery

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Introduction: Gastrointestinal tumors are commonly presented for surgical resections. According to Global Cancer Statistics 2020: GLOBOCAN estimates colorectal carcinoma ranks third in terms of incidence, but second in terms of mortality for overall carcinomas worldwide. At the same time, obesity is a fast growing disease with of pandemic proportions with a current global prevalence of 39% according to the World Health Organisation (WHO). Many patients presented for major abdominal resections of gastrointestinal tumors are obese. Obesity is chronic disease with complex pathophisiology. Adipose tissue besides being a storage site is responsible for secretion of various adipokines with imunometabolic role. Adipokines (also called adipocytokines) are cell-signaling molecules (cytokines) produced by the adipose tissue that play many functional roles in energy/metabolic status of the body, and inflammation. Among adipokines, adiponectin is predominantly antiinfflamatory adipokine which inhibits production of infflammatory citokines (IL-6) and is decreased in obesity. Adipocyte dysfunction in obesity with altered adipokines release results in chronic low-grade inflammatory state. Surgical stress after major abdominal surgery in perioperative period causes neuroendocrine, metabolic and imunologic changes in organism with production of proinfflamatory citokines and results with appearance of systemic infflammmatory response syndrome (SIRS). Dysregulated and overrated SIRS in early postoperative period can lead to complications with additional comorbidities, longer hospital stay and poorer outcome. A low grade chronic infflammatory state in obesity and hypoadiponectinemia can enable the cytokine storm and exaggerated / dysregulated SIRS in obese patients after surgery. Obesity according to this knowledge presents independent risk factor for developing more severe systemic infflamatory response syndrome in early postoperative period after major abdominal surgery.

Hypothesis: Lower blood adiponectin levels are associated with higher systemic infflamatory response in patients after major abdominal surgery.

Aim of this study is to:

1. Investigate correlation between perioperative blood levels of adiponectin and markers of systemic infflamation in patients after major abdominal surgery.
2. Investigate correlation between perioperative blood levels of adiponectin and appearance of systemic infflamatory response in patients after major abdominal surgery.
3. Investigate correlation between body mass index (BMI), waist circumference (WC), ultrasound measurments of abdominal fat thickness and perioperative adiponectin levels in patients presenting for major abdominal surgery.
4. Investigate correlation between perioperative blood levels of adiponectin, body mass index (BMI), waist circumference (WC), ultrasound measurments of abdominal fat thickness and appearance of postoperative complications, intensive care unit (ICU) length of stay, overall hospital length of stay and final outcome after discharge in patients after major abdominal surgery.

Materials/Participants and Methods: Single-center prospective observational clinical study.

Inclusion criteria: age >18 yeras, patient presenting for major elective abdominal surgery of gastrointestinal system according to tumor.

Exclusion criteria: age<18 years, BMI<18.5kg/m2, patients with acute surgical conditions, patients with established acute systemic/local infection, patients with chronic corticosteroid therapy and/or immunomodulation therapy, patients with allergies to used anestehetics/analgetics in study.

Research plan: After ethical approval and written informed consent, demographic, antropometric and comorbidities data will be taken from all patients included in study. BMI, WC and ultrasound measurments of abdominal fat thickness will be taken preoperatively. Patients will bi divivded in two groups according to BMI: obese (O) and nonobese(N) group.

Blood collections for determining adiponectin levels, IL-6, Complete Blood Count with Differential Count, lactats in arterial blood, C-reactive protein(CRP), procalcitonin(PCT), plasma cholinesterase(PCE), albumins (ALB), neutrophil/lymphocite ratio (NLR), platelet/lymphocyte ratio (PLR), CRP/ALB ratio will be taken before surgery (1), 24 hours after surgery (2) and 72 hours after surgery (3). All patients will bi given the same technique of balanced general endotracheal anesthesia with the same drugs and the same postoperative multimodal analgesia regimen. Appearance of SIRS in first 72 hours postoperative period will be detected and documented according to major clinical SIRS criteria. Complications in early postoperative period during hospitalisation will

be including: anastomotic dehiscence, wound infection, reoperation, appearance of sepsis, pneumonia, uroinfection, noncardiac respiratory failure, (need for noninvasive oxygen therapy), mechanical ventilation >24 hours, atrial fibrillation, congestive heart failure, myocardial infarction, acute kidney injury/failure. Length of ICU and overall hospital stay with final outcome after discharge from hospital will be documented.

Significance/Expected scientific contribution: Understanding of the underlying mechanisms which contributes to the appearance and severity of SIRS in early postoperative period is important for developing more predictive diagnostics and possible treatment options for postoperative complications. The adipocytokines have important role in many aspects of inflammation and immunity. This study can help in better understanding the role od adiponectin in pathophysiology of SIRS after major surgery.

Dissertation Proposal Title: Pancreatic stone protein as a sepsis biomarker

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Introduction: Sepsis is life-threatening condition that results from dysregulated host response to infection, which can lead to multiple organ dysfunction. Incidence and mortality of sepsis are high, thus early diagnosis and treatment are important in order to improve the outcome. Pancreatic stone protein (PSP) is a protein for which was originally believed that its function is inhibition of calcium carbonate precipitation in pancreatic juice. However, recent studies suggested its role as a biomarker of infection and sepsis. PSP can differentiate, more accurately than C reactive protein (CRP) and procalcitonin (PCT), infection from sepsis and an inflammatory state from sepsis in intensive care patients.

Hypothesis: Increased PSP serum concentration in patients with bacteremia indicates the development of sepsis. Higher concentrations of PSP are associated with more severe clinical presentation and higher mortality in sepsis.

