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TABLE OF CONTENTS

Foreword

Dies doctorandorum is an annual event which we have to good pleasure of organizing again, now in 2020. This is an open and public event so we invite people from all spheres of life to join in. But the focus is mainly on our doctoral students whose work is opened to critique and debate.

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

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

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

As for the public, please keep in mind that some of our PhD candidates will get the opportunity to practice presenting their own ideas, concepts and research results for the first time. So, I would consider it positive if the audience could create an encouraging and inspiring atmosphere for our young candidates so they can feel satisfaction and pride related to their sometimes difficult and valiant efforts to further scientific development.

Professor Jure Mirat, M.D., Ph.D.

Dean, Faculty of Medicine Osijek

Abstracts of annual seminars

Dissertation proposal title: Stroke lipidomics under the condition of GM1 depletion

PhD candidate: Irina Bagić, M.D., Department of Neurosurgery, University Hospital Centre Osijek, Croatia

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

Introduction: Ischemic stroke is one of the main causes of death worldwide caused by a loss of blood flow to the brain resulting in cerebral hypoxia and irreversible neuronal damage. Currently, there is no targeted ischemic stroke therapy based on the stroke metabolics and lipidomics in every day clinical use. There is a potentially a benefit from use of ganglioside GM1 in ischemic stroke therapy based on its neuroprotective properties.

Hypothesis: Ischemic stroke caused by the middle cerebral artery occlusion in the *B4Galnt1* knockout mice will have a greater extent and more extensive oxidative lipid damage in comparison to the wild-type mice due to the lack of neuroprotective effect of the GM1 ganglioside.

Aims:

1. Induce an ischemic stroke (60 min) of the middle cerebral artery of one cerebral hemisphere in *B4Galnt1* knockout mice (KO), heterozygotes (HET) and wild-type mice (WT)

2. Make a lipidomic analysis of the affected and unaffected hemispheres 3 and 7 days after ischemic stroke with the help of mass spectroscopy imaging (MSI)

3. Compare the lipid profiles in non-affected hemispheres and in stroke-affected hemispheres

4. Determine the extent of ischemic lesion by immunohistochemical methods

Materials and methods: The experiments will be carried out on 3 months old mice placed in 3 groups of 16 animals based on their genotype (KO mice with deficiency in synthesis of GM1, WT mice expressing normal amount of GM1 and HET with one silent allele). For inducing ischemic stroke animals will be subjected to tMACAO method (transient middle cerebral artery occlusion), they will be sacrificed and the dissected brain will be preserved. Immunohistochemical and lipidomics analysis will be performed using MSI. Brain sections will be blocked in blocking solution and incubated in primary antibodies against GAP43, Trl2 and CASP3. Afterwards, sections will be incubated in corresponding secondary antibodies, and analyzed using fluorescent microscope.

Research plan: All animals from the three groups will be subjected to unilateral ischemia using tMACAO method. The impact of ischemia on motor and sensory functions will be evaluated using behavioral tests. Ischemic animals will be sacrificed on the 3rd or 7th day and dissected tissue will be stored at -80°C. Lipidomics analysis and immunohistochemical analysis with specific markers to determine brain lesion will be used.

Expected scientific contribution: The ganglioside GM1 could potentially be neuroprotective in cerebral ischemic conditions and its therapeutic properties are being considered for ischemic stroke management. Currently available KO mice present an opportunity to investigate the effect of this ganglioside in ischemic stroke recovery.

MeSH/Keywords: stroke, GM1 ganglioside, mice, brain ischemia, lipids

Dissertation proposal title: Changes in the thickness of the neurosensory retina in five macular areas after cataract surgery by ultrasound (phacoemulsification) depending on the amount of ultrasound energy consumed in healthy individuals and diabetics

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

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

Introduction: Gray cataract surgery by ultrasound (phacoemulsification) is a common method for cataract surgery. Ultrasound surgery has been shown to cause structural changes in the neurosensory retina of the posterior segment of the eye, more specifically in the region of the macula of the posterior half-eye, which is responsible for central visual acuity and a part of peripheral vision. Any pathological event in the posterior pole of the eye results in visual impairment and can create serious difficulties in the day-to-day functioning of the individual as a person. There are papers showing the relationship between the ultrasound method of gray cataract surgery and the thickening of the neurosensory retina in the center of the macula. However, there is no mention of the relationship between the ultrasonic energy consumed in phacoemulsification and the change in retinal thickness in the macula or the presentation of the change in retinal thickness outside the center itself, macules called foveol in healthy subjects, as well as subjects with diabetes.

Hypothesis: The change in the thickness of the neurosensory retina in the five measuring areas of the macula depends on the amount of ultrasonic energy expended during the ultrasound method of cataract surgery.

Aims:

1. Define the amount of ultrasonic energy used during the operation of gray cataract by ultrasonic method
2. Define change in neurosensory retinal thickness in five defined macular areas
3. To establish the relationship between the amount of ultrasound energy consumed and the change in the thickness of the neurosensory retina in the macula by defined areas in healthy subjects
4. To establish the relationship between the amount of ultrasound energy consumed and the change in the thickness of the neurosensory retina in the macula by defined areas in subjects with diabetes

Research plan: The study is planned as a prospective study that compares the thickness of the neurosensory retina in five defined areas of the macula and the amount of ultrasound energy consumed during gray cataract surgery by ultrasound. The measurement of the thickness of the neurosensory retina will be done by "optical coherence tomography - OCT" on the OCT Spectralis Heidelberg device, while the amount of ultrasound energy delivered will be read from the Oertli OS3 ultrasound surgery device. Patients from the Dr. Balog Polyclinic, a teaching base of the Osijek medical mchool, regularly scheduled for cataract surgery will be included in the study.

The order of study:

1. Hypothesis
2. Organization of the study
3. Data source
4. Method of sampling (according to the inclusion and exclusion criteria)
5. Pattern formation
6. Grouping data by groups (age, gender)
7. Archiving data
8. Statistical data processing
9. The results
10. Analysis of the obtained results

Materials/Participants and methods: The subjects will be patients in the regular program planned for ultrasound surgery according to the previously defined inclusion and exclusion criteria. Criteria for inclusion of respondents are the presence of an old gray barbell, structurally neat posterior half of the eye, diabetes, and exclusion criteria are diabetic maculopathy, diabetic retinopathy, vitreomacular traction, glaucoma, any other retinal pathology of the posterior half of the eye, posttraumatic eye movements, infection of the eye and systemic diseases with an impact on the eye other than diabetes.

Methods: Measurements of neurosensory retinal thickness measurements expressed in microns will be collected through a Spectralis OCT Heidelberg device, while ultrasound energy values during cataract surgery will be read on the Oertli OS3.

Statistic methods: There will be a systematic statistical analysis of the collected data in order to confirm the hypothesized work. The numerical data will be described by the arithmetic mean and standard deviation, in the case of distributions that do not follow the normal distribution by the median and interquartile ranges. The statistical methods we will use are to determine the normality of the Kolmogorov-Smirnov test, the methods of descriptive statistics and frequency, the methods parametric (and non-parametric as needed) simple analysis of variance i.e. ANOVA and multiple analysis of variance MANOVA. In the case of ANOVE or MANOVE significance, post hoc tests would be used to determine which pairs of groups were statistically significantly different from one another. Finally, a discriminant analysis will be conducted on all numerical indicators in order to eventually identify groups of variables that together may represent some discriminatory group differentiation factor. The program that would be used is Statistic for Windows 12.0. (DELL Computers, USA). A significance level of 5 % will be used in this study. The required number of respondents to the survey will be determined by a priori tests in G * Power free a total of at least 120 subjects divided into four groups should be selected for the moderate effect factor (effect size = 0.25) and for the four groups in the analysis of variance, for the required minimum power of 0.80 (power $\geq$ 0.80).

Significance/expected scientific contribution: Complete insight and contribute to understanding the relationship of ultrasound energy used and the change in the thickness of neurosensory retina in five areas of the macula in healthy subjects as well as in subjects with diabetes without diabetic maculopathy.

MeSH/Keywords: cataract, retina, macula, phacoemulsification, OCT, diabetes mellitus

Dissertation proposal title: Prognostic value of the cancer stem cells in rectal cancer

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Mentor 2: Assoc. Prof. Goran Kondža, M.D., Ph.D., University Hospital Centre Osijek, Croatia; Faculty of Medicine Osijek, University of Osijek, Osijek, Croatia

Introduction: The CRCs are a very heterogeneous group of diseases driven by a vast array of mutations and mutagens. For that reason, it is still difficult to find a 'catch-all' molecular therapy. The standardized principle of the therapy od CRC is neoadjuvant chemoradiotherapy, surgical resection, and adjuvant chemotherapy, if necessary, according to pathological staging. Despite this kind of therapy protocols, 40 % of patients have recurrent disease. Also, most of the treatments, especially surgical treatment, become inefficient in disease dissemination, which occurs in 25 % of cases. Five-year survival rate in this, stage IV, disease is less than 15 %. As mentioned above, due to the heterogeneity of mutations and still not fully known carcinogenic pathways, research of the development of colon cancer and prediction of its behavior should be our primary interest. In the last decade, the major focus has been placed on cancer stem cells (CSC) theory in CRC development.

Hypothesis: Cancer stem cells shape the heterogeneity of tumor, and in that way predict its behavior and prognosis.

Aims:

1. To describe CSC characteristics based on CD44 expression
2. To describe their number, position, relation with tumor size, and TNM staging
3. To describe the CD44 expression with microsatellite stability of the tumor
4. To describe the CD44 expression with tumor budding
5. To compare the CD44 expression cells with the control group, which is pathologic samples of the patients with colorectal adenoma.

Materials/Participants and methods: Samples of the rectal tumor tissue of 112 patients operated in abdominal surgery department during the 2017 and 2018 will be analyzed. Tumor tissue will be stained in the standard manner with HE and MLH1, MSH2, MSH6, PMS2 to be examined under the light microscope for histological gradus, pathological TNM stage, microsatellite stability, and tumor budding. Immunohistochemical staining with CD44 antibody will be performed in a standardized manner to analyze the characteristics of cancer stem cells in the samples. CD44 expression, which is presented as brown staining in the cytoplasm or on the membrane, will be analyzed in two areas of the tumor – whole tumor area and center of the tumor, both as the intensity of staining and percentage of the stained cells. Expression in the whole tumor area will be described as an immunoreactive score, max. score of 8, and in the center of the tumor it will be described whether to be mostly in the invasive front, the central front, or heterogeneously. Data will be statistically analyzed. Correlation of CD44 with an answer to neoadjuvant chemoradiotherapy, microsatellite stability, and tumor budding will also be analyzed.

Research plan: After getting permission from the Department for Pathology, the research will be presented to the Ethics Committee of the University Hospital Centre Osijek and the Faculty of Medicine Osijek. The samples will be retrieved from the archive, stained and analyzed. During the conduction of this research, minimal two papers will be published in indexed journals.

Significance/Expected scientific contribution: This research will contribute to the new theories in the understanding of colorectal cancer development. It will provide a better understanding of the cancer stem cell theory, their influence on the colorectal cancer characteristics, based on CD44 expression, which can be used as a new biomarker for prediction of the tumor behavior. In that way, it will be possible to personalize the treatment of each patient.

MeSH/Keywords: stem cell, rectal cancer, CD44, tumor marker, survival.

Dissertation Proposal Title: Diagnostic significance of carbohydrate sulfotransferase 7 in lung cancer

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

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

Introduction: Lung cancer represents a significant diagnostic challenge. Stages 1 and 2 are especially difficult to diagnose.The fact that carbohydrate sulfotransferase 7 (CHST7) controls biosynthesis of chondroitin sulfate proteoglycans (CSPG) involved in the carcinogenesis and metastatic processes shifts the focus from the well established markers like hyaluronan, versican and CD44 to the CHST7. All overall (prognostic) stages of primary lung tumors were associated with the significantly increased serum CHST7. According to previous research, CHST7 is a promising marker of lung cancer which needs to be evaluated for early stages of lung cancer.

Hypothesis: Serum or peripheral mononuclear blood cells` (PMBC) CHST7 may discern lung cancer from other pulmonary inflammations.

Aims: To evaluate diagnostic performance (efficacy) of CHST7 in differential diagnostics of lung cancer and other pulmonary inflammations

Materials/Participants and methods: Clinical case control study will be performed at University Hospital Center Osijek and Clinical Hospital Center Zagreb. It will include at least 100 participants suffering from lung cancer and other pulmonary inflammations. Blood samples will be taken from all enrolled subjects for the determination of serum and PMBC CHST7 and routinely used diagnostic panel of lung cancer biomarkers including serum CEA, CYFRA 21-1 and NSE. Cancer

types will be established using cytological and histological samples according to the World Health Organization classification of lung tumors. Serum CHST7 concentrations (μg/L) of the undiluted samples will be determined using ELISA. Concentrations of CEA, CYFRA21-1 and NSE will be measured using the electrochemiluminescence immunoassay. Mann-Whitney U (MWU) test will be applied for the unmatched pairwise comparisons. Together with wellknown diagnostic parameter performance metrics, the difference in receiver operating characteristic (ROC) area-under-curve (AUC), i.e. Δ ROC AUC, will be used in this study.

Research plan: Blood samples will be taken from all participants, serum and PMBC CHST7 content will be determined together with other lung cancer specific tumor marker, carcioembryonic antigen (CEA), neuron specific enolase (NSE) and cytokeratin fragment 21-1 (CYFRA) concentrations will be determined in serum while lactate dehydrogenase (LDH), ferritin and NSE content will be determined in PMBC.

Significance/Expected scientific contribution: We expect that the possible diagnostic significance of CHST7 in lung cancer will be reliably determined.

MeSH/Keywords: CHST7, lung cancer, carbohydrate sulfotransferase, serum, PMBC

Dissertation Proposal Title: Treatment and quality of life in postmenopausal women with vulvovaginal atrophy

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

Introduction: It is estimated that vulvovaginal atrophy (VVA) affects between 50 % up to 90 % of postmenopausal women, and it can be more severe in cancer patients. With longer life expectancy and contemporary gynecological cancer treatments it is to be expected that there will be an increased VVA morbidity. One of the main problems with VVA is that, even though there are many affected women, most of them do not report VVA symptoms and therefore are not treated, which affects their quality of life and sexual health. Today there are several treatment options for VVA which include topical lubricants and moisturizers, topical and systematic hormone therapy and laser therapy.

Hypothesis: Women suffering from VVA have poorer quality of life and poorer sexual health.

Aims:

1. To determine incidence and severity of VVA
2. To investigate clinical characteristics of VVA
3. To design a clinical tool for assessment of VVA

4. To investigate severity and impact of VVA symptoms and related sexual matters and quality of life

5. To evaluate different therapy options and their results in treating VVA

Materials/Participants and Methods: In this non-randomized clinical trial, after giving consent to participate in the research, patients will be divided into three groups according to their treatment options (topical therapy, systemic hormone therapy and laser therapy) and they will undergo a pre-treatment gynecological examination which will include Vaginal and Vulvar assessment scales, life quality and sexual health questionnaire as well as laboratory tests. After the treatment, patients will undergo the same process they did pre-treatment.

Research plan: Prior to the main study a pilot study will be carried out to translate, adapt and validate Clinical Tools for VVA (Vaginal and Vulvar assessment scales), quality of life and sexual health. After the validation of research tools, the main study will be carried out.

Significance/Expected scientific contribution: Determination of incidence of VVA, severity of its symptoms, impact it has on their quality of life and sexual health as a way of better understanding risk factors for the development of this condition and a way to create successful prevention programs.

MeSH/Keywords: quality of life, sexual health, vulvar diseases, laser therapy, menopause

Abstract Title: The coccoid form of Helicobacter pylori is correlated to histopathological characteristics of stomach mucosa

Part of the Disertation Proposal: The coccoid form of Helicobacter pylori is correlated to histopathological characteristics of stomach mucosa in Helicobater pylori associated gastritis

PhD candidate: Nikolina Brkić, M.D., General Hospital Vinkovci, Croatia

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

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

Aims: To evaluate is it the coccoid form of HP correlated to pathohistological characteristics of stomach mucosa by immunohistochemical method.

Materials and methods: The material are the samples of gastric mucosa obtained by ambulatory gastroscopic examination of patients with dyspeptic disorders in the period between January 2018. and May 2020. This material is collected from the archives of the Department of Pathology and Cytology at a general hospital. Paraffin blocks of samples, prepared by standard histological preparation and stained with hemalaun-eosin, are stained by immunohistochemical method on HP antigen. Statistical data processing was done using MedCalc Statistical Software version 19.2.1.

Results: In this study data from 214 patients were analyzed, of which 52.3 % are women. The median age is 57 years with an interquartile range of 12 and 83 years. HP is mainly present in both spiral and coccoid forms: in the gastric antrum od 63.6 % of patients and in the gastric corpus of 92.5 % of patients. Only the coccoid form of HP has been described in 6.5 % of patients in the antrum and 1.4 % of patients in the gastric corpus. Gastric atrophy has been reported in 7.6 % of patients in the antrum and 6.1 % of the patients in the gastric corpus. Gastric mucosal metaplasia has been described in 21.5 % of the patients in the antrum and 8.5 % of patients in the corpus. The coccoid form is associated with atrophy in the corpus (Spearman correlation coefficient rho = 0.136, p = 0.046).

Conclusion: Describing the coccoid form of HP with histopathological changes of the gastric mucosa would be useful to clinicians in the patient’s treatment process.

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

Dissertation proposal title: Efficacy of percutaneous laser disc decompression versus epidural steroid and local anesthetic by transforaminal approach in the treatment of lumbar radicular pain

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

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

water evaporation, thereby reducing the pressure in the intradiscal space and achieving disc decompression. Later, a stable scar is created at this site, which prevents the re-herniation of the intervertebral disc.

Hypothesis: There will be no differences in effectiveness of reducing pain intensity, improving quality of life, reducing degree of disability, reducing neuropathic pain, reducing anxiety and depression, and improving sleep quality in treating lumbar radicular pain caused by disc herniation without discoradicular contact, but PLDD will be more effective in patients where there is discoradicular contact.

PLDD will lead to a greater reduction in serum levels of inflammatory markers in patients where is nerve compression caused by intervertebral disc herniation.

ESI TF will lead to a greater reduction in serum levels of inflammatory markers where is no nerve compression by intervertebral disc herniation.

The group of patients underwent PLDD will have significantly higher retraction of disc herniation compared to the ones in ESI TF.

