Dan doktorata 2024
Phd Day 2024

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1. Preliminary research results – basic medical sciences

Poster Title: Does Low Dose Injections of Tetanus Toxin into the Rat Basal Ganglia Induce Parkinsons Like Motor Impairments?

PhD candidate: Patrik Meglić

Part of the thesis: Central excitatory and inhibitory neurotransmitters in hyperkinesia and spasticity in rats

Mentor(s): Ivica Matak, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Nuclei of the basal ganglia exert a primary role in extrapyramidal motor control, but also have broader roles encompassing motor learning, executive functions, behavior and emotions. Abnormalities within neuronal circuits of the basal ganglia system are key to the development of movement disorders such as dystonia and Parkinsonism. In the central nervous system, tetanus toxin (TeNT) induces neuronal disinhibition by selectively blocking inhibitory synapses in neuronal projection-connected brain regions, undergoing axonal and trans-synaptic transport. This study aimed to investigate the behavioral impact of neuronal disinhibition in the basal ganglia and their interconnected regions, induced by low, non-convulsive doses of TeNT.

Materials and methods: Male Wistar rats (N=9/group) were unilaterally injected in the caudate putamen (CPu) with 2x0.4 ng of TeNT (distributed to 2 injection sites) while smaller regions like globus pallidus internus (GPi) and substantia nigra (SN) received single injections of 0.4 ng of TeNT. Various motor behavioral tests including beamwalk, gait analysis, open field exploration, and swimming tests, were conducted repeatedly (days 0, 7, 10, 14) to assess the effect of TeNT-induced disinhibition on normal motor performance. On the tenth day after receiving TeNT, rats were subjected to a D-amphetamine-induced rotation test (1 mg/kg i.p.), which typically induced ipsilateral circling in hemiparkinsonism models. Finally, prepulse inhibition and audiogenic seizure tests were conducted on the last day of the experiment to examine possible deficits in sensorimotor gating caused by TeNT injections which might indicate hyperexcitabilityrule and to rule out any potential epileptogenic effects of TeNT. Brain tissue was collected for immunohistochemical staining and western blot analysis.

Results: Disinhibition of CPu with TeNT induces visible hind limb contralateral motor impairment during the beamwalk test, a relatively complex motor task requiring precise hind-paw placement prediction. Animals who received injection to the CPu or GPi exhibited ipsiversive circling behavior in the open field and swimming tests. Circling behavior in the open field test was exaggerated by D-amphetamine. Animals that received injections in the CPu and GPi showed an increase in stride frequency and a decreased swing time in catwalk gait analysis. Evident recovery of motor symptoms towards the end of study suggested reversibility of TeNT-evoked impairments. No signs of epileptogenic behavior were observed in the audiogenic seizure test, while tyrosine hydroxylase regional content suggests lack of TeNT-evoked excitotoxic degeneration of dopaminergic projection within the basal ganglia. Animals injected in GPi and CPu exhibited hemiparkinsonism / Parkinsonism-like motor impairments, showcasing comparable rotational behavior, gait-related issues, and a proprioceptive deficit after CPu injections. These findings underline the complex interplay between inhibitory and dopaminergic transmission in the basal ganglia, a critical factor for motor control and function.

Discussion: TeNT, primarily entering GABA-ergic neurons in supraspinal brain regions, may be used as a tool to study the intricate system of GABA-ergic circuits and projections, as well as the significant interaction of inhibitory and dopaminergic transmission within basal ganglia-shaping extrapyramidal motor regulation and function. Further investigations are needed to pinpoint the exact affected neurotransmission pathways, which could potentially shed light on the general mechanism behind Parkinsonism-like motor impairments.

MeSH/Keywords: Tetanus toxin, Motor impairments, Parkinson's disease

Poster code: R-01-03-004

Poster Title: Effect of pentadecapeptide bpc 157 on the progression of calcium chloride-induced abdominal aortic aneurysm in rats

PhD candidate: Helena Žižek

Part of the thesis: Effect of pentadecapeptide BPC 157 on the progression of calcium chloride-induced abdominal aortic aneurysm in rats

Mentor(s): Associate Professor Alenka Boban Blagaić, MD PhD, Assistant Professor Tomislav Meštrović, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Abdominal aortic aneurysm (AAA) is defined as dilation of the abdominal aortic (AA) segment ≥1.5 times or ≥50% of the diameter compared to the diameter of the adjacent, intact segment. AAA is caused by an inflammatory process that leads to elastin and collagen fibers degradation and weakening of the vessel wall. We investigated the effect of pentadecapeptide BPC 157 therapy in an experimental rat model of AAA. The aim of this study is to determine the effect of BPC 157 administered perorally on the progression of calcium chloride-induced abdominal aortic aneurysm in rats.

Materials and methods: An established calcium chloride-induced rat model of AAA was used. Rats were divided in two groups, BPC 157 treated group (10 ng/mL in drinking water, ad libitum every day after AAA induction) and control group (pure drinking water) and evaluated 8 weeks after AAA induction. AA and AAA macroscopic presentation was recorded using USB microcamera and AA and AAA diameter measurement using imageJ software was performed before AAA induction and 8 weeks after AAA induction. Dilation ratios (DR) were calculated as follows: DR=(largest diameter/start diameter)x100. Histopathological examination of AAA specimens was performed 8 weeks after AAA induction.

Results: Rats treated with BPC 157 showed lower increase in AA diameters in contrast to the control rats. DRs were as follows, control group: 184±6 at 8 weeks; and treated group: 126±4 at 8 weeks. Histopathological examination of AAA specimens at 8 weeks revealed undular lamina elastica interna, no thinning or detachments in tunica media, and few solitary leukocytes in treated group, whereas control group showed linear lamina elastica interna, thinning of tunica media with areas of detachment and leukocyte aggregates.

Discussion: Peroral BPC 157 therapy slows the progression of AAA induced by calcium chloride in rats. Further research is needed, especially establishing the effect of BPC 157 through multiple time points, i.e. at 2 and 4 weeks after AAA induction, which would clarify the effect of BPC 157 on the dynamics of AAA progression. Also, it's necessary to clarify the substrates through which BPC 157 exerts an influence on the progression of AAA, i.e. evaluating the expression of genes of interest (Mmp-2, Mmp-9, Timp-1, Timp-2, Nos2, Nos3, Il-1, Il-6, Tnf-α) and the concentration of reactive oxygen species and NO in the vessel wall at all time points after AAA induction. Everything mentioned will be done within the framework of the proposed doctoral dissertation.

MeSH/Keywords: abdominal aortic aneurysm, BPC 157

Poster code: R-01-03-007

Poster Title: OCCLUSION LIKE SYNDROME IN RATS ARRISING AFTER APPLICATION OF CATALEPTOGENIC DOSE OF L-NAME AND THE EFFECT OF PENTADECAPEPTID BPC 157

PhD candidate: Ivana Jurca

Part of the thesis: Effect of pentadecapeptide BPC 157 abd L-arginine in occlusion like sindrome induced by cataleptogenic dose of L-NAME

Mentor(s): Professor Ivica Sjekavica, MD PhD, Assistant Professor Ivana Jurjević, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: L-NAME (N(G)-Nitro-L-arginine methyl ester), the NO syntases antagonist, causes catalepsy when administered in high doses (40 mg/kg intraperitoneally) 1. In the previous study 2, it was shown that pentadecapeptide BPC 157 antagonizes catalepsy symptoms caused by L-NAME. Syndrome occurring before, parallel and after clinical signs of catalepsy remains unexplored. The aim of this study is to investigate pathophysiological changes leading to and following L-NAME-induced catalepsy and the effect of BPC 157 on these changes. To investigate the impact and correlation with the NO-system, the effect of L-arginine, applied alone and concurrently with L-NAME and BPC 157, will also be investigated.

Materials and methods: Male Wistar rats will be divided in 6 rats/group/time interval (5 minutes, 30 minutes, 1 day and 3 days). The rats will be treated with intraperitoneally administered L-NAME (40 mg/kg) and L-arginine (100 mg/kg) alone, both medicines concurrently, and in combination with BPC 157. The macroscopic and pathohistological analysis of the brain, heart, lungs, kidneys, digestive system, inferior cava vein, portal vein, aorta and azygos vein will be performed, as well as venography using digital subtraction angiography, with invasive intracranial, portal and caval pressure measurement, and thrombosis and ECG evaluation. For initial evaluation, macroscopic analysis of the brain volume was performed after craniotomy and on ex vivo brains, via USB microcamera recordings, 5 minutes, 30 minutes and 1 day after medicatin aplication (L-NAME, and L-NAME+ BPC 157). Venography was performed using 1mL of iodine isoosmolar nonionic contrast media via direct IVC cannulation, 5 minutes after medication application.

Results: Control rats exhibited greater brain swelling, in comparison to treated rats. Venography showed IVC dilation and congestion with significantly delayed arterial flow and very pale parenchimal opaciffication of abdominal organs and intestinal wall in control rats. In treated rats IVC was normal in diametar, with much faster blood flow in arterial sistem and significantly better opacificiation of abdominal parenchimal organs and bowel wall.

Discussion: Preliminary results show BPC 157 counteraction of brain swelling as well as IVC dilatation and congestion before and after L-NAME induced catalepsy. Considering the changes observed and analyzed in previous studies 3, 4,5 , the influence of NO on the circulatory system and the central control of motor activity, we assume that with the application of a cataleptogenic dose of L-NAME, in addition to brain swelling and an increase of ICV diameter and congestion, will also develop intracranial , caval and portal hypertension and multiorgan failure (brain damage, heart failure, lung congestion, kidney, liver and gastrointestinal tract lesions, thrombosis), also known as ''occlusion-like'' syndrome. We hypothesize the therapeutic effect of BPC 157 through the activation of central and peripheral collateral venous circulation, with consequent antagonism of intracranial, caval and portal hypertension, tachycardia and thrombosis, with reduction of organ damage. Also, by separately applying a cataleptogenic dose of the NOS antagonist L-NAME and the NOS agonist L-arginine and their simultaneous combined application, we want to show whether and how the mentioned syndrome is mediated via NO. Described study will be performed within the framework of the proposed doctoral dissertation.

MeSH/Keywords: L-NAME, catalepsy, syndrome, BPC 157

Poster code: R-01-03-018

Poster Title: Neuroprotective effect of pentadecapeptide BPC 157 on 3-nitro proprionic acid-induced model of Huntington's disease in rats

PhD candidate: Ivan Krezić

Part of the thesis: Neuroprotective effect of pentadecapeptide BPC 157 on 3-nitro propionic acid-induced model of Huntington's disease in rats

Mentor(s): Assistant Professor Ivana Jurjević, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Huntingtons disease (HD) is an autosomal neurodegenerative disease associated with severe degeneration of basal ganglia neurons, especially the striatum, and characterized by motor, cognitive, and psychiatric disorders and has no existing cure for the disease. Stable gastric pentadecapeptide BPC 157 has been proven as a potent cytoprotective agent at healing of different injuries. Its neuroprotective effects were shown in models of Parkinson disease and multiple sclerosis as well as in model of spinal cord injury. We wanted to demonstrate the neuroprotective effect of pentadecapeptide BPC 157 on a model of 3-nitro proprionic acid (3-NPA) induced model of HD in rats.

Materials and methods: Male Albino Wistar rats will be used to prepare the doctoral dissertation. Rats will be administered 3-NPA (20 mg/kg body mass, by intraperitoneal injection) once a day for 1, 2, and 3 days (depending on treatment schedule); and BPC 157 (10 µg/mL or 10 ng/mL) will be administered intraperitoneally before (pretreatment) or after (treatment) with 3-NPA. Functional tests (locomotor activity), measurement of body weight, neurological scoring, measurement of oxidative stress parameters (measurement of lipid peroxidation), gene expression of proteins involved in the pathogenesis of Huntington's disease: involved in damage of the blood-brain barrier (MMP2, MMP9, TIMP-1, TIMP-2); in the regulation of inflammation (NF-B, IL-6, GFAP, caspase-3, TNF-, COX-2) in striatum; and pathohistological analysis of striatum will be used to determine the degree of damage.

Results: 3-NPA resulted in a marked locomotion activity and deterioration of the neurological status. BPC 157 application significantly alleviated (treatment regime) or prevent the occurrence (pretreatment regime) of neurological and motor deficit.

Discussion: We demonstrated the protective effect of BPC 157 on preventing and reducing damage (measured by neurological score) in the animal model of 3-NPA induced HD and its potential for clinical use in HD to halt disease progression

Acknowledgments: None to declare.

MeSH/Keywords: BPC 157; Huntington's disease; 3-nitroproprionic acid; brain; oxidative stress

Poster code: R-01-03-056

Poster Title: Assessing the cognitive effects of methylphenidate in a rat model of sporadic Alzheimer's disease using a home-cage-based approach

PhD candidate: Davor Virag

Part of the thesis: Changes of the circadian locomotor activity rhythm in a rat model of sporadic Alzheimer's disease

Mentor(s): Professor Melita Šalković-Petrišić, MD PhD

Affiliation: Laboratory for Molecular Neuropharmacology, Department of Pharmacology, University of Zagreb School of Medicine; Institute of Fundamental Clinical and Translational Neuroscience, Research Centre of Excellence, Croatian Institute for Brain Research, Univer

Introduction: Epidemiological and animal studies have highlighted an Attention Deficit Hyperactivity Disorder (ADHD)-like phenotype as a risk factor for later development of Alzheimer's disease (AD), with some smaller clinical studies in AD patients showing a potentially beneficial effect of methylphenidate (MPH) treatment. We aimed to investigate effect of oral MPH on cognitive performance in a rat model of AD induced by intracerebroventricular streptozotocin (STZ).

Materials and methods: Three-month old male Wistar rats (n=40) were injected intracerebroventricularly with citrate buffer (control/CTR) or STZ (3 mg/kg) split in two doses 48 hours apart after which MPH therapy was initiated daily in a dual-bottle dosage regimen (4 and 10 mg/kg) for 6 weeks to half of CTR and STZ groups. Cognitive ability was assessed in the classical Novel Object Recognition (NOR) test at 2, 4, and 6 weeks after STZ. Baseline and 6-week continuous measurement of cognitive performance was performed in an operant conditioning task using VlaDiSlav, a custom home cage apparatus.

Results: At 2 weeks, STZ demonstrated worse performance than CTR (NOR), but this deficit was ameliorated by MPH. No change in NOR were observed at 4 and 6 weeks between the groups. Learning curves in continuous testing using VlaDiSlav show a severely diminished learning rate in STZ group, however, STZ+MPH learning rate was similar to CTR values.

Discussion: VlaDiSlav continuous measurement gives insight into the learning process suggesting that STZ induced a severe learning deficit that might be normalized by MPH treatment. These findings also highlight the benefits of supplementing conventional behavioural testing with continuously operating, automatised, programmable home cage-based devices.

Acknowledgments: Supported by: Croatian Science Foundation (HRZZ IP-2018-01-8938; DOK-2021-02-6419) and MEDICE Arzneimittel Pütter GmbH & Co. KG (methlyphenidate donation)

MeSH/Keywords: Alzheimer Disease; Streptozocin; Disease Models, Animal; Methylphenidate

Poster code: R-01-03-071

Poster Title: Efficacy of an emollient plus formulation during a 4-week follow-up in subjects with mild atopic dermatitis

PhD candidate: Dora Hrestak

Part of the thesis: Utjecaj novog emolijens plus pripravka na bazi ektoina, bakterija Lactobacillus spp. i Bifidobacterium spp. na sastav kožne mikrobiote te procjena njegove sigurnosti, učinkovitosti i podnošljivosti u liječenju blagog atopijskog dermatitisa

Mentor(s): Professor Suzana Ljubojević Hadžavdić, MD PhD, Mihaela Perić, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Atopic dermatitis (AD) is a chronic, recurring inflammatory skin condition known for its specific symptoms, such as dryness, intense itching, redness, skin excoriations, and lichenification. The use of emollient plus preparations (EPP) is crucial in AD management offering both symptomatic relief and quality of life improvement. The goal of this clinical study was to assess the safety and efficacy of a new emollient plus lotion consisting of a cosmetic base (water-in-oil inverse emulsion with a high proportion of lipids) with three active ingredients based on microbiota (ectoin and a combination of inactivated Lactobacillus and Bifidobacterium bacterial strains preparations) designed for alleviating AD symptoms.

Materials and methods: The study was a prospective, multicentered, controlled and double-blinded, comparing a new emollient plus with a control emollient (no active ingredients) in adult patients diagnosed with mild AD. Both treatments were applied twice daily, each on one side of the body (left/right on the same patient) during 4 weeks. Efficacy was assessed by determining changes between groups and 3 time-points (day 0, week 2 and 4). Parameters measured were: modified objective SCORAD index, transepidermal water loss, hydration, erythema, redness, lichenification, elasticity, pH, scaliness and skin microtexture.

Results: Of the initial 84 patients screened, 32 adhered to the study protocol until its completion. During a 4-week administration period, both EPP and the control emollient demonstrated a significant decrease of the oSCORAD index, most notable in the subset with a more severe form of mild AD (SCORAD ≥17). Furthermore, a significant decrease in redness and erythema was observed after the application of EPP during 4 weeks, accompanied by differences between the groups when compared to the control emollient, changes being more pronounced in the subset with more severe forms of mild AD (SCORAD >17). Both emollients significantly increased skin hydration and roughness, while a significant decrease in skin elasticity was observed in the control group.

Discussion: During the observation period, both the EPP and the control emollient demonstrated efficacy in alleviating signs and symptoms associated with AD. This comprehensive study assessed various parameters to gauge the effectiveness of the treatments, with notable findings revealing a significant decrease in the objective SCORAD (oSCORAD) index over the 4-week administration period. A reduction in the oSCORAD index was particularly pronounced in individuals with a more severe form of mild AD, as indicated by a baseline SCORAD score of ≥17. Moreover, the application of the EPP led to a significant decrease in redness and erythema, two hallmark characteristics of AD. Interestingly, there were discernible differences between the groups when comparing the effects of the EPP with the control emollient. These differences were more pronounced in individuals with a more severe form of mild AD, further emphasizing the potential benefits of the EPP formulation in AD management. Additionally, both emollients significantly improved skin hydration and roughness. This finding underscores the importance of maintaining adequate skin hydration in individuals with AD, as dryness and roughness are common features of the condition. However, it is worth noting that a significant decrease in skin elasticity was observed in the control group, highlighting a potential limitation of certain emollient formulations in addressing all aspects of skin health in individuals with AD. Overall, these results demonstrate that the microbiota-based EPP could offer significant benefits for patients by reducing inflammation, improving hydration, and potentially enhancing overall skin health compared to traditional emollients.

MeSH/Keywords: emollient plus, atopic dermatitis, clinical study

Poster code: R-01-03-078

Poster Title: The effect of pentadecapeptide BPC 157 on ethyl alcohol-induced damage of breast and surrounding tissue in rats

PhD candidate: Mariam Samara

Part of the thesis: The effect of pentadecapeptide BPC 157 on ethyl alcohol-induced damage of breast and surrounding tissue in rats

Mentor(s): Professor Predrag Sikirić, MD PhD, Assistant Professor Rado Žic, MD PhD, research advisor

Affiliation: University of Zagreb School of Medicine

Introduction: Methodologically speaking, damage of breast and surrounding tissue still remains practically unresolved issue because of the lack of a simple and easily reproducible model. Potential solution could be translation of well-known established model. The effect of alcohol by direct application to the gastric mucosa and the consequences of direct contact of alcohol with the cell is described in Andre Robert's work on cytoprotection. According to the paper, the cytoprotective effect would be to antagonize these lesions. One of the agents that has been shown to be a potential essential mediator of Robert's cytoprotection is the stable gastric pentadecapeptide BPC 157, which has shown direct endothelial protection, anti-inflammatory activity - most likely due to its angiomodular effect, and high healing potential. Existing models of breast tissue damage are complicated, expensive and almost exclusively focused on the effect of milk secretion and quality which limits the capacity of studies. Also, administration of live bacteria can cause sepsis, restricting the physiological relevance of the model. Therefore, seeing the previously mentioned effects of pentadecapeptide BPC 157, and taking in account the shortcomings of existing models, the idea was to transfer the model shown in gastric tissue to mammary gland and adjacent tissue

Materials and methods: In this study, 12-week-old female albino rats weighing 200g, randomly divided into groups, will be used. Each group contains 6 animals. There are control groups, groups to which the pentadecapeptide BPC 157 or NOS substrate / blocker will be administered subcutaneously to the mammary region. Different intervals will be done, intervals of 15min, 30min, 60 min, 7 days and 14 days. The total number of experimental groups will be 8 and the number of animals in the experiment will be 240. After performing anesthesia, 0.1 ml of 96% ethyl alcohol will be administered intramammary with a 30G insulin injection, followed immediately by various agents intraperitoneally (0.9% NaCl solution, pentadecapeptide BPC 157, NOS substrate L-arginine, NOS blocker L-NAME) . The state of experimental animals will be assessed regularly and depending on results, analgesia will be administered if necessary. If there's no progress in an animal's wellbeing, the animal will be euthanized. After administrating ethyl alcohol subcutaneously to the mammary region, the animals will be euthanized in the following intervals: 15 min, 30 min, 60 min, 7th day and 14th day, using double doses of anesthetics. After confirming the animal’s death, tissue samples will be collected. The lesions will be assessed using macroscopical changes and patohistological analysis. The macroscopic scoring system will be from 0 to 4 (0– no change, 1– erythema, 2– oedema and erythema, 3– mild, shallow ulcer, 4– severe, deep ulcer). Patohistological analysis will consist of semi-quantitive evaluation of congestion level, fatty-tissue necrosis grade and number of inflammatory cells and perivascular infiltration. Statistics: ANOVA test with sample size 48, 8 groups, significant level $\alpha = 0,05$ and size of effect f= 0,7 power > 90

Results: Macroscopic changes: The pilot project showed that lesions in animals receiving pentadecapeptide BPC 157 were significantly less remarkable than in control groups. Microscopic changes: In shorter intervals the pilot project showed minor dilation of ducts, discrete exfoliation of epithelial cells with discrete dilation of blood vessels in groups receiving pentadecapeptide BPC 157 while control groups had more pronounced dilation, exfoliation and inflammatory cells infiltration. In longer intervals the pilot project showed ductal dilation with the whole circumference epithelial exfoliation, significant blood vessel dilation, inflammatory cell infiltration and necrotic areas in control group while results in groups receiving BPC 157 were significantly less remarkable.

Discussion: Since the topic has yet to be approved from ethics committee all conclusions are based upon the results of the pilot project. That being said, animals that received pentadecapeptide BPC 157 were less affected by administration of cytotoxic agent ethyl alcohol. Macroscopically speaking, lesions were smaller, less profound, showing no necrotic areas. Microscopically speaking, depending on the interval, BPC 157 groups had less pronounced dilation of ducts, blood vessels, fewer inflammatory cells and necrotic areas.

MeSH/Keywords: ethanol, cytoprotection, pentadecapeptide BPC 157, mammary gland

Poster code: R-01-03-082

Poster Title: SYNDROME ENCOMPASSING SUPEROR SAGITTAL SINUS OCCLUSION, ALCOHOL-INDUCED GASTRIC LESIONS AND THE TRANSECTION OF THE SPINAL CORD

PhD candidate: Slaven Gojković

Part of the thesis: SYNDROME ENCOMPASSING SUPEROR SAGITTAL SINUS OCCLUSION, ALCOHOL-INDUCED GASTRIC LESIONS AND THE TRANSECTION OF THE SPINAL CORD

Mentor(s): Associate Professor Alenka Boban Blagaić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: In previously published papers we estabilshed the effects of the BPC 157 on the 1. ligation of the superior sagittal sinus in rat and their systemic repercusions; 2. Transection of the spinal cord in rat and their systemic repercusions; and 3. Ethanol-induced gastric lesions and their systemic repercusions. With these three basic interactions we approached a more general problem, hoping to intertwine this previous research work with the already published papers in the area of brain-gut, gut-brain and peptide interactions. There have been other publocations from our group focusing on the specific molecular and systemic effects of the BPC-brain-gut interaction that were also a major motivator for further studies on this subject.