Aims: To measure PSP serum concentration in patients with bacteremia to examine if it correlates with sepsis development. To examine differences in changes of PSP in comparison with changes in CRP and PCT in sepsis and if higher PSP is related with sepsis severity. To examine if mortality is higher in patients with high PSP.

Materials/Participants and Methods: The research will be performed in 102 patients with positive blood culture test, at the Department of Infectology of the Clinical Hospital Centre in Osijek.

Research plan: In patients that fulfil all criteria for systemic inflammaytory response syndrom (SIRS) two sets of blood culture will be obtained. In those with positive blood culture test, serum PSP will be measured using Enzyme-Linked Immunosorbent Assay (ELISA). Demographics, anamnesis and clinical status, complete and differential blood

count, coagulation and biochemical blood tests, urine, urine culture andchest X - ray examination will be collected. Patients will be followed if they fulfil criteria for sepsis. As criteria for sepsis, the increase of two or more points in Sequential organ failure assessment (SOFA) criteria will be used. Ultimately, PSP will be compared between sepsis patients and those who have not fulfilled criteria for sepsis.

Significance/Expected scientific contribution: This research could prove that PSP can be used as an important biomarker for sepsis, which may facilitate early diagnosis of sepsis.

MeSH/Keywords: bacteremia; biomarker; pancreatic stone protein; systemic inflammatory response syndrome; sepsis

Disertation Proposal Title: Effects of atorvastatin and rosuvastatin on serum makers of endothelial dysfuction, lipid profile and reaction time in postmenopausal women

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Introduction: The average age at which menopause occurs is 50 years so women spend almost a third of their life in postmenopause. In postmenopause, there are significant changes in the lipid profile that favor increased atherogenesis: increase in the level of total and LDL cholesterol and decrease in the level of HDL cholesterol. Prevalence of dyslipidemia in premenopause is 35%, and in postmenopause is 65.2%. Dyslipidemia represents a high risk factor for the occurrence of cardiovascular disease.

Hypothesis: Use of atorvastatin and rosuvastatin in postmenopausal women causes a decrease in the concentration of serum markers of endothelial dysfunction, endocan and endoglin, and improves the reaction rate and cognitive functions compared to postmenopausal women who do not use statin therapy.

Aims:

1. To quantify the concentration of serum markers of endothelial dysfunction, endocan and endoglin, in postmenopausal women before and after use of atorvastatin and rosuvastatin.
2. To quantify the speed of reaction in postmenopausal women before and after administration of atorvastatin and rosuvastatin.
3. To examine whether there is a difference in the effect of statin therapy depending on the type of statin therapy.
4. To quantify the concentration of estrogen in postmenopausal women before and after the use of statin therapy.

Participants and Methods: Female persons, aged between 45 and 70, will be included. The study is a prospective study with two groups of subjects (using atorvastatin or rosuvastatin). Each group will contain 64 respondents, total of 128. On the basis of anamnesis and electronic health record we will collect demographic informations, informations about smoking status, alcohol consumption and physical activity, time of entering menopause, antropometic measures. Laboratory measurement will include: serum concentration of endocan and endoglin, lipid profile, urate and glucose in fasting plasma, glucose tolerance test, creatinine, AST, ALT, GGT, CK and CRP. The cognitive functions will be tested with the Montreal Cognitive Assessment (MoCA) and reaction time will be estimated with online Human Benchmark test and the survey on the behavior of risk groups in traffic**.**

Research plan: After the first measurement, treatment in subjects will begin with the dose of statin therapy that was recommended. Further measurements will be performed every 4 weeks, up to a total of 4 measurements. In each of the 4 points, all the measurements will be repeated, except cognitive function tests and the measurement of serum concentrations of endocan and endoglin (only at first and last measurement).

Expected scientific contribution: Serum concentration of endocan and endoglin in previous studies corresponded to the expression in the endothelial cells of blood vessels, so it can be used to monitor the atherosclerosis process. The results will contribute to a better understanding of the importance of the use of statin therapy, especially in primary prevention, in postmenopausal women who do not have cardiovascular incidents in their personal history and the impact of the use of statin therapy on a cognitive functioning and reaction time.

Keywords: cardiovascular risk, endoglin, hyperlipidemia, postmenopause, reaction time

Dissertation Proposal Title: Early biomarkers of the risk of developing a severe and complicated course of the disease in patients with SARS-CoV-2 pneumonia compared to pneumonia of other etiology

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Introduction: The rapid spread of SARS-CoV-2 in early 2020 marked a significant global health challenge. Acute kidney injury, occurs in SARS-CoV-2 positive patients, likely through microthrombosis rather than direct viral action. This study aims to validate the predictive value of urinary NGAL as an early biomarker for acute kidney failure in COVID-19 pneumonia and other pneumonias, as well as examining other relevant biomarkers.

Hypothesis: The predictors of severe and complicated disease course in patients with pneumonia are elevated urinary NGAL, leukopenia, thrombocytopenia, elevated IL-6, and elevated ferritin. These predictors are statistically significantly more common in patients with SARS-CoV-2 pneumonia compared to patients with pneumonia of other etiologies.

Aims: This study aims to identify biomarkers that may predict the severity and complication of SARS-CoV-2 and non-SARS-CoV-2 pneumonia. It also aims to investigate the correlation between elevated urinary NGAL as an early marker of acute kidney failure and the development of a clinically unfavorable disease course in these patients.