Aims:

1. To compare effectiveness in reducing the intensity of pain with epidural steroid (ESI) and local anesthetic using transforaminal approach (ESI TF) and percutaneous laser disc decompression (PLDD) in patients with lumbar radicular pain caused by intervertebral disc herniation.
2. To compare a difference between ESI TF and PLDD in intervertebral disc herniation with and without discoradicular contact.
3. To compare the effect of ESI TF and PLDD on serum levels of inflammatory markers interleukin 6 (IL-6), leukocytes, C-reactive protein and differential blood count.
4. To compare the association between inflammatory marker levels and pain intensity reduction in ESI TF and PLDD in the treatment of lumbar radicular pain caused by intervertebral disc herniation.

5. To compare the impact of PLDD and ESI TF in the treatment of lumbar radicular pain caused by disc herniation on quality of life, degree of disability, neuropathic pain, degree of anxiety and depression, and quality of sleep.
6. To compare the effect of PLDD and ESI TF on disc herniation retraction.

Materials/Participants and methods: Criteria of inclusion: examinees will be persons between 18 and 60 who agree to participate in the studies which they will confirm by signing the informed consent. Examinees have unilateral lumbar radicular pain, pain duration of 0 - 6 weeks so that we can exclude patients who have chronic pain, which does not respond to conservative treatment, disc herniation at one level, magnetic resonance verified disc hernia, pain intensity measured by VAS scale from 0 - 10, > 5. Exclusive criteria: patients younger than 18 and older than 60 years, patient refusal to participate in the study, central lumbar canal stenosis, lumbar radicular pain caused by causes other than intervertebral disc herniation, pregnancy. Furthermore; steroid, local anesthetics, fentanyl, midazolam and contrast agent allergy, a positive history of prolonged bleeding, anticoagulant therapy, local or systemic infection, previous lumbar spine surgery, opioid abuse, proven inflammatory rheumatic disease or inflammatory intestinal disease in the acute phase, and other infections. To observe the medium effect in the difference of numerical variables between four independent groups of examinees, with a significance level of 0.05 and a strength of 0.80, the minimum required sample size is 115 subjects (29 examinees per group).

Patients will undergo four measurements. The first measurement will be before the procedure, the second measurement will be at the control examination after one month, the third measurement at the control examination three months after the procedure and the fourth measurement six months after the procedure. Control MR LS of the spine will be done six months after the procedure. The VAS scale (Visual Analogue Scale) will be used to assess pain intensity. The questionnaires that will be used are SF-36 (Short form health survery -36), Oswestry Disability Index (ODI), Pain Detect, Hospital Anxiety and Depression Scale (HADS) and the Pittsburgh Sleep Quality Index (PSQI). From the clinical examination, a Lasegue test, a toe-heel test and a forward bend will be performed. Laboratory tests will determine the levels of interleukin 6 (IL-6), C-reactive protein (CRP), leukocyte count, and differential blood count (DKS). These parameters will be analyzed on

three occasions: before the procedure, one month after the procedure and six months after the procedure.

Research plan: The research is designed as a randomized controlled experiment, and will be done at the Department of Anesthesiology, Reanimatology and Intensive Medicine at the Department of Pain Management of the University Hospital Centre Osijek after obtaining the Ethics Committee of the Clinical Hospital Center Osijek and Faculty of Medicine Osijek approval. All patients will receive written informed consent with described performed procedure that will also be explained to them orally. After signing the informed consent, patients will be divided into four groups using random number generator. One group of patients will undergo epidural application of steroids and local anesthetics by transforaminal aproach and the other will undergo percutaneous laser disc decompression. Within each of these two groups patients will be divided into groups depending on contact of the disc hernia and nerve. All examinees will be selected at the Department of Pain Management. Basic demographic, social data and MR findings will be collected during the course of the research. Visual Analogue Scale (VAS), Pain detect, Lasegue test, Oswestry Low Back Disability Questionnaire, Hospital Anxiety and Depression Scale, SF - 36 Questionnaire will be measured at baseline and one, three and six months post-procedure.

Significance/Expected scientific contribution: This study will investigate which form of treatment, PLDD or ESI TF, is more successful in the treatment of lumbar radicular pain caused by intervertebral disc herniation in patients with or without discoradicular contact. We will investigate the importance of inflammatory markers as prognostic biomarkers in the treatment of patients with lumbar radicular pain caused by intervertebral disc herniation with and without discoradicular contact by comparing initial and control measurements.

MeSH/Keywords: disc herniation, radiculopathy, percutaneous laser disc decompression, transforaminal epidural steroid injection, interleukin – 6, interventional treatment

Dissertation proposal title: The link between lifestyle habits and level of hemoglobin within the population of volunteer blood donors

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

Mentor: Assoc. Prof. Marina Samardžija, M.D., Ph.D., Department for Transfusion Medicine, University Hospital Osijek, Croatia

Introduction: In today's hectic pace of life people tend to adopt certain lifestyles which facilitate everyday life, while not considering consequences on health. When planning the necessary quantities, accredited healthcare institutions that collect and produce blood products are required to factor the number of possible temporarily deferred or permanently rejected blood donors in order to collect sufficient quantities of blood and ensure self-sufficiency. The most often reason for deferring the donating of blood is actually of a temporary nature due to reduced or increased levels of hemoglobin. This research will investigate key factors contributing to this cause.

Hypothesis: H1: Compared to non-smokers, smoking causes increased levels of hemoglobin in volunteer blood donors. H2: Volunteer blood donors who are more intensively involved in physical activities, consumer various types of foods and drink ample quantities of fluids will not be hemoconcentrated but will have normal levels of hemoglobin.

Aims:

1. To investigate factors which affect hemoglobin levels in volunteer blood donors.
2. To investigate whether there is a clear link in lower levels of Hgb or increased levels of Hgb with respect to specific lifestyles and habits in various institutions involved in collecting blood from volunteer blood donors.

Materials/Participants and methods: Respondents comprise a sample of volunteer blood donors who came to donate blood at the Clinical Department for Transfusion Medicine, University Hospital Osijek (Croatia) and the Belgrade Institute for Transfusion Medicine (Serbia). The research will include a total of N = 2000 respondents (1000 from Serbia and 1000 from Croatia). Participation in the research will be voluntary and anonymous. The materials to be used in the research will include a survey. Consent from respondents for participation in the research is an integral part of the questionnaire.

Research plan: Commence research at the University Hospital Osijek. Research at the Belgrade Institute for Transfusion Medicine in Serbia is to commence by the end of 2020.

Significance/Expected scientific contribution: The results of this research will provide new insights into the effects of lifestyles on reducing or increasing hemoglobin values in volunteer blood donors. The obtained results will provide the opportunity to more precisely plan for sufficient number of volunteer blood donors necessary for the operation of transfusion healthcare services.

MeSH/Keywords: volunteer blood donors, hemoglobin, lifestyle, transfusion medicine

Abstract title: Improving access to palliative and end-of-life care conversations with older people with chronic obstructive pulmonary disease

Part of the dissertation proposal: Palliative and end-of-life care conversations with older people with chronic obstructive pulmonary disease

PhD candidate: Petra Čičak, M.D., Department of Pulmonology, University Hospital Centre Osijek; Faculty of Medicine, University of Osijek, Croatia

Mentor: Prof. Sanja Popović-Grle, M.D., Ph.D., Clinical Department for Lung Diseases Jordanovac, University Hospital Center Zagreb; School of Medicine, University of Zagreb, Croatia

Co-Mentor: Prof. Jerko Barbić, M.D., Ph.D., Department of Nephrology and Dialysis, University Hospital Centre Osijek; Faculty of Medicine, University of Osijek, Croatia

Introduction: Despite the progressive nature of chronic obstructive pulmonary disease (COPD), association of high morbidity and mortality with severe COPD, and the view that discussions between patients and clinicians about palliative care plans should be grounded in patients' preferences, many older patients do not receive timely end-of-life care (EOLC) discussions with healthcare professionals (HPs), potentially risking inadequate care at the advanced stages of the disease.

Aims: To evaluate clinicians' present practice and older patients with COPD in EOLC communication/palliative care as well as the cause of poor communication about EOLC.

Participants and Methods: Two groups of participants, patients at least 65 years old with COPD and HPs, were interviewed anonymously. A self-administrated questionnaire contained various questions relating to patients previous experience about EOLC communication with HPs related to COPD and future wishes related to such conversations. Before joining the research, the abbreviated mental test score (AMTS) was carried out on all patients.

Results: In total, 83 participants (22 HPs and 61 patients with COPD) were included in the study. According to the results, 77 % of patients reported that they had not had EOLC discussions with HPs, 64 % expressed the opinion that they would like such conversations, and the best timing for such discussion would be during frequent hospital admissions. Furthermore, 77 % of HPs thought that EOLC communication is important, but only 14 % actually discussed such issues with their patients because most of them felt uncomfortable starting such a topic.

Conclusion: Our current practices do not facilitate satisfactory conversations about palliative and EOLC in older patients with severe COPD. The majority of older patients with COPD did not have the chance to discuss advanced care planning with their HPs, including any kind of patient-clinician communication about EOLC. The majority of HPs interviewed felt uncomfortable approaching such a discussion, while the majority of older patients would like to have such a discussion. Improving communication represents an important opportunity for the improvement of the quality of COPD care in these patients.

MeSH/Keywords: COPD, end of life care, palliative care, communication, healthcare personnel

Dissertation proposal title: Value of sST2/GLS combination in predicting unwanted cardiovascular events in patients on hemodialysis

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

Mentor: Assist. Prof. Irzal Hadžibegović, M.D., Ph.D., University Hospital Dubrava, Zagreb; Faculty of Medicine Osijek, University of Osijek, Croatia

Introduction: Hemodialysis (HD) patients have significantly elevated risk of cardiovascular disease (CVD) and death. Different parameters have been investigated for their predictive power in identifying patients at highest CVD risk, e.g. AROii mortality risk score (patient, laboratory and dialysis data), N-terminal pro-brain natriuretic peptide (NT-proBNP) level and cardiac biomarkers. Elevated NT-proBNP is associated with left ventricle (LV) dysfunction, heart failure symptoms and poor prognosis, but its level may be influenced by the HD procedure. Soluble ST2 (sST2), a marker of "cardiac stress", shows the presence and severity of cardiac remodeling and fibrosis. It is a strong predictor of CV endpoints in heart failure, believed to be superior to NT-proBNP in risk stratification and independent of renal function. Global longitudinal strain (GLS) is an ultrasound marker of LV systolic function, and a prognostic factor in general population.

Hypothesis: sST2/GLS combination is superior in prediction of unwanted cardiovascular events in HD patients to NT-proBNP.

Aims:

1. Main: Assessing the predictive value of sST2/GLS combination in the prognosis of unwanted CV events in HD patients.
2. Secondary: Comparing the predictive value of sST2/GLS combination, with each method alone, NT-proBNP, and AROii score.
3. Comparison of the sST2/GLS combination in patients with high/low average interdialytic weight gain in the last six months.
4. Comparison of unwanted CV events and parameters in diabetic/non-diabetic patients.

Materials/Participants and methods: 70 patients on HD will be enroled. Their demographic data, medical history data, HD data, comorbidities, medications, BMI, blood pressure, ECG, sST2, GLS, NT-proBNP, AROii score would be collected at baseline. Patients will be followed for twelve months during which unwanted CV events (myocardial infarction, hospitalization for unstable angina, heart failure, stroke, peripheral vascular event, CV death) and all-cause mortality will be assessed.

Research plan: Measurements will be done at baseline with a 12-month follow-up period. After collecting the data statistical analysis will be done.

Significance/Expected scientific contribution: The results may help to identify HD patients with the highest CV risk, who may require interventions in order to improve their prognosis.

MeSH/Keywords: ESRD, ST2, GLS, CVD, NT-proBNP

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

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

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

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

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

Aims:

1. To determine levels of WNT inhibitors in GCF and serum of periodontal patients with or without statin therapy
2. To investigate the correlation of serum and GCF indicators of bone remodeling and statin levels of the participants
3. To evaluate expression of genes involved in bone remodeling pathway in periodontal participants with or without statin therapy.

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

Research plan: Following recruitment, participants will be involved in dental treatment to detect the presence of periodontitis. Patients diagnosed with periodontitis who are or are not on statin therapy will be included in the study. Sampling of GCF will be carried out at the Specialist Periodontal Clinic in Tuzla. Dental examination will detect the area in which the gingival pockets are expressed, and these teeth will be isolated (water, abrasion, optradame or cofferdam) and the field of work dried. With the sterile forceps, a sterile paper point of appropriate size (20 or 25 in diameter) based on the depth of the periodontal pocket and to the minimum resistance limit will be applied and held for 30 seconds to soak with GCF. A blood sample will be taken in the Biochemical Laboratory of the Tuzla Health Center. Total of 4 ml of whole blood will be sampled and separated from cells by centrifugation for 15 min at 3000 RPM and then stored at - 20°C until analysis. An additional 2 ml of whole blood will be permanently stored at - 20°C until RNA isolation. The determination of gingival (GCF) and serum levels of SOST and DKK1 will be performed in the Laboratory of Pharmacology at the Faculty of Medicine in Osijek using a commercially available ELISA kits (Biomedica, Vienna, Austria) according to the manufacturer's instructions. RNA will be isolated from peripheral blood samples using commercially available kit RNAeasy Mini Kit (QIAGEN, Hilden, Germany) according to manufacturer’s protocol.

Significance/Expected scientific contribution: This study will master and optimize the technique of GCF sampling. To the best of our knowledge, this will be the first study examining circulating levels SOST and DKK1 as antagonists of the WNT signaling pathway in GCF and their correlation to statin therapy in chronic periodontal patients. This study will evaluate whether statin therapy interacts with periodontal disease, and whether the difference is significant with respect to patients not administered statins.

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

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

Dissertation proposal title: Clearance of glucoregulatory peptide hormones during hemodialysis in prediabetic and diabetic end-stage renal disease patients

PhD candidate: Ivan Feldi, M.D., General hospital Našice, Croatia

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

Co-Mentor: Assoc. Prof. Tatjana Bačun, M.D., Ph.D., Faculty of Medicine Osijek, University of Osijek; University Hospital Centre Osijek, Croatia

Introduction: Type 2 diabetes mellitus (DM) is the leading cause of end-stage renal disease (ESRD) in developed countries. Effect of hemodialysis (HD) on glucoregulatory hormones level has been investigated up to some extent. It is known that HD ameliorates insulin sensitivity and decreases insulin level. Divergent effect of HD on glucagon, insulin and glucose-dependent insulinotropic polypeptide (GIP) was noticed in earlier studies. A recent study showed that HD significantly decreases the level of other glucoregulatory hormones such as glucagon, GIP, glucagon-like peptide - 1 (GLP- 1), C-peptide and insulin in non-diabetic ESRD patients. The levels of glucoregulatory hormones are expected to be different in patients with DM then in non-diabetic patients. To our knowledge, effect of HD on glucoregulatory hormones in patients with DM has not been investigated.

Hypothesis: Glucoregulatory peptide hormone levels are dissimilar in patients with DM then in non-diabetic patients, and hence their clearance by HD might be different.

Aims:

1. To evaluate the impact of high flux HD and high-volume hemodiafiltration (HDF) on fasting and postprandial plasma levels of pancreatic (insulin and glucagon) and gut (GIP and GLP-1) glucoregulatory peptide hormones in prediabetic and diabetic ESRD patients compared to non-diabetic patients.
2. To establish the fasting and postprandial levels of glucoregulatory hormones in prediabetic and diabetic ESRD patients and patients with grade IV chronic kidney disease (CKD) compared to non-diabetic patients.
3. To further contribute to pathophysiology of HD-induced hypoglycemia.

Participants and methods: Study will include 60 patients. Ten patients with ESRD and DM, ten patients with ESRD and prediabetes, and ten non-diabetic ESRD patients undergoing chronic HD at Department for Nephrology at University Hospital Centre Osijek will be included in the main study. Also, 30 patients with grade IV CKD, of which ten with DM, ten prediabetic and ten non-diabetic will be included.

Research plan: Research will be done on three separate days. On the first research day during HD process and on the second research day during HDF. All participants will be given a standardized liquid meal 1 hour into the dialysis session. On the third day only meal test without dialysis will be performed. The group with grade IV CKD will be included in the research only on the third day. Baseline blood samples will be collected before dialysis, and during the dialysis session blood samples will be drawn from the arterial line of the dialyzer at time 30, 60, 75, 90, 120, 150, 180, 210 and 240 min. Blood samples from the venous line of the dialyzer will be drawn at time 60, 120, 180 and 240 min. Level of plasma GIP, GLP-1, glucagon, glucose, insulin and C-peptide will be attained from the collected samples and fractional removal from the blood, clearance, and fractional appearance in dialysate will be calculated.

Expected scientific contribution: Our study would further contribute to the knowledge of pathophysiology of DM in patients with ESRD on chronic HD.

MeSH/Keywords: hemodialysis, chronic kidney disease, diabetes mellitus, glucagon, incretin hormones

Disertation proposal title: Personality and insight into illness in early-stage psychosis patients and their parents

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

Mentor 1: Assoc. Prof. Branka Restek Petrović, M.D., Ph.D., Department of Psychiatry, Psychiatric Hospital „Sveti Ivan", Zagreb, Croatia

Mentor 2: Prof. Ivan Požgain, M.D., Ph.D., Department of Psychiatry, University Hospital Centre Osijek; Faculty of Medicine Osijek, University of Osijek, Croatia

Introduction: The ability of a person to reflect upon his illness is called Insight into illness. Impaired insight is common during early stage of psychosis, causing treatment delays, poorer treatment adherence, involuntary admissions, agressive behaviour, poorer social functioning and poorer long-term outcomes. Clinically insight is defined as a multidimensional construct comprising of: awareness of having mental illness, its social consequences, compliance with treatment and ability to relabel unusual mental events (i.e., delusions, hallucinations) as pathological. Insight is multifaced concept that reflects complex interaction of biological, personal and social factors. The traditional belief that an individual's personality is altered or even destroyed by psychotic experience (Bleuler, Kraepelin) has been changed. Personality patterns may specifically affect how patient perceive information regarding their state either from subjective experience or from information imported by parents. However, it is still unclear to what extent personality organization (PO) may play a role in a complex concept of insight.

Hypothesis: Insight into illness of patients in early stage of psychosis and their parents is related to the personality organization. There is a positive correlation between the parents' insight and their child's insight.