Materials and methods: To achieve the aim of this research, to recognize, evaluate and investigate the specific and general interactions between the brain-gut axis and the pentadecapeptide BPC 157, we will examine the results of three previously published papers. These papers focus on the effects of the BPC 157 on the 1. ligation of the superior sagittal sinus in rat and their systemic repercusions; 2. transection of the spinal cord in rat and their systemic repercusions; and 3. ethanol-induced gastric lesions and their systemic repercusions. The data was compiled and analysed together, specifically aiming to recognize the BPC-brain-gut interaction and to examine the effects of the BPC 157 on the healing processes. We tried to achieve this by reviewing the previously published literature on the subject and comparing it with our own study results. We are also broadening the scope of our research as we gather more data from this cross-analysis, which we will continue to publish in the future.

Results: There is a significant interaction of the BPC 157 and the brain-gut axis. Furthermore, in the state of various noxes, BPC 157 can act beneficially on the healing processes.

Discussion: In order to achieve a more profound understanding of the brain-gut, gut-brain and peptide interactions, especially in the context of stress and pathological processes, extensive cross-analysis of the previously published data from other groups as well as our own was undertaken. Preliminary results show that this research may give further understanding of the inflammation processes and their interaction with biologically active peptides and show the nervous-systemic adaptations to healing and stress-response. On the other hand, it could provide more insight into the fine peptide-neural axis interactions with the gastric mucosa in the healing of gastric ulcers.

MeSH/Keywords: BPC 157, brain-gut axis, gut-brain axis, stress, therapy, rat

Poster code: R-01-03-112

Poster Title: Therapeutical effect of pentadecapeptide BPC 157 on Occlusion like syndrome and lower leg fracture in rats.

PhD candidate: Andreja Prtorić

Part of the thesis: Therapeutical effect of pentadecapeptide BPC 157 on Occlusion like syndrome and lower leg fracture in rats.

Mentor(s): Associate Professor Ivan Dobrić, MD PhD, Assistant Professor Ivana Jurjević, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: The effect od stable pentadecapeptide BPC 157 on tissue healing has been confirmed in many studies so far, including its beneficial effect on wound healing like burns, mucosal fistulas, muscle tissue damage, tendon, ligament and bone injury. Its cytoprotective effect with mucosal ulcus healing has been described in cases of inflamatory bowel diseases. Studies also show pseudarthrosis healing in rabbits, bone resorption reduction in femoral head necrosis in rats, stimulating effect on tendocytes after Achilles tendon transsection and quadriceps muscle transsection in rats. Likewise, it has been proven that BPC 157 prevents trombotic incidents and modulates angiogenesis in wound healing. Recent rat studies have shown the therapeutical effect of BPC 157 in case of Occlusion and Occlusion like syndrome. The syndromes are described consequently to isolated occlusion of superior mesenteric artery, superior saggital synus and suprahepatic occlusion od inferior vena cava. In case of Occlusion like syndrome, physical occlusion is absent, but the result is equal to result in Occlusion syndrome. The syndromes were described in cases of abdominal compartment, miocardial infarction, lithium intoxication and alcohol induced gastric lesions. They are a result of endotel damage and vessel thrombosis, and present as hypertension in superior saggital synus, portal vein, inferior caval vein and aortal hypotension. The syndrome effect was described in gastrointestinal and circulatory system, but not in case of osteomuscular injury.

Materials and methods: A tibia and fibula fracture was induced in, properly anesthetized, male Wistar albino rats that were randomized divided in treated and control group. In treated group BPC 157 (10ng/kg or 10mcg/kg) was applied directly at the fracture site, while in the control group 1mL of saline was applied. In the next 30 days treated group was given BPC 157 (0,16ng/mL/12mL/day or 0,16mcg/mL/12mL/day) diluted in water orally, and the control group drank regular water. We directly examined the hematoma formation after the fracture and in the next 30 days measured functional recovery observing contractures and gait analysis. After 30 days bone healing was recorded using Core Beam CT 3D. In another group of animals, we examined the onset of Occlusion-like syndrome in lower-leg fracture, with (treated group) or without (control group) BPC 157 therapy. After inducing a fracture and directly applying BPC 157 or saline, we recorded the animals' ECG and, using microcamera, recorded the brain and abdominal organs (stomach, intestines, liver, pancreas, aorta, superior and inferior vena cava and superior mesenteric artery and vein). After 15 minutes we measured the pressure inside the superior sagital synus, portal vein, vena cava inferior and aorta. The stomach and duodenum were inspected for ulcus formation, and lastly the thoracic organs were recorded including heart, lungs, vena cava superior and azygos vein.

Results: In treated group we found hematoma formation inside the fracture gap within first 5 minutes, while in the control group the bleeding was rapidly extending outside the fracture gap to the soft tissues. In 30 days we found better fracture healing and functional outcome in treated group. After the lower leg fracture Occlusion-like syndrome was found in control group, but was absent in treated group.

Discussion: These preliminary results show the Occlusion-like syndrome as a result of lower leg fracture in rats. The syndrome is counteracted by pentadecapeptide BPC 157, which also leads to better fracture healing and functional outcome.

MeSH/Keywords: pentadecapeptide BPC 157, lower leg fracture, Occlusion like syndrome

Poster code: R-01-03-155

Poster Title: Effect of BPC 157 on hemodynamic changes as a consequence of acute severe colitis induced by acetic acid enema in rats

PhD candidate: Dinko Bekić

Part of the thesis: Effect of BPC 157 on hemodynamic changes as a consequence of acute severe colitis induced by acetic acid enema in rats

Mentor(s): Associate Professor Anita Škrtić, MD PhD, Assistant Professor Krešimir Luetić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Ulcerative colitis is a chronic inflammatory bowel disease characterized by inflammation of the colon mucosa, and the course of the disease is characterized by phases of remission and relapse. The pathogenesis of the disease is still unknown, but it is known to be of multifactorial etiology, which includes the interaction of external factors with a disturbed immune response in genetically predisposed individuals with altered gut microbiota. Considering that the exact etiology and pathogenesis are still unknown, the cure of the disease is not yet possible.

Materials and methods: Randomly divided rats (6 rats/group/interval) will be anesthetized by intraperitoneal administration of 40 mg/kg thiopental and 10 mg/kg diazepam. In craniotomy and laparotomy rats, 1 ml of 9% acetic acid will be administered intrarectally, and 5 minutes after that medication (BPC 157 in a dose of 10 ng/ kg, intragastric; 5 mg/kg L-arginine, intraperitoneal; and 0.9% NaCl (5 ml/kg) intragastric. (total of 144 rats).After the application, intra-aortic, intracaval, intraportal, intracranial pressure will be measured at different time intervals (1 min, 15 min, 60 min) using a cannula that will be connected to a pressure transducer .Animals will be euthanized with a double dose of anesthetic after 1 min, 15 min and 60 min after medication. Also, in order to specifically monitor the effect on hemodynamics and heart function, an electrocardiographic record will be recorded. The observation will include a macroscopic analysis of the intestines, brain, lungs and heart, liver, spleen, stomach, spleen, large blood vessels with a micro camera and pathohistological examination of the mentioned organs.

Results: Preliminary results are showing that acute severe colitis induced by acetic acid enema in rats causes systemic syndrome characterized with systemic hypotension, intracaval, intraportal and intracranial hypertension. Pentadecapeptide BPC 157 was given intraperitoneally in defined time intervals counteracted severe colitis development. In addition, all systemic effects were reversed by BPC 157 in preliminary results.

Discussion: Ulcerative colitis is a chronic inflammatory bowel disease characterized by inflammation of the colon mucosa, and the course of the disease is characterized by phases of remission and relapse. With the experimental model of acute severe colitis induced by a 9% acetic acid enema in rats and by proving its systemic effect, we want to show that severe colitis is a systemic disease even in its earliest stages. Considering the severity of the disease, the number of patients and the speed of development of the clinical picture, rat studies are the main model for studying the pathophysiology of severe ulcerative colitis. The model of ulcerative colitis caused by intestinal barrier injury by rectal application of acetic acid proved to be representative, and the degree of injury depends on the concentration and dose of acetic acid in which 4-50% acids were used, as well as the exposure time. The initial epithelial injury in colitis caused by acetic acid is not immunologically mediated, but leads to a secondary inflammatory response as a reaction to severe injury to the mucosa and deeper structures of the colon wall. The methods of applying acetic acid in the works are heterogeneous, including the performance of a double colostomy without a specified start of application or volume of acetic acid. Contrary to the above, this research is about an experimental model induced by 9% acetic acid that monitors the changes that occur within the first hour after the application of acetic acid, and is based on previously described models. It is an acute trial, and we do not expect mortality in the treated and control groups, but rather differences in the monitored parameters. We expect BPC 157 to reverse systemic effects as described in earlier studies (preliminary results are showing expected results). Regardless of the therapeutic options for ulcerative colitis, the rate of colectomy is still high, which imposes the need for further research into the pathophysiology of severe ulcerative colitis, as well as new therapeutic models and options. An experimental model of acute severe colitis induced by acetic acid enema could show that systemic hemodynamic changes occur in the earliest phase and that BPC 157 could be additional tmedical therapy for severe cases.

MeSH/Keywords: BPC 157; Hemodynamics; Rat; Colitis.

Poster code: R-01-03-162

Poster Title: NOTCH SIGNALING PATHWAY INHIBITS OSTEOCLAST DIFFERENTIATION IN PATIENTS WITH RHEUMATOID ARTHRITIS

PhD candidate: Sara Aničić

Part of the thesis: The role of the Notch signaling pathway in the differentiation of osteoclast progenitors in rheumatoid arthritis

Mentor(s): Professor Danka Grčević, MD PhD, Assistant Professor Alan Šućur, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Rheumatoid arthritis (RA) is a chronic joint-affecting autoimmune disease characterized by periarticular and systemic bone loss, as a result of increased differentiation of osteoclasts. Osteoclasts are multinuclear cells with bone resorbing ability, formed by fusion of osteoclast progenitors (OCPs), derived from myeloid/monocytic lineage. Circulating OCPs express CD45+CD15−CD3−CD19−CD56−CD11b+CD14+ immunophenotype; which corresponds to classical monocytes – a subpopulation expanded in RA. Their further differentiation is dependent on various external signals. Since previous studies have shown the importance of Notch signaling pathway in hematopoietic cells differentiation, we examined this juxtacrine pathway's role in osteoclast differentiation.

Materials and methods: Peripheral blood was collected from RA patients and healthy controls. Peripheral blood mononuclear cells (PBMC) were extracted using Ficoll density gradient. Classical monocytes were isolated with FACS Aria cell sorter, as cells expressing CD45+CD15−CD3−CD19−CD56−CD11b+CD14+ immunophenotype. Expression of Notch signaling pathway components was analyzed using flow cytometry and quantitative PCR. Same procedure was performed with joint tissue from RA patients undergoing knee or hip replacement surgery. Clinical data were collected from patients with blood samples, and correlated with Notch receptor expression. Peripheral blood OCPs were differentiated in osteoclastogenic culture, with addition of macrophage colony-stimulating factor, M-CSF, and receptor activator of nuclear factor kappa beta ligand, RANKL. Expression of Notch receptors, ligands and transcriptional targets was assessed during osteoclast differentiation using quantitative PCR. OCPs were seeded in control wells and wells coated with Notch ligands Jagged-1 (JAG-1) or Delta-like Ligand (DLL-1), with or without addition of anti-Notch-1 or 2 antibodies. Cells were quantified using TRAP (tartrate-resistant acid phosphatase) staining.

Results: Substantial proportion of OCPs within peripheral blood and joint tissue of RA patients express Notch receptors. Our results showed decrease in gene expression of the Notch receptors on blood OCPs in RA compared to control, with an even greater decrease observed in joint OCPs. Similar effect was observed at the level of protein expression. There was no significant difference in Notch ligand expression. Increased percentage of circulating OCPs was detected in RA compared to control, with further increase in joint OCPs. Negative correlation was found between Notch-1 gene expression and DAS28(CRP) (disease activity score according to C-reactive protein). RNA expression of Notch pathway components changed during osteoclast differentiation in vitro. The most prominent changes were increased expression of Notch-3 and DLL-1. Furthermore, continuous in vitro exposure to fixated Notch ligands, JAG-1 and DLL-1, altogether inhibited M-CSF and RANKL-induced osteoclastogenesis in a dose-dependent manner, with visible shift towards proliferation of undefined mononuclear cells, especially with DLL-1. This inhibitory effect was partially reversed by anti-Notch-1 antibodies, while equivalent dose of anti-Notch-2 antibodies further inhibited osteoclastogenesis.

Discussion: We have shown that it is possible to isolate cells with osteoclastogenic potential from peripheral blood and joint tissue of RA patients, and to differentiate them into mature TRAP+ osteoclasts in vitro. Overall, results indicate inhibitory role of Notch activation in human osteoclast differentiation. However, not all the results are consistent with this, which can possibly be explained by complex time and context-dependent nature of Notch signalization.

Acknowledgments: The work was supported by Croatian Science Foundation (IP-2018-01-2414, IP-2020-02-2431 and DOK-2021-02-6365).

MeSH/Keywords: Notch signaling pathway; osteoclast progenitors; rheumatoid arthritis; bone resorption

Poster code: R-01-04-100

Poster Title: Genetic background of immune response and inflammasome activation in Parkinson's disease

PhD candidate: Sarah Meglaj Bakrač

Part of the thesis: Genetic background of immune response and inflammasome activation in Parkinson's disease

Mentor(s): Professor Fran Borovečki, MD PhD, Assistant Professor Antonela Blažeković, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Parkinson's disease (PD) is a progressive neurodegenerative disorder that predominantly affects the population of people over 60 years of age. The symptomatology of the disease is heterogeneous, characterized by various motor and non-motor symptoms. Neuropathologically, the disease is characterized by the degeneration of dopaminergic neurons in the brain and intraneuronal accumulations of α-synuclein protein. Accumulation of this protein is thought to lead to an inflammatory response in the brain, thereby disrupting neuronal homeostasis and synaptic function. Long-term inflammation in the patient's brain is associated with increased permeability of the blood-brain barrier. This enables the entry of immune cells into the brain and accelerates the gradual degeneration of dopaminergic neurons.

Materials and methods: Up to this point, this study included 18 subjects with PD and 10 healthy control subjects. Peripheral blood samples were collected from each subject and used for DNA isolation. Immune cells, i.e. dendritic cells (DCs) and CD4+ T lymphocytes, were isolated from peripheral blood mononuclear cells (PBMC) using magnetic separation. Isolated DNA was further used for next-generation sequencing (NGS) of clinical exome, and descriptive statistical analysis was performed on obtained sequences. After the separation, the immune cell suspension of each subject was used in library preparation for single-cell sequencing (scSeq). The prepared libraries were sequenced by NGS, and the data obtained was analysed using the Cell Ranger and Loupe Browser software (10x Genomics, USA). Reference-based annotation was used to identify and separate different cell-type clusters.

Results: By clinical exome sequencing, a total of 2365 different variants were obtained, out of which 1150 variants were exclusively present in PD subjects. Variants are divided into three categories according to the predicted outcome of the change: pathogenic (P), likely pathogenic (LP), and variant of unknown significance (VUS). Their share to the total number of variants is 3%, 5%, and 92% respectively. Several P and LP variants appear exclusively in PD subjects. These variants are located within genes involved in numerous biological processes such as cellular transport, cellular metabolism, and immune response. After conducting scSeq for each sample, the data was processed using Cell Ranger. Finally, an aggregated dataset was generated containing all cells of all samples. The aggregated dataset was used for visualization in the Loupe Browser. Using the PBMC reference dataset, all obtained cells were annotated and sorted into a total of seven clusters: CD4 T cells, CD8 T cells, DC, Monocytes, NK cells, other, and other T cells. Additional data normalisation is currently being performed, after which the differential expression analysis will be conducted.

Discussion: Preliminary data indicate that certain genes and variants play a critical role in various biological pathways, particularly in the immune response. However, it's important to note that not all samples have been sequenced and analysed yet, and that the differential expression analysis still needs to be conducted. Therefore, it is too early to draw any conclusions.

Acknowledgments: This study is a part of the research project "Molecular mechanisms of immune response and inflammasome activation in Parkinson's disease" (Immune PD, IP-2020-02-8475), funded by the Croatian Science Foundation.

MeSH/Keywords: Parkinson disease, alpha-synuclein, Inflammasomes, Next-generation sequencing, Dendritic cells, CD4-positive T-lymphocytes, Single-Cell Gene Expression Analysis

Poster code: R-01-05-039

Poster Title: The pharmacogenetic risk factors for apixaban associated bleeding and inefficacy

PhD candidate: Jozefina Palić

Part of the thesis: The role of pharmacogenetics in an individualised approach to apixaban treatment

Mentor(s): Professor Martina Lovrić-Benčić, MD PhD, Assistant Professor Tamara Božina, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Apixaban is a direct oral anticoagulant which has good safety and efficacy. However, there are cases of bleeding and thromboembolic events among users in practice. Apixaban is a substrate of CYP3A enzymes and ABCB1/ABCG2 transporters. The polymorphisms in the genes that encode them could affect apixaban pharmacokinetics. The objective of the study is to determine association between relevant polymorphisms and occurrence of apixaban-related adverse events.

Materials and methods: These are preliminary results of the larger cohort (N=470 expected). Adults with a new indication for apixaban treatment were genotyped for CYP3A4*22, CYP3A4*1B, CYP3A5*3, ABCG2c.421C>A, ABCB1c.1236C>T, c.2482-2236G>A, and c.3435C>T variants. Analysis was performed with specific TaqMan® Assays on ABI7500 Real-Time PCR system. Clinical and laboratory data were collected. Associations between carriers/noncarriers of variant alleles or haplotypes/phenotype and bleeding/thromboembolic events were analysed. Statistical analysis was performed by JASP0.17.1 software.

Results: 140 patients (Mdn age=66,5 (20-89); f=59, m=81) were treated in dosages of 2x5 mg (n=113) and 2x2,5 mg (n=27). Eleven patients had apixaban associated bleeding (serious=3, anaemia=3, epistaxis=2, haematoma=2, haematuria=2, haemoptysis=1) and six thromboembolic events (insult=4, thrombosis=2). Genotype distribution is consistent with Hardy Weinberg's equilibrium. Based on the univariate analysis, a significant association between ABCB1 c.1236C>T variant carriers and smaller bleeding occurrence (OR=0.27, 95%CI: 0.06-1.13, p=0.046) was found.

Discussion: There is no association between the CYP3A4*22, CYP3A4*1B, CYP3A5*3, ABCG2c.421C>A, ABCB1c.2482-2236G>A, and c.3435C>T variants nor phenotype with the apixaban-related side effects, only for ABCB1 c.1236C>T variant carriers. These results should be re-evaluated on the larger cohort.

MeSH/Keywords: pharmacogenetics, ADRs, DOAC, drug-drug-gene interactions, personalised medicine

Poster code: R-01-05-090

Poster Title: Proteomic Analysis of Mouse Cerebral Cortex After Ischemic Brain Injury - Revealing Potential New Dual-Function Biomarkers of Damage and Repair

PhD candidate: Dominik Hamer

Part of the thesis: Identification of molecular markers of damage and repair of mouse cerebral cortex after ischemic injury using proteomic analysis

Mentor(s): Assistant Professor Anton Glasnović, MD PhD

Affiliation: University of Zagreb School of Medicine; BICRO BIOCentre

Introduction: Ischemic stroke is one of the leading causes of death and disability in Croatia and Europe. It is caused by disorder in the blood supply of certain brain regions which reduces or completely cuts off the supply of nutrients and oxygen. Since in vivo radiological diagnostics for ischemic stroke is often untimely applied, financially demanding and can be contraindicated in some patients, modern focus in preclinical research is shifted in the direction of discovering new stroke-related molecular markers for rapid and non-invasive diagnostics.

Materials and methods: Ischemic brain injury was induced by Koizumi tMCAO method in which the 30-minute occlusion of the left middle cerebral artery was achieved using siliconized monofilament. Magnetic resonance in vivo imaging of mouse brain was performed on 7T MR system (BioSpec 70/20 USR Bruker Biospin, Germany) at three time points: 7th day before tMCAO (baseline), 1st (acute phase) and 35th day (chronic phase) after inducing ischemic injury. Lesion size and localization was determined by manual delineation of ipsilateral and contralateral hemisphere using ImageJ 1.53d software for image analysis. At same time points assessment of neurological deficit score (NDS) was performed by assigning points in several categories: weight, appearance, motility, spontaneous activity, gait disturbances, postural signs, lateral resistance, ipsilateral and contralateral forelimb placing. Finally, animals were anesthetized and transcardially perfused with 30 mL of cold phosphate buffered saline (PBS, pH 7.4). Cerebral cortex was isolated and tissue was prepared for non-targeted proteomic analysis using short-GeLC–SWATH protocol. Total protein concentration was measured using PierceTM 660 nm Protein Assay (Thermo Fisher Scientific, Illinois, USA). After protein separation and in-gel trypsin digestion, peptides were analyzed by NanoLC™ 425 System coupled to a Triple TOF 6600 mass spectrometer (Sciex, United States). Data processing was performed using SWATH processing plug-in for PeakView (v2.0.01, Sciex®) and statistically analyzed using MetaboAnalyst online platform.

Results: Based on MR images, all animals suffered cortical ischemic lesions and in total 30 samples of mouse cerebral cortex were used in this research (5 ipsi- and 5 contralateral from each time point). Using ipsilateral cortex samples from all groups, we were able to identify and quantify 2496 proteins in total of which 616 were statistically significant based on Kruskal–Wallis analysis ($p<0.05$), Bonferroni correction (FDR<0.05) and partial least squares-discriminant analysis (VIP>1). Bioinformatic analysis and gene ontology (GO) of differently expressed proteins revealed most significant changes in proteins which are associated with regulation of: trans-synaptic signaling, localization, immune system process, tissue development, cellular component biogenesis, cellular component organization, cell-cell signaling, cell projection organization and cell population proliferation. Analysis of contralateral cerebral cortex revealed 2398 identified proteins in total, but statistical analysis showed no differentially expressed proteins. Based on behavioral analysis, animals showed good neurological recovery in chronic phase with ND score similar to baseline.

Discussion: In this experiment, a non-targeted proteomic analysis has been conducted on a mouse tMCAO model aiming to reveal the proteomic changes of cerebral cortex in the acute and chronic phases of brain ischemic injury. The tMCAO proved to be well-suited model for proteomic characterization, dynamics and molecular changes after ischemic injury. Although we were able to identify 616 differently expressed proteins in several GO categories, there is still the need to reveal which of them and in what measure can cross blood-brain barrier in order to be identified in blood or saliva, so that they can be categorized as good biomarkers for rapid and non-invasive stroke diagnosis.

Acknowledgments: Study was supported by the European Union through the European Regional Development Fund as project - Sinergy of molecular markers and multimodal in vivo imaging during preclinical assessment of the consequence of the ischemic stroke (SineMozak).