Participants and Methods: This study will enroll hospitalized patients with confirmed SARS-CoV-2 infection and radiologically confirmed pneumonia, along with patients with pneumonia but without SARS-CoV-2 infection. Patients with chronic renal failure, those receiving daily corticosteroid therapy, and those with disseminated malignant disease will be excluded. A minimum of 64 patients per group (128 total) will be enrolled. Patient medical history will be collected on admission, and serum

and urine biomarkers will be analyzed. Serum analysis will be repeated on day two of hospitalization to monitor whether the patient has developed a need for a higher degree of oxygenation or if clinical improvement occurs.

Research plan: This prospective cohort study will be conducted over 1-2 years at the Clinic of Infectious Diseases, University Hospital Centre Osijek. The study will monitor biomarker dynamics, hospitalization duration, and disease outcomes. Additionally, it will assess the incidence of acute kidney injury throughout hospitalization.

Significance/Expected scientific contribution: The contribution of this study lies in the early identification of patients developing severe and complicated pneumonia. This will enable timely and effective treatment, and provide insights into the unique features of COVID-19 compared to other causes of pneumonia.

MeSH/Keywords: Acute Kidney Injury; COVID-19; Lipocalin-2; Pneumonia; SARS-CoV-2

Dissertation Proposal Title: The effects of consumption of chicken meat enriched with carnosine on microvascular function and inflammation in patients with chronic coronary artery disease

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Introduction: There are a large number of factors that promote the development of endothelial dysfunction which underlies the development of cardiovascular diseases such as hyperlipidemia, arterial hypertension, diabetes, oxidative stress, and secretion of pro-inflammatory cytokines. Since the increased level of oxidative stress and activation of the endothelium underlies the development of endothelial dysfunction in cardiovascular diseases, food enrichment with carnosine could contribute to the improvement of vascular reactivity in the microcirculation.

Hypothesis: A diet that includes consumption of chicken meat enriched with carnosine will have a positive effect on reducing oxidative stress and inflammatory responses in patients with chronic coronary artery disease.

Aims: The aim of this study is to investigate the effects of consumption of functionally enriched chicken meat on oxidative stress and pro- and anti- inflammatory cytokines in patients with chronic coronary artery disease.

Materials/Participants and Methods: The study will include at least 30 participants, male and female with chronic coronary artery disease over age of 18 years. The markers of endothelial dysfunction and oxidative stress (CRP vWF, pro-inflammatory and anti-inflammatory cytokines, the activity of antioxidant enzymes, oxidative stress and antioxidant capacity) will be measured in serum before and after the dietary protocol.

Research plan: The study will be prospective, interventional and randomized. Thirty patients with chronic coronary artery disease will be divided into two groups, the experimental group, which will eat enriched chicken meat and a control group which will eat regular chicken meat for three weeks. After collecting the data, a statistical analysis will be done.

Significance/Expected scientific contribution: The expected scientific contribution of this research is to investigate the mechanisms, effects and possible benefits of the consumption of chicken meat enriched with carnosine as a functional food in improving the cardiovascular health of patients with chronic coronary artery disease.

MeSH/Keywords: carnosine; endothelial dysfunction; functional food; inflammation; oxidative stress

Dissertation Proposal Title: The significance of obesity and the influence of pepsin on the hypertrophy of lymphatic tissue of adenoids and palatine tonsils in children with obstructive sleep apnea

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Introduction: Hypertrophy of the adenoids in the nasopharynx and palatine tonsils is one of the most common causes of obstructive sleep apnea (OSA). The gold standard in diagnosing OSA is polysomnography. Adenotomy and tonsillectomy are the first line of treatment for children with OSA. One of the etiological factors of OSA nowadays is obesity. Changes in eating habits and increased physical activity are recommended. The role of pepsin as a promoter of the inflammatory response with the consequent hypertrophy of the lymphatic tissue of adenoids and palatine tonsils has been point out more and more.

Hypothesis: In children with obstructive sleep apnea with an increased BMI and by percentile indicating obesity, there is a difference in the level of pepsin expression on the palatine tonsils and adenoid vegetations compared to children with obstructive sleep apnea and a normal BMI.

Aims: To examine the presence of pepsin in children's saliva using protein electrophoresis and Western-blot methods; to examine the degree of pepsin expression in palatine tonsils and adenoids by immunohistochemical method; to compare the level of pepsin expression in the lymphatic tissue of palatine tonsils and adenoids with the results of the PSQ (Pediatric Sleep Questionnaire); to examine the association of BMI, body mass and percentile with the level of pepsin expression in palatine tonsils and adenoids

Materials/Participants and Methods: The study will include 100 patients under the age of 12 with an indication for tonsilloadenotomy. The sources of the obtained data are heteroanamnesis, height, body mass, body mass index (BMI), PSQ (Pediatric Sleep Questionnaire) and ENT status after clinical examination.

Research plan: Adenoids and palatine tonsils after tonsillectomy, together with the preoperative saliva sample, will be taken for further analysis. Lymphatic tissue will be subjected to immunohistochemical staining, which would identify pepsin-positive cells in the adenoid tonsil tissue and palatine tonsils, with grading from weak to strong positive. From the saliva, the presence of pepsin would be determined by electrophoresis and the Western blot method.

Significance/Expected scientific contribution: The scientific contribution would be in discovering the influence of pepsin as proinflammatory factor on the damage of the lymphatic tissue of adenoids and palatine tonsils and as a promoter of the cascade inflammatory response. Confirmation of pepsin as an etiological factor. Education of parents about children's eating habits. Introduction of antireflux therapy in preservation of the healthy lymphatic tissue and reduction of surgical procedures - tonsilloadenotomy.