Aim: To examine the correlation of insight with the personality organization (PO) of patients in the early stages of psychosis and their parents, as well as relatedness between parental and patient's insight.

Material/Participants and methods: A cross-sectional study will be conducted on a consecutive sample of 132 early-stage psychosis patients (disease duration < 5 years) hospitalized at the Psychiatric Hospital "Sveti Ivan" and their parents. The inclusion criteria will be diagnosis of the early stage of psychotic spectrum disorders (F20-29) according to the ICD 10th Revision, signed informed consent, both sexes, age 18 to 35. Non-inclusion criteria will be: severe brain damage, mental retardation, severe drug dependence, acute suicidality and inability to complete the tests without assistance. The study will be conducted in accordance with ethical principles and with the approval of the Hospital Ethics Committee.

Research plan: The purpose and objectives of the research will be explained to all participants and as they sign informed consent they will be subjected to a clinical interview, questionnaires and measurement scales. Instruments to be used: for patients - sociodemografic questionnaire, the Positive and Negative Syndrome Scale (PANSS), Clinical Global Impression - severity (CGI-S), the Scale to assess Unawareness of Mental Disorder (SUMD), Kernberg Inventory of Personality Organization (IPO), and for parents - sociodemografic questionnaire , the modified version of SUMD, and Kernberg Inventory of Personality Organization (IPO).

Significance/Expected scientific contribution: A greater understanding of the course and correlates of insight at early phases of psychosis may improve our understanding of a complex phenomenon of insight with significant implications for treatment adherence, treatment outcome and their quality of life.

MESH / keywords : insight into illness, clinically insight, personality organization, early-stage psychosis

Dissertation proposal title: Effectiveness of a multidisciplinary biopsychosocial approach on the quality of life of patients with chronic low back pain

PhD candidate: Dijana Hnatešen, MSc, RN, University Hospital Centre Osijek; Faculty of Medicine Osijek, Faculty of Dental Medicine and Health Osijek, University of Osijek, Croatia

Mentor: Prof. Roman Pavić, M.D., Ph.D., University Hospital „Sisters of Mercy", Clinic for Traumatology, Zagreb; Faculty of Medicine Osijek, University of Osijek, Croatia

Introduction: Chronic pain (CP) is a global problem that can affect all aspects of a patient's life. For more than twenty years, the International Association for the Study of Pain (IASP) defined chronic CP as "pain that persists beyond normal tissue healing time, which is assumed to be 3 months" and as "an aversive sensory and emotional experience typically caused by, or resembling that caused by, actual or potential tissue injury." The World Health Organization (WHO) incorporated chronic pain as a chronic disease in the International Classification of Diseases (ICD-11) in May of 2019.

The overall prevalence of chronic pain in the general population is around 20 %. Chronic pain of moderate to severe intensity has been estimated to occur in 19 % of European adults and 40 % of them reported inadequate management of their pain. As a major public health concern it is estimated that 10.6 million U.S. adults have "high impact" chronic pain, defined as pain that interferes with important life activities (eg, performing household tasks, participating in work or school). The worldwide cost of pain is enormous and its burden is huge in terms of personal and socioeconomic costs.

A thorough review of the literature showed that physical, psychological and social factors including somatisation, psychological distress, fear avoidance beliefs, catastrophising, illness perceptions, and coping strategies that may be implicated in CLBP disability. Disability, emotional imbalances

and social isolation are frequently associated with chronic pain often resulting in a vicious circle that compromises the quality of life (QoL) of affected patients. In addition, chronic conditions not only affect the patient but also their families, communities and the health systems, as they are also stakeholders in the struggle to control the disease and its complications.

Low back pain (LBP) is the leading cause of years lived with disability worldwide than any other health condition. Chronic non-specific low back pain (LBP) mostly affects working populations and is a costly contemporary health problem in terms of absenteeism, lost productivity, substantial healthcare costs, and personal suffering. Along with pain and impaired function, chronic LBP patients frequently experience anxiety, depression and reduced quality of life (QoL).

Chronic pain is best understood through a biopsychosocial lens taking into account the complex interaction between physiological and social factors that influence the experience of pain. Multidisciplinary pain management using a biopsychosocial approach is considered the gold standard.

Multidisciplinary treatment is defined as multimodal treatment provided by practitioners from different disciplines. For example: the prescription of an anti-depressant by a physician alongside exercise treatment from a physiotherapist, and cognitive behavioral treatment by a psychologist, all the professions working separately with their own therapeutic aim for the patient and not necessarily communicating with each other. In addition, to be considered as a multidisciplinary program, the program must be delivered with at least two different health care specialties including medicine, nursing, psychology, social work, physiotherapy, and/or occupational therapy. Multidisciplinary biopsychosocial rehabilitation (MBR) programs for LBP include physical therapy combined with psychological, social, and occupational therapies, and they aim to improve back-related physical function, address psychological issues, and target social or work-related behaviors. The multidisciplinary pain management team members are often located within the same clinic with frequent contact through team meetings to discuss unified goals and values for the program and for patients. Patients reaching chronic stages of LBP seem to benefit from multidisciplinary approaches and several recent guidelines have advocated MBR for chronic back pain. The efficiency of the multidisciplinary programs was found to be better than the standard medical treatment and other non-multidisciplinary treatments.

Hypothesis:

- Participants with LBP included in the multidisciplinary program will report significant improvement than participants in the control group with improvements in reducing pain severity, reducing level of disability, reducing pain-related life interference, reducing physical health–related and mental health–related quality of life, reducing anxiety, depressed mood, reducing of neuropathic pain and reducing insomnia symptoms
- Participants with LBP included in the multidisciplinary program will report significant changes in psychological process measures, including decreased pain catastrophizing
- Participants with LBP included in the multidisciplinary program will have better sleep quality and a higher level of physical activity during the four-week active treatment followed by Fitbit technology.
- Participants with LBP included in a multidisciplinary program using a biopsychosocial approach to acquire competencies will improve the quality of life and life with chronic low back pain compared to the control group.

Aims:

1. Study the impact of multidisciplinary program in the treatment of low back pain on degree of pain severity, degree of disability, improving physical health–related and mental health–related quality of life, degree of neuropathic pain, degree of anxiety and depression, degree of pain catastrophizing and improving sleep quality before treatment, after treatment (4 weeks) and after three months.
2. Study the impact of physical therapy in the treatment of low back pain on degree of pain severity, degree of disability, improving physical health–related and mental health–related quality of life, degree of neuropathic pain, degree of anxiety and depression, degree of pain catastrophizing and improving sleep quality before treatment, after treatment (4 weeks) and after three months.

3. Identify differences in the assessment of pain intensity, disability, physical and mental health associated with quality of life, presence of neuropathic pain, levels of anxiety and depression, degree of catastrophizing, and sleep quality between patients enrolled in a multidisciplinary program and patients enrolled in physical therapy before treatment, after treatment (4 weeks) and after three months.
4. Evaluate the quality of sleep and physical activity of patient with LBP using Fitbit technology during a four-week active treatment in both groups.
5. Educate participants with LBP through a multidisciplinary biopsychosocial approach during a four-week active treatment of competencies that will improve quality of life and life with chronic low back pain.

Materials/Participants and methods: Criteria included: participants will be between the ages of 18 and 70 who agree to participate in the studies that they confirm by signing the informed consent. Participants with chronic low back pain.

Exclusive criteria: patients younger than 18 and older than 70 years, patient refusal to participate in the study, pregnancy, inability to use Fitbit technology.
Sample size is 80 subjects (40 participants per group). Patients will be given tree measurements. The first measurement will be before the four week active treatment, the second measurement will be after four week active treatment, the third measurement at the control examination after 3 months from the treatment. The VAS scale (Visual Analogue Scale) will be used to assess pain intensity. The questionnaires that will be used are Short form health survery-36 (SF-36), Oswestry Disability Index (ODI 2.1a), Pain Detect, Hospital Anxiety and Depression Scale (HADS), Pain Catastrophizing Scale (PCS) and the Pittsburgh Sleep Quality Index (PSQI).

Research plan: The research is designed as a randomized controlled experiment, and will be conducted at the Department of Anesthesiology, Reanimatology and Intensive Medicine at the Department of Pain Management of the University Hospital Centre Osijek after obtaining the Ethics Committee of the Clinical Hospital Center Osijek and the Faculty of Medicine Osijek approval. All patients will receive written informed consent in which there will be described performed research

that will be also explained to them orally. After signing the informed consent, patients will be divided into two groups depending on the planned treatment. One group of participants will be included in a multidisciplinary approach to treating chronic pain and the other group will be included in physical therapy. All participants will be selected at the Department of Pain Management. Basic demographic and social data will be collected during the course of the research. Short form health survery-36 (SF-36), Oswestry Disability Index (ODI 2.1a), Pain Detect, Hospital Anxiety and Depression Scale (HADS), Pain Catastrophizing Scale (PCS) and the Pittsburgh Sleep Quality Index (PSQI) will be measured at baseline, after completion of active treatment and after 3 months post-active treatment.

Significance/Expected scientific contribution: Considering that in the Republic of Croatia multidisciplinary treatment of chronic pain is performed only at the Department of Pain Management of the University Hospital Centre Osijek and that no research has been conducted on the impact of multidisciplinary treatment of chronic pain on the Croatian population we believe that this research will show the importance and advantage of this approach in the treatment of LBP and will be an incentive for its organization in other hospitals in the Republic of Croatia.

MeSH/Keywords: chronic pain, low back pain, multidisciplinary program, quality of life

Abstract title: Perioperative monitoring and postoperative outcomes in patients undergoing abdominal cancer surgery

Part of the disertation proposal: Use of extended haemodynamic monitoring in abdominal cancer surgery and impact on patient outcome

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

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

Introduction: Perioperative hemodynamic (HD) monitoring, depth of anaesthesia and tissue oxygen saturation monitoring (StO2) facilitate the assessment and adjustment of anaesthesia and the amount of fluids and drugs according to the main determinants of adequate oxygen delivery and utilisation. No studies on the effect of the simultaneous use of perioperative HD monitoring, depth of anaesthesia and StO2 monitoring on the patient outcome were found.

Aims: To determine whether the simultaneous use of extended HD monitoring, depth of anaesthesia monitoring and StO2, compared to conventional approach, may have positive effects on perioperative complications and the patient outcome after major abdominal cancer surgery.

Materials/Participants and methods: A total of 80 patients ASA II and III class undergoing abdominal carcinoma surgery were randomised into two groups. Group 1 patients had fluid therapy based on LiDCO Rapid monitoring and anaesthesia depth was controlled with a Bispectral index monitoring. In group 2 a conventional approach to perioperative monitoring and fluid therapy was

applied. StO2 was measured in all patients by the INVOS sensor above the brachial muscle and thenar eminence. Postoperative complications, need for reoperation, readmission to the ICU, the length of hospital stay, and postoperative mortality were recorded up to 30 days. Statistical analysis was done using Mann-Whitney and Fisher's exact test.

Results: The mean values of StO2 were 61.12 vs 59.12 above the brachial muscle, and 71.11 vs. 69.87 on thenar in group 1 and group 2 respectively (ns). Hospital stay in group 1 was 10.6 days vs 12.5 in group 2. Three patients were reoperated in group 1, compared to 4 patients in group 2. Two patients in group 1 were readmitted to ICU, and 1 patient in group 2. Complications were observed in six patients in group 1 and seven in group 2, one patient in group 1 and two patients in group 2 died (ns).

Conclusion: We have not confirmed any differences in the outcomes between two study groups. Further research with more patients involved may confirm whether extended monitoring may improve patient outcome.

MeSH/Keywords: hemodynamic monitoring, depth of anaesthesia, tissue oxygen saturation, abdominal cancer surgery, postoperative outcome.

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

PhD Candidate: Marul Ivandić, M.D., University Hospital Centre Osijek, Croatia

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

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

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

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

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

Preliminary results: We measured median glucose values at 6 AM night after training. After aerobic training median glucose value was 4,6 mmol/l. After strength training glucose median value was 10,0 mmol/l. There were three hypoglycemic incidents after aerobic training. There were no hypoglycemic incidents after strength training.

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

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

Abstract title: Radiosurgery (Stereotactic ablative radiotherapy) of locally advanced pancreatic cancer using Calypso Extracranial Tracking© intrafractional motion managerment

Part of the disertation proposal: Patients with unresectable, locally advanced pancreatic cancer (LAPC) have generally poor outcome. There are evidences that addition of the local Stereotactic ablative radiotherapy (SABR) to standard systemic therapy contributes to the local, and even distal control improvement in those patients. With more accurate intrafractional tumour motion management provided by Calypso Extracranial Tracking© during SABR, and consequent local dose escalation, this improvement could be even greater, and lead to overall better outcome.

PhD candidate: Hrvoje Kaučić, M.D., Special Hospital Radiochirurgia Zagreb, Croatia

Mentor: Prof. Dragan Schwarz, M.D., Special Hospital Radiochirurgia Zagreb, Zagreb, Croatia

Introduction: The pancreatic cancer that has spread to the organs or large blood vessels near the pancreas is called locally advanced pancreatic cancer (LAPC). According to the international guidelines, LAPC is treated with systemic therapy. Overall survivall (OS) of those patients is six to nine months. Only highly selected patients with LAPC are candidates for Stereotactic ablative radiotherapy (SABR). Primary goal in SABR is obtaining a local control (LC), which relates highly to the biological efective dose (BED). The intrafractional motion tracking provides the BED escalation. Recent publications emphasize that only $BED_{10} > 80$ Gy can achieve long-term LC.

Aims: To determine the long term prognostic role of SABR using Calypso Extracranial Tracking© (CET) in treatment of LAPC, compared to standard treatment of the disease in terms of LC, OS and toxicities.

Materials/Participants and methods: 34 selected patients with LAPC were treated with SABR in our institution from April 2017 - October 2019. Inclusion criteria were - MSCT or MR confirmed LAPC, PHD confirmation of adenocarcinoma or malignant neuroendocrine tumour, ECOG 0-2, no prior RT and no synchronous malignancy. SABR was performed on Varian EDGE© linear accelerator using RapidArc© or IMRT, and CET for intrafracional motion tracking. Every patient had Calypso beacon bean elecromagnetic fiducials implanted by interventional radiologist. Follow up (FU) was scheduled every 3 months using MSCT, and local and/or distal relapse was evaluated by radiologist.

Fractionation were 1, 3 or 5 fx, and calculated BED_{10} was used for distinctive comparison. SABR was applied highly heterogeneously, with prescribed mean dose, calculated on 80 % isodose to the tumour border. Application of systemic therapy was left to discretion of a reffering medical oncologist. FU was calculated from the time of PHD.

Results: All 34 patients completed SABR. Median FU was 15 months (ranging 5 - 39). Median BED_{10} was 85.5 Gy (ranging 48 - 129.5). Eleven patients received gemcytabine based chemotherapy (32 %), and five received FOLFIRINOX (15 %). Preliminary results show that 14 patients had distant relapse (41 %). One patient had local relapse after 27 months. One-year survival was 62 %, and there is a subgroup of eight patients (24 %) that had survival > 24 months. There was no grade 3 or higher toxicities.

Conclusion: SABR using CET may be superior to the standard treatment for LAPC, primary in LC, OS and toxicity.

MeSH/Keywords: LAPC, SABR, Calypso extracranial tracking, BED, tumour motion management.

Abstract title: Positive effect of *n*-3 PUFAs consumption on serum levels of anti-inflammatory cytokines in young healthy participants

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

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Introduction: Unbalanced, nutrient-poor diet followed by sedentary lifestyle and harmful habits leads to an increased risk of developing diabetes, obesity and cardiovascular disease. Previous studies have shown positive effect of *n*-3 polyunsaturated fatty acids (PUFAs) on microvascular reactivity, lipid metabolism and reduction of oxidative stress.

Aims: To determine serum levels of inflammatory and anti-inflammatory cytokines, resolvins (RvE1), leukotrienes (LT) B4, B5, prostaglandins (PG) E2, E3 and Th17/Treg cells ratio in young healthy individuals after three-week consumption of *n*-3 PUFAs enriched hen eggs.

Materials/Participants and Methods: 38 young healthy participants of both sexes (age 23.8 ± 2.4 years) consumed *n*-3 PUFAs enriched eggs (experimental group, n=17) or standard eggs (control group, n=21), three eggs per day for three weeks. Cytokine concentrations were determined by the Luminex method; RvE1, LTB4, B5 and PGE2, E3 levels were measured by ELISA kits; Th17 and Treg ratio/levels were determined by flow cytometry method. All participants kept a diet diary throughout the protocol. The study was approved by the Ethics Committee of the Faculty of Medicine, University of Osijek, and all respondents signed informed consent.

Results: Subjects from control group had significantly increased end-point serum concentrations of IL-17A and VEGF-A when compared to baseline, while subjects from experimental group had significantly reduced serum IFN-gamma concentration. The end-point concentration of IL-10 increased significantly in both groups. End-point serum levels of IL-10 and IL-21 were significantly higher in the experimental group compared to the control group.

End-point levels of PGE2 and LTB4 were significantly increased in control group when compared to baseline levels. LTB4/B5 and PGE2/E3 ratios were significantly decreased in experimental group after the protocol. End-point RvE1 serum levels were significantly increased in experimental group compared to baseline levels.

Compared to baseline, end-point levels of CD25+FOXP3+ T lymphocytes (Tregs) were significantly decreased in both groups. Levels of CD4+IL17A+ T cells (Th17) were significantly decreased in control group after protocol. Th17/Treg ratio was increased in experimental group after protocol.

Conclusion: Consumption of *n*-3 PUFA enriched eggs resulted in increased levels of anti-inflammatory cytokines and decreased levels of serum inflammatory cytokines, indicating that functional foods may have an immunomodulatory effect.

MeSH/Keywords: *n*-3 PUFAs, cytokines, inflammation, functional foods, diet

Acknowledgement: The work is supported by grant to Scientific Centre of Excellence for Personalized health care, University of Osijek, Croatia, #KK.01.1.1.01.0010.

Abstract Title: Assessment of the mechanisms of flow-induced dilation of carotid artery in low salt and high salt fed *Tff3*$^{-/-}$/C57BL/6N mice and their wild type controls

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Introduction: Impaired vascular endothelium-dependent responses to various physiological stimuli are caused by high salt (HS) dietary intake. Some of those responses are mediated by arachidonic acid (AA) metabolites. Transgenic *Tff3*$^{-/-}$/C57BL/6N knockout mice have changes in lipid metabolism which may affect vascular function.