MeSH/Keywords: mouse brain, ischemic injury, magnetic resonance imaging, proteomic analysis, tissue biomarkers

Poster code: R-01-08-021

Poster Title: Anatomical and physiological role of the capillary network of the cranial and spinal dura in the protection of central nervous system tissue

PhD candidate: Slavica Martinović

Part of the thesis: Capillary network anatomy and aquaporin distribution in the cranial and spinal dura mater in humans and pigs

Mentor(s): Professor Marijan Klarica, MD PhD, Assistant Professor Marija Baković, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Examining the morphological characteristics of the capillary system of the dura mater within the skull and the spinal canal has not been the subject of significant research until now. Also, the possible existence, distribution, and quantity of aquaporins, which could answer the question of the water circulation mechanism in that area, was not examined. The results of this research would help in understanding the physiology of neurofluids and the pathophysiology of bleeding in the epidural space, as well as opening up new possibilities in using this knowledge for the therapeutic purposes of various intracranial pathologies

Materials and methods: The materials are samples of the parietal part of the cranial dura, falx and thoracic part of the spinal dura of deceased persons autopsied at the Institute of Forensic Medicine and Criminology, and postmortem samples of pig cranial and spinal dura taken as part of experimental research at the Faculty of Veterinary Medicine. Subjects were adult persons autopsied at the Institute for Forensic Medicine with the informed consent of the family, and experimental pigs used in research at the Faculty of Veterinary Medicine with the permission of the Ethics Committee. The inclusion criteria are the age of the person and the time of death < 24 hours from the moment of sampling, while the exclusion criteria are head trauma and the existence of intracranial or intraspinal pathological processes (inflammation, bleeding, tumors, anomalies). After the autopsy, samples of the parietal dura, falx and thoracic part of the spinal dura will be extracted and placed in formalin for fixation. At the end of this process, histological slides will be made and will undergo a standardized protocol for fluorescent immunohistochemistry. The finished preparations will be analyzed on a laser confocal microscope. The preparation and analysis process will take place at the Croatian Brain Research Institute.

Results: All necessary pig samples and most of the human samples were collected. Tests of antibodies (CD31, AQP1, and AQP4) were performed on the samples in order to find the correct fluorescent immunohistochemistry protocol. After the establishment of the best protocol, preliminary test recordings on the confocal microscope showed the presence of both AQP1 and AQP4 channels in all parts of porcine and human dura.

Discussion: Until a few years ago, the dura was not an area of great interest. This changed with the emergence of the theory about the presence of a lymphatic system inside the skull. This research further expands the knowledge about dura anatomy and provides new potential explanations for intracranial physiology, primarily about the transfer of fluids such as plasma and cerebrospinal fluid, ie whether the dura participates in the creation and absorption of fluids. Also, this research should show whether there are anatomical differences between the cranial and spinal dura, which has a richer capillary network, and whether there is a difference in the expression of AQP1 and AQP4 in different regions (parietal, falx, spinal). We hope that the results could help us explain the physiological attachment of the cranial dura to the periosteum while the spinal dura is free, and whether this affects the physiology of the cerebrospinal fluid in combination with pressures (subatmospheric pressure intracranially in an upright position). The limitation of the research itself is the influence of the postmortem period on the expression of the investigated proteins and the possibility of dependence of age-sex-related characteristics on the number of capillaries and the expression of aquaporin, for which it would be necessary to conduct an extended study with a larger number of samples

MeSH/Keywords: cranial dura mater, spinal dura mater, aquaporins, capillaries, fluorescent immunohistochemistry

Poster code: R-01-08-035

Poster Title: Insights into bradykinin receptor type 2-mediated murine cerebral glucose metabolism

PhD candidate: Marta Pongrac

Part of the thesis: Role of bradykinin type 2 receptor in glucose metabolism in mouse brain

Mentor(s): Associate Professor Marina Radmilović, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Bradykinin is a vasoactive peptide with a prospective role in glucose metabolism. Through its interaction with the bradykinin type 2 receptor (B2R), bradykinin enhances glucose uptake by regulating the expression and activity of glucose transporters in various organs and tissues. However, the impact of B2R on cerebral glucose metabolism remains unexplored, therefore, this study aims to investigate whether B2R deficiency affects murine cerebral glucose uptake and the expression of glucose transporters and insulin receptor.

Materials and methods: Five months old male C57BL/6J (WT, n=26) and C57BL/6J/Bdkrb2tm1Jfh/SmiJ (B2R-KO, n=26) mice were subjected to measurements of body weight, basal, and 6-hour fasting blood glucose levels, as well as glycated hemoglobin. Then, intraperitoneal tolerance tests were conducted using 1 g/kg D-glucose and 0.75 IU/kg insulin. The first cohort of animals consisting of 5 mice per group underwent optical in vivo and ex vivo imaging using the IVIS Spectrum imaging system. Mice were intravenously administrated 50 µM fluorescently labeled glucose and imaged to observe the glucose uptake into the brain. Mice were imaged for 60 min in vivo, after which they were perfused, brains were isolated and then ex vivo imaged. From the second cohort of animals with 6 mice per group, glucose transporters, and insulin receptor expression were determined using quantitative real-time PCR analysis.

Results: B2R-KO mice exhibited higher basal ($p=0.003$) and fasting ($p=0.008$) glucose levels compared to controls. However, no significant differences were found in intraperitoneal glucose and insulin tolerance tests, as well as in body weight or glycated hemoglobin levels. In vivo optical imaging showed increased cerebral glucose uptake in B2R-deficient mice compared to controls ($p<0.0001$), while ex vivo imaging 70 minutes post-injection showed no difference between genotypes. B2R-KO mice had lower expression of slc2a3/GLUT3 ($p=0.02$) and slc2a4/GLUT4 ($p=0.006$) mRNA compared to control group.

Discussion: Our preliminary findings indicate that B2R affects the early kinetics of cerebral glucose uptake without altering total brain glucose uptake. Moreover, we showed that B2R-deficient mice exhibited lower slc2a3/GLUT3 and slc2a4/GLUT4 mRNA expression, contrary to the initially performed analysis. These findings are in accordance with the previously shown role of B2R in GLUT4 translocation and enhanced glucose uptake in other cells and tissues, suggesting its involvement in cerebral glucose metabolism.

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MeSH/Keywords: glucose, brain, bradykinin receptor type 2, glucose transporters

Poster code: R-01-08-087

Poster Title: System for Early Neurological Deviation Detection

PhD candidate: Goran Kuzmac

Part of the thesis: General movements assessment using artificial intelligence methods

Mentor(s): Professor Milan Radoš, MD PhD, David Neubauer, PhD

Affiliation: University of Zagreb School of Medicine

Introduction: The General movement assessement, based on visual gestalt perception of infant movements, has become one of the most valuable tools for the detection of motor impairment in early infancy. However, methods based on rating videos requires trained profesionals, they are time-consuming and thus expensive. In recent years, approaches based on machine learning have become more available and gained increased attention. In this research, we compare results of the general movement assessmets by trained human profesionals with the results obtained by machine learning models.

Materials and methods: The software solution SENDD (System for Early Neurological Deviation Detection) was developed in collaboration of the IT company TIS from Zagreb and the Dr. Sabol Children's Clinic for the collection and analysis of videos of general movement of infants. In the period of 3 years (2020-23), we collected videos of 326 infants aged 8 to 15 weeks post-term. The videos were obtained voluntarily, via SENDD online platform or application for mobile devices. The collected videos were evaluated by at least two trained professionals and divided into 4 categories according to the Hadders Algra (normal optimal, normal suboptimal, mildly abnormal and definitely abnormal). In the process of training machine learning models and later evaluation, both categories of normal movement are merged into one category as well as the categories of abnormal movement (due the binary decision making of some models). One set of evaluated videos served as a basis for training machine learning models. The second, independent, set of videos were evaluated by the selected and trained machine learning algorithms and neural networks, precisely logistic regression (LR), k nearest neighbors (KNN) and convolutional neural network (CNN).

Results: For the training and validation of the machine learning models, we used 492 videos (262 videos with normal movement, 230 with abnormal) from 194 recorded children (119 with normal movement, 75 with abnormal). The test set included different 151 videos (109 with normal movement, 42 with abnormal movement) of 39 children (29 with normal movement, 10 with abnormal movement). The LR showed an agreement between the model and the human examiners of 76.92% with a tendency to more often assess abnormal movement in a child assessed to have normal movement by human examiners (8 children out of 29 with normal movements). The LR had positive predictive value of 53% and a negative predictive value of 95%. The KNN model showed an agreement of 64.10% with a tendency to often estimate abnormal movement in a child assessed to have normal movement by human examiners (10 children out of 29 with normal movement). In this model, the assessment of normal movement in children that were assessed as abnormal by human examiners (4 children out of 10 children with abnormal movement) is noticeable. The positive predictive value of this model is 38% and the negative predictive value is 83%. The CNN showed an agreement of 58.97%. The tendency of frequent assessment of abnormal movement in children who were assessed to have normal movement by human examiners was again expressed (16 children out of 29 with normal movement). In this model, there was not one misestimation of normal movement in children with assessed abnormal movement by human examiners. The positive predictive value of this model is 38% and the negative predictive value is 100%.

Discussion: The preliminary results of the general movement assessment by machine learning models show a significant tendency to attribute abnormal movement in children with normal movement according to trained human examiners, while the attribution of normal movement in children with abnormal movement is significantly less frequent. The result is a relatively high negative predictive value, which points to the potential of using these machine learning models as a screening method where it is important not to miss an abnormality, i.e. it is less harmful to falsely assess abnormality than to falsely assess normality.

MeSH/Keywords: artificial intelligence, general movement assessment, machine learning, neural networks

Poster code: R-01-08-097

Poster Title: Rostro-caudal differences in the ratio of GABAergic neurons subtypes through the rat neocortex

PhD candidate: Andrea Blažević

Part of the thesis: Specificities of GABA-ergic neurons in the rat cerebral cortex

Mentor(s): Associate Professor Ana Hladnik, MD PhD, Professor Zdravko Petanjek, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: GABAergic interneurons exhibit significant diversity within the rat cerebral cortex, with a higher prevalence observed in parietal and occipital regions compared to frontal areas. While numerous studies have explored rodent interneurons, a comprehensive evaluation of their abundance and laminar distribution across cortical regions remains elusive. Among rodent interneuron populations, parvalbumin (PV) neurons are recognized as the most prevalent, followed by somatostatin (SOM) and calretinin (CR) neurons, with calbindin (CB) neurons typically categorized as a subset of SOM neurons. However, the extent of overlap between these populations remains contentious, with conflicting findings from different studies.

Materials and methods: We conducted double-labeling immunofluorescence analysis on free-floating sections of the rat cerebral cortex, focusing on frontal, parietal and occipital regions. Antibody combinations included calretinin-calbindin, calretinin-parvalbumin and calretinin-somatostatin. A systemic evaluation was carried out to assess the abundance and laminar distribution of interneuron subtypes, aiming to ascertain the relative proportions of different subclasses and investigate the extent of overlap between calretinin neurons and the other three subpopulations.

Results: A comprehensive qualitative analysis of double-labeled immunofluorescent histological sections showed that there were no major rostro-caudal differences in the number and laminar distribution of calbindin, parvalbumin and somatostatin neurons, while calretinin neurons were more abundant in occipital region. No overlap between calretinin and other major interneuron populations was observed. Parvalbumin, somatostatin and calretinin neurons were evenly distributed within a cortical column, while calbindin neurons were more numerous in upper cortical layers.

Discussion: CR neurons are generally more abundant in caudal than in rostral regions of the rat cerebral cortex contradictory than in primates. In rodents, associative areas are in occipital while it is known that in humans and primates are in frontal region. This study contributes that shift in CR neurons number could play a key role in advanced parallel processing between cortical areas. Well understanding of differences between rodents and human neural circuits will contribute to understanding of the biological basis of the highest cognitive functions and etiopathogenesis of neural diseases associated with GABAergic interneurons.

MeSH/Keywords: interneurons, rat cerebral cortex, executive functions

Poster code: R-01-08-098

Poster Title: Sex specific differences in the response of mouse astrocytes and neurons to enhanced expression of the Spry2 gene induced by transduction with lentiviral vectors

PhD candidate: Monika Berecki

Part of the thesis: Sex specific differences in the response of mouse astrocytes and neurons to enhanced expression of the Spry2 gene induced by transduction with lentiviral vectors

Mentor(s): Assistant Professor Anton Glasnović, MD PhD, Professor Marija Heffer, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Lentiviral vectors (LVs) offer promising avenues for gene modification in the central nervous system (CNS), with minimal inflammatory responses and potential to avoid unintended transduction. Sex differences influence glial cell function and inflammation, crucial for understanding brain function and effective treatment. The Sprouty2 (Spry2) protein plays a significant role in cell differentiation, migration, and proliferation, particularly in neural stem cell differentiation and CNS regeneration. We hypothesized that upregulation of Spry2 gene expression would affect neuronal morphology more than astroglia and result in lower neuroinflammation in females compared to males.

Materials and methods: Plasmids were multiplied in E. coli using the heat-shock method for LV production. LV concentration was measured using the One-Wash, HIV-1 p24 ELISA kit. Experiments were conducted on 64 mice, with LV suspension injected into the brain. Brain tissue was adequately stored (in protease inhibitors for proteomic and genomic analysis, or OCT medium for IHC analysis) on minus 80 degrees Celsius, untill further analysis. Tissue will be analyzed using the freefloating IFIHC method to analyze colocalization with fluorescent marker proteins from LV, and tissue clearing and light-sheet microscopy for morphology analysis. Molecular analyses (WB and qPCR) will be performed on brain tissue to investigate LV effectiveness. Statistical analysis will be done using the IBM SPSS Statistics version 26.0.0 program. Experimental design: D0 Intracranial injection of LVs (LVSpry2, LVcontrol, PBScontrol) D7 Brain isolation for immunohistochemistry (IHC) D14 Brain isolation for IHC D21 Brain isolation for WB, qPCR, LSM

Results: Our preliminary results indicate partially successful production of LVs using a plasmid multiplication method in E. coli via heat-shock, with LV concentration measured using the OneWash, HIV1 p24 ELISA kit. However, we encountered issues with only one type of LV, necessitating repetition of production. All mice (except the group intended for the unsuccessful LV type) were treated and analyzed for potential neurological damage at each experiment timepoint. Female mice underwent vaginal swab analysis for estrus determination. All experimental mice survived stereotactic injection and were sacrificed as planned. Tissue storage was adequate for further analysis. Some of the tissue has already been cut on a cryostat to a thickness of 35 µm, and the injection area was confirmed by means of a fluorescent confocal microscope, visualizing GFP and mCherry markers.

Discussion: The successful modification of genes within the central nervous system (CNS) using lentiviral vectors (LVs) holds great promise for advancing our understanding of brain function and developing therapeutic interventions. Our study aimed to investigate the effects of upregulating Sprouty2 (Spry2) gene expression on neuronal morphology and neuroinflammation, with consideration of sex differences. Preliminary results showed partially successful LV production, with issues encountered in one type of LV necessitating further production. All mice underwent treatment and were analyzed, demonstrating survival and successful stereotactic injection. Tissue confirmation via cryostat sectioning and fluorescent confocal microscopy ensured accurate targeting of injection sites. Our findings highlight the complexity of LV production and the need for careful selection of vectors. The observed sex differences emphasize the importance of considering biological variables in neuroscience research. Further analysis of tissue samples will provide insights into the effects of Spry2 gene upregulation on neuronal morphology and inflammation. These results contribute to the ongoing research into CNS gene modification and underscore the significance of understanding sex-specific responses in neurological studies.

Acknowledgments: This study was supported by the EU through the ERDF, under grant agreement No. K.01.1.1.04.0085, project Genomic engineering and gene regulation in cell lines and model organisms by CRISPR/Cas9 technology CasMouse; as the Scientific Centre of Excellence f

MeSH/Keywords: Lentiviral Vectors, Gene Expression Regulation, Sex Dimorphism, Neurons, Astrocytes

Poster code: R-01-08-099

Poster Title: The effect of cellular aging on selective degradation of misfolded proteins in yeast Saccharomyces cerevisiae

PhD candidate: Mihaela Pravica

Part of the thesis: The effect of cellular aging on protein quality control in yeast Saccharomyces cerevisiae

Mentor(s): Mirta Boban, PhD, research associate

Affiliation: Croatian Institute for Brain Research, School of Medicine, University of Zagreb

Introduction: The neuropathological feature of several neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's is the accumulation of misfolded proteins in brain cells. While aging is a major factor in the development of these diseases, the impact of cellular aging on the molecular processes that control the accumulation of misfolded proteins is poorly understood. To better understand the development of these diseases, it is important to investigate how cells maintain protein homeostasis under normal conditions and how these processes are disrupted during aging. To prevent the detrimental effects of misfolded proteins, cells possess highly conserved protein quality control pathways, including selective degradation via the ubiquitin-proteasome system, selective autophagy, and protein sequestration into specialized cellular compartments. The aim of the proposed research is to investigate the impact of cellular aging on the protein quality control of model misfolded proteins through evolutionarily conserved protein quality control pathways in Saccharomyces cerevisiae.

Materials and methods: The experimental model used in this study is the yeast Saccharomyces cerevisiae. Strains isogenic to wild-type S288C strain were obtained from Euroscarf, Bad Homburg, Germany, or constructed in this study. Chronological aging of yeast cells was achieved using the standard method of cell growth in a complete medium, for 2 to 5 days without media change, during which cell culture enters the stationary phase. Cell fractionation was performed to separate quiescent and non-quiescent cells from the stationary phase culture by the Percoll density gradient centrifugation. Protein degradation was investigated by cycloheximide chase followed by Western blot analysis, where cells were collected at specific time points after cycloheximide addition, which inhibits protein synthesis de novo. Furthermore, total cellular lysates were prepared and levels of misfolded proteins were examined by Western blot. The signal intensity of the model proteins was normalized to the total amount of protein in the cell lysates using the Stain-free imaging method (BioRad). Intracellular localization of proteasomes was examined using gene fusions of proteins with fluorescent proteins and fluorescence microscopy. The viability of yeast cells was assessed by a semi-quantitative method of analyzing colony formation from decimal dilutions of the culture inoculated onto a solid medium.

Results: We noted that the model misfolded proteins tGnd1-HA and stGnd1-HA persist to be degraded during later phases of glucose deprivation in both quiescent and non-quiescent cell fractions. Additionally, we confirmed the re-localization of the proteasomes into the proteasome storage granules in both quiescent and non-quiescent cells. Lastly, we observed no significant difference between the reproductive capacity of the mutant strains for ubiquitination and wild-type strain.

Discussion: We observed that misfolded protein degradation endures during glucose starvation in quiescent and non-quiescent cell fractions, suggesting the importance of elimination of the misfolded protein by selective protein quality control pathways, particularly in aged cells. Despite the relocalization of the proteasomes to proteasome storage granules, where they are considered not to be active, our results indicate that cells maintain a sufficient level of active proteasomes for the selective degradation of misfolded proteins. The viability of yeast cells is unaffected in san1Δ, ubr1Δ or double mutant san1ubr1Δ during chronological aging, suggesting that ubiquitination of the misfolded proteins may not be a limiting factor for cells to retain reproductive capacity.

MeSH/Keywords: aging, protein quality control, selective degradation, ubiquitin proteasome system, misfolded proteins, yeast Saccharomyces cerevisiae

Poster code: R-01-08-101

Poster Title: Electrophysiological and molecular characteristics of subthalamic nucleus neurons.

PhD candidate: Tin Luka Petanjek

Part of the thesis: Molecular profile of electrophysiologically defined neuron subpopulations in rat subthalamic nucleus.

Mentor(s): Professor Miloš Judaš, MD PhD, Nikola Habek, PhD

Affiliation: University of Zagreb School of Medicine

Introduction: The subthalamic nucleus (STN) is a small diencephalic nucleus and part of the extrapyramidal motor system. The electrophysiological profile of the STN is often studied in the context of Parkinson's disease. Alterations in STN activity, such as increased burst firing or abnormal oscillations, are associated with the motor symptoms of the disease. Recognising different molecular and morphological characteristics of tonic and phasic neuron subpopulations would enable better translation of electrophysiological data between rat and human STN.

Materials and methods: Experiments were performed on STN neurons in slices obtained from juvenile male rats anesthetized with sevoflurane and decapitated, the brain was carefully extracted and placed in an ice-cold oxygenated artificial cerebrospinal fluid (ACSF). Coronal brain slices were prepared using a vibratome in an ice-cold slice cutting solution then transferred immediately to constantly circulating oxygenated ACSF maintained at room temperature and allowed to recover. Slices were then transferred to a recording chamber. Electrophysiological properties of STN neurons were recorded in whole cell patch clamp configuration and data were analyzed using the pClamp software. For morphological analysis juvenile male rats were anesthetized with a ketamine and xylazine solution and perfused transcardially with paraformaldehyde. Brains were postfixed and then placed in 20% sucrose solution. Coronal brain slices were cut using a cryostat and subsequently rinsed then incubated overnight in primary antibodies. Subsequently slices were rinsed, incubated in secondary antibodies mounted on slides and coversliped. Slices were recorded using a confocal microscope and pictures subsequently analysed using software.

Results: Two types of neuronal firing patterns in the STN were identified. Most neurons (72.2 %) displayed a rhythmic continuous tonic discharge of single spikes and were labelled as tonic neurons. The rest of the neurons (28.8%) were capable of occasional irregular burst firing discharges followed by inactivity periods and were labelled as phasic neurons. Tonic neurons also appeared to have a smaller cell body with more primary dendrites compared to phasic neurons with larger cell bodies and less primary dendrites after being filled during recording. Strong immunoreactivity was observed throughout the subthalamic nucleus for VGLUT2 (glutamatergic neuron marker), synaptophysin (presynaptic terminals), nNOS (abundant neuronal marker in STN), PV (specific interneuron marker) and GAT-3 (GABA reuptake transporter). Synaptophysin and GAT-3 staining specifically have demonstrated significant density throughout the STN suggesting potential astrocytic synaptic regulation.

Discussion: Currently molecular analysis is done on perfused tissue slices as molecular analysis of electrophysiologically recorded slices and subsequent classification of specific neuronal markers has proven a technically challenging step that still needs to be overcome.

MeSH/Keywords: subthalamic nucleus, tonic, phasic, neuron

Poster code: R-01-08-109

Poster Title: Molecular and morphological characteristics of the extracellular matrix in the adult human prefrontal cortex

PhD candidate: Matija Vid Prkačin

Part of the thesis: Molecular and morphological characteristics of the extracellular matrix in the adult human prefrontal cortex

Mentor(s): Professor Zdravko Petanjek, MD PhD, Assistant Professor Ivan Banovac, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Perineuronal nets (PNNs) are a type of extracellular matrix (ECM) that play a significant role in synaptic activity and plasticity of interneurons in health and disease. We researched PNNs' regional and laminar distribution, and their molecular composition in Brodmann areas (BA) 9, 14r, and 24, in 5 adult human postmortem brains.