Keywords: Adenoids, Palatine tonsils, Pepsin, OSA, food habits

Dissertation Proposal Title: "Effect of apneic oxygenation of nondependent lung during one lung ventilation on local and systemic inflammatory response"

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Mentor: Assist. Prof. Tatjana Šimurina, General Hospital Zadar, Zadar, Croatia

Introduction: During lung surgery anesthesiologist need to isolate side of lung that will be operated and that lung is not ventilated. In these conditions alveolar hypoxia develops. Low alveolar pO2 levels activate resident lung cells (macrophages, monocytes, epithelial cells) which then initiate pulmonary inflammation. Apneic oxygenation is method used to maintain oxygenation in a patient who is not breathing. Sometimes during one lung ventilation hypoxemia develops and apneic oxygenation is used in nondependent lung to increase arterial partial pressure of oxygen. There are no researches that analyse effect of apneic oxygenation during one lunge ventilation (OLV) on inflammatory response.

Hypothesis: Apenic oxygenation of nondependent lung during one lung ventilation decreases local and systemic inflammatory response and improves postoperative recovery.

Aims: To determine the influence of apneic oxygenation of nondependent lung on local and systemic inflammatory response, postoperative recovery and postoperative cognitive function.

Materials/Participants and Methods: Inclusion criteria: Patients older than 18 years with lung tumor scheduled for thoracotomy. Exclusion criteria: Patients younger than 18 years, use of drugs that could affect the stress response (corticosteroids and other immunosuppressants).

Research plan: Patients will be divided in two groups. One group will receive apneic oxygenation of nondependent lung during one lung ventilation and control group will not get it. Before surgery blood sample will be taken for complete and differential blood count, CRP, PCT, albumins, fibrinogen, interleukin 6. All patients will receive same technique of balanced anesthesia. After anesthesia induction double lumen tube will be inserted. Position of double lumen tube will be checked with fiberoptic bronchoscope. During bronchoscopy from distal bronchoalveolar tree bronchoalveolar lavate (BAL) will be taken. In BAL we will analyse cell count and interleukin 1 beta value. Before reexpansion of lung we will take BAL again for

analysis. After surgery patients will go to ICU. Blood samples will be repeated after 24 hours. Postoperative complications, recovery and cognitive function will be analysed.

Significance/Expected scientific contribution: To determine whether there are differences in local, systemic proinflammatory response, postoperative recovery and cognitive function in between patients who get apneic oxygenation of nondependent lung and control group that don't get it.

MeSH/Keywords: One lung ventilation, Apneic oxygenation, Proinflammatory response, Postoperative recovery, Lung surgery.

Dissertation Proposal Title: Prognostic significance of QuantiFERON test in predicting the severity of clinical picture in SARS CoV2-positive patients

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Introduction: In addition to early and rapid diagnosis of the disease, it is also crucial to detect patients with the potential development of a more severe form of the disease and to start active treatment in time. The need to determine early indicators of possible complications of the disease has been shown. Severe patients showed decreased Hb% and hematocrit, increased TLC, polymorph and monocyte count along with increased ferritin level, thus reflecting the acute phase response to viral infection. D-dimer levels have been reported as a reliable prognostic indicator of in-hospital mortality in patients admitted for COVID-19. The Qunatiferon test (interferon gamma release test (IGRA)), which is used for quantifying the immune status of patients against tuberculosis. QFT-Plus is discovery based on interferon-gamma (IFN-γ) which is released by the immune response mediated by T-cells after in vitro stimulation of human whole blood with Mycobacterium complex-specific antigens for tuberculosis. Furthermore, the QFT-Plus test non-specifically elicits a T-cell response and thus determines the immune capacity of the examined individuals. Given that a more severe form of the disease is expected in immunocompromised patients, the Quantiferon test could be a predictor of the severity of the clinical picture in COVID 19 the patient.

Hypothesis:A negative QunatiFERON test is an early indicator of clinical and laboratory worsening of the health condition in patients with acute SARS CoV2 infection and may indicate earlier hospitalization.

Aims: To examine the correlation of the results of the QunatiFERON test and the degree of severity of the clinical aspect of patients with acute SARS CoV2 infection. To examine the diagnostic utility of the QunatiFERON test as an early indicator of deterioration of clinical condition in patients with COVID 19 in group of patients with

comorbidities and in group of previously healthy patients. To determin the level of the QuantiFERON test in patients vaccinated against SARS CoV2 and ruled out of COVID 19 disease with a negative PCR for SARS CoV2 and vaccinated and acutely ill with COVID 19 disease confirmed by a positive PCR result for SARS CoV2.

Materials/Participants and Methods: The research will be conducted on patients with confirmed SARS Co2V acute infection and patients with confirmed COVID 19 vaccination, at Clinic for infectious diseases, Clinical hospital center Osijek, Croatia.