Aims: This study aimed to assess the influence of HS diet on the mechanisms of flow-induced dilation (FID) in isolated, pressurized carotid arteries of *Tff3*$^{-/-}$ knockout mice and the C57BL/6N mice, used as wild type controls.

Methods: Ten-weeks-old transgenic *Tff3*$^{-/-}$/C57BL/6N knockout mice and WT/C57BL/6N (WT) (parental strain) healthy male mice were divided to low salt (LS; 0.4% NaCl in rodent chow) and HS (4% NaCl in rodent chow) groups, fed for 1 week and drink water *ad libidum*. The mice were anesthetized with ketamine-chloride (100 mg/kg) and midazolam (5 mg/kg) and decapitated. Carotid arteries were isolated, cannulated and pressurized for 60'at 100 mmHg to assess basal diameter and then subjected to flow at pressure gradients Δ10-Δ180 mmHg (DMT pressure

myograph, Danmark). The endothelium-independent response was tested using the NO donor sodium nitroprusside (SNP; 10^{-6}M) and endothelium-dependent vasodilation was tested using acetylcholine (ACh; 10^{-6}M mol/L). FID was determined in the presence of Nω-nitro-L-arginine methyl ester (L-NAME; 10^{-4}M; NO synthase (NOS) inhibitor), the cyclooxygenase inhibitor indomethacin (INDO; 10^{-5}M) and the CYP450-epoxygenase inhibitor (MS-PPOH; 10^{-5}M). Statistical analyses were performed with Two-way ANOVA tests; $p<0.05$ was considered significant. All experimental procedures conformed to the European Guidelines for the Care and Use of Laboratory Animals (directive 86/609) and were approved by the local and national Ethical Committee (No. 2158/61-02-139/2-06 and No. 2158/61-07-14-119).

Results: FID was similar between the *Tff3*$^{-/-}$_LS and *Tff3*$^{-/-}$_HS groups, while WT_ HS mice exhibited significantly reduced FID compared to WT_LS group. FID was significanty reduced in *Tff3*$^{-/-}$_LS compared to WT_LS mice. ACh-induced dilation was significantly decreased in *Tff3*$^{-/-}$_LS mice compared to WT_ LS mice. High salt intake reduced the ACh-induced dilatation in WT group and in *Tff3*$^{-/-}$ group of mice compared to respective LS control groups, while vasodilation in response to SNP was preserved at all study groups, and there was no significant difference in the response among the groups. In *Tff3*$^{-/-}$_ LS group L-NAME, INDO and MS-PPOH significantly reduced FID at each pressure gradient. In *Tff3*$^{-/-}$_HS group only INDO reduced FID at Δ120, 140 and 160 mm Hg. In WT_LS group and WT_HS group all inhibitors reduced FID at Δ100-180 mm Hg

Conclusion: The vasorelaxation in WT mice is considerably impaired by the HS diet. The deletion of *Tff3*$^{-/-}$ gene may attenuate vasorelaxation, mechanisms which are affected by HS intake.

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

Acknowledgements: This study was supported by the Croatian Science Foundation under the project IP-2014-09-6380 (V-ELI Athero), VIF-2018-MEFOS-09-1509 grant and Faculty of Medicine Osijek Institutional grant #IP-1 (2019; PI Ines Drenjančević).

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

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Introduction: Chronic overnutrition, common nowadays in developed countries, results in obesity, increased risk of metabolic diseases and interference of normal cellular signaling via alteration of lipid environment in the cell membrane or downstream molecules in key signaling pathways. Insulin (IR) and insulin-like growth factor 1 (IGF-1R) receptors, commonly expressed in the hippocampus, are affected as well by such lifestyle. Changes in their signaling pathways may underlie neuroendocrine dysfunction and neurodegeneration. Identifying lipids in affected signaling pathways may be key to understanding the pathophysiology of insulin resistance-related neurodegeneration and whether or not commonly used antidiabetic drugs have any effect on slowing this process.

Hypothesis: The hippocampal lipidome of adult Sprague Dawley (SD) rats on a high-fat, high-sugar diet (HFHSD) is different than the lipidome of healthy rats. Early antidiabetic pharmacotherapy reduces the HFHSD effects on the hippocampal lipidome.

Aims:

1. To identify the hippocampal lipidome of healthy rats and rats on a HFHSD with and without antidiabetic pharmacotherapy
2. To determine affected metabolic pathways
3. To associate possible alterations with expression levels of IR and IGF-1R.

Materials and methods: The research will be carried out on the brain tissue archive of 24 adult SD rats, randomly separated into four groups of six – a control group and three groups on a HFHSD, two of which were treated with metformin and liraglutide, respectively. Mass spectrometry imaging (IMS) will be performed to analyze the hippocampal lipidome using online databases. Immunohistochemical (IHC) analysis will be performed to determine the expression levels of IR and IGF-1R and the expression of the four most common gangliosides. The IMS images will be compared with the IHC images to determine and analyze the location and cells with the most significant changes.

Research plan:

1. Brain tissue preparation for IMS;
2. IMS data acquisition and analysis;
3. Perfoming free floating immunohistochemical (IHC) analysis using 25 um coronary hemispheral cerebral slices;
4. Comparison of IMS and IHC analysis results.

Expected scientific contribution: The study has translational potential, which can be accomplished using modern imaging methods, which could pivot the lipidome analysis to in vivo imaging. Ellucidation of hippocampal insulin resistance effects, with or without

pharmacotherapeutics, can provide valuable information for pharmacotherapeutic alterations in high-risk patients for neurodegenerative disease.

MeSH/Keywords: Lipidome, hippocampus, metabolic syndrome, imaging mass spectrometry, antidiabetic drugs

Dissertation proposal title: Phonatory movement of the pharyngoesophageal mucosa in laryngectomy patients

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Introduction: High-speed videoendoscopy (HSV) provides the most reliable and accurate objective quantification of the vocal mucosa vibratory behaviour regardless of whether this behaviour is periodic or aperiodic. Although HSV is the only method that visualizes and measures vibration of pharyngoesophageal mucosa (PEM) after laryngectomy, there is lack of clinical data about it in current literature.

Hypothesis: There is a difference in phonatory movement of the PEM between tracheooesophageal and oesophageal voice. Characteristics of phonatory movement differ among patients with good and poor quality tracheoesophageal and oesophageal speech, between patients who had a cricopharyngeal myotomy and among those who did and did not undergo radiotherapy.

Aims:

1. Describe phonatory movement of PEM in laryngectomy patients with HSV

2. Determine the connection between the acoustic quality of the voice and the visual characteristics phonatory movement of the PEM

3. Determine whether radiotherapy and cricopharyngeal myotomy affects the phonatory movement of the PEM

4. Assess the impact of loss of voice in terms of psychological and socioeconomic problems in laryngectomy patients.

Material/Participants and methods: Adult male laryngectomised patients who completed oncological treatment and underwent voice rehabilitation are included in study. Non-eligibility criteria: acute respiratory infection, other primary cancer, another surgical procedure which includes the pharyngeal area. Subjects will undergo ENT examination, subjective and objective perceptual evaluation of voice quality, acoustical voice assessment, HSV during voice production and endoscopic evaluation of swallowing. In order to spot the difference in numerical variables of two independent subject groups, with effect size of 0.8, level of significance of 0.05 and power of 0.8, the minimum sample size required is 52 subjects.

Research plan: Planned research duration is one year. Each subject during one visit will undergo an ENT examination and subjective and objective perceptual evaluation of voice quality. Voice production time will be measured, as well as endoscopic evaluation of swallowing, and HSV during voice production.

Expected scientific contribution: Description would be provided of still insufficiently undescribed phonatory movement of PEM in laryngectomy patients. New parameters would be proposed for potential use in creating an evaluation algorithm. An optimal surgical reconstructive method of pharyngoesophageal segment formation during laryngectomy could potentially be proposed and also better planning and conducting voice rehabilitation.

MeSH/Keywords: high-speed videolaryngoscopy, total laryngectomy, tracheoesophageal speech, oesophageal speech, voice handicap

Dissertation proposal title: Health related quality of life in children with chronic constipation and their parents

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Introduction: More than 25 % patients followed by pediatric gastroenterologists are constipated. Constipation can be defined as infrequent bowel movements or difficult passage of stool that persist for several weeks or longer.

Frequency of normal bowel movements varies with age, up from 2 to 4 stools per day in newborns, to 1 stool per day in the first years of life.

Constipation can be devided into functional (95 %) and organic (5 %). „Slow transit" and „outlet obstruction" are two main forms of functional constipation. The first one is reflection of colonic motility disorders. The second one is due to uncontrolled function of pelvic muscles.

Organic causes are specific structurals, neurologic, toxic/metabolic or bowel disorders. They are rare but very important to be recognized. The most common is Hirschprung disease. Other organic causes presented in newborn period or later are anorectal malformations, cystic fibrosis, metabolic disorders like hypothyroidism, hypercalcaemia, hyperkalemia and vertebral disorders.

Early diagnostic is very important in order to prevent unnecessary, unpleasant and harmful procedures and to develop the strategy of treatment. Beside laboratory and biochemical tests, anorectal manometry, rectal biopsy, colonic transit study, colonic manometry and contrast enema represent very important diagnostic procedures in defining the form of constipation.

Specific organic causes should be treated. Functional constipation is treated with specific dietary measures and changing the habits which include meals with the right ammount of fibers, plenty of

water and toilet training. Inefficiency of the above measures demands stool softeners and rectal enemas.

Chronic constipation presents a great burden in everyday life in both children and their parents. Many of them, depending of etiology, have problems regarding social life, everyday activities.

They suffer from soiling, pain and need rectal enemas on daily base and very often manual desimpaction of faecaloma. Eventually, some of them can be cured only with surgical intervention, with or without colostomy formation.

Health related quality of life (HRQOL) describes patient perception of health. It measures the impact of chronic disease on physical, social and emotional behavior in both children and parents. It has a huge clinical interest especially in children. Disability or limitation in children represents a huge stress for parents, which can have a great impact on both physical and mental health.

Hypothesis: There are significant difference in specific segments of life depending on chronic constipation etiology. Determining quality of life, using specific questionnaires, is valuable indicator of course of the disease and treatment success.

Aims:

1. To determine the quality of life in children suffering from chronic constipation so as the quality of life of their parents and to compare the results with the quality of life in healthy children and their parents
2. To define correlation of quality of life in children suffering from chronic constipation and quality of life of their parents
3. To determine the difference in quality of life in children and their parents depending of etiology of chronic constipation and treatment measures.

Materials/Participants and methods: Participants will be children, 4 - 18 years of age, suffering from chronic constipation determined by Rome III criteria, and their parents. Control group consists of healthy children and their parents. Excluding criterias are children who are not capable to fill the information form due to their mental status or educational problems. To observe the mean effect in the difference of numerical variables between two independent groups of subjects, with a significance level of 0.05 and a strength of 0.9, the minimum required sample size is 47 subjects per group. Including the unresponsive and fallout rate of 25 %, the estimate number of 60 participants in each group should be enough for bringing out the conclusion.

Research plan: Using including criterias and confirming the diagnose of chronic constipation the initial value of quality of life in children and their parents will be determined using PedsQL 4.0 and PAC-QOL questionnaires with special versions for children (depending on age) and parents. SF-36 and WHOQOL-BREF will be additionally used to determine the quality of life in parents. Chronic constipation etiology will be determined using diagnostic tests: contrast enema, rectal biopsy, anorectal manometry, colonic transit study based on Metcalf formula. After defining the normal data distribution (Kolmogorov-Smirnov), identifying differences between two groups will be based od parametric (Student t-test, ANOVA) and nonparametric tests (Mann-Whitney, Kruskal-Wallis, ANOVA). Correlation will be determined using Pearson or Spearman methods. P values less than 0.05. will be considered statistically significant.

Significance/Expected scientific contribution: The results of the study will show the extent of different types of chronic constipation in correlation with health status of affected children with emphasis on importance of treatment and correlation with quality of life of children and their parents. The study will show the values of specific questionnaires as future predictors of possible complications and treatment success. Eventually, the results will contribute to taking a stand, based on scientific evidences, about questionnaires used for quality of life prediction and reduce negative impact of chronic constipation on children health.

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

Dissertation proposal title: Prognostic significance of Nrf-2 factor and correlation with activity of ferroptosis in mechanical ventilated patient with COPD exacerbation

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Mentor 2: Assoc. Prof. Robert Smolić, M.D., PhD., University Hospital Centre Osijek; Faculty of Medicine Osijek, University of Osijek, Croatia

Introduction: Chronic obstructive pulmonary disease (COPD) is a common disease worldwide with significant morbidity and mortality. An exacerbation of COPD is defined as an acute worsening of respiratory symptoms that results in additional therapy such as mechanical ventilation in the most severe forms of AECOPD. Ferroptosis is a recently recognized form of regulated cell death which is related with disrupted iron homeostasis and accumulation of cellular reactive oxygen species (ROS) and has been implicated in the pathogenesis of AECOPD. Abnormal increases in Fe2+ and H2O2 (oxidative stress) triggers the Fenton reaction which oxidizes membrane lipids to lipid peroxides leading to cell damage. The Fenton reaction can be inhibited by glutathione peroxidase 4 (GSH-Px4) whose reduced activity indicates the severity of ferroptosis. Increased production of reactive oxygen species (ROS) in response to mechanical stress such as mechanical ventilation may additionally contribute to ferroptosis by oxidative stress which can be measured by estimating total oxidation status (TOS). Nuclear factor-erythroid 2 related factor 2 (Nrf2) is a ubiquitous master transcription factor which upregulates antioxidant response elements (AREs)-mediated expression of antioxidant enzyme and cytoprotective proteins. Nrf-2 regulates hundreds of genes, of which many are either directly or indirectly involved in modulating ferroptosis,

including metabolism of glutathione, iron and lipids, and mitochondrial function. This potentially positions Nrf2 as an important deterministic component of pathogenesis AECOPD which is modulating the severity and outcomes of AECOPD, especially in patients on mechanical ventilation.

Hypothesis: Low concentration of Nrf-2 factor is associated with increased activity of ferroptosis in mechanically ventilated patients with COPD exacerbation which resulted in prolonged mechanical ventilation.

Aims:

1. To determine the level of Nrf-2 factor in patient`s serum
2. To determine the total oxidant status (TOS) in patient`s serum
3. To determine the activity of glutathione peroxidase 4 (GSH-Px4) in patient`s serum
4. To determine the correlation among concentration of the Nrf-2 factor, TOS and activitiy GSH-Px4
5. To determine the correlation between concentration of the Nrf-2 factor and duration of mechanical ventilation
6. To determine the correlation between activity GSH-Px4 and duration of mechanical ventilation.

Materials/Participants and methods: The study will include 100 patients with COPD egzacerbation (GOLD guidelines) who are divided into two groups: patients with COPD exacerbation treated with mechanical ventilation and patients with COPD exacerbation not treated with mechanical ventilation (control group). Each group will consist of 50 patients. The study will not include patients with advanced malignancy and patients with multiorgan failure. For the purposes of this study, patients will have their venous blood taken to determine the level of next parameters: Nrf-2 factor concentration (ELISA test), total oxidant status (colorimetric method), glutathione peroxidase 4 activity (spectrophotometric method) using commercially available tests

and basic laboratory parameters (complete and differential blood count, biochemical inflammatory factors and arterial blood gas).

Research plan: It is a cross-sectional study. The research will be conducted at the University Hospital Centre Osijek and Faculty of Medicine Osijek. The planned study time is 12 months. The study will include two groups of patients with egzacerbation of COPD: patients who will be treated conservatively and patients who will be treated with mechanical ventilation. Blood sampling for the purposes of this study will be performed within 48 hours from establishing of diagnosis. In the group of patients who will be treated with mechanical ventilation blood sampling will be repeated after 96 hours of mechanical ventilation.

Significance/Expected scientific contribution: The target of this study is to determine the impact of Nrf2-factor and its correlation with ferroptosis activity on clinical course and outcomes of the COPD egzacerbation suffering patients. This could be clinically significant for the development of the drugs with impact on this correlation.

MeSH/Keywords: nuclear factor erythroid 2-related factor 2, ferroptosis, total oxidant status, glutathione peroxidase 4, COPD egzacerbation, mechanical ventilation

Dissertation proposal title: Diagnostic value of brain SPECT with ^{123}I-ioflupane in confirming Parkinson's disease

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Introduction: Parkinson's disease is a chronic, multisystem and progressive neurodegenerative disease that most typically occurs after the sixth decade of life and is more common in male patients. Older age, gender, genetics and the influence of environmental factors are the most significant risk factors. Parkinson's disease is clinically manifested by a triad of symptoms: muscle rigidity, general hypokinesia and resting tremor, but also loss of postural reflexes. This disease predominately affects neurons, which play an essential role in transmitting signals and integrating information into the central nervous system. The formation and deposition of proteins (Lewy bodies) is toxic and causes degeneration or death of dopaminergic neurons, leading to dysfunction of the nervous system.

Hypothesis: Nuclear medicine uses radionuclides whose accumulation can be monitored and measured for the purpose of diagnostic confirmation of Parkinson's disease, specific treatment and disease control.

Aims:

1. To investigate a possibility for early and accurate detection of Parkinson's disease by

reviewing the findings obtained from brain SPECT ^{123}I-ioflupane and a combination of visual method and semiquantitative analysis of radioactivity accumulation

2. To determine whether there are differences in ^{123}I-ioflupane accumulation, and to which extent, in regions of interest between striatum (caudate and putamen nucleus).

Materials/Participants and methods: At the Clinical Institute of Nuclear Medicine and Radiation Protection, University Hospital Centre of Osijek, 926 patients with clinically uncertain Parkinson's disease participated in the study, 526 of whom were male and 400 female. Monitoring the accumulation of ^{123}I-ioflupane in dopaminergic neurons using single-photon emission computed tomography (SPECT) imaging will provide insight into neuronal functionality.

Research plan: Investigate by visual interpretation the possible differences in the accumulation of radiopharmaceuticals in striata, confirm/dismiss Parkinson's disease and differentiate it from other neurodegenerative diseases. Demonstrate by semiquantitative analysis of radiopharmaceutical accumulation in regions of interest that the progression of Parkinson's disease reduces the accumulation of ^{123}I-ioflupane in the basal ganglia due to loss of dopaminergic neurons.