Materials and methods: The cytoarchitectonic regions were delineated using Nissl staining. Anti-calretinin/calbindin/parvalbumin/somatostatin antibodies were used for the detection of GABAergic interneurons. Anti-versican/neurocan antibodies and Wisteria floribunda agglutinin (WFA) were used to show the condensed ECM. Anti-NeuN antibodies were used to visualize cortical neurons. Histological sections processed by immunofluorescence were be imaged on a confocal microscope. Quantitative analysis of immunoreactive cells and condensed perisomatic ECM was done using Neurolucida 2020 (MBF Bioscience, Williston, Vermont, USA) and Neurolucida Explorer (MBF Bioscience) on confocal images of double-labelled immunofluorescent histological slides (NeuN/ECM markers and PV/WFA). NeuN immunolabeling was used to delineate the cortical layers. To determine cortical layers in PV/WFA double labelled sections, contours from neighbouring NeuN-labelled slides were used. For each marker combination (NeuN/WFA, NeuN/NCAN, NeuN/VCAN and PV/WFA), three slides from each region of each brain specimen (n = 5) were quantified using the Detect cells function in the Neurolucida software, after which manual correction was performed by two independent observers. Condensed perisomatic ECM was counted as a PNN only if it met all the following criteria: 1) condensed ECM surrounded a visible soma outline, 2) clear demarcation between the perisomatic ECM and background staining was present, 3) perisomatic ECM surrounded at least 50% of the soma's outline, 4) condensed ECM enveloped at least the proximal part of the cellular processes. Condensed ECM surrounding visible cellular outlines that did not meet these criteria were counted as ECM aggregates. On NeuN/ECM markers double labelled slides, only PNNs or ECM aggregates surrounding NeuN-immunolabelled (NeuN+) cells were counted. The results of the quantitative analysis are presented as: (a) the proportion of NeuN+ cells surrounded by a specific PNN or ECM aggregate, and (b) the proportion of total PNNs or ECM aggregates found in a specific cortical layer. (c) the proportion of PV+ cells surrounded by WFA+ PNNs, and (d) the proportion of WFA+ PNNs surrounding PV+ cells. The statistical analysis was done using GraphPad Prism, version 10.0.2 (GraphPad Software, La Jolla, USA). Data were shown as arithmetic mean ± standard deviation (SD). Data from the same brain were analysed as paired data. Repeated measures (RM) one-way ANOVA with Tukey's multiple comparison test were used to test the differences between BA9, BA14r and BA24. A P-value of less than 0.05 was considered statistically significant.

Results: Each of the ECM molecular markers we analysed (WFA, NCAN and VCAN) formed two types of condensed ECM – PNNs and aggregates. These two forms of condensed ECM could be distinguished based on the degree of demarcation between the signal and background, and based on the degree to which they envelop cellular processes. The ratio between PNNs and ECM aggregates differed substantially between different markers, with WFA staining predominantly visualizing PNNs and NCAN staining predominantly visualizing aggregates. Each ECM marker had a characteristic laminar distribution with WFA+ PNNs being most numerous in layers III, IV and V, and NCAN+ and VCAN+ PNNs being most numerous in layers I and VI. Regional differences were most pronounced in WFA staining where BA24 differed significantly from BA9 and BA14r in the total proportion of WFA+ PNNs and aggregates. Finally, the co-localization with different interneuron markers (PV, CR and SOM) was highly specific for each ECM marker.

Discussion: The presented data point towards a high degree of ECM specialization, possibly related to different cortical microcircuits. Understanding the basis of such specialization is of great importance for determining the possible significance of ECM alterations in various neuropsychiatric conditions, such as schizophrenia, bipolar disorder, and major depressive disorder.

MeSH/Keywords: anterior cingulate cortex, PNNs, interneurons

Poster code: R-01-08-116

Poster Title: Denervation-induced changes of synaptopodin in dentate gyrus granule cells following transection of the perforant pathway

PhD candidate: Fran Božić

Part of the thesis: Reorganization of mouse hippocampal granule cell dendritic spines after entorhinal cortex lesion in vivo

Mentor(s): Professor Mario Vukšić, MD PhD, Thomas Deller, PhD

Affiliation: University of Zagreb School of Medicine

Introduction: This research uses layer-specific innervation of the dentate gyrus molecular layer as the granule cells get selectively denervated only in the distal dendritic segments by transecting the perforant path using the entorhinal cortex lesion model (ECL). It induces pronounced structural changes in the dendritic arbour and its spines. Synaptopodin is a protein tightly linked to the dendritic spines. It interacts with calcium signalling molecules in the spines and promotes actin molecule polymerisation. These functions are the basis of spine structural plasticity by promoting dendritic spine growth and structural stability.

Materials and methods: This research uses C57BL/6-J male mice aged 10-24 weeks perfused at several time points following the stereotaxic entorhinal lesion (3, 7, 14, and 28 days post-lesion (dpl)) (n = 5 per group). Additionally, sham-operated mice are the negative controls for the study (n = 5 in total). Firstly, the mouse is intraperitoneally anaesthetised, and the head is placed and fixed in a stereotaxic frame to which a retractable wire knife carrier is affixed. The skull is re-aligned, and the tip of the wire knife is placed on the lambda vertex of the skull. A cranial window is created using a dental drill at previously obtained coordinates, and the wire knife is lowered into the brain to perform the lesion. The skin is then disinfected, and surgically stitched, and the antibiotic prophylaxis is administered intraperitoneally. The sham-operated mice undergo the same procedure except for lowering the wire knife. Upon reaching the planned time point, the mice are transcardially perfused, and the brains are then sectioned on a vibratome in 100 µm thick coronary slices in the dorsal part of the hippocampus. The brains are stained with Fluoro-Jade C dye that identifies the degeneration process in the outer molecular layer in the case of a complete lesion. The slices are placed in a chamber and placed on the fluorescent microscope stage. Borosilicate glass micropipettes are pulled using a vertical puller, loaded with the fluorescent dye, and then guided into the hippocampal granule cell layer, where the cells are impaled under visual control. Slices are then stained using the standard immunohistochemistry protocol for synaptopodin puncta. The imaging of the dendritic segments is performed on a confocal microscope. The analysis of images is conducted with the researcher blind to the analysed data. Only the spines connected to their dendritic segment and projecting laterally in the X-Y plane by inspecting the entire z-stack are included in the analysis. Objectified criteria are applied when measuring spine geometry. Furthermore, the synaptopodin cluster is attributed to a specific spine if positioned inside its boundary.

Results: The preliminary results of this research show that the changes in spine head size and spine density follow the pattern previously exhibited by several other research groups. Additionally, synaptopodin seems to show its specific dynamics in correlation with other structural changes, and that might imply its role in the structural plasticity of dendritic spines in vivo, a feature previously only shown in in vitro conditions. Furthermore, additional analysis will confirm whether synaptopodin-positive spines suppress the formation of new spines in their vicinity and whether this feature applies to every time point after denervation.

Discussion: Previous research has shown that spines after dentate granule cells' denervation exhibit a specific pattern of geometric changes and that their density rapidly drops in the first three days and then gradually recovers in the following three weeks. It has also been revealed that synaptopodin is a marker that correlates with spine stability and survival post-denervation and is predominantly associated with spines of larger volume. This research follows this path by examining whether synaptopodin displays the same behaviour in an in vivo model of dentate gyrus denervation. Further analysis of the obtained data is necessary to make any claims regarding the effects of synaptopodin on spine stability and survival, as well as the effects of synaptopodin-positive spines on their immediate neighbouring spines.

Acknowledgments: Dinko Smilović, MD PhD, Mario Zelić, MD, Leonarda Grandverger, Ivana Janić

MeSH/Keywords: dendritic spines, synaptopodin, denervation, spine geometry

Poster code: R-01-08-126

Poster Title: Reorganization of the rat brain cortical structure after mild perinatal hypoxic exposure: a correlative in vivo MRI - histological study

PhD candidate: Matea Drlje

Part of the thesis: Histological and in vivo MR markers of extracellular substance reorganization and cytoarchitectonics in the cerebral cortex of a rat model of moderate perinatal hypoxia

Mentor(s): Professor Nataša Jovanov Milošević, MD PhD, Diana Cash, PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Premature birth is the leading cause of neonatal brain injuries, most frequently complicated by hypoxia-ischemia. While the effects of severe hypoxic injuries on the brain are widely researched, the consequences of mild hypoxic events are largely underinvestigated. In order to explore the resistance of the brain during the perinatal period and disclose possible developmental reorganization in cortical differentiation after a single mild perinatal hypoxic event in rats, we cross-referenced histological and immunohistochemical findings with in vivo magnetic resonance imaging (MRI) data

Materials and methods: A total of 28 Wistar Han (sexes equally represented) rats were subjected to either moderate hypoxia (8% O2, 92% N2/2h, n=14) or normoxia (21% O2, 79%N2/2h, n=14) on postnatal day one (P1). Structural and diffusion in vivo MRI were performed in 16 rats at age P15, and immediately after the rats were sacrificed, the brains were weighed and processed further for histological (Nissl modification of cresyl-violet staining, and immunohistochemical (Purified Anti-Neurofilament H (SMI-32) and Microtubule-associated protein 2 (MAP2) evaluation.

Results: In rats that underwent hypoxia, the most significant elevation in fractional anisotropy (FA) values was observed in the anterior cingulate cortex (ACC), which was further evaluated immunohistochemically. The observed changes in cytoskeletal elements of the ACC were intermediate filament and microtubule reorganization, as revealed by SMI32 and MAP2. The immunohistochemical findings between the control and the hypoxic group correlate well with FA changes. No brain or body mass differences between hypoxic and control rats were detected at P15. However, MRI volumetric analysis revealed an indication of regional brain volume changes.

Discussion: The aforementioned gives additional data and validates our model of mild perinatal hypoxia. Further research will be focused on elucidating the molecular pathophysiological mechanisms of the described perinatal cortical reorganization. That would lay ground for the development of new MRI modalities for diagnostic purposes in perinatal medicine.

MeSH/Keywords: hypoxia; plasticity; DTI

Poster code: R-01-08-149

Poster Title: SPHINGOID BASE AND GANGLIOSIDE CONTENT IN GLIOMAS WITH DIFFERENT PROLIFERATIVE INDEX

PhD candidate: Mia Jurilj Sajko

Part of the thesis: Determination and comparison of the sphingolipid profile in primary brain tumors of different degrees of malignancy

Mentor(s): Associate Professor Krešimir Rotim, MD PhD, Assistant Professor Dragana Fabris, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Gliomas are the most common primary brain tumors. Sphingolipid metabolites including free and phosphorylated sphingoid bases (S1Ps) are involved in glioma pathogenesis. By phosphorylation, their roles shift from pro-apoptotic towards promoting tumor cell survival and proliferation. Gangliosides (GG) are sialic acid-containing glycosphingolipids, the most abundant in the nervous system, representing potential therapeutic targets. Sphingoid base content was analyzed in different malignancy grade glioma patients' serums (PSs) and healthy control serums (HSs) using liquid chromatography/mass spectrometry (LC/MS) profiling. GG content and characterization were performed in tumoral (T) and peritumoral (PT) tissues.

Materials and methods: Serum samples from patients with primary brain tumors (glioblastomas - GBM, diffuse astrocytomas, oligodendrogliomas) were analyzed and compared to HSs. Sphingoid bases were extracted and purified using the modified method by Sullards et al. [1]. Samples were analyzed by Agilent 6550 iFunnel Q-TOF LC/MS by MRM analysis using d14:1 as an internal standard. Complex ganglioside (GG) mixtures were isolated and purified from T and PT tissue samples in parallel using the modified Svennerholm and Fredman method. Total GG concentrations were determined spectrophotometrically, while qualitative analysis of GG mixtures was performed by high-performance thin-layer chromatography (HPTLC) followed by densitometric scanning.

Results: Sphingosine 1-P (d18:1-P) was quantified in PSs with approximately 1.5 times lower concentrations than in HSs. Sphinganine 1-P (d18:0-P) was quantified in PSs with approx. 27 times higher concentrations than in HSs. The highest concentrations of d18:0-P were found in glioblastoma PSs but also, interestingly, in two lower-grade glioma PSs (diffuse astrocytoma and oligodendroglioma). Total GG content (expressed as μg GG-SA/g tissue wet weight) in tumoral tissue was approximately one-and-a-half times lower than in peritumoral tissue (considering both lower and higher-grade gliomas entering the equation) and approximately two times lower than in normal brain tissue.

Discussion: According to our knowledge, this is the first time that sphinganine 1-P (d18:0-P) was determined in serums of different malignancy-grade glioma patients. Concentrations in PSs were higher in GBM patients than lower-grade gliomas and significantly higher than in HSs, suggesting a potential role as a glioma-associated biomarker. Further research must be conducted to clarify the role of d18:0-P in different-grade gliomas. In all glioma tissue samples total GG content was lower than in normal brain tissue, which is according to the research done so far. Total GG content, as expected, was lower in the tumoral than in the peritumoral tissue and significantly lower than in normal brain tissue (including average ganglioside content of white and grey matter).

Acknowledgments: This research was supported by the Adris Foundation to D.F. and by the European Fund for Reg. Development to the Institute for Anthropological Research (BIOANT, KK.01.1.1.02.0002).

MeSH/Keywords: gliomas, sphingoid bases, gangliosides, liquid chromatography, mass spectrometry

Poster code: R-01-08-156

2. Preliminary research results – clinical medical sciences

Poster Title: The association of serum concentrations of lipocalin-2, interleukin-8 and calprotectin with the presence of hidradenitis suppurativa

PhD candidate: Joško Miše

Part of the thesis: The association of serum concentrations of lipocalin-2, interleukin-8 and calprotectin with the presence of hidradenitis suppurativa

Mentor(s): Professor Zrinka Bukvić Mokos, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease characterized by recurrent nodules, abscesses and tunnels in skin folds reflecting a neutrophilic infiltration and inflammation in the deep dermis. This study aims to examine the association between the serum concentrations of lipocalin-2 (LCN-2), interleukin-8 (IL-8), and calprotectin and the presence of mild and moderate-to-severe and severe forms of hidradenitis suppurativa (HS). Recent studies have shown elevated LCN-2 levels in several skin diseases, such as psoriasis, eczema, skin wounds, keratoacanthoma and squamous cell carcinoma. It is being investigated as a potential biomarker in over 70 studies. IL-8 is a pro inflammatory cytokine whose role in the pathophysiology of HS is being investigated. Calprotectin is a biomarker whose serum levels could correlated with the inflammatory conditions, not solely related to bowel diseases.

Materials and methods: 60 HS patients will be included in the study: 30 patients with mild (stage I) and 30 patients with moderate-to-severe HS (stages II and III), with 30 healthy controls. The values of serum concentrations of LCN-2, IL-8, and calprotectin will be determined using commercially available ELISA tests.

Results: 60 eligible HS patients were identified and included in the study (30 patients with Hurley stage I or II, and 30 patients with Hurley stage III) and their sera were obtained and stored at -80 C. 30 healthy controls were included whose sera were collected and stored at -80 C. The kits to examine the serum levels of LCN-2, IL-8 and calprotecin were purchased. First preliminary results of ELISA testing are expected in the next month.

Discussion: The results of the thesis could contribute to identification of serum biomarker levels for disease severity assessment. Determining predictive values of their serum concentrations for the presence of the disease, we could be able to add an objective parameter in the existing disease severity classifications and the selection of the most appropriate treatment. The analysis of IL-8 role could offer a new potential therapeutic target molecule.

MeSH/Keywords: hidradenitis suppurativa, lipocalin-2, interleukin-8, calprotectin

Poster code: R-02-02-077

Poster Title: The interrelationship between disability and psychological distress in chronic nonspecific low back pain among adults of working age population

PhD candidate: Jelena Marunica Karšaj

Part of the thesis: Association between depression disorder symptoms and functional disability in working active patients with chronic non-specific low back pain

Mentor(s): Professor Simeon Grazio, MD PhD

Affiliation: University Hospital Center Sestre milosrdnice

Introduction: Chronic non-specific low back pain (LBP) is conceptualized on the biopsychosocial model and is generally accepted that the experience of the CNLBP could significantly contribute to psychological distress. The BDI-II (Beck Depression Inventory-II) is commonly used self-assessment instrument for screening and diagnosis of depression. The BDI-II has 21 items, is well-known for its reliability and validity in assessing symptoms of depression in various clinical and non-clinical populations. As it can reliably discriminate between chronic pain patients with and without symptoms of clinical depression; it is used to assess level of depression in chronic pain within the last two weeks. The RMDQ (Roland Morris Disability Questionnaire) has a content and construct validity and reflects the concepts of mobility in activities of daily living. The maximum score of RMDQ is 24 points (one point per statement) and represents maximum disability and is substratified into no disability, mild, moderate and severe. Our aim was to investigate the interrelationship between the result of the BDI-II and the result of functional disability measured with RMDQ among adults of the working-age population with chronic non-specific LBP regarding age, sex, BMI, length of work, and its duration.

Materials and methods: We analyzed 64 patients' data (10 men and 54 women). Men were older than women (49.55 vs. 44.80 years; P=0.01). The influence of age (mean 48.81 ± 5.47), length of working expectancy (mean 25.64 ± 7.73), body mass index (mean 26.52 ± 4.55), duration of chronic non-specific LBP in months (mean 96 [26-180]), and the overall result of RMDQ (without disability, mild, moderate, and severe disability), as non-dependent variables on the total result of BDI-II, was analyzed by logistic regression. Conducting this study was approved by the Ethical Committee of the University Hospital Center "Sestre milosrdnice" in February 2023 identified by code number 003-06/23-03/003. Written informed consent was obtained from patients who fulfilled the criteria to participate in the study.

Results: The interrelationship between depression (assessed with BDI-II) and disability (assessed with RMDQ) was stronger for women than for men (p=0.08). There is a tendency that higher results in RMDQ will assume higher results in BDI-II (p<0,115). No significant interrelationship was indicated among other variables and perceived depression and disability due to chronic non-specific LBP. Overall, the reported strength of the interrelationship between measures of psychosocial distress and measures of self-reported disability cannot precisely predict which group within BDI-II a patient with a disability would match.

Discussion: In our sample, there was a tendency that higher results in total RMDQ score will assume higher results in the total BDI-II score, especially in women, although overall the significance wasn't demonstrated. A bigger sample size and more detailed analysis among constituents in both questionnaires are needed, which could lead to different treatment approaches in patients with chronic non-specific LBP.

MeSH/Keywords: chronic non-specific low back pain, disability, depression, working age

Poster code: R-02-03-017

Poster Title: Association of diaphragmatic ultrasound characteristics and disease activity in patients with axial spondyloarthritis

PhD candidate: Hana Skala Kavanagh

Part of the thesis: Association of diaphragmatic ultrasound characteristics and disease activity in patients with axial spondyloarthritis

Mentor(s): Professor Simeon Grazio, MD PhD, Assistant Professor Marija Gomerčić Palčić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Axial spondyloarthritis (axSpA) is a chronic inflammatory rheumatic disease predominantly affecting the spine and sacroiliac joints having impact on thoracic function and diaphragmatic dynamics. The utility of ultrasound in assessing diaphragmatic function offers a promising avenue for objective evaluation that provides insight into disease activity and potential therapeutic responses.

Materials and methods: A cross-sectional survey is being conducted. Ninety axSpA patients, diagnosis based on the validated ASAS classification criteria aged 18 to 65 years will be divided into the tested (active and very active disease) and control (inactive and low-active disease) groups according to structured questionnaire for measuring disease activity ASDAS-CRP (Ankylosing Spondylitis Disease Activity Score). The planned duration of the research is one year (from January 2, 2024 to January 2, 2025, that is, until 45 patients in each group are included in the study). The exclusion criteria are: proven other acute or chronic obstructive/restrictive lung disease, pulmonary hypertension, surgical interventions in the area of the chest and abdomen and thoracic spine, acute infection, scoliosis or congenital deformation of the chest, proven neuromuscular disease, obesity (BMI >40 kg /m2), malnutrition (BMI <18.5 kg/m2), misunderstanding of the instructions or inability to perform a particular segment of the test. The tests that are being performed on the subjects are: complete blood count, SE, CRP, radiogram of the heart and lungs (PA and profile), spirometry, diffusion capacity for carbon monoxide (DLCO), ultrasound evaluation to assess the thickness of the diaphragm and mobility during the breathing cycle. Assessment of functional capacity for physical activity will be carried out with a 6-minute walk test (6MWT), shortness of breath will be assessed with the mMRC (Modified Medical Research Council) and modified Borg scale, and the degree of exhaustion after the 6MWT with the modified Borg perception of effort scale.

Results: Initially, 110 patients were included in the study. All data were collected for 90 patients, 45 male (50%) and 45 male (50%) patients. Reasons for dropping out of the study: newly diagnosed COPD (2), newly diagnosed asthma (1), acute respiratory infection (1), loss of consciousness during test (1), life relocation (1), misunderstanding of the instructions (1), failure to meet the ASAS classification criteria for diagnosis of axSpA (1), dropped out from the study-no show (12). Now a database of collected data is being created by entering data into an Excel so that statistical analysis of the data can be done in the next phase.

Discussion: The results of the research could for the first time determine the connection between the features of the diaphragm using ultrasound and disease activity in patients with axial spondyloarthritis, whereby diaphragm ultrasound (available, non-invasive and non-ionizing diagnostic method) can serve as a parameter for assessing disease activity.

MeSH/Keywords: spondyloarthritis, ultrasonography, diaphragm, respiratory mechanics, disease activity

Poster code: R-02-03-052

Poster Title: Psoriatic arthritis and sarcopenia

PhD candidate: Sanda Špoljarić Carević

Part of the thesis: Psoriatic arthritis and sarcopenia

Mentor(s): Associate Professor Nadica Laktašić Žerjavić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Psoriatic arthritis (PsA) is a chronic inflammatory rheumatic disease belonging to the spondyloarthritis group. Sarcopenia, a generalized muscle disorder, can result in decreased physical capacity and increased risk of poor outcomes.Studies on sarcopenia in PsA are scarce, with recent reviews indicating a high prevalence of reduced muscle mass but not necessarily confirming sarcopenia according to the latest criteria. No published studies to date have investigated the prevalence of sarcopenia and its association with disease duration, activity, functional status, vitamin D levels, and outcomes in PsA patients using the EWGSOP2 criteria.

Materials and methods: This cross-sectional study was conducted at Naftalan Special Hospital. The Ethical Committee of Naftalan Special Hospital approved the research: 238/10-111-1016-3/22, as well as the Ethical Committee of the Faculty of Medicine, University of Zagreb. All participants provided informed consent prior to inclusion in the study. Participants included were both male and female patients over the age of 18 years diagnosed with psoriatic arthritis according to the CASPAR criteria. Exclusion criteria: under 18 years of age, other inflammatory rheumatic diseases, malabsorption, eating disorders, inflammatory bowel diseases, a history of malignant disease in the past five years, long-term immobility, neuromuscular diseases (myasthenia gravis), multiple sclerosis, Parkinson's disease, cerebrovascular diseases (stroke), pregnancy, the presence of a metal foreign body, epilepsy. Clinical parameters of the disease were measured using the DAPSA (Disease Activity index for Psoriatic Arthritis) and ASDAS (Ankylosing Spondylitis Disease Activity Score) indices for quantifying disease activity. Skin disease was assessed using the PASI (Psoriasis Area and Severity Index) scale. Body mass and height were measured to calculate BMI (Body Mass Index), and fatigue was assessed with the FACIT-F questionnaire. Muscle function was evaluated using a handgrip strength test with a dynamometer,physical functionality was measured using the TUG (Timed Up and Go) test. Appendicular muscle mass was measured through bioelectrical impedance analysis (BIA). Levels of C-reactive protein (CRP) and vitamin D were also determined.