Research plan: Patients with positive PCR test for SARS CoV2 will be examined by a medical doctor. Basic anamnestic data will be taken from these patients: personal anamnesis: age (years), gender (M/F), existence of comorbidities (previous respiratory, cardiac, endocrinological, hematological, oncological, etc. diseases with special reference to previously cured TB). Data about current illness collected: Positive PCR test for SARS CoV2, day of illness upon admission to hospital, symptoms of illness (fever, respiratory, urinary and gastrointestinal complaints). Patients will be questioned abut their epidemiological history: information on vaccination against SARS CoV2, known contact with a SARS COV2 positive person, etc. Laboratory-diagnostic findings will be performed: PCR test for SARS Co2V (if positivity has not already been confirmed earlier), X-ray of the lungs, laboratory findings (leukocytes, erythrocytes, hemoglobin, hematocrit, platelets, prothrombin time, d- dimers, aspartate aminotransferase, alanine aminotransferase, gamma glutamyltransferase, urea, creatinine, total proteins, albumins, c reactive protein, lactate dehydrogenase, interleukin 6 and ferritin),Quantiferon test. The development of the clinical disease as well as the dynamic of laboratory and X-ray findings will be monitored. The Quantiferon test will be performed at the Institute of Public Health Osijek, and other laboratory-diagnostic methods will be performed at Clinical hospital center Osijek.

Significance/Expected scientific contribution: Early detection of COVID 19 patients who have a tendency to develop a more severe clinical picture, thereby enabling earlier adequate treatment. A clearer insight into the connection between infection with the SARS CoV2 virus and the level of interferon gamma in blood.

MeSH/Keywords: SARS CoV2; respiratory insufficiency; COVID 19; pneumonia

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

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Mentor: Assist. Prof. Sandra Šarić, Clinical Hospital Center Osijek, Osijek, Croatia

Introduction: Mortality and morbidity of patients with ST elevation myocardial inarction(STEMI) si not only because of mechanical obstruction of coronary artery but also result of biochemical disturbances. Vascular endothelial growth factor(VEGF-A) 165 is important factor of angiogenesis through inducing migration and proliferation of endothelial cells, increased vascular permeability, and modulating trombogenesis. In alternative ways of genetic cutting it could be changed in isoform - VEGF-A165b, that has the same affinity for combining with vasculoendothelial growth factor receptor(VEGFR-2), but it makes protein phosphorylation unsuccesful, resulting in antiangiogenesis. It has been shown in previus studys that despite complet coronary revascularisation in acute coronary infarction 50 % of patients have lower myocardial perfusion.4

Hypothesis: Increased plasma VEGF-A 165/VEGF-A 165b ratio is a marker for sucessfull angiogenesis after STEMI

Aims:

1. Compare levels of VEGF-A 165 and VEGF-A165b before, 24 hours after, and one month after primary percutaneus coronary intervention(pPCI) in STEMI patients with angiologycal marker of successful revascularisation TIMI flow (Thrombolysis in myocardial infarction), and clinical signs of successful revascularisation(simptoms, ST segement elevation resolution)
2. Evaluate global and regional longitudinal myocardial deformation by echocardiography(STRAIN rate deformation) in acute and chronic state after STEMI and compare result with levels of VEGF-A 165 and VEGF-A165b
3. Evaluate mayor adverse cardiovascular events 6 months after STEMI(worsening of heart failure, hospitalization because of heart failure, reinfarction, need for coronary revascularisation, death from heart failure, death from cardiovascular cause, all cause death) with levels of VEGF-A 165 and VEGF-A165b

Materials/Participants and Methods: We will involve 45 consecutive patients with STEMI without any exclusion factors. Procedural success after pPCI will be evaluated

by TIMI grade. Plasma levels of VEGF-A 165 and 165b will be detected with ELISA kits. With echocardiography global and regional longitudinal myocardial deformation will be evaluated.

Research plan: In all patients without exclusion criteria, blood samples will be taken before, 24 and 96 hours after, and month after PCI. Echocardiography exam will be done before hospital discharge and one month after STEMI. All patients will be followed by invastigators predischarge, one month and 6 months after discharge ambulatory or by phone.

Significance/Expected scientific contribution: We are expecting to show imbalans of proangigenic and antiangiogenic factors of vascular endothelial growth factor family in STEMI patients despite optimal pPCI and optimal medical terapy. Finaly, we are expecting to prove that reduction od heart function postinfarctly is the result of imbalanced corellation of VEGF-A165/VEGF-A165b, and it could be messured through clinical adverse events. The inhibition of antiangiogenic factor could ultimatly reduce mortality and morbidity burden in STEMI patients.

MeSH/Keywords: ST elevation myocardial infarction, vascular endothelial growth factor A 165, vascular endothelial growth factor A 165b, percutaneus coronary intervention, neoangiogenesis, proangiogeness

Abstract Title: Sodium-to-Potassium Ratio as an Indicator of Diet Quality in Healthy Pregnant Women

Part of the Disertation Proposal: The effect of table salt intake on the vascular function of pregnant women in the third trimester of pregnancy

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Introduction: High dietary sodium together with insufficient potassium intake are related to an increase in cardiovascular (CV) risk in general, that is the reason why sodium-to-potassium (Na-to-K) ratio has been introduced as a more reliable index to assess CV risk and CV-related mortality than either sodium or potassium intake alone. Excessive gestational weight gain (GWG) in pregnancy was recognized as a wide spread health issue especially because almost 75% of pregnant women exhibit excess or insufficient GWG.

Aims: This study aimed to investigate diet quality in healthy pregnant women at the third trimester of pregnancy based on the assessment of Na-to-K ratio from both 24-hour urine sample and food frequency questionnaire (FFQ), to compare dietary micro- and macronutrient intake with current nutritional recommendations (RDA), and to investigate whether gestational weight gain (GWG) is associated with Na-to-K ratio, and diet quality during pregnancy in general.

Participants and Methods: This cross-sectional study involved 65 healthy pregnant women between 37 and 40 weeks of gestation. Subjects body mass index, GWG, body composition, and arterial blood pressure were measured. Molar Na-to-K ratio was calculated based on 24-hour urine sodium and potassium excretion, as well as based on data obtained by EPIC-Norfolk food frequency questionnaire (FFQ). Data on average daily total energy, micro- and macronutrients, as well as food groups intakes were obtained by FFQ. Data were processed using appropriate software (FETA, FFQ EPIC Tool for Analysis).