Significance/Expected scientific contribution:

To determine a contribution and diagnostic value of brain SPECT with ^{123}I-ioflupane in early confirming of Parkinson's disease

To demonstrate the sensitivity and exactness of SPECT brain with ^{123}I-ioflupane in diagnosing Parkinson's disease in regard to other diagnostic methods.

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

Abstract title: The influence of hyperthyroidism and antithyroid drugs treatment on changes of bone density, bone metabolism and inhibitors of Wnt pathway sclerostin and dickkopf 1

Part of the disertation proposal: The influence of Graves'disease and antithyroid drugs treatment on changes and bone density and bone metabolism

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Introduction: Graves'disease (GD) is an autoimmune disease in which excesive production of thyroid hormones can accelerate bone metabolism and decrease bone mineral density (BMD). Since loss of BMD became an important public health issue, extensive researches of various pathophysiological mechanisms responsible for bone remodeling are being investigated, among them wingless integrated (Wnt) pathway and its inhibitors, like sclerostin and dickkopf 1 (Dkk1). Activating Wnt pathway leads to BMD improvement and conversely to its decrease.

Aims:

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

2. to determine BMD at the time of diagnosing GD and beginnig of treatment with ATD and after a period of 1 year

3. to determine concetration of sclerostin and Dkk 1 at the time of diagnosing GD and beginnig of treatment with ATD and after a period of 1 year

Materials/Participants and methods: 42 patients with diagnosed GD were included in the study. Diagnosis of GD was based on elevated concetrations of thyroid hormones, free triiodothyronine (FT3) and free thyroxine (FT4), suppressed thyroid stimulating hormone (TSH), positive TSH-R antibodies (TRAb), positive thyroid scan done with Tc99m pertechnetate and ultrasound characteristics matching GD. BMD at lumbar spine and left hip was determined as well as OC, Dpd and β-Ctx, sclerostin and Dkk1 in serum and urine samples. All patients filled a questionary regarding osteoporosis. Patients were then treated with ATD for a period of one year. After one year control findings of BMD and control measurments of the above mentioned bone markers and Wnt inhibitors were performed.

Results: The research included 42 patients. 5 of them (12 %) were male and 37 (88 %) were female. Average age was 47 years, ranging from 20 to 75 years. In comparison with baseline values, control findings of FT3, FT4, TRAb, OC, beta – CTx and Dpd were significantly lower, while significantly higher values of BMD of lumbar spine and left hip were registered. Concentrations of sclerostin and Dkk 1 still remain to be analyzed.

Conclusion: These findings suggest that decreased BMD of lumbar spine and bone remodeling changes can be of secondary etiology due to GD and that bone loss is of reversible nature after initiation of ATD. Thus, changes in BMD should be monitored since treatment of transitory osteopenia/osteoporosis may not be necessary

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

Abstract title: Expression of $CD163^+$ cells, collagen deposition and adipocyte morphometry in subcutaneous and visceral adipose tissue of young male children

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

PhD candidate: Robert Mujkić, Faculty of Dental Medicine and Health Osijek, University of Osijek, Croatia

Mentor: Assoc. Prof. Kristina Selthofer Relatić, M.D., Ph.D., University Hospital Centre Osijek; Faculty of Medicine Osijek, University of Osijek, Croatia

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Introduction: Adipose tissue (AT) has the ability to adapt to a dynamically changing nutritional environment which is critical to maintain metabolic control. In response to changes in the nutritional status, the AT undergoes through dynamic remodeling, including quantitative and qualitative alterations in adipose tissue-resident cells that indicates that AT remodeling in obesity is closely associated with AT function. Little is known about extracellular matrix (ECM) remodeling and immune cells in AT in children, possibly due to the difficulties in obtaining AT samples. The ECM is essential for tissue architecture and has an important role in adipogenesis and thus AT growth. Adipose tissue macrophages (ATMs) are the dominant leukocyte population in AT in lean and obese state. Resident ATM population has properties that overlap with alternatively activated macrophages and is associated with suppression of the immune response and with ECM remodeling.

An understanding of how AT remodeling differs between visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) may unlock the links between visceral adiposity and metabolic disease.

Aim: The aim of this study was to examine if there are some differences between collagen deposition, adipocyte morphometry and number of $CD163^+$ cells in SAT and VAT of healthy young male children depended on their age.

Participants and methods: The research is based on 40 young male children for this preliminary part of the research, who were hospitalized for elective abdominal surgery at the Department of Pediatric Surgery of the University Hospital Osijek. Prior to the beginning of the research every parent of the subject was given written explanation of the purpose of the research and signed an informed consent. Subjects were divided in two sub-groups by age (up to 5 years and ≥ 5 years). Samples of SAT and VAT were gained during surgical procedure. Immunohistochemistry for $CD163^+$ cells was performed and number of positive cells was counted per mm^2 of adipose tissue. Histological staining of extracellular components was performed with Masson's trichrome stain and histomorphometric analysis of digital images of histological tissue sections was conducted using the free online image analysis program Fiji, a distribution of ImageJ.
Inclusion criteria for the research: anamnestically healthy subjects who do not suffer from diabetes, cardiovascular, neurovascular, genetic, hormonal and malignant diseases. Exclusion criteria for the research: the presence of a positive history of severe and chronic disease, and of previously known diseases (e.g., malignant and genetic disorders). Excluded are individuals who were operated due to entrapped inguinal or ventral hernia.

Results: Tissue sections staining with Masson's trichrome stain for ECM content showed increased collagen deposition in SAT in the group where subjects were ≥ 5 years of age which was statistically significant compared to the group up to 5 years of age ($p = 0,016$, Mann-Whitney U test). There was no statistical significance among groups when comparing adipocyte surface area in SAT and VAT. There was a positive correlation between BMI, hip and waist diameter, weight, Z-score and number of $CD163^+$ cells in VAT (Spearman correlation). In children ≥ 5 years of age more $CD163^+$

cells were counted in VAT compared with children up to 5 years of age ($p = 0,044$); and also hip and waist diameter ($p < 0,001$ and $p = 0,001$) was statistically higher compared to the group up to 5 years of age (Mann-Whitney U test).

Conclusion: In healthy and growing young male children, increased deposition of collagen content is age related. Accumulation of $CD163^+$ cells in adipose tissue is linked with anthropometric measures. For more and detailed conclusions, further analytical methods need to be done such as RT-PCR and ELISA on a larger sample size.

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

Dissertation proposal title: Specifics of the immunological profile in gestational diabetes

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

Introduction: Gestational diabetes mellitus (GDM) is an independent type of diabetes defined as glucose intolerance with first recognition during pregnancy, mostly between 24th and 28th week of gestation. Today 1 – 36 % of pregnant woman have GDM, depending on population and tests used in studies. GDM is associated with an inadequate immune response. Previous analyses of the immune system in GDM include predominantly T lymphocytes. T lymphocytes comprise several subpopulations. CD4+ T lymphocytes include Th1 cells that have proinflammatory activity and Th2 cells that have anti-inflammatory activity. The prevalence of Th2 is characteristic of a normal pregnancy. In the case of GDM or insulin resistance (IR), there is prevalence of Th1 cells which can lead to more fatal pregnancy outcomes. Metabolic syndrome is an inflammatory condition that can lead to IR and consequently to diabetes. Inflammation of adipose tissue is a chronic inflammation and B lymphocytes play a significant role in its regulation. B1/B2 lymphocyte ratios have been shown to be lower in bone marrow, spleen, and subcutaneous adipose tissue than in visceral adipose tissue, which is responsible for the development of metabolic syndrome and IR. Studies have shown that TNF-alpha and IL-6 are proinflammatory factors that play an important role in the development of IR and GDM. IL-10 is an anti-inflammatory cytokine that is lowered in GDM. Adiponectin is an anti-inflammatory hormone secreted by adipose tissue cells and its levels are reduced in pregnant women with GDM.

Hypothesis: Pregnant women who develop GDM have a specific immune profile that can give us insight into the immune processes involved in the onset of GDM and reveal new potential early biomarkers of GDM.

Aims: To investigate the specifics of the antenatal immune profile of pregnant women with GDM by analysing various immune markers from the blood of pregnant women during the 1st and 3rd trimesters of pregnancy.

Participants and methods: Participants will be pregnant women divided into two groups: women with GDM and women with normoglycemia. Analysis of routine laboratory parameters related to GDM (glucose, insulin, c-peptide, lactate, triglycerides, HDL and LDL cholesterol, CRP, HbA1C) and analysis of lymphocyte profiles by flow cytometry and quantification of cytokines, adipokines and immunoglobulins will be performed during the 1st and 3rd trimester.

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

Significance/Expected scientific contribution: Find potential predictive and/or diagnostic markers of GDM that will contribute to earlier diagnosis and better obstetric care of women with GDM.

Keywords: pregnancy, gestational diabetes, immune response, lymphocytes, inflammation

Abstract Title: Serum neurotrophins levels after genicular nerves neurolysis performed with different temperatures as a prognostic factor in the treatment of osteoarthritic knee joint pain

PhD candidate: Ivan Omrčen, M.D., University Hospital Centre Osijek, Croatia

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

Introduction: The nerve ablation using radiofrequency or cryoneurolysis causes iatrogenic neural degeneration aiming only for sensory or sympathetic denervation without motor deficits. According to Sunderland's classification, current neural ablations produce reversible second-degree peripheral nerve injury to the myelin, axon, and endoneurium without any disruption of the fascicular arrangement, perineurium, and epineurium. The molecular mechanisms related to axonal regeneration after injury include production of neurotrophic as a Nerve Growth Factor (NGF), Brain-Derived Neurotrophic Factor (BDNF), Neurotrophins-3 (NT3) and Neurotrophins-4/5 (NT4/5).

Hypothesis: The different temperature of genicular nerves ablation during radiofrequency neurolysis and cryoneurolysis, result in dissimilar neurotrophins serum levels in patients with knee joint osteoarthritis.

Aims: To determine the difference in serum levels of NGF, BDNF, NT3, and NT4/5 during neurolysis performed at different temperatures, and the possibility of using it as a prognostic factor in the treatment of osteoarthritic knee pain. To compare the effectiveness and duration of

radiofrequency neurolysis versus cryoneurolysis of genicular nerves on analgesia, the knee joint function improvement, prevalence of neuropathic pain after each procedure in patients with osteoarthritic knee joint pain.

Materials/Participants and methods: The survey will be a randomized prospective cohort study. It will be performed in University Hospital Centre Osijek. The sample size of the subjects will be determined using the G-power test. Including criteria: patients with knee joint osteoarthritis, pain lasting more than three months, weak response to conservative treatments, age 18+. Excluding criteria: patients with severe psychiatric comorbidities, total knee endoprosthesis, oral anticoagulant medications, implanted electrical devices, pregnancy, and allergic reactions to medical equipment.

Research plan: All patients will undergo a diagnostic block of the superior medial, superior lateral, and inferior medial genicular nerve. After a positive diagnostic block (50% reduction in their numeric rating score (NRS), we will randomize patients to the radiofrequency neurolysis group or the cryoneurolysis group. Both methods are performed under combined ultrasound and fluoroscopic guidance. Blood samples for ELISA diagnostic of NGF, BDNF, NT3, and NT4/5 will be taken at baseline and 1, 7, 21, 30, 60, and 90-day post-procedure. Numeric rating score (NRS), Western Ontario and McMaster Universities (WOMAC) Index of Osteoarthritis, PAIN detect, Global Perceived Effect, The Depression Anxiety Stress Scale will be measured at baseline and 30, 60 and 90 days post-procedure.

Expected scientific contribution: Contribution to the understanding of the pathophysiology of regeneration after peripheral nerve injury. Contribution to creating a clinical algorithm for treating chronic pain in knee joint osteoarthritis.

Keywords: nerve growth factors, genicular nerves, radiofrequency neurolysis, cryoneurolysis, knee osteoarthritis pain.

Abstract Title: Examination of the effects of selected antibiotics and new amidinobenzimidazole derivatives on the multiresistant bacteria ability to produce biofilm based on MALDI/TOF mass spectrometry

Part of the Disertation Proposal: Antibacterial acivity of newly synthetized amidinobenzymidazole and coumarine compounds

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

Mentor: Assoc. Prof. Domagoj Drenjančević, M.D., Ph.D., University Hospital Centre Osijek; Faculty of Medicine Osijek, University of Osijek, Croatia

Introduction: Over the last 20 years, we have faced a steady decline in the detection and registration of new antibiotics despite multiple institutional support. Of particular concern are "ESCAPE" pathogens: *Enterococcus faecium, Staphylococcus aureus, Clostridium difficille, Acinteobacter baumannii, Pseudomonas aeruginosa,* and species from the Enterobacteriaceae family (*Klebsiella pneumoniae* and *Escherichia coli*).

Benzimidazole and coumarine derivatives are structural isosters of purines which allow them to interact with biopolymers and, therefore, can have different biological and clinical applications. Numerous efforts have been focused in recent years to discover new antibacterial agents based on the structure of benzimidazoles with the aim of shaping substances that have therapeutic applications aimed at binding to DNA. The increasingly important role of benzimidazole and triazole derivatives has been demonstrated in in vivo studies against Gram-positive and Gram-

negative bacteria and the bis-benzimidazole compound (ridinylazole, SMT-19969) was recently included in a phase III clinical trial in the treatment of *Clostridium difficile*.

Aims:

1. Test the susceptibility of bacteria to newly synthesized amidinobenzimidazole and coumarin compounds by microdilution method.

2. Interpret sensitivity results.

Materials/Participants and methods: The study was performed on three ATCC (American Type Culture Collection; reference microbial strains) strains of bacteria: *Acinetobacter baumannii* ATCC 19606, *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 and on three bacterial strains of clinical isolates: *Acinetobacter baumannii* 9768 , Methicillin-resistant *Staphylococcus aureus* (MRSA) ESBL 11710 and *Escherichia coli* ESBL 26001 from the collection of bacterial strains of the Department of Microbiology, Parasitology and Clinical Laboratory Diagnostics of the Faculty of Medicine in Osijek. The research was performed in the laboratory of the Department of Microbiology, Parasitology and Clinical Laboratory Diagnostics at the Faculty of Medicine in Osijek.
The newly synthesized amidinobenzimidazole and coumarin derivatives were provided in cooperation and courtesy of prof. dr. sc. Silvana Raić Malić (Department of Organic Chemistry, Faculty of Chemical Engineering and Technology, University of Zagreb) and are part of a long-term cooperation.

Testing of antibacterial activity was conducted in accordance with the standard broth microdilution method as recommended in the guidelines of the Clinical and Laboratory Standard Institute (CLSI) method for diluting antimicrobial susceptibility to aerobic growth of bacteria, M7-A9 and performance standards for antimicrobial susceptibility testing, M100-S26).

Results: In accordance with CLSI, the minimum inhibitory concentration is the concentration at which the antimicrobial substance completely inhibits the growth of the microorganism. The results are read visually - it is first necessary to check whether the compound controls and strain growth controls (located in the first and last well) are valid, ie that there has been no contamination. For the test to be valid, a growth of ≥ 2 mm "buttons" or turbidity of the strain growth control well is acceptable. If the conditions are met, the microtiter plate can be read. by comparing control and tested wells, the minimum inhibitory concentration is read.

After several months of testing, we discovered that at least 58 newly synthetized compounds have shown antibacterial activity, determined as stated in previous sections of this paper.

Conclusion: As our results so far have shown, there are realistic possibilities that some of the compounds we have tested (and will continue to test) will contribute to solving the burning problems of bacterial resistance. Future tests that we will perform on selected compounds will give us additional insight into the possibilities of inhibiting bacterial growth as well as possible biofilm formation.

MeSH/Keywords: amidinobenzimidazole, coumarine, resistance, bacteria, MIC

Dissertation proposal title: Impairment of eye movements as a diagnostic and prognostic biomarker in Parkinson`s disease

PhD candidate: Zvonimir Popović, M.D., Department for Neurology, University Hospital Centre Osijek; Faculty of Medicine Osijek, University of Osijek, Croatia

Mentor: Assist. Prof. Svetlana Tomić, Department for Neurology, University Hospital Centre Osijek; Faculty of Medicine Osijek, University of Osijek, Croatia

Co-Mentor: Prof. Silva Butković Soldo, M.D., Ph.D., Department for Neurology, University Hospital Centre Osijek; Faculty of Medicine Osijek, University of Osijek, Croatia

Introduction: Parkinson's disease is a slow-progressive neurodegenerative disease characterized by the pathological accumulation of α-synuclein and the formation of Lewy bodies. The risk of acquiring disease increases with age and is caused by a complex combination of environmental factors, genomics, and epigenomics. Diagnosis of the disease is reached at the onset of motor symptoms, such as bradykinesia, tremor, rigor, and postural instability. Depression, anxiety, sleep disorder, dementia and several other non-motor symptoms are also present.

Disease can be divided according to age of disease onset on young (< 50 years) and older onset (≥ 50 years). Patients with older onset of the disease are characterized by complex clinical image, faster progression of dementia, and shorter post-diagnose survival. Regarding dominant motor symptoms, patients can be divided into tremor dominant (with a more benign course), intermediate and PIGD type (postural instability and gait disorder; characterized by harsher clinical symptoms on the onset of disease and faster progression of cognitive changes).

One of the motor symptoms in Parkinson's disease is eye movement impairment, present in 75 % of patients. This can be evaluated using eye-tracker, with lesions in basal ganglia causing abnormalities in different eye movements. None of the studies have tried evaluating the significance of analysis of eye movements as a sensitive biomarker of Parkinson's disease subtypes differentiation.

Hypothesis: Patients with older onset of Parkinson's disease and with PIGD variant will have more impaired eye movements, especially in terms of delayed saccade initiation and reduced smooth pursuit gain.

Aims: Research aims to associate the impairment of eye movements with a type and duration of Parkinson's disease, examine the correlation between impairment in eye movements and motor and non-motor test, evaluate the role of eye movement impairments as a potential diagnostic and prognostic biomarker.

Materials/Participants and methods: Study will be conducted on patients with the diagnosis of idiopathic Parkinson's disease, which will be set according to UK Brain Bank criteria. Exclusion criteria are dementia, parkinsonism of other causes and vision disturbances.

Research plan: Patients will be surveyed for motor and non-motor symptoms and their eye movements will be evaluated using an eye tracker. They will be divided in two subtypes according to age of disease onset and motor-dominant symptoms. The goal is to have at least 30 examinees in each group. Differences between groups will be analyzed using SPSS.