Results: The study involved 15 patients with psoriatic arthritis, with the majority of participants being female (93.3%, n=14), while the male gender was represented in 6.7% (n=1). The average age of participants was 58 years (SD=9.58), with a median age of 63 years and an age range from 45 to 77 years. Different levels of disease activity were recorded among the participants. Active disease according to the DAPSA index was noted in 26.7% of women (n=4). According to ASDAS criteria, very high disease activity was registered in 93.3% of participants (n=14; 13 women and 1 man), while high activity was recorded in one woman (6.7%). Skin disease was moderately active in 33.3% of women (n=5). Excessive body mass (BMI > 30) was registered in 60% of participants (n=9), overweight was recorded in 26.7% of women (n=4). Significant fatigue (value > 30) was noted in 20% of women (n=3). Reduced grip strength, which may be an indicator of sarcopenia, was present in 20% of women (n=3). An extended TUG test, which may indicate reduced physical function associated with sarcopenia, was recorded in 6.7% of women (n=1). Measurements of appendicular muscle mass using bioelectrical impedance showed normal values in all participants, suggesting the absence of sarcopenia despite the presence of other factors that could indicate its risk.

Discussion: This study reveals various aspects of the psoriatic arthritis among participants. The majority were female, with an average age of 58 years. Disease activity varied, with most participants showing high activity. Moderate activity of skin disease was observed in 5 patients. Obesity was common, as well as fatigue and there was found reduced grip strength, which may indicate sarcopenia, but measurements of muscle mass showed normal values, suggesting the absence of sarcopenia despite other risk factors. These results show the need for individualized treatment approaches in patients with psoriatic arthritis to improve patient outcomes. It's worth noting that while we did not demonstrate sarcopenia in psoriatic arthritis, the sample size was small.

MeSH/Keywords: psoriatic arthritis, sarcopenia,

Poster code: R-02-03-081

Poster Title: PRELIMINARY OUTCOMES OF vNOTES AUTOLOGOUS TISSUE RECONSTRUCTION VRS STANDARD VAGINAL SURGERY

PhD candidate: Luka Matak

Part of the thesis: Comparison of the effectiveness of vNOTES pelvic organ prolapse reconstruction using autograft with a standard surgical approach

Mentor(s): Professor Slavko Orešković, MD PhD, Professor Jan F. Baekelandt, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Pelvic organ prolapse (POP) is a common condition in women. During their lifetime up to 40% of all women will develop POP. In this study we aim to demonstrate a new technique for pelvic organ prolapse repair, harvesting the autologous posterior rectus fascia sheath via vaginal natural orifice transluminal endoscopic surgery (vNOTES), as an alternative for a synthetic mesh

Materials and methods: Patients older than 18 with no desire to preserve fertility, POP-Q (Pelvic Organ Prolapse Quantification) 3 or 4 stages of prolapse with indication for hysterectomy and prolapse repair will be selected and randomly divided into two groups. One group will be operated on with standard technique (vaginal hysterectomy with anterior and/or posterior vaginal repair) for pelvic organ prolapse and in the other autologous graft will be used using vNOTES (PREFAP). Objective evaluation of the effect of postoperative results will be done using transperineal ultrasound before the operation, 6 and 12 months after the procedure.

Results: A total of 45 patients have been operated on and are being followed up for one year. Of these, 25 in the PREFAP group and 20 in the group operated with the standard method. The average duration of hospitalization was 4 days. The total average BMI is 28.41kg/m2. In the PREFAP group, one recurrence was detected within a year that required rectocele repair, while in the vaginal group, two recurrences required re-repair. No major complications were reported during surgery.

Discussion: Several warnings and restrictions have been delivered to urogynecological surgeons in order to avoid the implants of prosthetics meshes. Autologous tissues show satisfying outcomes in terms of safety and efficacy. Our results show that the efficiency of a new method in the treatment of pelvic organ prolapse using autologous tissue that is prepared and fixated by the vNOTES approach is the same. Longer follow-up is needed to examine the long-term effect of autologous tissue.

Acknowledgments: The completion of my dissertation would not have been possible without support of my wife.

MeSH/Keywords: urogynecology, pelvic organ prolapse, autologous tissue

Poster code: R-02-05-094

Poster Title: Influence of determinants of polycystic ovary syndrome and hormonal contraceptive treatment on quality of life and incidence of anxiety and depression symptoms

PhD candidate: Olga Miloš

Part of the thesis: Influence of determinants of polycystic ovary syndrome and hormonal contraceptive treatment on quality of life and incidence of anxiety and depression symptoms

Mentor(s): Professor Dinka Pavičić-Baldani, MD PhD, Marko Ćurković, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Women with polycystic ovary syndrome (PCOS) have increased incidence of anxiety and depressive symptoms and a lower quality of life (QoL) compared to the general population.1-4 It is unclear whether anxiety and depression symptoms are results of phenotypic and biochemical determinants of PCOS, consequence of hormonal contraceptive (HC) use or it is an independent feature of PCOS.

Materials and methods: It is planned to include 120 women aged 18-45 with diagnosis of PCOS according to the Rotterdam criteria. The PCOS group will be classified into 4 PCOS phenotypes and each phenotype will have 30 women. The control group will consist of 30 healthy women with previously excluded PCOS. After inclusion in the research and completed questionnaires (SF-36- Short Form 36, MPCOSQ -The modified polycystic ovary syndrome questionnaire, GAD7-Generalised Anxiety Disorder Scale, HADS- Hospital Anxiety and Depression Scale, PHQ9- Patient Health Questionnaire-9, sociodemographic questionnaire) and laboratory measurements, all subjects will be prescribed HK. Laboratory measurements and questionnaires will be repeated three and six months after initial use of HK.

Results: From May 2023 to May 2024, in Clinic for Women's Diseases and Obstetrics, Petrova we collected about 50 women who belong to the PCOS group. Laboratory processing, ultrasound, biometric measurements, questionnaires were filled out, and hormonal contraception was introduced, which for now all women tolerated well. Based on the mentioned parameters, the women were calssified according to PCOS phenotypes. So far, not a single woman has given up on research. Samples for determination of platelet serotonin and allopregnanolone due to delayed analysis were stored at -23 C.

Discussion: After included 120 women in research we are planning to analyze platelet serotonin and allopregnanolone from stored samples of blood.

MeSH/Keywords: polycystic ovary syndrome, quality of life, depression, anxiety, hormonal contraceptives

Poster code: R-02-05-107

Poster Title: The analysis of immunoglobulin G glycosylation in diffuse large B cell lymphoma, not otherwise specified (NOS)

PhD candidate: Vibor Milunović

Part of the thesis: The analysis of immunoglobulin G glycosylation in diffuse large B cell lymphoma, not otherwise specified (NOS)

Mentor(s): Professor Slavko Gašparov, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Diffuse large B cell lymphoma (DLBCL) NOS is the most common lymphoid malignancy. By gene expression profiling and the cell of origin theory, it is divided in germinal like B cell (GCB) and activated B cell like (ABC) with GCB subtype conferring better prognosis. Due to the lack of the methods, immunohistochemistry by Hans is used to divide these lymphomas in GCB and non-GCB subtypes. Glycosylation represents the co- and posttranslational modifications of proteins where sugar moieties, so called “glycans”, attach covalently to various proteins. The changes in glycosylation have been implied in various malignant and benign disorders

Materials and methods: 80 patients suffering from newly diagnosed DLBCL-NOS and 80 age and sex-matched healthy controls were included. The main inclusion criterion was the ability to tolerate anthracycline-based immunochemotherapy. The patients were recruited from the Division of Hematology, Clinical Hospital Merkur, while the diagnosis was established in the Clinical Department of Pathology and Cytology, Clinical Hospital Merkur, according to Croatian lymphoma consensus. After the diagnosis and prior the initiation of immunochemotherapy, the blood sample was taken from patients for the analysis of N-glycosylation of immunoglobulin G performed in the Division of Glycobiology Genos D.O. according to established standards. The results of N-glycans analysis are to be represented in chromatogram peaks. The hypothesis was tested by T-test while the other variables were analysed with different tests based on categorical or continuous characteristics. Due to unknown effect-size, interim power analysis after the inclusion of 40 subjects with effect-size being 0.63 (power=80%, p=0.05).

Results: 40 patients suffering from DLBCL-NOS were included. Majority of patient received CHOP-R immunochemotherapy (cyclophosphamide, doxorubicin, vincristine, methylprednisolone) while those patients with high-risk feature (pathological, stage, localization) received continuous infusion with R-DA-EPOCH (etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab). Those with high risk for central nervous relapse received prophylactic high dose methotrexate (3.5 g/m2, cycles 7 and 8). Patients were irradiated in case of bulky disease. The final analysis of association of N-glycosylation will be carried out upon the accrual of the whole cohort.

Discussion: Historically, CHOP-R in Division of Hematology Clinical Hospital Merkur was the standard of care in this population. However, in recent years the approach to DLBCL is changing. R-DA-EPOCH immunochemotherapy was introduced for a subset of high-risk patients. Furthermore, intrathecal methotrexate in patients with high risk for central nervous system relapse was abandoned and replaced with high dose methotrexate. This trend shows ever evolving paradigm of DLBCL-NOS treatment.

Acknowledgments: I acknowledge professor Damir Nemet and KROHEM foundation for financially supporting this thesis.

MeSH/Keywords: non-Hodgkin lymphoma, diffuse large B cell lymphoma, immunoglobulin G, glycomics

Poster code: R-02-09-010

Poster Title: Vascular complications of transbrachial compared to transfemoral and transradial approach in cardiac and peripheral catheterization

PhD candidate: Krešimir Kordić

Part of the thesis: Vascular complications of transbrachial compared to transfemoral and transradial approach in cardiac and peripheral catheterization

Mentor(s): Associate Professor Matias Trbušić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: The transfemoral approach has long been the standard for endovascular procedures, but it's associated with frequent complications. The transradial approach is now the most common for coronary angiography and percutaneous coronary interventions, offering advantages like fewer vascular complications, lower costs, patient comfort, and shorter hospital stays. However, it involves longer procedure times, higher radiation doses, and a steeper learning curve and is not always feasible. The transbrachial approach retains some of the advantages of the transradial approach (suitable for patients with severe aortoiliac occlusive disease, easier patient mobility, and shorter hospitalization. However, its safety record is mixed in the literature. Some studies report complications in up to 36% of cases, while others indicate that it has the same or fewer complications compared to the transfemoral approach.

Materials and methods: We are conducting a prospective observational study. Patients undergoing diagnostic or therapeutic procedures via transbrachial, transradial, or transfemoral approach are being included. The choice of vascular approach is at the operator's discretion. Each vascular approach has its specific postprocedural follow up. Access complications are being recorded, categorized as major (requiring blood transfusion or surgery, vessel occlusion, pulse loss, or permanent neurological deficit of the upper or lower extremities) and minor (subcutaneous hematomas <10 cm, pseudoaneurysms, and arteriovenous fistulas not requiring surgery). Pain is assessed using a visual analog scale. The study aims to include 141 patients in each study group and has defined inclusion and exclusion criteria to ensure consistency.

Results: By March 1, 2024, 105 patients have been included in the brachial, 117 in the femoral, and 145 in the radial approach group. In the brachial approach group, we recorded 2 major complications (1 hematoma and 1 pseudoaneurysm requiring surgical treatment) and 4 minor complications (smaller hematomas). In the femoral approach group, we recorded 3 major complications (2 retroperitoneal bleedings requiring surgical treatment and 1 hematoma requiring blood transfusion) and 6 minor complications (5 smaller hematomas and 1 retroperitoneal hematoma that did not require blood transfusion or surgical treatment). In the radial approach group, we recorded 1 major complication (compartment syndrome requiring surgical treatment) and 3 minor complications (smaller hematomas).

Discussion: Overall, we found a numerically small number of major complications. The smallest number of complications was in the transradial group, which is expected since it is the most used vascular approach today. We found only 2 major complications in the transbrachial approach group. We believe that the reason for the relatively small number of complications in the transbrachial approach group is the operators experience with transradial access, the use of modern materials, and a standardized algorithm for postprocedural monitoring. The transfemoral approach group had the numerically highest number of complications, although the overall number is small. Once we have included all intended participants and carried out the statistical analysis, we expect to demonstrate that transbrachial access is a safe and effective alternative to the most commonly used transradial approach, and that it has advantages over the transfemoral approach.

MeSH/Keywords: percutaneous interventions, transradial approach, transbrachial approach, transfemoral approach.

Poster code: R-02-09-031

Poster Title: Plasma cytokine profiling in patients with chronic graft-versus-host disease

PhD candidate: Antonija Babić

Part of the thesis: Subpopulacije monocita i citokini monocitnog odgovora u perifernoj krvi bolesnika s kroničnom bolesti presatka protiv primatelja

Mentor(s): Assistant Professor Hrvoje Lalić, MD PhD, Professor Drago Batinić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Chronic graft-versus-host disease (cGVHD) is a significant allo- and autoimmune complication following allogeneic hematopoietic stem cell transplantation (allo-HSCT), affecting multiple organ systems. Monocytes, cells of innate immunity, exhibit diverse subpopulations characterized by their functions, ranging from phagocytic to inflammatory, anti-inflammatory, or variable roles. Their roles in cGVHD are unclear, with studies showing differences in activation markers and chemokine receptors but inconsistent cytokine secretion. Unlike certain chemokines as cGVHD biomarkers, the role of monocyte-related cytokines remains unexplored. Our primary objective was to assess the relationship between monocyte-associated cytokines and cGVHD, differentiating between de novo and established cases. We also aimed to compare these cohorts with allo-HSCT patients without cGVHD.

Materials and methods: We enrolled 62 adult cGVHD patients (de novo=31, established=31) and 31 control allo-HSCT patients at University Hospital Centre Zagreb from 2017 to 2023. Plasma samples were analysed using microsphere bead array technology to quantify 12 cytokines (IL-4, IL-2, CXCL10, IL-1β, TNF-α, MCP-1, IL-17A, IL-6, IL-10, IL-γ, IL-12p70, IL-8). Age and sex distributions were evaluated among groups.

Results: Age and sex did not significantly differ between groups. CXCL10 levels were significantly elevated in de novo cGVHD patients compared to controls ($p<0.001$). Moreover, both CXCL10 ($p<0.001$) and immunosuppressive IL-10 ($p=0.049$) were higher in de novo cGVHD patients than those with established cGVHD. IL-6 demonstrated a positive correlation with the global NIH cGVHD score ($\rho=0.448$, $p<0.001$) and a negative correlation with the Karnofsky score ($\rho=-0.524$, $p<0.001$) across all cGVHD patients, indicating its association with disease severity.

Discussion: This study provides preliminary evidence supporting CXCL10 as a potential biomarker for cGVHD, with marked elevation in de novo cases. Additionally, our findings highlight a link between elevated IL-6 levels and increased cGVHD severity. These results underscore the clinical relevance of these cytokines in understanding and managing cGVHD, warranting further validation in larger cohorts.

Acknowledgments: This work has been funded by Croatian Cooperative Group for Hematological Diseases Foundation and Croatian Science Foundation (project IP- 2016-06-8046)

MeSH/Keywords: chronic graft-versus-host disease, monocytes, cytokines

Poster code: R-02-09-036

Poster Title: Superior vena cava isolation in paroxysmal atrial fibrillation - mind the phrenic!

PhD candidate: Vedran Pašara

Part of the thesis: Electrical isolation of superior vena cava in the invasive treatment of paroxysmal atrial fibrillation

Mentor(s): Assistant Professor Vedran Velagić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Pulmonary vein isolation (PVI) is the standard of care for patients with atrial fibrillation (AF), but medium-term success rates are far from ideal. Therefore, new methods are being developed, with emphasis on ectopic foci outside the pulmonary veins. Superior vena cava (SVC) is one of the most important non-pulmonary origins of AF and some studies suggested that empiric SVC isolation could improve outcomes in ablation of paroxysmal atrial fibrillation (PAF). Although complications have been reported in SVC ablation, most studies found complication rates of SVC ablation similar to conventional cryoballoon PVI. This prospective, single-center study aimed to assess the complication rate and success rate of PVI followed by SVC isolation by cryoballoon application in comparison with conventional cryoballoon PVI.

Materials and methods: We designed an unblinded, randomized clinical trial and recruited consecutive patients with PAF who were randomized to a conventional cryoballoon PVI or PVI and additional SVC cryoballoon isolation using single 180 s freeze.

Results: A total of 140 patients (61.4% male, mean age 62.1 years) with PAF were included and 67 (47.9%) were randomized to SVC isolation in addition to conventional ablation. Planned 180-s freeze in the SVC could be completed in 15 (22.4%) patients, while 27 (40.3%) patients received at least 120-s freeze. The mean time to SVC isolation was 40.2 ± 37.9 s. Real-time recording of SVC isolation was observed in 39 (58.2%) patients. Right phrenic nerve palsy (PNP), impending or transient, occurred in 6 (8.2%) patients in the PVI-only group and in 25 (37.3%) patients in the SVC-group (Chi-square test $p < 0.001$). There were no persistent PNP cases. Bradycardia or junctional rhythm was observed during a procedure in 15 (22.4%) patients who received SVC isolation. One patient eventually received a permanent AAI pacemaker due to a sinus node injury.

Discussion: Our data suggest that SVC isolation can be successfully achieved in the majority of PAF patients using conventional cryoballoon. However, this significantly increases the rate of transient or impending PNP and sinus node injury in comparison to conventional cryoballoon PVI. Therefore, careful phrenic nerve function monitoring and attention to sinus bradycardia or junctional rhythm are important to mitigate this risk. Follow-up data are being collected to determine whether this risk could be outweighed by a lower rate of PAF recurrence.

MeSH/Keywords: atrial fibrillation, superior vena cava, phrenic nerve, cryoablation

Poster code: R-02-09-046

Poster Title: The effect of parenteral semaglutide on intestinal iron absorption in patients with type 2 diabetes

PhD candidate: Petra Meliš

Part of the thesis: The effect of parenteral semaglutide on intestinal iron absorption in patients with type 2 diabetes

Mentor(s): Assistant Professor Srećko Marušić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Diabetes mellitus is a public health problem. Glucagon-like peptide 1 (GLP-1) is an endogenous hormone used to regulate blood glucose levels in patients with type 2 diabetes. A group of drugs that target GLP-1 receptors is called GLP-1 receptor agonists. Within this group of drugs, there are differences in pharmacokinetic and pharmacodynamic properties. Semaglutide belongs to a group of long-acting GLP-1 receptor agonists. Elemental iron is absorbed in the first part of the small intestine. Many drug-drug interactions have been found to interfere with iron absorption. Previous studies have not focused on the relationship between parenteral administration of semaglutide and intestinal iron absorption. The purpose of this study was to determine whether concomitant parenteral administration of semaglutide and iron supplements affects iron absorption in patients with type 2 diabetes.

Materials and methods: This prospective study was conducted in the endocrinology clinic at the University Hospital Dubrava in Zagreb, Croatia. Both the Medical School Zagreb Ethics Committee and the University Hospital Dubrava Ethics Committee approved the study's protocol. Prior to recruitment, all patients who participated in the trial gave written informed consent. All diabetic patients who met the inclusion criteria were admitted to the outpatient endocrinology clinic between November 2023 and April 2024. Patients between the ages of 40 and 70 with poorly managed type 2 diabetes (HbA1c ≥ 7%) who were candidates for treatment intensification and beginning of parenterally administered semaglutide were eligible to participate in the study. All participants underwent the following procedures prior to the study's commencement: patient demographic and clinical data was collected and entered into a database made specifically for the study. Prior to the beginning of parenterally administered semaglutide, all participants completed an oral iron absorption test (OIAT). As described in previous studies, OIAT was conducted in an outpatient setting. A single dose of 350 mg ferrous fumarate capsule, which is equal to 115 mg of elemental iron, was given orally following an overnight 12-hour fast in order to obtain a venous sample for various parameters prior and 2-hour after capsule ingestion. During the two hours of the OIAT, participants weren't allowed to eat or drink anything. All individuals received parenterally administered semaglutide following the initial OIAT. To enhance glycemic control, the therapy was up-titrated every four weeks. After reaching the maximum tolerable maintenance dosage for two weeks, each participant completed a follow-up OIAT at week 10 of the study. Data from the first and subsequent OIATs were analyzed statistically. In order to detect a statistically significant difference in iron absorption following semaglutide administration, we assumed that 15% of patients included in the study will have reduction in absorption after semaglutide administration. Type I error was set at 0.05 and Type II error was set at 0.2 (80% power). Using the power analysis test we calculated needed total sample size of 50 patients. Each subject served as their own control. Previous studies have indicated that a 30% reduction in iron absorption would be considered a significant difference in iron absorption in the same individual prior to and two weeks after the maintenance dosage of semaglutide was attained. Data were analysed using MedCalc Statistical software, version 17.9.6 (MedCalc Software bvba, Ostend, Belgium).

Results: The investigation has so far included all 50 of the study's required participants. Out of the 50 individuals, 30 have finished the monitoring period, and all data for analysis are accessible. Given the dynamics of data collection and the participants' monitoring periods, all data required for statistical analysis is likely to be obtained within the next month. After that, statistical data processing will start. Based on current data, approximately 70% of participants will experience a significant reduction in iron absorption following the start of parenteral semaglutide therapy.

Discussion: Parenteral semaglutide administration and iron absorption have not been the subject of any prior research. This work adds to our understanding of the pharmacokinetics and pharmacodynamics of parenterally administered semaglutide, pointing us in the direction of a deeper analysis of potential interactions.

MeSH/Keywords: type 2 diabetes, semaglutide, iron, oral iron absorption test

Poster code: R-02-09-057

Poster Title: Risk factors for rivaroxaban-related bleeding events – possible role of pharmacogenetics: case-series

PhD candidate: Ana Marija Slišković

Part of the thesis: The role of pharmacogenomics in an individualized approach to rivaroxaban treatment

Mentor(s): Associate Professor Joško Bulum, MD PhD, Livija Šimičević, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Rivaroxaban variability may be associated with age, liver and kidney function, concomitant illness and therapy, and pharmacogenetic predisposition. We evaluated possible risk factors for rivaroxaban-associated bleeding.

Materials and methods: Sixteen patients with rivaroxaban-associated bleeding were analysed. Thirteen patients were carriers of ABCB1, ABCG2, CYP2J2, and/or CYP3A4/5 gene polymorphisms. Possible drugdrug interactions with increased bleeding risk were identified in 9 patients. Six patients had eGFR<60. The presented cases are subjects of a more extensive prospective nested case-control study. For the investigation of drug-drug interactions (DDIs), the Lexicomp® Clinical Decision Support System was applied.

Results: These sixteen patients (nine females, seven males; median age 73 years, range 61-80) have been consequently included in the study as RIVA related bleeding cases: gastrointestinal (GI) (N=9), epistaxis (N=5), haematuria (N=1) and gynaecological (N=1). After cessation of rivaroxaban all bleedings ceased. The average RIVA daily dose was 17.5 mg (range 5-20 mg), and was administrated according to the indications: AF (N=13); deep vein thrombosis-DVT (N=2) and PAD (N=1). Bleedings occurred between 1 to 12 months following the introduction of RIVA. Renal function was assessed based on the estimated glomerular filtration rate (eGFR) with the CKD-EPI equation. All presented subjects were genotyped for relevant ADME gene variants: CYP2J2*7(rs890293) and rs11572325, CYP3A4*1B (rs2740574), CYP3A4*22 (rs35599367), CYP3A5*3 (rs776746), ABCB1 (c.1236C>T [rs1128503], c.2677G>T/A [rs2032582], c.3435C>T [rs1045642], c.2482-2236G>A [rs4148738], ABCG2 c.421C>A (rs2231142). Pharmacogenetic analyses were performed by specific TaqMan® DME and SNP Assays on a 7500 Real-Time PCR System. Laboratory data show that 13 subjects had at least one variant allele that may possibly influence RIVA pharmacokinetics in terms of higher bleeding risk. Four subjects were CYP2J2*7 heterozygous carrier, six were CYP2J2 rs11572325 heterozygous carriers, two patients were CYP3A4*22 carriers (*1/*22 and *22/*22), three were CYP3A5*3 heterozygous (expressers), two patients had ABCB1 1236T-2677T-3435T-rs4148738A homozygous variants haplotype, three ABCG2 421CA (heterozygous) and one AA genotype (homozygous variant carrier). Only three patients had all wild-type investigated pharmacogene variants. Regarding DDI one patient experienced haematuria due to RIVA-propafenone interaction along with pharmacogene variants, in second patient with extreme gynaecological bleeding RIVA-ASA interaction was observed, while the third patient presented with epistaxis 6 months after the introduction of RIVA due to same interaction. In patient with melena possible influence of RIVA-amiodarone and RIVA-clopidogrel interactions were noticed.