Results: A Na-to-K ratio of 2.68 (1.11–5.24) does not meet nutrition quality and is higher than the WHO recommendations due to excessive sodium and insufficient potassium intake. According to FFQ, Na-to-K ratio was associated with a higher daily intake of soups, sauces, cereals, fats, and oils and a low intake of fruit and non-alcoholic beverages. A total of 49% of pregnant women exhibited excessive GWG, which was attributed to the increase in adipose tissue mass ($r = 0.323$, $P = 0.010$). Reported total energy intake (EIrep) was below the resting metabolic rate (RMR) ($p<0.001$). There was no significant correlation between GWG and EIrep, due to almost 41.5% of participants classified as low energy reporters (LER), with the lowest proportion of LERs found in the group of pregnant women with optimal GWG. Daily intake of vitamin D, vitamin E, folate, niacin, riboflavin, calcium, iron, and zinc was suboptimal compared to RDA.

Conclusion: The results of the present study indicate that molar Na-to-K ratio assessed by both 24-hour urine analysis and FFQ of healthy pregnant women in the third trimester of pregnancy does not meet recommendation set by WHO, due to excessive sodium and insufficient potassium intake. Also, FFQ results showed insufficient dietary intake of the substantial number of micronutrients compared to RDA. 49% of examined pregnant women exhibited excessive GWG, that was significantly attributed to the increase in adipose tissue mass.

MeSH/Keywords: pregnancy; sodium-to-potassium ratio; micronutrients; gestational weight gain; 24-hour urine; food frequency questionnaire

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Dissertation Proposal Title: The importance of serum levels of glial fibrillary acidic protein (GFAP), ubiquitin, ubiquitin C terminal hydrolase L1 (UCH-L1) and S100B protein as a diagnostic biomarker in acute traumatic brain injury

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Introduction: Traumatic brain injury is a world-wide important public health problem, causing an increase in the number of emergency department visits and hospitalizations in the USA, as well as lethal outcomes. In the USA there are more than 1.7 million traumatic brain injuries annually, with a tendency to rise. The assessed global incidence is 69 million annually, with a increasing prevalence in the las 25 years (11). According to a meta-analysis by Frost et all., which analyzed 15, the prevalence of traumatic brain injury was 12% (11). Due to large numbers of patients with head trauma in emergency departments and surgical emergency clinics, there is a great demand for neuroradiologic assessment (brain CT), which has developed into a key diagnostic tool in patients with head trauma and suspected brain injury. According to research, less than 10% of patients with head trauma have a positive finding in their neuroradiological assessment (8). With the goal of protecting patients from harmful ionizing radiation, the American academy of neurology revised its guidelines for treatment of concussion in 1997. (8). According to the new guidelines there is no need for a brain CT in head trauma during sporting activities, if there is no clear history of loss of consciousness (GCS<15), post-traumatic amnesia and focal neurological findings.

According to a retrospective study from 2012, the use of CT as a diagnostic tool in children, the cummulative dose of about 50 mGy could tripple the risk of developing leucemia, and doses of about 60mGy could tripple the risk of brain cancer. Eaven though the medical benefits should outweigh the low absolute risk from the radiation, the doses recieved fromt the CT should be as low as posible, and alternative procedures without ionising radiation should be considered (12).

According to scandinavian guidelines fot treating minor, minimal and mild traumatic head injury in adults, a great majority of patients had minimal brain injury with a GCS od 13-15 (95%), with a low prevalence of positive brain CT findings. Out of this small number of patients a very small number of patients will need accute neurosurgical treatments. Considering the possible harm from the ionising radiation, the economic and human resuorce burden it poses, guidelines for the diagnostic work-up in head trauma have been written with the goal of rationalising the use of CT imaging (7).

The nervous system of vertebrates consists of two heterogenous cell types: neurons and glia cells. Glia cells, of which astrocytes are an important group, were described by Virchow as the structural support elements of the nervous system during the 19th century. Astrocytes are a heterogenous family of morphologically and functionally diverse cells, whose structural plasticity is mostly maintained by a filament network made up from mostly vimentine and GFAP. The evidence that had been gathered during the previous years have shown that astrocytes fulfill various active roles in the maintenance of normal brain physiology, such as: secreting several compounds, building the blood-brain barrier, metabolizing several neurotransmitters, and maintaining the ion balance of the inter-cell matrix. Radial glia, an astrocyte precursor is involved in neuronal migration during embryonal development of the brain. Glia cells are also involved in the supporting of neurons through neurotrophic signaling required for their survival, proliferation, and differentiation.

Apart from their physiological role, astrocytes play an important role in the pathology of the nervous system. Glial fiber accumulation is an important histological sign of the astrocytes' response to central nervous system (CNS) injury, which is aptly called reactive gliosis. This kind of response is characterized by intensive astrocyte proliferation and a strong expression of GFAP. The presence of glial scars in several neurological disorders, such as multiple sclerosis, is a strong implication of the astrocytes' role in the physiological and pathological processes of the CNS.

Glial fibrillar acidic protein (GFAP), a protein initially isolated from MS plaques, is widely known as a marker of astrocyte differentiation, and is one of the main components of intermediary filaments of mature astrocytes. Aside from astrocytes, GFAP is present in non-myelinating Schwan cells in the peripheral nervous system and intestinal glial cells. The activation of the GFAP gene seems to play a key role in astroglial cell activation (astrogliosis) in neurodegeneration and after brain injury (1).