Significance/Expected scientific contribution: Analysis of different eye movement impairments that have its own specific pathoanatomical basis and its correlation with PD subtypes, motor, and non-motor symptoms could provide a better understanding of the pathophysiology of certain subtypes of PD and clinical presentation. This could help us as a diagnostic and prognostic biomarker for PD subtypes. The method itself is simple, non-invasive, and doesn't require large material investments.

Keywords: Parkinson’s disease, eye movements, biomarker, saccade, motor

Dissertation proposal title: Relation of interleukin 6, genetic polymorphisms of interleukin 6 and interleukin 23 receptor with phenotype characteristics in inflammatory bowel disease

PhD candidate: Ines Rajkovača Latić, M.D., Department of Gastroenterology, General Hospital „Dr. Josip Benčević", Slavonski Brod, Croatia

Mentor: Prof. Silvio Mihaljević, M.D, Ph.D., Department of Gastroenterology and Hepatology, University Hospital Centre Osijek, Croatia

Introduction: Inflammatory bowel diseases (IBD) are chronic relapsing inflammatory disorders of gastrointestinal system including Crohn's disease and ulcerative colitis caused by multiple pathogenic factors including environmental changes, genetic susceptibility, gut microbiota and dysregulated immune response. Complex interactions between pro-inflammatory and anti-inflammatory cytokines play a crucial role in IBD immunopathogenesis. For example,one of the most studied IL-6 is known as a potent pro-inflammatory and acute response cytokine that affects Th2 and Th17/reg cells differentiation depending on antigen stimulation. Through IL-23 receptor (IL-23R) stimulation it promotes Th 17 cells differentiation. Also, recent studies have identified an important role of IL-23 in the pathogenesis of IBD suggesting that single nucleotide polymorphisms in the IL-23R gene affect disease susceptibility.

Hypothesis: The hypothesis states that there is a significant difference between IL-6 serum levels, selected genetic polymorphisms of IL-6 and IL-23R in IBD patients and healthy controls and in different IBD patients subgroups according to phenotype characteristics of the disease.

Aims: The aim of the study is to measure IL-6 serum levels and to examine genetic polymorphisms of IL-6 rs1800795 and IL-23R rs11209026 in IBD patients and healthy controls. Another aim is to examine if there is a difference between these parameters in IBD patients subgroups according to endoscopic features and localisation of disease, therapeutic strategies, corticosteroid response and extraintestinal manifestations.

Materials/Participants and methods: Study will include 140 IBD patients and 100 healthy controlos. IL-6 levels will be measured by chemiluminescent sequential immunometric method, while genetic polymoprhysms of IL-6 and IL-23R will be measured by a real-time fluorescence resonance energy transferbased method.

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

Significance/Expected scientific contribution: This research should provide better understanding of IBD pathogenesis and it`s relation to phenotype characteristics of the disease.

MeSH/Keywords: colitis, ulcerative; Crohn's disease; inflammatory bowel diseases; interleukin-6; polymorphism

Dissertation proposal title: Methodology and clinical validity of electrophysiological parameters posterior roots reflexes caudea equinae

PhD candidate: Julija Rimac, M.D., National Memorial Hospital Vukovar, Croatia

Mentor: Prof. Davor Jančuljak, M.D., Ph. D., Clinical Hospital Centre Osijek, Croatia

Introduction: Technique for evoke monosynaptic reflexes of the spinal cord is in use as a monitoring tool of nerve function in lumbosacral and hip surgery. It can be used in placing epidural electrodes for electrical stimulation of spinal cord after spinal injury. PRMR (posterior root muscle reflex) has not been used in clinical practice. The F wave and H reflex are electrodiagnostic methods for evaluation conduction characteristics of proximal parts of peripheral nerves. They have limitations and abnormal result is not specific. PRMR is electrical equivalent of the monosynaptic stretch reflex elicited by epidural stimulation the same sensory axons like H wave at proximal sites adjacent to the posterior spinal cord. PRMR involving the Ia afferent (sensory) and efferent (motor) pathways. This methodology is non-invasive and safe. It can be helpful in diagnosis nerve roots demyelination, radiculopathy, functional integrity of lumbosacral plexus and proximal parts of the sciatic and femoral nerves.

Hypothesis: Transcutaneous electrical stimulation of posterior lumbosacral roots can be used as diagnostic tool for direct detection of proximal nerve conduction slowing and block in lower limbs.

Aims: The aim of this study is to modificate the metodology of PRMR, to validate electrophysiologic characteristics of PRMR in healty subjects and evaluate the ability to monitor functional integrity of lumbosacral roots and proximal nerves.

Materials/Participants and methods: The testing will be preformed with patient in laying position. For electric stimulation we will use self–adhesive electrodes placed over the skin in the Th12-L1 interspinous space (anode) and over the umbilicus (cathode). Aplication of paired stimulus from EMNG apparatus with an interstimulus interval (ISI) of 50 ms and duration of 0.5 ms, generate compound muscle action potential (CMAP) simultaneously, bilateral from low limb muscles in distribution of sciatic and femoral nerves. Latency and amplitude of the PRMR response will be studied.

Research plan: In first phase we will modificate methodology used in intraoperative monitoring to elicit PRMR in low extremity muscles. In second phase methodology will be used for validation normal values of PRMR in healty subjects. Last phase of research will include patients with proximal nerves pathology and comparation their PRMR characteristics with values in healthy subjects.

Significance/Expected scientific contribution: these non-invasive methodology have a good diagnostic potential for sensitive detection conduction failure in lumbosacral spinal roots and proximal nerves. It could give more specific information than H reflex and F wave and contribute to topographic diagnosis accuracy.

MeSH/Keywords: Posterior root muscle reflex, electrical spinal cord stimulation, monosinaptic spinal reflex, intraoperative monitoring, lumbosacral spinal roots

Dissertation proposal title: Therapeutic effect of phytoestrogens from hop extract and alendronate in rat model of osteoporosis

PhD candidate: Edi Rođak, Faculty of Medicine Osijek, University of Osijek, Croatia

Mentor: Assist. Prof. Nikola Bijelić, Faculty of Medicine Osijek, University of Osijek, Croatia

Co-Mentor: Prof. Nada Oršolić, Faculty of Science Zagreb, Croatia

Introduction: Osteoporosis (OP) is characterized by loss of bone mass and increased fragility. Primary OP is a result of menopause, while secondary OP is a result of another disease, nutrition disorder, certain medications etc. Complications of OP decrease the quality of life and may even be life-threatening.

In 1960s, estrogen supplementation was standard therapy for OP. Later research showed that hormone replacement therapy was associated with major adverse effects. Bisphosphonates (drugs slowing down bone resorption) have been used instead.

Novel research has shown that phytoestrogens (naturally occurring analogs of estrogen) may be used in OP therapy. Main phytoestrogens in hops are and 8-prenylnaringenin (8PN) and xantohumulol (a precursor for 8PN synthesis in liver). 8PN shows higher affinity for α than β estrogen receptor.

Hypothesis: Phytoestrogens from hop extract have a positive effect on bone quality and metabolism, reduce needed dose of bisphosphonates in OP therapy and have no major side effects in animal model of OP.

Aims:

- Investigate the impact of tested substances and their combinations on:
    1. structural parameters and mineral composition of bone
    2. bone metabolism and oxidative stress markers in serum
    3. microscopic morphology of target organs
    4. hematological and biochemical parameters

Materials and methods: Research will be conducted on 70 six month-old Wistar female rats. Rats will be divided in seven groups. Bilateral ovariectomy will be done in six groups (ovariectomy OP model) and sham operation in one group. A month after ovariectomy, treatment with alendronate (Alendox 70) and hop extract (XantoFlav), or a combination of the two will be started. The tested substance(s) or vehiculum will be given via intragastric administration daily for two weeks. Animals will be sacrificed and organs harvested for further analysis.

Research plan:

Year 1: Animal preparation, ovariectomy, 2-week treatment

Year 2: Performing analyses (bone density, structure and mineral composition; serum and blood; bone turnover markers; histology and immunohistochemistry)

Year 3: Statistical analysis and publishing

Expected scientific contribution: We expect to show the impact of phytoestrogens on bone quality and metabolism as a putative alternative or supplement to bisphosphonate therapy.

Keywords: phytoestrogens, hops, osteoporosis, bone – metabolism, 8-prenylnaringenin

Abstract title: Clinical characteristics of West Nile virus infections in eastern Croatia

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

PhD candidate: Dario Sabadi, M.D., Clinic for Infectious Diseases, University Hospital Centre Osijek, Croatia.

Mentor: Assoc. Prof. Ljiljana Perić, PhD, M.D., Clinic for Infectious Diseases, University Hospital Centre Osijek, Croatia.

Introduction: West Nile virus (WNV) is a single-stranded RNA virus that belongs to the genus Flavivirus of the family Flaviviridae. The virus was isolated in 1937. from the blood of a febrile woman in the West Nile region of northern Uganda. Today, WNV is one of the most widespread arboviruses. Based on phylogenetic analyzes, seven gene lines have been described so far, of which the most significant are lines 1 and 2. WNV infection is a zoonosis that occurs seasonally (summer, early autumn) related to the activity of mosquitoes (genus Culex). The primary hosts and reservoirs of the virus are different species of birds and the virus is maintained in a bird-mosquito-bird cycle. Also, other animals (horses) as well as humans can become infected.

The incubation period for infection is 2 - 6 (14) days. The clinical manifestations of WNV infection in humans are asymptomatic in 80 %, in about 20 % it manifests as WNV fever. Less than 1 % of patients develop a neuroinvasive form of the disease (meningitis, encephalitis, myelitis). Risk factors for the development of severe neuroinvasive forms of the disease are older age and immunosuppression, while the role of other underlying diseases such as arterial hypertension, diabetes, and cerebrovascular disease in the pathogenesis of WNV infection has not been fully elucidated. In such patients, the mortality rate can be 10 to 15 %, and survivors often have permanent neurological damage.

Although the onset of symptoms is preceded by a viremic period, viremia in humans persists for only a short period and the disease usually manifests after the virus is no longer detectable.

Therefore, the diagnosis of WNV infection is usually based on serological methods. There is no specific drug and the therapy is symptomatic.

In the last two decades, cases of WNV infection in humans and animals have been continuously recorded in Europe. In Croatia, the first clinical cases of neuroinvasive WNV infection were described in 2012 in Osijek-Baranja County, after which the disease appeared sporadically or epidemically in subsequent transmission seasons in eastern and northwestern counties. Because a large number of infections are asymptomatic, the exact prevalence of WNV infection is unknown. Furthermore, so far in Croatia, only two strains of WNV detected during the 2013 epidemic have been sequenced and genetically characterized in the area of Zagreb which belonged to line 2.

According to the data of the Reference Center of the Ministry of Health for Epidemiology, around 200 cases of aseptic meningitis/encephalitis are reported annually in Croatia (50 cases in the eastern Croatian counties), of which 80 – 90 % have no proven etiology. Because WNV is not included in routine diagnostic algorithms, the significance of this virus in the etiology of neuroinvasive diseases, clinical and laboratory features, as well as molecular epidemiology, have not been fully elucidated. Also, the impact of underlying diseases on the severity of the clinical manifestations and the outcome of WNV infection has not been elucidated.

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

Materials and methods: The study presents 24 patients with central nervous system infection caused by West Nile virus, hospitalized at the Clinic for Infectious Diseases, Osijek in the period from 2012 – 2020. Serum, cerebrospinal fluid (CSL), and urine samples were taken from patients with neuroinvasive infection. Clinical symptoms and laboratory parameters were collected using a survey questionnaire. CSL and urine samples were screened for the presence of WNV RNA by real-time RT-PCR. Positive samples were further tested by classical and “nested” RT-PCR method to obtain genome segments of sufficient length for genotyping. All samples were extracted after gel electrophoresis from the gel and purified, and sequencing and phylogenetic analysis will be

performed in the following period. Serological testing of serum samples and CSL (IgM and IgG antibodies, the avidity of IgG antibodies) have been made with commercially available enzyme-linked immunosorbent assays, and in case of cross-reactions, a confirmatory test by neutralization test was done.

Results: In the period from 2012 to 2020, 24 patients (14 women and 10 men) with WNV neuroinvasive form of the disease were hospitalized. The mean patient's age was 65. Twenty-one patients had a personal history of comorbidities (arterial hypertension, diabetes). In twenty-two patients, the neuroinvasive form of WZN was presented as meningoencephalitis, in one patient as acute cerebellitis, and one as polyradiculoneuritis. The most common symptoms of the neuroinvasive form of WNV infection were fever (22 patients), headache (21 patients), nausea (15 patients), and neck stiffness (12 patients). The mean cerebrospinal fluid pleocytosis was around 103 x 10 (6) / L. with an initial neutrophilia. Diffuse dysrhythmia in the EEG was noted in nineteen patients, and brain atrophy in six patients using brain CT. WNV diagnosis was confirmed by the detection of WNV IgM and low avidity IgG antibodies in serum and CSF. Molecular and phylogenetic WNV analysis is in progress and will be presented in the coming period.

Conclusion: Detection of WNV allows elucidation of the etiology in patients with neuroinvasive diseases. Defining clinical, laboratory and epidemiological/molecular characteristics (genotyping and sequencing) represents an original scientific contribution to the epidemiology of this infection and will enable the improvement of measures to prevent mosquito-borne infections.

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

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

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

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

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

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

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

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

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

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

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

The diagnosis of primary headache is made clinically. According to the ICHD classification of headaches in children, migraine can be diagnosed after five attacks of headache with migraine characteristics without aura or after two attacks of headache with migraine characteristics with aura, so sometimes the diagnosis requires months or even years of monitoring.

It is for this reason that there is a need to identify a specific and sensitive migraine biomarker that would be easy to determine even outside of a headache attack.

In the past 20 years CGRP (calcitonin gene related peptide) has been defined as a neuropeptide that has a clear role in the pathophysiology of migraine. It is secreted from the trigeminal ganglion and acts as a potent vasodilator of cerebral and dural blood vessels. Although the mechanism by which CGRP acts as a migraine trigger has not been fully elucidated, it is known that CGRP acts as a mediator of trigeminal vascular pain transmission from intracranial vessels to the central nervous system and is a vasodilating component.

It has been shown that the stimulation of the trigeminal ganglion induce CGRP release, and iatrogenic CGRP promotes migraine attack in migraine sufferers.

Elevated serum CGRP levels during migraine attacks have been found in both adults and children.

Further research has shown elevated serum CGRP values beyond the migraine attack itself, while this was not the case in primary tension-type headaches.

Elevated CGRP values normalized in patients with acute migraine after the introduction of sumatriptan therapy suggesting that triptans block the release of CGRP and thus lead to a reduction of discomfort.

CGRP has been defined as a new target for therapeutic options but also for migraine prevention, and monoclonal antibodies to CGRP and its receptors have been developed that show promising results in migraine prevention.

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

Aims:

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

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

To observe the mean effect in the difference of numerical variables between the three independent groups (control group, migraine headache, tension headache), with a significance level of 0.05 and strength 0.8, the minimum required sample size is 159 subjects (53 subjects per group).

All must meet the clinical and diagnostic criteria of primary headache, and would be divided into two main groups:

Group 1 - children with clinical criteria for migraine headache and

Group 2 - children with clinical criteria for tension headache.

After the diagnosis of primary headache, the serum CGRP value of both groups of subjects would be determined.

In order for a certain value of CGRP in serum to be informative, it is necessary to determine the reference values of the test depending on the age of the child, so it is necessary to have a control group of children in this study who have no anamnestic data on neurological problems.

Methods: The following data will be collected from the included subjects:

• Clinical characteristics of headache, age of onset of first headache attack, associated diseases and disorders, results of common clinical and paraclinical methods that are otherwise routinely used in the diagnosis of children with primary headaches (e.g. TCCD, MRI / MRA of the brain).

• All subjects would be tested for serum CGRP by ELISA using one of the commercially available kits. The measurements would be performed in the Biochemical Laboratory of the Children's Hospital Zagreb

• The CGRP values of children with migraine and children with tension headache would be compared with the values of CGRP in the control group, and we expect significantly higher values of CGRP in the group of children with migraine.

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

Research plan:

1. Examine the values of CGRP biomarker by ELISA method in the Biochemical Laboratory in Children's Hospital Zagreb on blood samples of patients diagnosed with primary headache

2. Investigate the reference values of CGRP biomarker by ELISA method in the Biochemical Laboratory in Children's Hospital Zagreb on blood samples of the control group of patients

3. Compare the values of serum concentrations of CGRP in three groups of patients (control group, patients with tension headache, patients with migraine headache)

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

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

MeSH/Keywords: CGRP, headache disorders, children, migraine, tension-type headache

Abstract title: Values of Pap smear and HPV status before and after LLETZ conization

Part of the disertation proposal: Influence of the immunological and microbiological status of a woman in the presence of premalignant changes of the cervical mucosa

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Mentor: Prof. Siniša Šijanović, M.D., Ph.D., University Hospital Osijek; Faculty of Medicine Osijek, University of Osijek, Croatia

Introduction: Human papillomavirus (HPV) is one of the most common causes of sexually transmitted disease in women, especially in sexually active young women, 18 to 30 years of age. 90 % HPV infections will regress spontaneously within 12 to 36 months as the immune system eliminates the virus. Chronic HPV causes premalignant and malignant changes of cervix.

Aims: To determine the correlation of Pap smear and HPV status before and six months after LLETZ conization.

Materials/Participants and methods: The study included 34 female patients treated at Sestre Milosrdnice University Hospital Centre. In all of them Pap smear and HPV test were performed

before and six months after LLETZ. McNemar test, McNemar-Bowker test and Kruskal-Wallis H test were used for statistical analysis.

Results: There were statistically significant more women with negative HPV six months after LLETZ ($\chi 2$ (1) = 22; $p < 0.001$). There were statistically significant more women with better and normal Pap smear six months after LLETZ ($\chi 2$ (3) = 31; $p < 0.001$). There was no difference in the age of women who were HPV negative six months after LLETZ and those who were HPV positive (H (1) = 0.030, p = 0.862). There was no difference in the age between women with different changes in PAP smear six months after LLETZ (P (3) = 1.628, p = 0.653). There was no difference in the duration of PAP smear abnormalities between HPV negative women six months after LLETZ and those who were HPV positive (H (1) = 1,248, p = 0.264). There was no difference in the duration of PAP smear abnormalities before LLETZ between women with different changes in the Pap smear six months after LLETZ (H (3) = 2.367, p = 0.500).