Discussion: This case series is the first, to our knowledge, that presents multiple risk factors for RIVA related bleeding including age, renal function, concomitant diseases, plus concomitant treatment and pharmacogenetics data. Pharmacogenetic data represent the cornerstone of personalised medicine. In conclusion, we should be aware that pharmacogenetic data have some impact on RIVA pharmacokinetics. NOACs are not free of DDIs, and DDIs have a impact on the risk of bleeding as an adverse event other than age and decreased renal function. Our data suggest a possible role of clinical and pharmacogenetic factors and their interactions in predicting bleeding on rivaroxaban treatment, however, they also indicate the need for further comprehensive research to enable safer use of this product based on a personalised approach.

Acknowledgments: None

MeSH/Keywords: Pharmacogenetic, Rivaroxaban, Rivaroxaban-Associated Bleeding

Poster code: R-02-09-084

Poster Title: Association between ECG changes and thromboembolic incidents in COVID-19,

PhD candidate: Petra Bistrović

Part of the thesis: Association of electrocardiographic characteristics with clinical outcomes of hospitalized COVID-19 patients depending on remdesivir treatment

Mentor(s): Professor Diana Delić-Brkljačić, MD PhD, Marko Lucijanić, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Thromboembolic events are a major complication of COVID-19 disease, largely due to endothelial injury and inflammation. (1) These include both venous (deep venous thrombosis and pulmonary embolism) and arterial thromboembolic (myocardial infarction, ischemic stroke, peripheral artery thromboembolisms) incidents. As the pandemic unraveled, it became clear practically all hospitalized patients required at least prophylactic doses of anticoagulation. Despite prophylaxis, some patients still developed thromboembolic incidents. All thromboembolic incidents can both originate from the cardiovascular system – specifically the heart, as well cause further cardiac complications. The easiest way to screen whether a person has an acute cardiac issue is to record a standard electrocardiogram (ECG). The aim of our research was to see whether specific ECG changes present at admission were associated with an increased risk of thromboses in severe and critical COVID-19 patients.

Materials and methods: We analyzed ECGs recorded at admission (available in medical archives) for 1752 severe and critical COVID-19 patients hospitalized at our institution, from March 2020 to June 2021. Patient data and outcomes were obtained from our institutions COVID registry. The ECGs were analyzed for rhythm abnormalities, heart rate, ST segment changes, interventricular conduction abnormalities, AV conduction, PR, QRS and QTc interval duration, P, QRS and T wave axis, QRS-T angle and Sokolow-Lyon hypertrophy criteria. We defined the outcomes as arterial thromboses (myocardial infarction, ischemic stroke, acute peripheral artery occlusion) and venous thromboembolisms (VTE) (deep vein thrombosis and pulmonary embolism).

Results: Our result show that bradycardia (OR 4.57, CI 1.81-11.54), ST elevation (OR 3.84, CI 1.11-13.31), left bundle branch block (OR 2.48, CI 1.03-5.98), prolonged QRS (OR 1.94, CI 1.04-3.60) and QTc (OR 2.03, CI 1.26-3.27), abnormal P wave (OR 1.01, CI 1.00-1.02) and T wave axis (OR 2.03, CI 1.26-3.27) were significantly associated ($P<0.05$) with an increased risk of arterial thromboses in severe and critical COVID 19 patients. Deep negative T waves were significantly associated with increased venous thromboembolisms (OR 2.56, CI 1.21-5.4, $p<0.05$). After multivariate analysis, bradycardia (OR 4.17, CI 1.64-10.62), ST elevation (OR 1.74, CI 0.19-3.34), QTc prolongation (OR 1.82, CI 1.12-2.97) and abnormal P wave axis (OR 2.44, CI 1.19-4.98) remained statistically significantly ($p<0.05$) associated with an increased risk of arterial thromboses, and deeply negative T waves remained significantly associated with VTE (OR 3.38, CI 1.57-7.29, $p<0.05$).

Discussion: Certain pathological ECG changes seem to be associated with an increased risk of thromboembolic incidents. Most of the changes associated with arterial thromboses can be typically present in myocardial infarction, while deeply negative T waves can be seen in pulmonary embolisms associated with right ventricle failure. However, biochemical markers typically associated with these conditions (high-sensitivity troponin and D dimers) were typically attributed to secondary elevation due to acute COVID-19. Identifying these changes early can help in early differentiation of patients, allowing for modification and individualization of anticoagulant and, if neccessary, antithrombotic therapy, thus potentially decreasing the number of thromboembolic complications.

MeSH/Keywords: COVID-19; SARS-CoV-2; thrombosis; Electrocardiogram

Poster code: R-02-09-085

Poster Title: Correlation of hand and quadriceps strenght to heart mass and heart remodelling in elite football players

PhD candidate: Matija Marković

Part of the thesis: The relationship between cardiac mass, hand grip and quadriceps strength assessed by dynamometer, in elite football players

Mentor(s): Associate Professor Tomislav Letilović, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Repetitive workload leads to changes in heart mass and, the so-called, athletic heart develops in sports. The heart mass differs between sports; different field positions within the same sport can have different remodelling of the heart, and they also have different quadriceps and hand muscle strength. The enlargement of the heart mass is often associated with endurance and strength and can also be the result of various cardiomyopathies. It is challenging to differentiate the athletic heart from the structural heart disease based on echocardiography.

Materials and methods: We will include professional football players from the First and the Second Croatian Football League and so-called free players who are members of the Croatian Association Football Union. We aim to include at least 46 forwards, 46 midfielders, 46 defenders, 46 goalkeepers or at least 184 respondents, according to the test strength analysis for correlation study. We will aim to analyze at least 218 football players in order to have a representative sample of all players in the analyzed football leagues. Handgrip strength was measured with hand dynamometer Camry, model EH 101 and all measurements are expressed in kilograms of force. Quadriceps strength, was measured with hand dynamometer MusTec – Almere, model BioFET, with a standard protocol. Initially, with dominant leg with a minimum interval of 30 seconds between each measurement and all measurements are expressed in Newton-meters of force. Echocardiography exam was performed in a standard protocol on Mindray DC-60 Shenzhen Bio-Medical ultrasound machine.

Results: So far, we have included 63 players. Six goal keepers, 22 back players, 20 mid-fielders, 15 attackers. The age is ranging from 18 do 37 years (mean 22 years), and they are of average body size 182,8 cm +/- 5,2 cm. Mean hand grip force 46.8 +/- 7.9 kg; 44.1 +/- 7.9 kg for dominant and non-dominant hands respectively. These force values are like those found in literature (Tavares 2019). Mean force knee extension 294 Nm +/- 21 Nm expressed for all legs combined, and there is a difference in standing and playing leg among pitch-positions. Defensive players have stronger standing leg while other position shooting leg was more powerful in this small sample, but the analysis at the moment is not completed. Mean heart mass in Deveraux method is 202,6 +/- 33,8 gr with indexed values of 102,7gr +/- 17,3 gr; mean diameter of septum in analysed group is 10,7mm+/-1,3mm, while mean relative wall thickness is 0,39 indicating that most of our in analysed players have normal geometry heart type. In doppler and tissue doppler analysis mean E wave is 79,9 cm/s, mean A wave is 42,1 cm/s, mean mitral septal e wave displacement 18,6 cm/s. Preliminary correlation test shows a non-significant weak correlation between non-dominant hand grip and heart mass (r = 0,297), a moderate correlation between hand grip test and longitudinal function of right heart (R 0,415, p=0,013; 0,377 p=0,026). But the significance of these preliminary results has yet to be profoundly analysed, as well as correlation among all other parameters that are stated in application of thesis have to be tested. Complete analysis and correlation of all measured heart functions and dimension has yet to be done.

Discussion: The data from the preliminary analysis on anthropometric and strength measurements are similar to those previously published in studies of elite football players. This similarity possibly suggests that the findings at the end of the trial could be applicable to similar players as well and other leagues. However, a significant challenge is the inclusion of a sufficient number of goalkeepers, since there are only three goalkeepers per club. We will need to include more than 16 clubs to ensure a robust sample size. Additionally, the preliminary data indicate differences in strength, heart size, and diastolic parameters across different pitch positions. Comparing these groups will likely yield interesting insights about specific heart remodeling to football. We expect these differences to provide more information about the various conditions and athletic capabilities among different pitch positions, and help better describe football as a sport that involves athletes of different types, such as endurance type and strength-type, as well as combined types among different field positions.

MeSH/Keywords: Hand Strength; Muscle Strength; Cardiac Volume; Cardiomegaly, Exercise-Induced; Myocardial Remodeling, Ventricular; Soccer

Poster code: R-02-09-113

Poster Title: Inflammatory Bowel Disease Is Associated with an Increased Risk for Covid-19-related Hospitalization, but Not with Mortality

PhD candidate: Ivan Kodvanj

Part of the thesis: Inflammatory bowel diseases as risk factors for illness and severity of the disease of COVID-19

Mentor(s): Professor Željko Krznarić, MD PhD, Professor Vladimir Trkulja, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: During the COVID-19 pandemic, concerns were raised regarding the vulnerability of inflammatory bowel disease (IBD) patients to severe outcomes od COVID-19 and susceptibility to COVID-19 infection. Our study is part of a larger research project that aims to evaluate the impact of COVID-19 on patients with IBD, i.e. risk of COVID-19 contraction, mortality, and hospitalization within the IBD population. To date, we have estimated the risk of hospitalization and mortality among COVID-19 patients affected with IBD.

Materials and methods: This study is based on several primary healthcare administrative databases maintained by the Croatian Institute for Public Health. To assess mortality and hospitalization risk, we included all individuals that tested positive for SARS-CoV-2 in Croatia from the onset of the pandemic until August 15, 2021. IBD patients were exactly matched to non-IBD patients based on several key parameters, including age, sex, vaccination status, pandemic timeframe, Charlson comorbidity index, pertinent pharmacological therapies, and relevant comorbidities. Subgroup analyses were conducted for ulcerative colitis (UC) and Crohn's disease (CD) patients, employing an optimal full algorithm utilizing Mahalanobis distance and exact matching algorithm for similar set of covariates. Log-binomial regression with a robust sandwich variance estimator was used to calculate relative risks (RR) and corresponding 95% confidence intervals (CI) for outcomes. Additionally, since disease activity and administration of biological therapy is not available in primary healthcare databases we explored the potential influence of disease activity and biological therapy on hospitalization and mortality risks by investigating hospital healthcare records of all IBD patients treated at the Clinical Hospital Centre Zagreb (UHCZ) and creating a comprehensive database for further research.

Results: In this population-based study, among 433,609 COVID-19 patients, 3067 IBD patients. Analysis revealed comparable crude proportions of COVID-19-related mortality between IBD and non-IBD patients (2.8% vs. 2.7%), with a relative risk (RR) of 0.85 (95%CI 0.60 – 1.19) after matching, indicating no significant difference in mortality risk. However, the hospitalization rate was notably higher in the IBD population based on crude proportions (6.1% vs. 4.5%) and adjusted RR of 1.43 (95%CI 1.17 – 1.75) after matching. Subgroup analysis within the IBD cohort identified 2061 patients with ulcerative colitis (UC) and 797 patients with Crohn's disease (CD), with 209 patients having unspecified IBD subtype. Crude comparisons indicated an elevated risk of COVID-19-related death (3.2% vs. 1.8%) and hospitalization (6.1% vs. 5.8%) among UC patients. However, subsequent matching using two distinct algorithms revealed no discernible disparity in mortality risk [RR_primary = 0.55 (95%CI 0.18 – 1.61) and RR_sensitivity = 1.14 (95%CI 0.57 – 2.30)] or hospitalization risk [RR_primary = 1.14 (95%CI 0.72 – 1.80) and RR_sensitivity = 0.92 (95%CI 0.62 – 1.35)] between UC and CD patients. For further research, we identified 547 patients treated with biological therapy at the UHCZ. Some of the patients were treated with multiple biological therapy during the study period, including adalimumab (168), golimumab (18), infliximab (275), tofacitinib (11), ustekinumab (70), and vedolizumab (68).

Discussion: Our findings suggest that IBD patients face a slightly higher risk of COVID-19-related hospitalization compared to the general population with no increased mortality risk. Furthermore, no significant disparities in outcomes are found between UC and CD patients. Future investigations will explore the influence of biological therapy and disease activity on hospitalization and mortality risks in IBD patients, along with assessing their susceptibility to contracting COVID-19.

MeSH/Keywords: COVID-19, inflammatory bowel disease

Poster code: R-02-09-119

Poster Title: Agreement between X-ray phase contrast imaging and conventional histopathology in the assessment of graft acute cellular rejection grading following heart transplantation

PhD candidate: Nikola Škreb

Part of the thesis: Graft rejection detection in heart transplantation recipients by synchrotron X-ray phase contrast imaging

Mentor(s): Associate Professor Maja Čikeš, MD PhD, Professor Bart Bijnens, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Acute cellular rejection (ACR) of the cardiac allograft is clinically the most relevant complication after heart transplantation (HTx). Light microscopy-based pathohistological diagnosis (PHD) of endomyocardial biopsy tissue (EMB) is the golden standard in post-HTx surveillance. Recent studies have demonstrated the application of X-ray phase-contrast imaging (X-PCI) for cardiac tissue imaging, enabling 2D and 3D virtual histopathology.

Materials and methods: A total of 100 EMB samples were collected prospectively at multiple timepoints from 62 post-Htx patients followed-up in two clinical centres, following centre-specific protocols. Study samples were imaged by X-PCI at the Paul Scherrer Institute TOMCAT beamline (Villigen, Switzerland) with an established imaging protocol that produced digital imaging datasets with an average of 6321 images per sample at 0.65 µm pixel size. The EMB samples were then prepared for PHD analysis with light microscopy. The analysis resulted in three digital datasets: conventional 2D histology images, X-PCI 2D images, and X-PCI 3D whole sample scans. The ACR grade was assessed by an experienced pathologist in a blinded fashion based on the ISHLT 2004. criteria, and agreement between methods was assessed using Cohen's weighted kappa, with clinically relevant grades (2R and 3R) carrying increased magnitude of weight in the calculations.

Results: The majority of samples did not show a clinically relevant grade of rejection (0R in 76% and 1R in 8% of samples, confirmed in all three datasets), whereas 15% of samples showed some 0R and 1R grade differences between datasets, and only one sample had a significant 2R grade confirmed in all three datasets. Grading based on conventional PHD showed substantial agreement with grading based on both 2D X-PCI ($\kappa = 0.71$) and 3D X-PCI virtual pathohistology ($\kappa = 0.68$), while the highest level of agreement ($\kappa = 0.77$) was seen when comparing 2D and 3D X-PCI analyses.

Discussion: A high level of agreement was achieved when comparing both 2D and 3D X-PCI virtual histopathology with conventional PHD analysis in graft rejection grading, especially when ruling out significant grades of rejection. X-PCI represents a non-destructive imaging method enabling visualisation of the whole EMB sample to assess ACR grading, demonstrating potential for application in HTx follow-up of selected patients.

Acknowledgments: This study has been fully supported by the Croatian Science Foundation under the trial registration no. IP-2020-02-5572.

MeSH/Keywords: heart transplantation, graft rejection, synchrotron imaging, histopathology

Poster code: R-02-09-143

Poster Title: Plasma concentration of miRNA-21 and miRNA-150 as predictors of paroxysmal atrial fibrillation recurrence after pulmonary vein isolation

PhD candidate: Sanda Sokol Tomić

Part of the thesis: Plasma concentration of miRNA-21 and miRNA-150 as predictors of paroxysmal atrial fibrillation recurrence after pulmonary vein isolation

Mentor(s): Associate Professor Nino Sinčić, MD PhD, Nikola Pavlović, PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Atrial fibrillation (AF) is the most common persistent arrhythmia in the population. The estimated global prevalence is 2,5 – 3,2 % and epidemiological studies have shown that people with FA have an almost fourfold higher risk of mortality than the general population. Leading cuase of death is still a stroke. The diagnosis of FA is established by electrocardiography. One of the methods for rhythm control is pulmonary vein isolation. Despite the development of ablation techniques, the AF recurrence rate remains significant. Non-coding micro RNAs (miRNAs) play an important role in pathophysiology of AF, but their association with AF recurrence has not been investigated.

Materials and methods: Prospective study for a period of 2 years. We intend to include 90 patients in the research, in order to collect a sufficient sample for appropriate statistical processing, taking into account the frequency of AF recurrence after ablation and eventual loss of patients in follow up. The statistical program MedCalc Software (v. 14.8.1,2014) was used to calculate the sample size. Including criteria: patients with an ECG-confirmed diagnosis of paroxysmal AF, in whom AF ablation (pulmonary vein isolation with a cryoballoon) is planned as a rhythm control method. Patients will be admitted to the Dubrava Clinical Hospital, Department of Cardiovascular Diseases. The exclusion criteria: left atrium larger than 5 cm measured echocardiogrphically from the long parasternal axis, age > 75 years, BMI > 30 kg/m2, significant valvular disease and inability to take oral anticoagulant therapy. Sampling peripheral blood: on the day of the cryoablation procedure. miRNA-21 and miRNA-150 will be isolated at the Laboratory for Epigenetics and Molecular Medicine, Department of Medical Biology. The statistical analysis will be conducted with statistical tests such as Kolmogorov – Smirn test, t-test, Mann-Whitney U test and Fisher's exact test. All P values less than 0.05 will be considered significant. In the analysis the GrandPad Prism 7 program will be used (No. lic.: GPW6-278912-RLMU-966F4).

Results: At this time, we enrolled 35 patients who underwent pulmonary vein isolation with cryoablation. Those patients are now in follow up. Blood sample was taken and forzen and analysis will be done when we collect all the participants.

Discussion: Circulating and tissue miRNAs regulate determinants of AF pathophysiology and have emerged as biomarkers of this disease. In this study, we present a supporting evidence for the role of miRNAs in AF, but the inconsistencies among the explorative and functional studies cannot be denied. So far, no distinct miRNA has been identified as a clinically useful biomarker or as target for AF treatment. The goal of this study was to gain insights into the mechanism responsible for miRNA expression associated with AF. MiRNA expression changes are linked to the pathophysiological continuum of myocardial strain, dilatation, tissue fibrosis, and the appearance of AF. Functional links between miRNAs and cellular pathways involved in cell growth, inflammation, or differentiation are well characterized. Therefore, understanding associations between miRNA expression and clinical determinants of atrial cardiomyopathy is relevant for identifying links to underlying cellular processes that can be targeted using modulators of the involved cellular pathways.

MeSH/Keywords: paroxysmal atrial fibrillation, microRNA, pulmonary vein isolation

Poster code: R-02-09-148

Poster Title: Early introduction of sodium and glucose cotransporter 2 inhibitors in the treatment of patients with acute heart failure with reduced ejection fraction: effects on short-term and long-term outcomes

PhD candidate: Marija Radić

Part of the thesis: Effects of early introduction of sodium-glucose co-transporter 2 inhibitors into therapy on short-term and long-term outcomes in the treatment of patients with acute heart failure with reduced ejection fraction

Mentor(s): Associate Professor Tomislav Letilović, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Heart failure is a clinical syndrome characterized by signs and symptoms resulting from structural and/or functional cardiac abnormalities. It can be classified based on ejection fraction into heart failure with preserved ejection fraction (HFpEF), mildly reduced ejection fraction (HFmrEF), and reduced ejection fraction (HFrEF). Standard therapies for HFrEF, such as ACE inhibitors (ACEi), angiotensin receptor blockers (ARB), mineralocorticoid receptor antagonists (MRA), and angiotensin receptor-neprilysin inhibitor (ARNI), have shown limited efficacy in treating patients. Recently, sodium-glucose co-transporter 2 (SGLT2) inhibitors have demonstrated favorable outcomes in heart failure patients with reduced ejection fraction, making them a recommended therapy due to their positive effects on mortality and clinical events.

Materials and methods: Materials and Methods: This prospective study will include patients aged 18-95 years hospitalized for acute heart failure. Patients with valvular or pericaridal disease, chronic kidney disease, type 1 diabetes mellitus, or active malignant disease will be excluded. Patients will be divided into two groups: those hospitalized for the first manifestation of acute heart failure and those hospitalized due to an exacerbation of chronic heart failure. SGLT2 inhibitors will be initiated immediately in the first group's therapy, while they will be added to existing heart failure therapy in the second group. Laboratory tests, heart ultrasounds, and physical examinations will be conducted, with follow-up assessments at 6 and 12 months. Statistical analysis will be performed using MedCalc.

Results: The study aims to evaluate the impact of early initiation of SGLT2 inhibitors on short-term and long-term outcomes in heart failure patients.

Discussion: Given the established benefits of SGLT2 inhibitors in patients with HFrEF who have not responded adequately to standard pharmacological therapy, it is anticipated that these benefits will also be observed with early initiation of therapy. The study seeks to determine if there are differences in outcomes between patients receiving immediate versus delayed SGLT2 inhibitor therapy. The goal is to assess whether early initiation of SGLT2 inhibitors reduces hospitalization rates, decreases mortality, and improves laboratory and clinical parameters, including NT-proBNP levels, ejection fraction, and renal function. The study also aims to explore short and long-term outcomes in heart failure patients treated with different subgroups of SGLT2 inhibitors.

MeSH/Keywords: heart failure, SGLT2 inhibitors, mortality, hospitalization

Poster code: R-02-09-153

Poster Title: Large artery stiffness in patients with abdominal aortic aneurysm before and after open surgical procedure

PhD candidate: Maja Vizjak Keretić

Part of the thesis: Large artery stiffness in patients with abdominal aortic aneurysm before and after open surgical procedure

Mentor(s): Academician Bojan Jelaković, Assistant Professor Predrag Pavić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Arterial stiffness is a physiological property that explains mechanical properties of elastic arteries. Aorta and large arteries play an important role in attenuation of pulsatile blood flow. When arterial stiffness increases, pulsatility attenuation lowers. Greater amounts of energy are transferred to microcirculation and consequently terminal organs are impaired. Hence, arterial stiffness is considered an indicator of cardiovascular, cerebrovascular and renovascular morbidity. Gold standard for measuring arterial stiffness is carotid-femoral pulse wave velocity. Since 2004, cardio-ankle vascular index is used as a novel index of the overall arterial stiffness. It is known from the literature that the arterial stiffness is increased in patients with aortic aneurysms. Moreover, patients after endovascular aortic aneurysm repair experience elevation in arterial stiffness. Impact of surgical replacement of abdominal aorta with vascular prosthesis on arterial stiffness is not yet investigated.