Despite some controversies surrounding GFAPs function within the brains physiology and pathophysiology, there has been a large amount of evidence regarding its active and relevant role in the development of the brain

GFAP is a monomeric protein of the intermediary filament that makes up the cytosceleton of astroglia (the same cell type where S100B is located) where it performs a structural role. Normally it's not present in blood, however during astrocyte death it's released into the cerebrospinal fluid, from where it can cross the blood-brain barrier due to its increased permeability (1). It's plasma levels are measurable for a prolonged period (half-life ≈ 48h) which enables its measurement in longer intervals after an injury (1). After a penetrant brain injury, as well as an injury caused by a high pressure pulse, its plasma and cerebrospinal fluid levels are elevated from 4 to 24h after the injury. Research has shown that substantial amounts of GFAP are released into the cerebrospinal fluid and plasma for hours and days after the initial brain injury (1). Considering the difficult distinction between a mild and medium brain injury in the emergency department, there are studies that have shown that GFAP plasma levels could be used to distinguish between them, and thus reduce the number of patients that would be candidates for neuroradiologic examination (brain CT) because of head trauma. The aforementioned characteristics make it an ideal biomarker for an emergency setting application.

Ubiquitin C terminal hydrolase L1 (UCH-L1) is a very common brain protein, it has been estimated to compose 1-5% of all neuronal proteins (2). Despite it only consisting of 23 amino acids, it possesses one of the most complicated 3D structures to be discovered. Apart from its expression in neurons, its expression in other healthy tissues is extremely limited, however its heavily expressed in some carcinomas (2). Despite the classification of UCH-L1 as a deubiquitinase enzyme (DUB), its specific function remains unclear. UCH-L1 is not necessary for neuronal development, however it plays an important role in axonal integrity and its dysfunction can influence neurodegenerative diseases (2).

Ubiquitine is a highly conserved protein consisting of 76 amino acids, that can be conjugated singularly or as an polyubiquitin chain to a target protein, thereby changing its function (2). Lysine is the most common target to be ubiquitinated, however in some cases some noncannonic side chains of serine, cysteine as well as N-terminal amino acids can be modified (2).

In neurons ubiquitination plays an important role in the regulation of development, function and neuron pathology. For instance it up- or down-regulates as well as affects the localisation and features of various proteins according to an increase or decrease of synaptic activity. The precise regulation of the ubiquitin systems remains a mystery, as well as the effects of specific functions or dysfunctions of the ubiquitin system on specific synaptic proteins and signal networks. The displacement of ubiquitin from its protein substrate is performed by deubiquitinase proteins (DUB). There are approximately 90 DUB-s in the human genome, out of which 4 are are in

the C-terminal hydrolase (UCH) subcategory. Each of the UCH has a N-terminal C12 peptidase domain formed from peptide-knot backbones, and a C-terminal extension and an unstructured loop that regulates access to the catalytic domain (2).

IT is also present in the sex glands, an to a lesser extent in fibroblasts during wound healing. Its presence in carcinoma cells is intriguing, considering the origin tissues of the carcinomas do not produce UCH-L1, such as pancreatic carcinoma, colorectal carcinoma and invasive breast carcinoma (2).

On a cellular level, UCH-L1 shows a strong, uniform cytoplasmic colouring in all brain neurons, as well as big sensor and motor neurons (2). This abundance of USH-L1 in neurons, together with its sparse appearance in other tissue has led to its clinical use as a neuron specific biomarker in brain trauma.

As an intracellular regulator, S100B has an effect on protein fosforilation, energy metabolism, cytoskeleton component dynamics, homeostasis, and cell proliferation and differentiation. As an extracellular messenger, in physiological concentration, S100B protects neurons from apoptosis, encourages axonal growth and astrocyte priloferation, while downregulating astrocytic and microglial reaction to neurotoxic substances, however high levels of S100B cause neuronal death (3).

S100B also serves a role outside of the brain: as an intracellular regulator it inhibits the post-infarction hypertrophy of cardiomyocytes, whereas as an extracellular messenger (in high levels) S100B causes cardiomyocyte death, activates endotelial cells and encourages vascular smooth muscle cells (3).

S100 is a multi-gene family of small (10kDa) Ca2+ binding proteins of the EF-hand type, it consists of 25 members and is exclusive to vertebrates. In humans the genes for S100A1-S100A16, S100A7L2, S100A7P1 i S100A7P2 proteins are present in the 1q21 chromosome and S100A11P, S100B, S100G, S100P i S100Z are in the e 7q22-q31, 21q22, Xp22, Xp22, Xp22, 4 chromosomes respectively. All members of this family, except S100G are Ca2+ sensor proteins who upon binding to Ca2+ start interactions with intracellular target proteins thus regulating their activity. The first member of the S100 family to be identified was S100B. This protein that has a brain-wide presence is mostly present in astrocytes (who seem to be its greatest source), however it seems to be secreted by other neuronal populations (3).

S100B is also secreted by other cells, outside of brain cells, such as melanocytes, adipocytes, chondrocytes, Schwann cells, glia cells, GI tract cells, adrenal gland matrix cells, smooth and striated muscle cells. Cardiomyocytes do not excrete S100B, however it is elevated in cardiomyocytes post-infarction (3).

As an intracellular regulator, S100B takes part in regulating protein fosforilation, energy metabolism, cytoskeleton component dynamics (thereby also reugulating cell shape and migration), Ca2+ homeostasis, and cell proliferation and differentiation (3).