Conclusion: LLETZ reduces the incidence of abnormalities in PAP smear and HPV positive tests. It doesn't affect the results of the HPV test and PAP smear with regard to age of women or to duration of PAP smear abnormalities before surgery. It is necessary to expand the study with imunological and microbiological status to confirm or reject the hypothesis.

MeSH/Keywords: PAP smear, HPV, LLETZ, HSIL, LSIL

Abstract title: The associations between religiosity and spirituality with psychopathology in patients with schizophrenia-preliminary results.

Part of the disertation proposal: cross-sectional data which are part of longitudinal study

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Mentor: Assist. Prof. Branka Aukst Margetić, M.D., Ph.D., Department of Psychiatry, University Hospital „Sisters of Mercy", Zagreb, Croatia

Introduction: Religiosity and spirituality are important in lives of patients with schizophrenia. Their associations with psychopathology are not clear. Religiosity and spirituality have been associated with better outcomes but also with higher psychopathology measures. We present our preliminary cross-sectional results, which are part of longitudinal study.

Aims: The aim of the study was to assess the associations between religiosity, spirituality and psychopathology in patients with schizophrenia.

Materials/Participants and methods: The study was conducted in Specialised hospital for psychiatry and palliative care St Raphael. Forty-five participants were included in the study and 24(53,3%) of those were female. Average age of the participants was 36,26 SD 10,66. All participants signed informed consent.

Religiosity was measured with Duke index of religiosity (DUREL) that consists of five items with five possibilities to answer on Likert scale. It measures three dimensions of religiosity: organised, nonorganized and intrinsic religiosity.

For the assessment of spirituality, we used Spiritual well-being questionnaire (SWBQ) that consists of twenty items with six possibilities to answer on Likert scale. In comprises of two subscales religious wellbeing and spiritual well-being. Sociodemographic data are assessed with questionnaire comprised for this study.

Results: The study did not show any associations between age, gender, marital status and religiosity, spirituality and psychopathology measured with PANSS and its subscales. Pearson correlations were performed and showed significant but negative correlation between existential spirituality and negative symptoms measured with PANSS (r=-0,357; P=0,022). Also, organised religiosity was correlated negatively with general psychopathology. Other correlations were not significant.

Conclusion: Higher existential spirituality is associated with lower negative symptoms and organised religiosity with less general symptoms in schizophrenia patients. Although our sample is small, it shows potential of supporting spirituality in schizophrenia patients may be associated with lower negative symptoms in schizophrenia.

MeSH/Keywords: spirituality, religiosity, schizophrenia, psychopathology, PANSS,

Abstract title: Influence of vestibular rehabilitation on the cupular and otolith organs in patients with unilateral vestibular hypofunction

Part of the dissertation proposal: Impact of vestibular rehabilitation on the recovery of all vestibular receptor organs and quality of life in patients with unilateral vestibular hypofunction

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Introduction: Previous studies have shown the benefit of vestibular rehabilitation (VR) on the recovery of patients with unilateral vestibular hypofunction (UVH). However, most studies mainly describe the impact of VR on the horizontal semicircular canal and only a few studies in the literature demonstrate the effect of VR on all vestibular receptor organs. Moreover, in recent years, there has been increasing awareness of the existence of various forms of UVH, depending on which part of the vestibular organ is affected. With the help of ocular and cervical vestibular evoked myogenic potentials (o and cVEMPs), Video Head Impulse Test (vHIT), and caloric test (CT), we are now able to detect various forms of UVH.

Aims: This study aimed to investigate the effect of VR on all vestibular receptor organs, and quality of life in patients with different types of UVH.

Materials/Participants and methods: A total of 80 patients (30 males and 50 females, median age 59.5 years) with three different types of UVH; combined and isolated loss of semicircular canal and otolith organ function, were included in the study. All patients performed a 12-week customized program of VR and received a full battery of vestibular function tests, before and after the VR. The DHI and SF-36 were performed before, after six weeks, and twelve weeks of the VR.

Results: Parameters of the CT, vHIT, oVEMP and cVEMP were significantly improved after VR. A total of 59 (74 %) patients fully recovered, with no significant difference in recovery regarding the type ($p = 0.13$) and stage of UVH ($p = 0.13$). All patients reported significantly lower disability and a better quality of life after the VR based on the DHI and SF-36 score.

Conclusion: Vestibular rehabilitation has a positive effect on the recovery of all vestibular receptor organs, and on the quality of life in patients with different types of UVH.

MeSH/Keywords: vertigo, unilateral vestibular hypofunction, vestibular rehabilitation, semicircular canals, otolith organs

Dissertation proposal title: Risk factors for allergic sensitization and atopic diseases development in children after respiratory syncytial virus infection

PhD candidate: Helena Tesari Crnković, M.D., General County Hospital Požega, Croatia

Mentor: Assoc. Prof. Mirjana Turkalj, M.D., Ph.D., Srebrnjak Children's Hospital, Zagreb, Croatia

Co-Mentor: Assist. Prof. Romana Gjergja Juraški, M.D., Ph.D., Srebrnjak Children's Hospital, Zagreb, Croatia

Introduction: Genetic, epigenetic and environmental risk factors have impact on atopic diseases development. High total Immunoglobulin E level (tIgE) as a marker of atopy and Respiratory Syncytial Virus (RSV) infection are risk factors for asthma development in children. In recent studies the emphasis is on discovery of new markers for atopic diseases, such as periostin.

Hypothesis: Children with risk factors in early life are at risk for development of atopic diseases. High periostin serum level is potential novel biomarker for childhood atopy and asthma.

Aims: To identify risk factors and markers for atopic diseases development in children 10 years after RSV infection.

Materials/Participants and methods: participants are children born in County Hospital Požega infected with RSV in the first two years of life. We analysed cord blood markers such as absolute eosinophil count (Eo) and tIgE and noted positive family history for atopy of a first-degree relative, prenatal risk factors as antibiotics and paracetamol usage, uterine complications, gestational age, birth weight, length and head circumference, breastfeeding duration, time of solid food introduction, tobacco smoke exposure, cat and dog allergens, household size, nursing care attendance. At first and second year of live we analysed Eo, tIgE and specific IgE (sIgE) (Dermatophagoides pteronyssinus, grass, weed, trees and ragweed pollen, cat and dog dander, milk, wheat, egg white and peanuts), and RSV sIgE, IgG, IgG3 and IgG4. After 10 years, participants will be divided in a group of children with positive and a second group with negative biomarkers for atopy (tIgE, sIgE). We will analyse fractionated exhaled nitric oxide (FeNO), spirometry, data from the ISAAC questionnaire, prick test on standard pallet of inhalation allergens (ISAAC II), tIgE, sIgE, periostin, and vitamin D.

Research plan: First phase of research (birth, first and second year of age) is conducted. Second stage of research will be a prospective study, conducted 10 years after first, in which we will enrol 70 subjects.

Significance/Expected scientific contribution: The research of risk factors and novel biomarkers for childhood atopy and asthma will contribute to their early prevention.

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

Dissertation proposal title: Influence of motivational interviewing and intensive education of insulin titration patients on basal oral therapy

PhD candidate: Goran Toplek, mag.med. techn., County Hospital Čakovec, Croatia

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

Introduction: The prevalence of diabetes has reached global proportions with 202 million cases projected by 2035. Croatian guidelines for the treatment of type 2 diabetes education is the cornerstone of treatment. In Croatia, continuous education is conducted when a patient visits for follow up. Studies show that 16 – 28 % of patients achieve adequate insulin titration at the beginning of therapy. The introduction of insulin therapy presents a risk for laps in titration and application, which can lead to development of complications and fatal outcomes. Poor patient motivation and uncertainty in titration is an important factor of non-adherence and poor glycemic control in patients on basal oral therapy (BOT). A unanimous agreement of healthcare professionals and patients exist to develop additional tools and models to help patients achive better glycemic control and prevent short and long term complications.

Hypothesis: Education and motivational interview, conducted every two weeks about insulin titration in patients with diabetes on BOT is associated with better adherence, glycemic control, quality of life and reduced hypoglycemia.

Aims:

1. Determine whether more intensive education and motivational interview of patients with type 2 diabetes on insulin titration on basal oral therapy, every two weeks, improves adherence compared to the standard form of education.

2. Examine the effect of motivational interview and intensive education on glycemic control and the reduction in the number of hypoglycemic events compared to the standard form of education

3. Examine the impact of motivational interview and intensive education on the quality of life of participants compared to the standard form of education

Materials/Participants and methods: Patients at the County hospital Čakovec meeting following criteria will be included in the study: diagnosis of type 2 diabetes for at least a year, age 18 years or older, treated with oral hypoglycemics and basal insulin. Hospital information system would be used for collecting HbA1c values. To observe the mean effect in the difference of numerical variables between two independent groups of subjects, with a significance level of 0.05 and a strength of 0.9, the minimum required sample size is 86 subjects per group, a total of 172 subjects.

Research plan: Patient would be referred by the physician to the nurse to recive education on titration and application of the prescribed therapy. During the initial examination and at the controls for three and six months, the participants would fill in the questionnaires on sociodemographic characteristics and WHOQOL-BREF as well as the questionnaire for adherence assesment. After the first education, one group would receive additional education and motivational interviews only at their regular follow up visits which is usually after three to six months, while the other group of patients would continue education by telephone and motivational interviews every two weeks.

Significance/Expected scientific contribution: Intensive education and motivational interview regarding insulin titration of patients on BOT conducted by a nurse every two weeks, leads to better glycemic control and adherence, less hypoglycemic events and improved quality of life.

MeSH/Keywords: adherence, basal oral therapy, diabetes, education, nurse

Dissertation proposal title: Correlation of tumor budding and expression of lipid biomarkers detected by MALDI TOF MS technology in colorectal cancer

PhD candidate: Bojan Trogrlić, M.D., Department of Digestive Surgery, University Hospital Center Osijek, Croatia

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

Introduction: Tumor budding has received increasing recognition as an important independent prognostic factor in colorectal carcinoma. Prominent tumor budding in adenocarcinoma arising in a polyp has been shown to be a risk factor for lymph node involvement. The variability in methods used for evaluating tumor budding in different studies and lack of standardized guidelines have impeded routine inclusion of tumor budding in pathology reports. Tumor budding has been shown to be an independent prognostic marker in colorectal carcinomas and the routine reporting of tumor buds is now advocated by using the approach outlined by the ITBCC guidelines. Tumor budding is included in the College of American Pathologists (CAPs) Colorectal Cancer Protocol as a recommended element. Presence of prominent tumor budding in an adenocarcinoma in a polyp may have implications for management, such as additional resection, while it serves as a prognostic factor in other settings. The evolution of mass spectrometry methods, such as MALDI-MS technology, has enabled the detection and identification of a wide variety of lipids with great potential to open new avenues for predictive and preventive medicine. Matrix-assisted laser desorption ionisation imaging mass spectrometry (MALDI-MSI) is a rapidly advancing technique for tissue analysis that allows simultaneous localisation and quantification of biomolecules in different histological regions of interest. The combination of MALDI MS technology and tumor

budding would contribute to a more individual approach to patients with CRC and has great potential especially in the prevention and prediction of this disease.

Hypothesis: Higher tumor budding will lead to higher expression of lipid markers detected by MALDI MS technology which will be applicable even in small biopsies of small polyps and small colorectal cancers which will suggest the biological behavior of the cancer, and its prognosis and possible additional treatment modalities.

Aims:

1. determination of lipid profile of tumor tissue using MALDI TOF MS technology
2. determining the correlation of lipid profile and histological grade of tumor tissue
3. determining the correlation of lipid profile and TNM classification of tumor tissue
4. determining the correlation of tumor budding, histological grade and lipid profile of tumor tissue

Materials/Participants and methods: The study will include all patients with colorectal cancer who have not undergone neoadjuvant chemoradiotherapy who will be treated at the Department of Abdominal Surgery of the University Hospital Center Osijek. All the patients were informed about the study and signed an Informed Consent term prior to their inclusion. Immediately after surgery, a patient suffering from colorectal cancer will have a fresh sample of tumor tissue and a sample of adipose tissue from a healthy part of the colon (which will be control group) removed from the removed part of the colon and frozen at -80 C for further lipids analysis on MALDI TOF MS. After that part, the remaining part of the resected part of the colon is treated with 10% buffered formalin and prepared for histological processing involving HE staining and tissue examination with an Olympus BX53 light microscope.

Research plan: Once the research hypothesis has been defined, a further research work plan will consist of forming informed consent for patients who will be invited to participate in the research work, and the research work together with the informed consent will be presented to the Ethics

Committee of the Osijek Clinical Hospital and the Osijek School of Medicine. This is followed by the next phase, which includes sample collection of these patient groups. When sufficient samples are collected (30), a pilot research study will be formed on which to base future doctoral dissertation.

Significance/Expected scientific contribution: The role of lipid synthesis in cancer metabolism and tumor development as well as in precancerous lesions still requires many studies to elucidate the numerous mechanisms and pathways of its development. In clinical practice, the lipid profile is measured based on the serum concentrations of total cholesterol, highdensity lipoprotein, low-density lipoprotein and triacylglycerols being poor information for the analysis of the lipid fractions. The mass spectrometry (lipid) technique allowed identifying the specific categories of lipids. Cancer cells are characterized by changes in lipid metabolism. Membrane alterations, rupture of energetic homeostasis, cell signaling, gene expression and protein distribution, affecting several cellular functions, such as apoptosis, autophagy, necrosis, proliferation, differentiation, growth, drug resistance. The significance of tumor budding in colorectal carcinoma, as an independent prognostic factor for adverse clinical outcomes, has now been well established. The routine reporting of tumor bud counts and scores is now advocated. The ITBCC guidelines represent the first international effort at a standardized methodology for assessing and reporting tumor buds in colorectal carcinoma. However, it is widely acknowledged that additional studies will be required to further refine the methodology and address the challenges in uniform reporting of tumor budding. Definite treatment options like chemotherapy in stage II disease, based on high tumor budding, need to be assessed. Studies such as this will enable further understanding of tumor progression and thus enable a more individual approach to patients with colorectal cancer, ie adequate treatment of precancerous lesions, and T1 and T2 tumors. In this way, we will enable the patient a better survival and a better quality of life.

MeSH/Keywords: colorectal cancer, tumor budding, maldi tof ms, lipid profile, histological grade

Dissertation proposal title: Comparison of stress response and postoperative recovery between total intravenous and inhaled anesthesia after colorectal cancer surgery using MALDI-TOF

PhD candidate: Hrvoje Vinković, M.D., University Hospital Centre Osijek, Croatia

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

Introduction: Colorectal cancer is one of the most common forms of malignancy in all developed countries. Each surgical procedure causes a stress response of the organism. The body's stress response is a set of metabolic, endocrine and immune changes that occur in response to a surgical procedure. It is characterized by an increase in stress hormones, primarily cortisol, growth hormone, catecholamines (noradrenaline and adrenaline), prolactin, CRP (C reactive protein), α-2 macroglobulin and other antiproteinases. Also, there is an increase in Heat Shock Proteins.

Hypothesis: The use of total intravenous anesthesia with continuous use of opioids will cause a lower stress response of the body in major surgery. When using total intravenous anesthesia with continuous use of opioids, there will be faster and better postoperative recovery and better postoperative analgesia.

Aims: To determine the influence of total intravenous anesthesia in comparison with balanced anesthesia with inhalation anesthetic on the stress response of the organism, postoperative recovery and postoperative analgesia.

Materials/Participants and methods: Inclusion criteria: Patients older than 18 years, ASA status I-III with colorectal cancer scheduled for laparotomy and bowel resection. Exclusion criteria: Patients under 18 years of age, ASA IV status, use of drugs that may affect the stress response (corticosteroids, azathioprine, immunosuppressants).

Two hours before the operation a blood sample will be taken from the patients to determine the basal values of stress hormones - cortisol, norepinephrine, adrenaline, and heat shock protein HSP 70. Patients will be divided into two groups.In patients in the first group, fentanyl 3 mcg/kg, propofol 2 mg/kg and rocuronium 0.6 mg/kg will be used for theinduction. Anesthesia will be maintained by continuous administration of propofol 150-200 mcg/kg/ min and fentanyl 1mcg/kg/h.

In patients in the second group, the inhaled anesthetic sevoflurane with fentanyl 3 mcg/kg and rocuronium 0.6 mg/kg will be used for induction of anesthesia. Anesthesia will be maintained with sevoflurane 1 MAC in an oxygen / air mixture. Intraoperative analgesia will be maintained by bolus administration of fentanyl 50 - 100mcg. Postoperatively, pain is assessed by a numerical pain scale (NRS) ranging from 0 - 10.

Four hours after extubation, a blood sample is taken to re-measure the levels of stress hormones - cortisol, norepinephrine, adrenaline, and heat shock protein HSP 70, and the increase is measured in relation to the basal values taken before the procedure.

Significance/Expected scientific contribution: To determine whether there are differences in the body's stress response to major surgery, in this case laparotomy with bowel resection by comparing two types of anesthesia, total intravenous anesthesia and balanced anesthesia with inhalation anesthesia.

MeSH/Keywords: colorectal cancer, stress response, postoperative recovery, MALDI-TOF, HSP 70, postoperative analgesia

Dissertation proposal title: Accurate assessment of hepatocellular carcinoma response to treatment using contrast-induced ultrasound

PdD candidate: Jelena Vlahović Puvača, M.D., Department of Diagnostic and Interventional Radiology, University Hospital Centre, Osijek, Croatia

Student advisor: Prof. Jerko Barbić, M.D., University Hospital Centre, Osijek; Faculty of medicine Osijek, University of Osijek, Croatia

Introduction: Hepatocellular carcinoma (HCC) is the sixth most common tumor worldwide and most common type of primary liver cancer in adults. It is closely linked to chronic viral infections (hepatitis B or C) or to exposure to toxins such as alcohol (cirrhosis) or aflatoxin.

Diagnostic methods for HCC detection are primary measuring of AF and medical imaging such as ultrasound, CT scan and MRI.

Contrast-enhanced ultrasound (CEUS) is an imaging modality that is inexpensive, used for non-invasive assessment of liver perfusion in real time throughout the vascular phase.

CEUS is superior to CT or MRI for detecting hypervascularity of HCC because of real-time evaluation of arterial phase enhancement.

Hypothesis: CEUS can evaluate and track hepatocellular cancer response to treatments and therapies accurately enough that it can minimize usage of imaging methods such as CT scan and MRI.