Materials and methods: Study is designed as a prospective observational study. In this study we will include patients admitted to the Department of Vascular Surgery at the University Hospital Centre Zagreb for an elective surgical repair of abdominal aortic aneurysm. Using sample size calculation methods, we have calculated that minimal required population sample is 34 patients (α=0.05; β=0.20). Exclusion criteria are: symptomatic or ruptured aneurysm, heart arrhythmia, cerebrovascular accident or myocardial infarction within three months, congestive heart failure (NYHA class III or IV), malignancy, dialysis, solid organ transplantation, life expectancy shorter than twelve months, peripheral arterial disease, pregnancy and breastfeeding. Upon patient's consent, medical history will be taken and physical examination and laboratory tests will be performed. Aortic aneurysm diameter will be measured on computed tomography angiography which is obligatory for a preoperative planning. Arterial stiffness will be measured with two different methods: carotid-femoral pulse wave velocity and cardio-ankle vascular index, before and after surgical procedure. In statistical analysis we will use descriptive and analytic statistical methods. Depending on normality of distribution of numerical variables we will use Student's t-test for dependent samples or the nonparametric Wilcoxon rank sum test to determine differences before and after surgery. The Pearson or Spearman correlation coefficient will be used to verify the connection between individual variables in the study. In all tests p value of <0.05 will be considered significant.

Results: At this time 29 patients have been enrolled and measured in the study. Mean age of participants is 66.2 and mean infrarenal aortic aneurysm diameter is 61.3mm, ranging from 83mm to 43mm. Preliminary results indicate that carotid-femoral pulse wave velocity and cardio-ankle vascular index are higher in postoperative measurements for most subjects. Further statistical analysis will be conducted in accordance with methods described earlier.

Discussion: Preliminary results are suggesting that there will be a difference in preoperative and postoperative measurement regarding arterial stiffness. If statistical analysis indeed confirms statistical significance further investigations should be supported. Nevertheless, based on our conclusions it could be wise to address this group of patients in regardance to their cardiovascular status resulting in more aggressive approach to diminishing their total cardiovascular risk after surgical replacement of diseased aortic wall.

MeSH/Keywords: vascular stiffness, aortic aneurysm, cardio ankle vascular index, carotid-femoral pulse wave velocity, vascular surgical procedures

Poster code: R-02-10-086

Poster Title: Relationship of vitamin D receptor polymorphisms and 25-hydroxy vitamin D concentrations in patients with psoriatic arthritis

PhD candidate: Alen Vrtarić

Part of the thesis: The relationship between the vitamin D (VDR) receptor genomic polymorphisms and the serum concentration of vitamin D variants (D2 and D3) with the activity of disease, functional ability, density and bone structure in patients with psoriatic arthritis

Mentor(s): Professor Simeon Grazio, MD PhD, Nora Nikolac Gabaj, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Psoriatic arthritis (PsA) is a chronic inflammatory rheumatic disease, which belongs to the group of spondyloarthritis. It is closely related to the skin disease, psoriasis. PsA typically affects the peripheral joints, rarely spine. In patients with ankylosing spondylitis (AS), which belongs to the group of spondylitis diseases as well as PsA, it was observed that the FokI polymorphism (rs2228570) in the VDR gene could be involved in bone density and the inflammatory response that occurs in ankylosing spondylitis.

Materials and methods: Patients diagnosed with psoriatic arthritis based on CASPAR (Classification Criteria for Psoriatic Arthritis) that were treated at the University Department for Rheumatology, Physical Medicine and Rehabilitation at University Hospital Center Sestremilosrdnice (age between 18-65 years) were included into study. Basic data on gender, age, type and duration of therapy, physical activity and eating habits were collected for each subject. Venous blood was sampled from each patient upon admission to the hospital: for the isolation of genomic DNA, one VACUETTE test tube with an EDTA anticoagulant and one VACUETTE tube with a clot activator to obtain serum (both Greiner Bio-One, Kremsmünster, Austria). Patients were divided into two categories according to the value of the DAPSA (Disease Activity Psoriatic Arthritis) index, which includes in the calculation the number of painful and swollen joints, CRP concentration and the patient's assessment of disease activity and joint pain. Genomic DNA from peripheral whole blood was isolated manually with commercially available High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany). Polymorphisms of the Vitamin D receptor (VDR) gene FokI(rs2228570) and TaqI (rs731236) were detected by real time polymerase chain reaction usingreagent TIB MOLBIOLSyntheselabor GmbH, Belin, Germany. The concentration of 25-OHD (25-hydroxy vitamin D) was measured on Abbott Alinityianalyser (Abbott, Abbot Park, IL, USA). Distribution of the data was tested by using Kolmogorov-Smirnov test. For the data that didnot follow normal distribution, results are presented as median and interquartile range (IQR). For group comparison, non-parametric Mann-Whitney test was used. For more than two groups Kruskall-Wallis test was used. For categorical data, Chi-squared test was used. Level of significance was set at 0.05.

Results: In total, 42 patients were included. Median concentration of 25-OHD was60 (40-79) nmol/L. Frequencies of TaqI polymorphism genotypes were: 23/42 TT (wild type), 17/42 heterozygous CT and 2/42 CC with homozygous mutation. Frequencies of FokI polymorphism genotypes were: 12/42 CC (wild type), 20/42 heterozygous CT and 10/42 with homozygous mutation. There were no differences in concentration of 25-OHD across tested polymorphisms: FokI CC=58(41-71);FokI CT=67 (34-82); FokI TT=70 (40-83)nmol/L; P=0.832 and TaqI TT=60 (31-72); TaqI CT+CC=70 (42-82) nmol/L; P=0.133. When polymorphisms were compared with categorized DAPSA, there was no statistically significant difference (TaqI P=0.129, FokI P=0.914).

Discussion: With these preliminary results we demonstrated that there is no significant difference in concentration of 25-OHD acrossVDR polymorphism genotypes. As we can see from our pilot study FokI polymorphism is more represented in our population. More data should be collected, analyzedand comparison of polymorphysms with clinical indicators of the disease should be performed. We cannot draw clear research conclusions yet. Currently publicationsshowed association of VDR polymorphisms with psoriasis, psoriatic arthritis and spondyloarthritis. Lower 25-OHD levels in patients with PsA were observed. Further analysis of VDR gene polymorphisms may help to obtain more information.

MeSH/Keywords: psoriatic arthritis, 25-OHD, vitamin D receptor, polymorphism

Poster code: R-02-11-013

Poster Title: Vitamin B1, B2, B6 concentration and LVEF in patients with chronic heart failure

PhD candidate: Marija Božović

Part of the thesis: Association of vitamins B1, B2, B6 and oligoelements Zn, Cu, Se, Mg concetrations with stage and course of illness in patients with chronic heart failure

Mentor(s): Associate Professor Matias Trbušić, MD PhD, Nora Nikolac Gabaj, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Chronic heart failure is the result of structural and functional changes in the heart, with the changes in energy production processes. The most common clinical indicator of heart failure is LVEF (left ventricular ejection fraction), which is determined by echocardiography and represents the ejection fraction of the left ventricle. According to ESC guidelines from 2016 (Acute and Chronic Heart Failure European Society of Cardiology Clinical Practice Guidelines), patients with heart failure can be divided into 3 groups: 1) LVEF greater than 50%, HFpEF (heart failure with preserved ejection fraction); 2) LVEF 40-49%, HFmrEF (heart failure with mid-range ejection fraction); 3) LVEF < 40%, HFrEF (heart failure with reduced ejection fraction). Specific aim of our research was to determine whether there is a connection between the concentrations of vitamins B1, B2, B6 and the stage of the disease expressed by LVEF and by NTproBNP concentration.

Materials and methods: The research included patients ages 18-80 from Clinical department of Cardiology, Sestre Milosrdnice University Hospital Center, Zagreb with diagnosis of chronic heart failure based on clinical picture and echocardiography findings according to the ESC guidelines. Two blood samples per patient were drawn, one VACUETTE® TUBE, 3 mL, K3EDTA (Greiner Bio-One, Kremsmünster, Austria) was used for vitamins B1, B2, B6 and one VACUETTE® TUBE, 4mL, Z No Additive (Greiner Bio-One, Kremsmünster, Austria) was used for NTproBNP concentration determination. Vitamin B1, B2, B6 concentrations were analyzed using the LC system Shimadzu Nexera and the MS/MS system Shimadzu LCMS-8050, Kyoto, Japan with commercial kit Recipe ClinMass® Complete Kit for Vitamins B1, B2 in Whole Blood and Vitamin B6 in Whole Blood, Munich, Germany. NTproBNP concentration was determined using Abbott Alinity i, IL, USA. Statistical analysis was performed using MedCalc® statistical software version 20.008 (MedCalc software, Ostend, Belgium).

Results: 42 patients were included in the research, 25 (0.60) were men aged 71 (30- 86) years. The concentration of vitamin B2 was statistically significantly higher in men than in women (408 (338-443) vs. 336 (326-388) nmol/L; P=0.047), while concentrations of vitamin B1 (181 (149-184) vs. 157 (148-184) nmol/L; P=0.183) and vitamin B6 (76 (64-119) vs. 66 (56-82) nmol/L; P=0.115) did not statistically significantly differentiate according to gender. Differences in vitamin B1, B2, B6 concentrations between patients subgroups, classified by LVEF (Group 1: ≥ 50% vs. Group 2:≤ 50%), were examined. No statistically significant difference was found in vitamin B1 (162 (142-194) vs. 182 (157-207) nmol/L); P=0.146), vitamin B2 (370 (330-416) vs. 408 (359-453) nmol/L; P=0.240) and vitamin B6 concentration (71 (57-91) vs. 79 (66-120) nmol/L; P=0.230) between subgroups. There is no statistically significant correlations between the concentration of NTproBNP and vitamin B1 (r=-0.07, P=0.767), vitamin B2 (r=-0.01; P=0.996) and vitamin B6 (r=-0.02; P=0.947).

Discussion: Previous studies have shown that the deficiency of vitamin B1 varies from 13% to 96%, vitamin B2 up to 27% and vitamin B6 up to 38% in patients with chronic heart failure. We wanted to determine whether there is a connection between the concentration of vitamin B and the stage of the disease expressed through LVEF. That is, whether patients in the worse LVEF category have a worse status of vitamins B1, B2 and B6. Our study showed that there is no statistically significant difference in the status of vitamin B and the stage of the disease expressed through LVEF. Also, there is no statistically significant difference between the concentration of NTproBNP and the concentration of vitamin B. Our results show that a worse status of vitamins B1, B2, B6 does not necessarily indicate a worse stage of the disease, expressed through LVEF, neither the higher NTproBNP concentrations.

MeSH/Keywords: chronic heart failure, vitamin B, LVEF, NTproBNP

Poster code: R-02-11-050

Poster Title: Plasmatic microRNA levels as a biomarker of hyperinflammation in COVID-19 patients - preliminary results

PhD candidate: Ivan Marković

Part of the thesis: Plasmatic microRNA levels as a biomarker of hyperinflammation in COVID-19 patients

Mentor(s): Associate Professor Filip Sedlić, MD PhD, Marko Lucijanić, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Some COVID-19 patients develop severe disease with respiratory failure. Approximately 15% of these patients develop hyperinflammatory responses and acute respiratory distress syndrome (ARDS). MicroRNAs (miRNAs) regulate gene expression through binding to target messenger RNA and inhibiting translation. MiRNA-146a and miRNA-126 inversely correlated with levels of proinflammatory cytokines. MiRNA-181b was downregulated in sepsis. MiRNA-887 acted as a biomarker of ARDS in patients with sepsis. Complement activation contributes to the pathogenesis of COVID-19. Lower expression of miRNA-5571-5p and miRNA-766-3p was related to hypocomplementemia in immune-mediated disease. MiRNAs are potential biomarkers in many diseases. In COVID-19, existing biomarkers lack accuracy in early identification of patients with high risk for critical illness.

Materials and methods: Three groups are included: 75 COVID-19 patients with critical illness (high-flow oxygen or mechanical ventilation), 45 COVID-19 patients with severe disease (oxygen supplementation), and 40 healthy controls. The patients were recruited at University Hospital Dubrava from December 2020 to June 2021, and the controls at Croatian Institute of Transfusion Medicine from February 2021 to June 2021. All blood samples were collected, and platelet-poor plasma was stored at -80 ^{o}C. The levels of complement components C3 and C4, and interleukin 6 (IL-6) have been determined in all COVID-19 patients. Total RNA has been isolated, and the expression of miRNAs is going to be determined by qPCR in all participants. A subgroup of 12 patients, who developed critical illness after the first blood sampling, underwent additional sampling. Statistical analysis was performed by Student's t-test, ANOVA, and Tukey test for post hoc comparison of mean ± standard error values, and Kruskal-Wallis and Mann-Whitney test for median with interquartile range values. Correlation between certain values was examined by Spearman's correlation test.

Results: Critically ill COVID-19 patients had significantly lower levels of C3 and C4, and significantly higher levels of IL-6, compared with patients with severe disease (0.98 ± 0.24 g/L, vs 1.37 ± 0.32 g/L, $p<0.001$ for C3; 0.21 ± 013 g/L, vs 0.39 ± 0.17 g/L, $p<0.001$ for C4; 90.7 (46.5-333) pg/mL vs 6.5 (3.3-13.5) pg/mL, $p<0.001$ for IL-6). In contrast, no significant differences were found in the levels of IgM ($p=0.814$), IgA ($p=0.133$), and IgG ($p=0.133$) between the groups. The determination of the expression of target miRNAs is pending.

Discussion: Discovery of new biomarkers for early identification of COVID-19 patients with high risk of developing critical illness would help in selecting patients who would benefit the most from timely initiation of immunomodulatory treatment, and hence improve the prognosis. So far, we have demonstrated significant differences in C3, C4, and IL-6 levels between patients with critical illness and less severe disease. Serial determination of these parameters may be useful in estimating the risk. However, we expect miRNAs to prove themselves as more sensitive biomarkers at earlier stages of the disease.

MeSH/Keywords: MicroRNA, COVID-19, inflammation, biomarker, respiratory distress syndrome

Poster code: R-02-14-067

Poster Title: Composition of the bacterial microbiota of donated human milk

PhD candidate: Jurjana Novoselac

Part of the thesis: Composition of the bacterial microbiota of donated human milk

Mentor(s): Assistant Professor Branka Golubić-Ćepulić, MD PhD, Associate Professor Zrinka Bošnjak, MD PhD

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Introduction: Previous research has proven the correlation between the bacterial diversity of human milk and different characteristics of the mother, the child, and the milk. Bacteria from the genera Bifidobacterium and Lactobacillus and other lactic acid bacteria (LAB) which act as probiotic bacteria are effective in the prevention of necrotizing enterocolitis (NEC), for which premature children are especially susceptible. There is not enough data on the presence and representation of bacterial microbiota in donor human milk (DHM). The aim of the research is to identify the presence of certain bacterial genera within DHM, with an emphasis on LAB.

Materials and methods: Archival samples from pools of raw donated milk expressed in the first 6 months after birth from 88 donors were included in the research. The samples met the microbiological quality requirements for clinical use. Two groups of samples were compared- those of donors who gave birth vaginally and were not exposed to antibiotics in the previous 6 months (N=53) and samples from donors who gave birth by Caesarean section or vaginally but were exposed to antibiotics in the previous 6 months (N=35). Data on donors, their children and milk characteristics were collected from the human milk bank records. Additional anaerobic cultures were made on selective media for LAB. Bacterial deoxyribonucleic acid (DNA) was isolated from the DHM samples and hypervariable region V1-V3 of the 16S ribosomal RNA (rRNA) gene was amplified. Next generation sequencing (NGS) was performed on Illumina platform and data on nucleotide sequences within the V1-V3 region were analyzed to obtain a taxonomic classification of bacteria within the samples.

Results: Staphylococcus was the most represented genus, present in all analyzed samples, in a percentage from 0.5 to 88 %, followed by genera Streptococcus, Burkholderia, Acidovorax, Ralstonia, Enterobacter, Lactobacillus, Acidithiobacillus, Klebsiella and Enterococcus. Lactobacillus was also present in all samples, in a percentage from 0.06 to 62%. Bacteria from genera Bifidobacterium were present in 62 (70%) of samples, in smaller relative percentage, with the largest within one sample being 3.6%. By comparing the groups of donors who were exposed to antibiotics and those who were not, no statistically significant difference was found according to the presence of LAB ($p=0.128$ for Lactobacillus, $P=0.152$ for Bifidobacterium). A comparison of other characteristics of mothers, children, and milk with the representation of bacterial microbiota is ongoing.

Discussion: According to the data processed so far, no correlation was found between the presence of LAB and the characteristics of milk donors. A better knowledge of the microbiota of DHM could be significant in the nutrition of the most vulnerable group of children. Maternal or raw DHM of a certain bacterial composition could be used in the future to restore the desired microbiota of pasteurized milk with the aim of personalized nutrition.

MeSH/Keywords: Bacteria, Microbiota, Human Milk, High-Throughput Nucleotide Sequencing

Poster code: R-02-16-006

Poster Title: Divergent association between resistance and virulence of Klebisella pneumoniae high risk clonal lineages ST86 and ST101

PhD candidate: Irina Pristaš

Part of the thesis: Virulence and resistance factors in invasive and non-invasive clinical isolates of Klebsiella pneumoniae bacteria

Mentor(s): Professor Branka Bedenić, MD PhD, Professor Arjana Tambić-Andrašević, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Recent reports indicate a rising incidence of hypervirulent Klebsiella pneumoniae (hvKp) in Europe, which, along with classic pathotype (cKp), represents a dual threat due to its potential to cause severe infections in otherwise healthy individuals and its possession of both virulence and resistance genes.

Materials and methods: In 2022, a cross-sectional study was conducted in three regions of Croatia, collecting 200 K. pneumoniae isolates: 100 invasive (blood and cerebrospinal fluid) and 100 non-invasive (urine) samples. One isolate per patient was included, obtained during routine clinical examinations for suspected indications. Laboratory analysis involved identification, string test, phenotypic and molecular detection of resistance mechanisms, and genome sequencing. Genomic DNA was extracted using the MasterPureTM Complete DNA and RNA Purification Kit (Epicenter, USA), followed by sequencing on the Illumina Miseq platform (Illumina, USA). Next generation seqencing (NGS) data was analyzed with BacPipe software package to detect genes linked to hypervirulence and antibiotic resistance. Sequence types (ST) were determined using multi-locus sequence typing (MLST) analysis, while ChewBBACA software determined cgMLST. Kleborate assessed virulence and resistance scores based on the presence of the virulence factors and resistance genes.

Results: Genomic data was obtained from 194 (97.0%) of collected isolates. The results of the ST profiling indicate a high level of diversity (Simpson Diversity Index = 0.926) among the analysed strains. The most prevalent clonal lineage in this K. pneumoniae dataset belonged to ST101 and it was detected in 45 (24.6%) isolates. Blood samples had lower ST diversity, with ST101 being the most prevalent (30.4%), and was linked to the presence of invasive devices, whereas urine samples exhibited higher ST diversity. Molecular typing employed cgMLST, an extension of traditional MLST, focusing on identifying alleles from a specific cgMLST scheme to generate a distance matrix. We highlighted 12 clusters, corresponding to STs found in more than 3 isolates. The Kleborate tool assessed antibiotic resistance and virulence factors in K. pneumoniae genomes. Virulence score of 0 indicates the absence of yersinabactin, colibactin, or aerobactin, whereas a score of 5 indicates the presence of all of them together. Resistance scores range from 0 to 3, based on detection of genotypes warranting escalation of antimicrobial therapy (ESBL < carbapenemase < carbapenemase plus colistin resistance). Most genomes (48.6%) had score 0, followed by score 2 (28.4%), score 1 (22.4%), and score 3 (0.56%) for resistance. Regarding virulence, 59.0% of isolates scored 1, while 34.4% scored 0. Strains with higher virulence scores were less prevalent. Six isolates (3.3%) were genotypically more virulent (virulence score 4), distributed among ST86 (2.2%), ST25 (0.5%), and ST290 (0.5%). ST86 exhibited a significantly higher likelihood of virulence score 4 (OR = 265, $p < 0.001$). Additionally, 45 isolates (24.6%) were ST101, associated with carbapenem-resistant clones, predominantly linked to carbapenemase production (OR = 27.6, $p < 0.001$).

Discussion: Through a comprehensive analysis of isolates collected from diverse clinical settings, the study offers valuable insights into the distribution of sequence types, resistance and virulence profiles within the K. pneumoniae population. K. pneumoniae ST101 is an emerging high-risk opportunistic pathogen, mostly reported in hospital-outbreak settings in several countries. In our study the predominance of ST101, especially in invasive isolates, suggests clonal expansion in the hospital setting, mostly related to the higher resistance scores and the presence of invasive device. The emergence of carbapenem-resistant ST101 strains is particularly concerning due to their link with increased morbidity and mortality rates. ST86 is associated with higher virulence scores, consistent with findings from similar studies. In our study we have found a few isolates with higher virulence score and most of them belonged to ST86. The study did not find convergence of resistance and virulence factors within the same strain, a phenomenon that poses a significant threat due to the spread of strains with heightened pathogenicity and resistance, which is already observed in some European countries.

MeSH/Keywords: Klebsiella pneumoniae, virulence factors, resistance, hypervirulence

Poster code: R-02-16-066

Poster Title: Association of atherosclerosis markers and diabetic retinopathy in patients with type 2 diabetes mellitus

PhD candidate: Albina Krasniqi

Part of the thesis: Association of atherosclerosis markers with the presence and severity of diabetic retinopathy in patients with type 2 diabetes mellitus

Mentor(s): Associate Professor Snježana Kaštelan, MD PhD, Professor Gani Bajraktari, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Diabetes Mellitus (DM) is a multisystemic disease associated with both micro-vascular and macro-vascular complications. DM is recognized as a key risk factor in the onset of macrovascular complications, primarily via the process of atherosclerosis. Different atherosclerosis markers, including serological inflammatory biomarkers and also imaging biomarkers by measuring carotid intima-media thickness (CIMT), carotid plaque, ankle brachial index (ABI), coronary artery calcium score (CACs) and central arterial stiffness have been used for assessment of atherosclerotic risk in different population groups. Diabetic Retinopathy(DR) is the most common microvascular complication of diabetes. In most cases microvascular and macrovascular complications of diabetes have been studied as independent disorders. However, current data suggest that these vascular complications may have common pathophysiological mechanisms.

Materials and methods: This study is a prospective clinical study. A total of 160 consecutive patients with type 2 Diabetes Mellitus with demographical, anthropometric, clinical and biochemical data will be included in the study. The inclusion criteria for our study subjects are as follows: all consecutive patients with DM type 2 that are older than 18 years; patients diagnosed with diabetes for at least 5 years; no previous known CV disease, stroke or peripheral vascular disease. We will exclude from our study patients with immunologic and infectious diseases, patients receiving corticosteroids or cytostatics, pregnant women and patients with eye diseases such as hypermature cataract, uveitis, age-related maculopathy, central or branch RVO and glaucoma. All patients will undergo a complete eye examination for assessment of the presence or absence of diabetic retinopathy. Diabetic retinopathy will be evaluated using digital retinal photography. According to retinopathy status, based on The International Clinical Disease Severity Scale, patients will be divided into three study groups: patients without DR (stage 1), patients with mild and moderate nonproliferative DR (stages 2 and 3) and patients with advanced DR (stages 4 and 5). Atherosclerosis will be assessed using imaging biomarkers as carotid intima media thickness (CIMT), carotid plaque, ankle brachial index (ABI), coronary artery calcium score(CACs) by CT angiography and central arterial stiffness. In addition to imaging biomarkers of atherosclerosis we will also evaluate inflammatory markers (including hs- CRP and IL-6). For statistical analysis differences between groups will be analysed using the unpaired Student t test following the analysis of variance, whereas the one-way ANOVA with Bonferroni correction will be performed to compare continuous variables between three study groups. The Chi-square test will be used to compare the categorical variables. Pearson correlations will be performed to identify simple correlations between variables and Cramer's V to measure the association between nominal or ordinal variables. In addition, logistic regression models will be performed to evaluate the relationship of diabetic retinopathy with demographical, anthropometric, clinical, and biochemical parameters.