There seems to be a strong regulation of S100B expression, with relatively strong upregulation in neuronal progenitor cells during proliferation and migration, followed by downregulation that coincides with glial cells differentiation and a re-expression in differentiated astrocytes.

S100B is mostly secreted by astrocytes, the secretion is regulated by numerous variables. It is also secreted by adipocytes, together with free fatty acids under the influence of cathecholamines. Furthermore, S100B is passively secreted from damaged neuronal cells (3).

The upregulation of S100B in astrogliosis is characteristic, in a process that consists of astrocyte proliferation and activation, and followed by their hypertrophy, an usual result of brain injury that can put brain tissue integrity at risk or during chronic brain inflammation. This leaves an open window for S100B to contribute to astrocyte reactivity after brain damage by favorising the migration of activated astrocytes to location of the injury, whilst also creating and/or stabilising the F-actine cytoskeleton of astrocytes. Probably through Src /PI3K/RhoA/ROCK and Src/PI3K/Akt/GSK3β/Rac1 pathways or by regulating still unknown intracellular activity through modules like Src/Pi3K (3).

The first evidence of S100B presence outside of neurons was found by Michetti et all. who had found measurable levels of S100B in the cerebrospinal fluid samples op people with multiple sclerosis. Since then S100B has been used as a marker of brain pathology (13). Later, Shashoua et all. had found S100B in brain tissue extracellular fluid, followed by Eldik and Zimmer who proved S100B in astrocyte cell lines during standard conditions (13).

Hypothesys:

1. There are increased serum levels of glial fibrillary acidic protein (GFAP), ubiquitin, ubiquitin C terminal hydrolase L1 (UCH-L1) and S100B protein after traumatic brain injury.
2. A positive report showing an increase serum level of glial fibrillary acidic protein (GFAP), ubiquitin C terminal hydrolase L1 (UCH-L1) and protein S100B can be compared to a positive finding on a brain CT, considering the time from the onset of the brain injury.

Aims: The aims of this study will be to establish the connection between neuroradiological findings (brain CT) due to acute brain injury with the dynamic of glial fibrillary acidic protein (GFAP), ubiquitin C terminal hydrolase L1 (UCH-L1) and protein S100B serum levels. Also the time difference between the brain injury and the increase of aforementioned biomarker serum levels, furthermore to compare the serum levels of the biomarkers to the extent of the brain injury. The aims are to determine the usefulness of routine laboratory tests in the assessment of acute brain injury.

Materials/Participants and Metods: The study will be conducted in the Emergency department (Surgical emergency clinic, Neurological emergency clinic, Radiology department) of the Clinical hospital "Sveti Duh". Trial candidates will be the patients that were examined in the surgical emergency clinic because of head trauma and had indication for a brain CT. The expected duration of the trial is 12 months. The trial protocol is expected to be approved by the ethical committee of the hospital. Trial subjects will be patients older than 18 years of age with a history of head trauma within the last 24h from admission. They will need to have been examined by a surgeon-traumatologist in the emergency surgical clinic and have a clear indication for a brain CT. Each patient will need to be examined by a neurologist to verify neurological deficits. Furthermore, each patient will have their consciousness graded by the Glasgow coma scale (GSC), where values of 15-13 will be classified as mild brain injury, values of 12-8 as medium brain injury, and values of 8-3 as severe brain injury. Regardless of neuroradiological (brain CT) findings each patient will have their serum levels of glial fibrillary acidic protein (GFAP), ubiquitin C terminal hydrolase L1 (UCH-L1) and protein S100B determined. The exclusion criteria consist of other brain pathology that has been found on the brain CT scan (ischemic or hemorrhagic stroke, brain tumors, brain anomalies, developmental anomalies of brain blood vessels), and more than 24h had had passed from the head trauma.

Research plan: An observational, prospective study is planned, according to the specific organization of prognostic accuracy studies. Patients will arrive at the Emergency department of the "Sveti Duh" clinical hospital: on their own accord, in an ambulance or after a visit to their general practitioner. There will be an initial examination performed by a surgeon in the Surgical emergency clinic. Each patient who will have undergone a neuroradiological examination will be examined by a neurologist and will have a blood sample taken for an analysis for the aforementioned biomarkers. Before the acquisition of the blood samples, the patients will have to sign an informed consent form to be a part of the study, the form will be approved by the ethics committee of the "Sveti Duh" clinical hospital. After reaching the expected number of samples a statistical analysis will be performed. The expected significance is $p<0.05$.

Significance/Exspected scientifitic contribution: Head trauma, and thusly brain injuries cause large numbers of emergency department visits. Most of those patients are candidates for a neuroradiologic examination (Brain CT). Through a initial physical examination, consciousness assessment, a neurology consultation and by determining the serum levels of GFAP, UCH-L1 and S100B the incidence of neuroradiologic examination (brain CT) can be lowered. By comparing the serum levels of the aforementioned biomarkers to the results of neuroradiological examinations, the biomarkers could be used as biomarkers for the ruling- out the presence of brain injury. Thus, a decrease of brain CT usage in head trauma could be achieved, leading to less exposure to harmful ionizing radiation in patients. Ionizing radiation can cause changes in metabolism, the physiological attributes of intracellular elements and can cause regeneration slowing. Furthermore, there could be a decrease in the financial and human resource burden of the healthcare system, and a decrease in the need for consultations of other specialists when examining head trauma.

MeSH/Keywords: head trauma, brain injury, biomarkers, brain CT, consciousness level.

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