Aims:

1. To determine whether CEUS as a diagnostic method can help clinicians to assess the quality and quantity of the therapy and/or treatment response.
2. To determine if there is a possibility of minimizing the use of imaging methods like CT and MRI in HCC follow-up.

Materials/Participants and methods: This research will be formed as a cross-sectional study, that is, as a typical method of research and prevalence and will be conducted among 35 patients with diagnosed and verified HCC. After patient consent is received, and patient medical history is revised, CT or MRI scan will be performed, as well as CEUS, before any kind of treatment or therapy administration. After 1 – 3 months, CEUS imaging, CT scan and MRI will be performed again to asses weather the results obtained from sophisticated imaging modalities are in correlation with the results obtained from CEUS imaging.

Research Plan: The research will be conducted at the Department of Diagnostic and Interventional Radiology (in collaboration with The Department of Oncology), Osijek University Hospital Center, in Osijek. After Ethics committee approval the research will be preformed on selected patients who correspond to the required characteristics.

Collected data will be analyzed by methods of descriptive and inductive statistics with table and graphic view of the results.

Expected scientific contribution: Researched, structured and processed theoretical material and collected and analyzed empirical material, will result in unified and systematized review of the need for the usage of CEUS as a diagnostic method in the assessment of therapeutic effect on HCC.

Keywords: contrast-enhanced ultrasonography, hepatocellular carcinoma, liver, ultrasonography, radiology

Dissertation proposal title: Axon regeneration after spinal cord injury in cuprizone demyelination or B4galnt1 and Sia8a knock out mice

PhD candidate: Vjenceslav Vrtarić, M.D., Department of Neurosurgery, University Hospital Centre Osijek, Croatia

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

Introduction: The mammalian central nervous system contains molecules responsible for inhibiting axon regeneration that also play a role in signalling connected to successful myelination (Nogo, MAG and OMgp). Axonal gangliosides GD1a and GT1b are receptors for the MAG molecules and inhibition of the synthesis of these gangliosides leads to demyelination. Temporary suppression of ganglioside synthesis could improve the success of regeneration.

Hypothesis: Gangliosides GD1a and GT1b are involved in inhibiting neuronal regeneration through interaction with MAG and NgR1 receptors and their complete or partial removal from the axon membrane will affect the degree of regeneration after spinal cord injury compared to cuprizone model of demyelination.

Aims:

1. To demonstrate the degree of regeneration of corticospinal pathway axons after a spinal cord lesion in wild-type mice compared to cuprizone-treated mice, or mice with excluded *B4galnt1* or *Sia8a* genes compared to wild-type mice using behavioral functional tests.
2. To determine the number of neurons and inflammation in critical regions after the regeneration period of 4 weeks.
3. To mark and quantify the axons that grow into the corticospinal tract in mice subjected to spinal cord lesions and mice that were not subjected to lesions with the BDA tracer injected into the motor cortex.

Materials and methods: The study will use 16 mice, 3 to 4 months old, of each model (*B4Galgt1* KO, *Sia8a* KO, WT C57black / 6 and WT C57black / 6 treated with cuprizone 20 days before the lesion), From each group, eight mice will be subjected to a spinal cord lesion and eight mice are controls. Spinal cord contusion will be followed by adequate postoperative care. Recovery will be monitored by a battery of behavioral tests (motor and sensory) for six weeks. Tissue sections will be perfused with buffer and fixative. Standard histology and immunohistochemistry will be performed on the samples to determine the degree of inflammation (GFAP, Iba), neuron survival (NeuN, TUNEL methods) and axon regeneration (SMI312, Tau, BDA).

Research plan: WT animals will be treated with cuprizone 20 days prior to contusion. Contusion of a spinal cord and injection of BDA tracer in motor cortex will be performed in eight mice and eight mice from control group will be subjected just to BDA injection followed by standard histology and immunohistochemistry analysis.

Expected scientific contribution: A study of axon regeneration after spinal cord injury in mice with excluded ganglioside synthesis genes or mice with demyelination will indicate differences that may be relevant for further research and clinical practice in the treatment of patients with spinal cord injury.

Keywords: gangliosides, axon regeneration, cuprizone, contusion, spinal cord injury

Dissertation proposal title: Survival of hemodialysis patients and mechanisms of the „obesity paradox“

PhD candidate: Dunja Vujčić, M.D., University Hospital Centre Osijek, Croatia

Mentor: Assist. Prof. Dubravka Mihaljević, M.D., Ph.D., Uniersity Hospital Centre Osijek; Faculty of Medicine Osijek, University of Osijek, Croatia

Introduction: Obesity, which is defined as body mass index (BMI) > 30 kg/m², is associated with a higher cardiovascular risk in the general population. However, in patients with end stage renal disease (ESRD) and in those on maintenance hemodialysis we can observe an „obesity paradox“, meaning that obesity in these patients is associated with better survival. Mechanisms of this paradox are still unclear. Since ESRD in itself is an independent cardiovascular risk factor, the „obesity paradox“ is still the topic of much research. The effect of an increased BMI in maintenance hemodialysis patients has shown differences regarding the age and sex of the patients, hemodialysis modality and volume status. Hypervolemia, which is a common problem in hemodialysis patients, has a negative effect on blood pressure, left ventricular hypertrophy and heart failure. It also induces a proinflammatory response and is predictive for all-cause and cardiovascular mortality in hemodialysis patients. Therefore, an increase in body mass with a higher lean muscle mass to fat tissue ratio is considered protective.

Hypothesis: Our hypothesis is that hemodialysis patients who have an increased BMI, but are not hypevolemic, will have a better survival.

Aims: Investigate the connection between fluid overload and biomarkers of heart failure (NT-proBNP and soluble ST2). Investigate the connection between fluid overload and anemia,

erythropoietin stimulating agent dosage and loss of muscle mass. Create a survival and cardiovascular morbidity curve regarding hypervolemia and obesity.

Materials/Participants and methods: The study would include all patients on maintenance hemodialysis in Clinical Hospital Centre Osijek. Patients with malignancy and patients on immunosuppressive medication would be excluded. The variables measured would be: anthropometric measurements, bioimpedance spectroscopy, hemoglobin, serum albumin, urea, creatinine, NT-proBNP, CRP, soluble ST2, erythropoietin stimulating agent type and dosage. A simple Protein Energy Wasting (PEW) Score would be calculated to assess nutritional status.

Research plan: A prospective cohort study which would follow patients on maintenance hemodialysis in Clinical Hospital Centre Osijek for two years. The monitored outcomes would be death, myocardial infarction, cerebrovascular accident, infection and lower extremity amputation.

Significance/Expected scientific contribution: The results of this research would improve the distinguishing of patients on hemodialysis who are obese and hypervolemic from those who are obese with no excess fluid. These two groups of patient require a different treatment approach and have a significantly different prognosis.

MeSH/Keywords: ESRD, dialysis, obesity, inflammation

Dissertation proposal title: Alzheimer's disease and diabetes - the effects of comorbidity on IL-6 and TNF-α serum levels

PhD candidate: Antonia Vuk, M.D., Psychiatric hospital "Sveti Ivan" Zagreb, Croatia

Mentor: Prof. prim. Igor Filipčić, M.D., Ph.D., Psychiatric Hospital "Sveti Ivan", Zagreb, Croatia

Introduction: Diabetes mellitus (DM) is a global pandemic disease. It is a heterogeneous group of metabolic diseases characterized by hyperglycemia, leading to harmful and costly micro and macro vascular complications, physical disability, and functional impairment.

Dementia refers to a clinical syndrome characterized by progressive cognitive decline that interferes with the ability to function independently. Alzheimer's disease (AD) is the most common form of dementia in older people.

Recent studies have demonstrated an association of etiopathology of AD and DM with elevated concentrations of inflammatory mediators, such as IL-6 (Interleukin 6), and TNF-α (Tumor necrosis factor alpha).

DM type 2 (DMT2) and AD are both associated with increasing age, each increases the risk of development of the other, nevertheless, the real relationship between remains controversial area. Although there are indications of shared pathogenic factors, some recent studies suggest that hyperglycemia itself is an inducer of oxidative stress and through several different mechanisms lead to elevated levels of various inflammatory mediators.

Hypothesis: AD and DMT2 comorbidity is associated with increased systemic inflammation and higher levels of IL-6 and TNF-α, independent of aging and other vital and clinical parameters.

Aims: To estimate prognostic value of DMT2 for IL-6 and TNF-α levels, independent of aging and other vital and clinical parameters.

Materials/participants and methods: The targeted population will be patients diagnosed with dementia in AD. The diagnosis of probable AD (ICD-11, NINCDS-ADRDA). Clinical assessment of cognitive impairment severity will be based on clinical picture of disorder and MoCA Test. The control group will be recruited from aged persons who were examined in our neurology clinic and did not reveal a cognitive impairment.

Research plan: a cross sectional study will be conducted at the Psychiatric Hospital "Sveti Ivan" in Zagreb. IL-6 and TNF-α serum levels will be measured in blood plasma of elderly patients with dementia in Alzheimer's disease. Diabetes will be ascertained according to previous medical records, and regulation will be measured with HbA1c values.

Significance/expected scientific contribution: If the hypothesis is not rejected, our study may emphasize the importance of DMT2 in systemic inflammation.

Keywords: Alzheimer disease, dementia, diabetes, IL-6, TNF-α

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

PhD candidate	Mentor	Title of abstract	Page number
Irina Bagić	Assist. Prof. Božidar Muršić, M.D., Ph.D.	Stroke lipidomics under the condition of GM1 depletion	
Slaven Balog	Assist. Prof. Antonio Kokot, M.D.	Changes in the thickness of the neurosensory retina in 5 macular areas after cataract surgery by ultrasound (phacoemulsification) depending on the amount of ultrasound energy consumed in healthy individuals and diabetics	
Ana Bednjanić	Assist. Prof. Valerija Blažičević, M.D, Ph.D.	Prognostic value of the cancer stem cells in rectal cancer	
Gramos Begolli	Assist. Prof. Zeljko Debeljak	Diagnostic significance of Carbohydrate Sulfotransferase 7 in Lung Cancer	
Terezija Berlančić	Assist. Prof. Ivan Miškulin, PhD.	Treatment and quality of life in postmenopausal women with vulvovaginal atrophy	
Nikolina Brkić	Assist. Prof. Dražen Švagelj, M.D., Ph.D.	The coccoid form of Helicobacter pylori is correlated to histopathological characteristics of stomach mucosa	
Dino Budrovac	Assist. Prof. Ivan Radoš, M.D., Ph.D.	Efficacy of percutaneous laser disc decompression versus epidural steroid and local anesthetic by transforaminal approach in the treatment of lumbar radicular pain	

Vladimir Cipek	Assoc. Prof. Marina Samardžija, M.D., Ph.D.	The link between lifestyle habits and level of hemoglobin within the population of volunteer blood donors	
Petra Čičak	Prof. Sanja Popović-Grle, M.D., Ph.D.	Improving access to palliative and end-of-life care conversations with older people with chronic obstructive pulmonary disease	
Ivan Durlen	Assist. Prof. Irzal Hadžibegović, M.D., Ph.D.	Value of sST2/GLS combination in predicting unwanted cardiovascular events in patients on hemodialysis	
Kristina Duspara	Assoc. Prof. Martina Smolic, M.D., Ph.D.	Evaluation of gingval crevicular fluid levels of sclerostin and dickkopf in periodontitis patients on chronic statin therapy	
Ivan Feldi	Prof. Lada Zibar, M.D., Ph.D.	Clearance of glucoregulatory peptide hormones during hemodialysis in prediabetic and diabetic end-stage renal disease patients	
Lada Goršić	Assoc. Prof. Branka Restek Petrović, M.D., Ph.D.	Personality and insight into illnes in early-stage psychosis patients and their parents	
Dijana Hnatešen	Prof. Roman Pavić, M.D., Ph.D.	Effectiveness of a multidisciplinary biopsychosocial approach on the quality of life of patients with chronic low back pain	
Višnja Ikić	Prof. Slavica Kvolik, M.D., Ph.D.	Perioperative monitoring and postoperative outcomes in patients undergoing abdominal cancer surgery	
Marul Ivandić	Assoc. Prof. Ines Bilić Ćurčić, M.D., Ph.D.	Differences in the incidence of hypoglycaemia after strength training and aerobic type exercise in patients with type 1 diabetes	

Hrvoje Kaučić	Prof. Dragan Schwarz, M.D., Ph.D.	Radiosurgery (Stereotactic ablative radiotherapy) of locally advanced pancreatic cancer using Calypso Extracranial Tracking© intrafractional motion managerment	
Nikolina Kolobarić	Assoc. Prof. Martina Mihalj, Ph.D.	Positive Effect of *n*-3 PUFAs Consumption on Serum Levels of Anti-Inflammatory Cytokines in Young Healthy Participants	
Nataša Kozina	Assist. Prof. Ivana Jukić, M.D., Ph.D.	Assessment of the mechanisms of flow-induced dilation of carotid artery in low salt and high salt fed *Tff3*$^{-/-}$/C57BL/6N mice and their wild type controls	
Marin Kuharić	Prof. Aleksandar Včev, M.D., Ph.D.	The effects of high-fat, high-sugar diet and early metformin and liraglutide treatment on the hippocampal lipidome of male and female Sprague Dawley rats	
Željka Laksar Klarić	Assist. Prof. Ana Đanić Hadžibegović, Ph.D.	Phonatory movement of the pharyngoesophageal mucosa in laryngectomy patients	
Marko Mesić	Assoc. Prof. Mario Kopljar, M.D., Ph.D.	Helath related quality of life in children with chronic constipation and their parents	
Damir Mihić	Assoc. Prof. Robert Smolić, M.D., Ph.D.	Prognostic significance of Nrf-2 factor and correlation with activity of ferroptosis in mechanical ventilated patinet with COPD exacerbation	
Katica Mijatović	Prof. Ivan Mihaljević, M.D., Ph.D.	Diagnostic value of brain SPECT with ^{123}I-ioflupane in confirming Parkinson's disease	
Dunja Mudri	Prof. Ivan Mihaljević, M.D., Ph.D.	The influence of hyperthyroidism and antithyroid drugs treatment on changes of bone density, bone metabolism and inhibitors of Wnt pathway sclerostin	

		and dickkopf 1	
Robert Mujkić	Assoc. Prof. Kristina Selthofer Relatić, M.D., Ph.D.	Expression of $CD163^{+}$ cells, collagen deposition and adipocyte morphometry in subcutaneous and visceral adipose tissue of young male children	
Jelena Omazić	Assoc. Prof. Jasenka Wagner Kostadinović, Ph.D.	Specifics of the immunological profile in gestational diabetes	
Ivan Omrčen	Assist. Prof. Ivan Radoš, M.D., Ph.D.	Serum neurotrophins levels after genicular nerves neurolysis performed with different temperatures as a prognostic factor in the treatment of osteoarthritic knee joint pain	
Marijan Orlović	Assoc. Prof. Domagoj Drenjančević, M.D., Ph.D.	Examination of the effects of selected antibiotics and new amidinobenzimidazole derivatives on the multiresistant bacteria ability to produce biofilm based on MALDI/TOF mass spectrometry	
Zvonimir Popović	Assist. Prof. Svetlana Tomić, Ph.D.	Impairment of eye movements as a diagnostic and prognostic biomarker in Parkinson`s disease	
Ines Rajkovača Latić	Assoc. Prof. Silvio Mihaljević, M.D., Ph.D.	Relation of interleukin 6, genetic polymorphisms of interleukin 6 and interleukin 23 receptor with phenotype characteristics in inflammatory bowel disease	
Julija Rimac	Prof. Davor Jančuljak, M.D., Ph.D.	Methodology and clinical validity of electrophysiological parameters posterior roots reflexes caudea equinae	
Edi Rođak	Assist. Prof. Nikola Bijelić, M.D., Ph.D.	Therapeutic effect of phytoestrogens from hop extract and alendronate in rat model of osteoporosis	

Dario Sabadi	Assoc. Prof. Ljiljana Perić, M.D., Ph.D.	Clinical characteristics of West Nile virus infections in eastern Croatia	
Jadranka Sekelj Fureš	Assist. Prof. Andrea Šimić-Klarić, M.D., Ph.D.	Association of alexithymia with laboratory values, sleep quality and datime sleepiness among hemodialysis patients The role of CGRP (calcitonin gene-related peptide) as a migraine biomarker in children	
Ivana Stojanović	Prof. Siniša Šijanović, M.D., Ph.D.	Values of Pap smear and HPV status before and after LLETZ conization	
Maristela Šakić	Assist. Prof. Branka Aukst Margetić, M.D., Ph.D.	The associations between religiosity and spirituality with psychopathology in patients with schizophrenia-preliminary results	
Anamarija Šestak	Prof. Siniša Maslovara, M.D., Ph.D.	Influence of vestibular rehabilitation on the cupular and otolith organs in patients with unilateral vestibular hypofunction	
Helena Tesari Crnković	Assoc. Prof. Mirjana Turkalj, M.D., Ph.D.	Risk factors for allergic sensitization and atopic diseases development in children after Respiratory Syncytial Virus infection	
Goran Toplek	Assoc. Prof. Ines Bilić Čurčić, M.D., Ph.D.	Influence of motivational interviewing and intensive education of insulin titration patients on basal oral therapy	
Bojan Trogrlić	Assist. Prof. Ksenija Marjanović, M.D., Ph.D.	Correlation of tumor budding and expression of lipid biomarkers detected by MALDI TOF MS technology in colorectal cancer	

Hrvoje Vinković	Assist. Prof. Tatjana Šimurina, Ph.D.	Comparison of stress response and postoperative recovery between total intravenous and inhaled anesthesia after colorectal cancer surgery using MALDI-TOF	
Jelena Vlahović Puvača	Prof. Jerko Barbić, M.D., Ph.D.	Accurate assessment of Hepatocellular carcinoma response to treatment using Contrast-induced ultrasound	
Vjenceslav Vrtarić	Assist. Prof. Božidar Muršić, M.D., Ph.D.	Axon regeneration after spinal cord injury in cuprizone demyelination or B4galnt1 and Sia8a knock out mice	
Dunja Vujčić	Assist. Prof. Dubravka Mihaljević, M.D., Ph.D.	Survival of hemodialysis patients and mechanisms of the „obesity paradox“	
Antonia Vuk	Prof. prim. Igor Filipčić, M.D., Ph.D.	Alzheimer’s disease and diabetes - the effects of comorbidity on IL-6 and TNF-α serum levels	