Results: This study is currently in the phase of data collection. Until now, 14 participants were included, their ophthalmologic status was evaluated and atherosclerosis markers collection is still ongoing. At this stage we did not conduct any statistical analysis as the data would not be sufficient for adequate analysis.

Discussion: The results of this study will be added as the evidence indicating whether atherosclerosis is associated with diabetic microvascular complications such as retinopathy, and will reveal the most appropriate markers to evaluate this association.To the best of our knowledge, this is the most comprehensive evaluation between atherosclerosis and diabetic retinopathy in patients with type 2 diabetes mellitus.

MeSH/Keywords: atherosclerosis, diabetic retinopathy, biomarker

Poster code: R-02-18-124

Poster Title: Saliva and plasma concentrations of TGF-β in cancer cachexia patients

PhD candidate: Ivan Vičić

Part of the thesis: Identification of potential protein biomarkers of cancer cachexia in saliva and plasma

Mentor(s): Professor Lovorka Grgurević, MD PhD, Associate Professor Borislav Belev, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Cancer cachexia is a condition that causes muscle and adipose tissue wasting in cancer patients. This condition significantly affects both the quality of life and the patient's resilience to cancer therapy toxicity. To standardize the evaluation process in cancer patients, identify the cause of weight loss, and detect cancer cachexia patients early in the treatment, a cancer cachexia biomarker is needed. Saliva is a sample that can be used as an alternative to plasma. Its advantages include being cheap, easy to collect, non-invasive and can be taken repeatedly. It can provide distinctive insights into a patient's condition, as it is a plasma ultrafiltrate. This study aimed to determine if saliva is an applicable sample for cancer cachexia research by determining the difference in concentration of transforming growth factor β (TGF-β) – a presumed mediator of cancer cachexia and tumor progression, between the cachectic and non-cachectic cancer patients.

Materials and methods: Seventy-eight patients were recruited during treatment at the University Hospital Center Zagreb. The most prevalent cancers were gastrointestinal cancers (70%), followed by other types of solid tumors. At the time of sample collection, all patients had metastatic disease and were divided into cachectic (per SCRINIO group criteria) and non-cachectic. There were no differences in age and gender between the groups. The patients matched none of the exclusion criteria. Medical history and laboratory results were retrieved from the hospital information system. Plasma samples were obtained by a venepuncture, and saliva samples were obtained by collecting whole unstimulated saliva. Sampling was performed at the same time of the day. The patients gave written consent before sampling, and the study was conducted according to the Declaration of Helsinki. The local ethics committee approved the study.

Results: The two groups did not differ regarding age, with a median age of 60 ($P= 0.170$). Laboratory tests were analyzed on the sampling day (or two days before or after). There were no significant differences in C-reactive protein, albumin, or transaminase serum concentrations between the groups ($P>0.05$). The serum concentration of TGF-β in the non-cachectic group was 73.5 pg/mL (22.1-196.3), and in the cachectic group, 168.3 pg/mL (28.3-224.5). The difference is statistically significant ($P<0.001$). The saliva concentration of TGF-β was 97.3 pg/mL (27.3-176.5) in the non-cachectic group and 216.6 pg/mL (66.3-283.5) in the cachectic group. The difference was also statistically significant ($P<0.001$).

Discussion: The effects of TGF-β in cancer progression and metastasis process have been previously established, and recently, a role in muscle wasting in cancer has been described. These results have shown a difference in the concentration of TGF-β in the plasma of our study population of metastatic patients with and without cachexia, as well as a difference in saliva concentrations. Our study confirms that saliva as a biological sample can reflect plasma concentration of the investigated protein. This is in accordance with the fact that saliva is a plasma ultrafiltrate, although a higher salivary concentration has not been expected. The finding that it is possible to measure a concentration difference in saliva between the groups offers the possibility to use this approach to investigate different aspects of cancer cachexia, a systemic metabolic condition, by a noninvasive and practical method. The limitations of this study include a wide age span in both groups, which is a known factor in saliva protein concentrations, the lack of objective measurement of sarcopenia, a defining trait of cancer cachexia, and the differences in therapy types between the groups that were not analyzed and could have defer the results.

MeSH/Keywords: cancer cachexia, saliva biomarker, cancer metabolism

Poster code: R-02-19-115

Poster Title: Immunohistochemical expression and predictive significance of sphingosine kinase 1 and cyclooxygenase 2 in neoadjuvant treated invasive breast cancer

PhD candidate: Jelena Prošev

Part of the thesis: Immunohistochemical expression and predictive significance of sphingosine kinase 1 and cyclooxygenase 2 in neoadjuvant treated invasive breast cancer

Mentor(s): Associate Professor Marija Milković Periša, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Breast cancer is the most frequently diagnosed cancer in women in Croatia and the world. Despite significant progress in treatment, the long-term prognosis of certain subtypes of breast cancer is not satisfactory, and the discovery of new prognostic and predictive markers is of particular importance. Sphingosine kinase 1 (SphK1) and cyclooxygenase 2 (COX2) are enzymes with a proven role in inflammation and carcinogenesis. Their elevated levels in breast cancer are associated with more aggressive features.

Materials and methods: A retrospective study is being conducted on archived biopsy tissue samples of breast cancer patients diagnosed and treated with a neoadjuvant procedure at KBC Zagreb in the period from January 1, 2018 to December 31, 2022. The expected duration of the research is 2 years. 3 subgroups are1) Her2 positive, 2) luminal B her 2 positive ,3) triple negative. Clinical data and data on the pathohistological features of the tumor are being downloaded from the hospital information system. The identity of the patients included in the research will be replaced by an identification number and will be known only to the researcher and the mentor. The level of SphK1 and COX2 will be read from samples obtained by a broad needle biopsy before treatment. For the purposes of this research, the degree of response measured by the RCB index will be determined in each tumor sample, and the expression of the enzyme in each individual degree of RCB response will be additionally analyzed. The immunohistochemical staining procedure will determine the expression of 2 investigated proteins - SphK1 and COX 2. The immunohistochemical staining process will be carried out in an automated Dako Autostainer at room temperature. Two additional slices of 3-5 µm thickness will be cut on tumor samples processed by standard pathohistological methods and processed immunohistochemically, with polyclonal SphK1 antibody and monoclonal COX-2 antibody.

Results: We have started analyzing the samples from the years 2020. and 2021. So far, we have identified 56 patients that fit the criteria from our research. 25 of them are triple negative, 21 are luminal B Her 2 positive, and 10 are only Her 2 positive. Median age of diagnosis was 56 years. Median size of tumor after surgery was 2,371 cm. 40 women (71%) had verified spread to the axillar lymph nodes before the start of neoadjuvant treatment.

Discussion: At the moment our research is still in the phase of collecting data. So far, we have analyzed some of the data from the years 2020 and 2021. We have observed that the median age of the patients is 56. That is younger than the average age of breast cancer diagnosis according to literature, but also expected considering that we are researching aggressive types that tend to affect younger women. The tumors were bigger in size (T2 stage) and 71% had spread to reginal axillary lymph nodes which is also expected considering that neoadjuvant treatment is optimal for locally advanced breast cancer. We have yet to start the immunohistochemical staining of the biopsy samples which is the main focus of the research.

MeSH/Keywords: breast cancer, SphK1, COX2

Poster code: R-02-19-130

Poster Title: Effect of surgical treatment in patients with moderate to severe obstructive sleep apnea on salivary cortisol concentration.

PhD candidate: Tena Šimunjak

Part of the thesis: Effect of surgical treatment in patients with moderate to severe obstructive sleep apnea on salivary cortisol concentration

Mentor(s): Professor Tomislav Baudoin, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Obstructive sleep apnea (OSA) is a common sleep disorder that affects millions of people worldwide. It is characterized by repetitive episodes of partial or complete upper airway obstruction during sleep, which results in frequent arousals and interrupted sleep.Patients with OSA are also at an increased risk for developing hypertension, cardiovascular disease, and stroke. Diagnosis of OSA is usually performed through a series of tests, including a sleep study, physical examination, and medical history review. The gold standard diagnostic test is polysomnography. The primary treatment of OSA is aimed at reducing the severity of symptoms and reducing the associated health risks. First-line treatment options include lifestyle modifications. In more severe cases, continuous positive airway pressure (CPAP) therapy, which delivers pressurized air to keep the airway open during sleep, is the treatment of choice for severe cases but more than 30% of patients have difficulties to tolerate CPAP. Surgical treatment options for OSA include upper airway surgeries, such as uvulopalatopharyngoplasty (UPPP) , expansion sphincter pharyngoplasty, maxillomandibular advancement and radiofrequency ablation procedures. Several studies have investigated the relationship between cortisol levels and OSA, reporting higher cortisol levels in moderate and severe OSA patients. The negative effects of increased cortisol levels in OSA patients are numerous. One of main concerns is its contribution to insulin resistance, higher blood pressure and higher heart rate. A Meta-analysis that included 637 patients demonstrated that there is a decrease in cortisol levels after conservative treatment with CPAP, however there is no publication that concerns cortisol levels after surgical treatment for OSA.

Materials and methods: This is an interventional prospective non-randomized comparative study. Participants included in study: those with moderate to severe obstructive sleep apnea confirmed by polysomnography. Excluded will be those with: other types of sleep disorders, psychiatric disorders, planned surgery within the next 3 months, and salivary gland pathology. In this study, cortisol levels will be measured in saliva. Participants will be divided into 3 groups with 30 patients in each group: Group 1. Patients with moderate apnea (AHI 15-30) and indication for surgery. Group 2. Patients with severe apnea (AHI>30) and indication for surgery. Group 3. Patients with severe apnea (AHI>30) treated conservatively with CPAP. For groups 1 and 2, the saliva sample for cortisol measurement will be taken 6 times in total. Twice before the surgery (10 days before the procedure and 1 day before the procedure) in the morning and evening. Last two samples will be taken 3 months after surgery in the same hours. For Group 3 the saliva samples will be taken the day before starting treatment with CPAP, and 3 months after the beginning of the treatment. The power analysis for sample size was done. The normality of the distribution will be analyzed by the Sharpio-Wilk test. The t-test of paired samples will be used to analyze the results. Cortisol values before and after treatment will be compared for each group separately and ANOVA will be used for differences between groups.

Results: Currently 48 patients are included in the study. Most of the patients in surgical group had on DISE-VOTE severe obstruction (>75%) in supine position on following levels: 88,9% soft palate, 49.1% oropharynx, 92.6% tongue and 74,1% on the epiglottis level. The concentric pattern of collapse was most frequently observed in patients with severe OSA. On average, participants experienced significant improvement in sleep quality and reduction of snoring Cortisol levels are still under analysis and are not ready yet to be discussed.

Discussion: The study has not yet been completed by a sufficient number of patients to draw the discussion. Expected scientific contribution is that surgical treatment of patients with moderate and severe obstructive sleep apnea leads to a drop-in cortisol levels, which, according to current knowledge, reduces risk for severe complications associated with OSA

MeSH/Keywords: cortisol, OSA, surgery, apnea, CPAP

Poster code: R-02-21-127

Poster Title: Testing and treatment olfactory disfunction in patients with post-acute COVID 19

PhD candidate: Iva Botica

Part of the thesis: Testing and treatment olfactory disfunction in patients with post-acute COVID 19

Mentor(s): Professor Drago Prgomet, MD PhD, Associate Professor Mario Bilić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: In the end of 2019, in Chinese county Wuhan an respiratory epidemic occured. Disease was called COVID 19 and it was caused by a newly discovered strain of the corona virus called SARS-CoV-2. The smell disappeared within 3-4 days after the onset of the disease, within 28 days it recovered in 98% of cases. Mechanism for developing olfactory disfunction after COVID 19 is still in research. If the presence of symptoms last longer than 3 months after acute infection, it is called post-acute COVID 19. A frequent symptom of post-acute COVID 19 syndrome is a long-term impairment of the sense of smell, present in 11% of patients.

Materials and methods: The research plan is to examine the sense of smell in patients suffering from post-acute COVID 19, to compare it after treatment with olfactory training, and to compare the results of the recovery of the sense of smell with the control group. The research started in October 2021. The examination is carried out in the Clinic for ENT and head and neck surgery. The respondents were divided into two groups. The first group consist of patients who have recovered from COVID 19 and notice an impairment of the sense of smell that lasts at least 3 months after infection. Subjects whose sense of smell recovered a little in one period, but still notice impairment of the sense, are also included. A standardized Sniffin Sticks test (Burghardt®, Wedel, Germany) were used. Three subtests were tested - the threshold, discrimination and identification. Olfactory training is carried out by targeted frequent exposure to familiar smells. After four months of olfactory training and mucous membrane washing, the subjects were contacted by phone and control testing were arranged. The results of the control test were compared with the initial test, and the dynamics of the sense of smell was recorded.

Results: Results of olfactory recovery in group of patients with post-acute COVID 19 were compared with olfactory recovery in control group. We had 44 patients with post-acute COVID-19. We performed initial and control testing after conducting 4 months of olfactory training. Average score of olfactory function on initial testing was 23, and on control testing was 29. Recovery of olfactory testing vary from 11 % to 37 %, average value was 26%. In control group, we have conducted both testing on 21 patients, on 16 patients we have conducted only initial testing, in next 2 months we are planning to perform testing after conducting full olfactory training. For those patients who conducted full olfactory training, average score of olfactory function was 28, and after olfactory training was 37. Recovery vary from 21% to 39%, average is 32 %.

Discussion: On our preliminary results, we have anticipated that olfactory training helps in olfactory recovery in patients with post-acute COVID-19. Statistical analysis is still in progress so we can't prove all dimension of recovery. Limited results in control group are similar to studies that has been conducted with patients with other sinonasal diseases that affect olfactory function. Long-term damage to the sense of smell in patients with post-acute COVID-19 is still unexplored, studies have conducted research in patients in the acute phase of the disease. The mechanism of long-term damage to the sense of smell is also unknown. After the rapid spontaneous recovery of the sense of smell after the acute phase, there is a significant percentage of patients who have long-term damage that does not recover with medical therapy. In this research, we apply treatment models that are used for already known viral diseases. We hope to be able to prove that long-term damage to the sense of smell can be recovered after recovering from COVID-19.

MeSH/Keywords: post-acute COVID-19, olfactory training, olfactory functio

Poster code: R-02-21-128

Poster Title: Effects of triple CFTR modulator therapy on chronic rhinosinusitis in cystic fibrosis

PhD candidate: Katarina Đurić Vuković

Part of the thesis: Chronic rhinosinusitis in patients on cystic fibrosis transmembrane conductance regulator therapy

Mentor(s): Assistant Professor Andrea Vukić Dugac, MD PhD, Ana Đanić Hadžibegović, PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Cystic fibrosis (CF) is characterized by multiorgan dysfunction and potentially fatal respiratory failure. Chronic rhinosinusitis (CRS) is among the most common CF comorbidities, with as many as 90% of CF patients showing radiological signs of CRS and over 60% having CRS symptoms. The triple cystic fibrosis transmembrane conductance regulator therapy Elexaftor - Tezacaftor - Ivacaftor (ETI) has been widely prescribed since its approval in 2020 in the European Union. Yet, the effect of triple CFTR modulator therapy on CRS is still unestablished. The aim of this study is to methodically evaluate the effects of an ETI treatment on clinical, biochemical data and quality of life of CF patients with chronic rhinosinusitis in order to demonstrate its efficacy and to compare the effect of this therapy between different CF related diseases and comorbidities.

Materials and methods: Pediatric and adult patients from CF center Zagreb eligible for triple CFTR-modulating therapy (ETI) are being recruited for this research. General and baseline data was collected from hospital information system prior to this recruitment, including: gender, age, age at the time of diagnosis, CF related diseases (e.g. CF related diabetes, pancreatic insufficiency), comorbidities (asthma), CFTR genotype, FEV1, ITM (Z-value for pediatric patients), sweat chloride levels, vitamin D3 levels, total IgE, blood glucose, HbA1c levels and microbiology results of sputum, throat and nasal swab samples. Anamnestic data regarding the number of acute exacerbations and conservative medication of chronic sinusitis in a year prior to recruitment was documented. Patients underwent ENT fiberendoscopy before the initiation of ETI therapy and clinical status of the nose was established using the modified Lund-Kennedy score (presence and extent of polyps, oedema and nasal secretion) and were divided in CRS and non-CRS groups. At the same ENT appointment, they completed a baseline quality of life questionnaire (SNOT-22 or SN-5) and were further subdivided in mild/moderate and severe CRS groups based on its score. Follow-up status of all of the afore mentioned parameters after one year of therapy is being assessed.

Results: At this point, 44 adult and 43 pediatric CF patients are enrolled in the study with most of the adult population (36/44) having completed one year of ETI therapy. We are still following a considerable number of pediatric CF patients (27/43) since the European Medicines Agency (EMA) age approval for initiation of this therapy just recently dropped from 12 to 6 years of age. In the next 6 months we are planning to assess a one-year follow-up status of the remaining pediatric CF patients enrolled and conclude the study. Our preliminary results in adult CF population showed clinical CRS prevalence of 67%. LK scores before initiation of ETI were 8/12 on average and after one-year follow-up they were 2/12 average. Initial SNOT scores in adult population were classified as severe with average score of 86 and after one year of therapy SNOT 22 scores dramatically declined, with an average score of 28 being classified as mild/moderate. Limited results from pediatric CF population showed clinical CRS prevalence of 41%, with initial LK score 5/12 average and 1/12 after one year. In pediatric population SNOT 22 scores were lower, with an average of 56 and 13, respectively. ETI therapy in both adult and pediatric patients that completed the study showed marked improvements in biochemical markers of systemic inflammation along with amelioration of lung function and sweat chloride concentration, comparable to other conducted studies. Since both follow up of pediatric CF patients and statistical analysis are still in progress we are unable to show complete and detailed results.

Discussion: Effects of triple CFTR modulator therapy (ETI) on CRS is still unexplored and not completely understood. Results of this study will contribute to a better understanding of therapy impact on local status, symptoms and control of CRS with regard to CF genotype, patient age and CRS severity. Moreover, results could expand our knowledge of indirect effects of ETI on quality of life, which should always be the ultimate treatment goal for CF patients.

MeSH/Keywords: cystic fibrosis, CFTR modulators, chronic rhinosinusitis

Poster code: R-02-21-141

Poster Title: Comparison of Bacterial Sinonasal Microbiota Depending on the Presence of Nasal Polyposis

PhD candidate: Ivan Raguž

Part of the thesis: Comparison of Bacterial Sinonasal Microbiota Depending on the Presence of Nasal Polyposis

Mentor(s): Associate Professor Zrinka Bošnjak, MD PhD

Affiliation: University of Zagreb School of Medicine; KB Sveti Duh, Klinika za infektivne bolesti Dr. Fran Mihaljević

Introduction: Through our research, we will assess the microbial diversity and composition of the middle nasal passage in patients with chronic rhinosinusitis with nasal polyps (CRSwNP) and compare them with a control population. By analyzing tissue samples, we aim to identify differences in bacterial microbiota that could hypothetically serve as a predictive factor for the development of nasal polyps requiring surgical treatment. This could provide insights for future studies evaluating the role of potential sinonasal mucosal transplantation from healthy donors to CRS patients or the application of sinonasal probiotics.

Materials and methods: The participants are divided into two groups. The first group consists of patients who have undergone surgery for chronic rhinosinusitis with nasal polyps. The second group includes patients for whom endonasal surgery is indicated, but no nasal polyps were detected during endoscopic examination. The total sample size is 32 participants (16 with nasal polyps and 16 without). With this sample size, the statistical power will be 92.4%, and the α error will be 1.5%. The bacterial microbiota of the sinonasal mucosa will be detected from tissue samples of nasal polyps and nasal mucosa using DNA extraction for 16sRNA gene sequencing. We will attempt to define the microbiota that could hypothetically be a predictive factor for the development of nasal polyps requiring surgical treatment through statistical cluster analysis.

Results: After obtaining a positive resolution from the Ethics Committee of KB Sveti Duh, tissue samples have been collected from 7 participants. These samples are stored at -80°C in the Microbiology Department. Once all planned samples are collected, they will be transported in a portable refrigerator to the Clinic for Infectious Diseases for PCR analysis.

Discussion: Numerous studies have attempted to identify connections between chronic rhinosinusitis (CRS) and specific bacteria. S. aureus, Streptococcus sp, Corynebacterium sp, Staphylococcus epidermidis, and Propionibacterium acnes are commonly found in patients with CRS. Additionally, Haemophilus influenza, Escherichia coli, Peptostreptococcus sp, Klebsiella sp, and Fusobacterium can also be observed, albeit in smaller numbers. With the exception of S. aureus, the association between individual bacterial species and CRS is weak. The hypothesis is that CRS does not arise solely from the presence of a single species but rather from changes in the composition or function of the microbial community. Recent literature provides consistent evidence that healthy individuals and CRS patients share similar overall bacterial burdens, but CRS patients exhibit reduced biological diversity. It appears that conservative treatment also impacts the sinonasal microbiota, and certain bacterial strains are associated with better treatment outcomes. The presence of microbial dysbiosis in CRS is supported by numerous studies, although it remains unclear whether this dysbiosis is a cause or merely related to the disease process. Endoscopic sinus surgery is an important treatment modality for patients with chronic rhinosinusitis with nasal polyps (CRSwNP) who have an unsatisfactory response to conservative therapy. Although long-term symptom control is achieved in most patients, recurrent polyps become visible in 40% of operated patients during endoscopic follow-up examinations after just 6 months. Despite endoscopic procedures and concurrent conservative therapy, there has been no progress in controlling polyps over the past 10 years. Apart from the severity of polyps and previous endoscopic surgery, there are no predictable factors. All of this highlights the need for innovative therapeutic approaches. The possibility of future sinonasal mucosa transplantation from healthy donors to CRS patients should be considered. Given the success of fecal transplantation in treating inflammatory bowel diseases, a similar transplantation procedure could potentially be developed for CRS patients.

MeSH/Keywords: microbiota, paranasal sinuses, rhinosinusitis, nasal polyps

Poster code: R-02-21-160

Poster Title: The effect of high glucose concentration on the expression of UPRmt genes LONP1, OMA1, YME1L, HSPD1 and HSPA9 in myocardial samples of patients with heart failure

PhD candidate: Vid Mirošević

Part of the thesis: The effect of high glucose concentration on the expression of UPRmt genes LONP1, OMA1, YME1L, HSPD1 and HSPA9 in myocardial samples of patients with heart failure

Mentor(s): Associate Professor Filip Sedlić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Emerging evidence suggests a link between mitochondrial dysfunction and HF pathophysiology. Despite this, insufficient data exists to elucidate the mechanism of cardiac injury induced by hyperglycemia, resulting in a lack of targeted therapeutic interventions. Cellular stress has the potential disrupt the mitochondrial network, ultimately leading to cell death. The mitochondrial unfolded protein response (UPRmt), a component of the mitochondrial quality control system (MQC), plays a vital role in preserving normal mitochondrial function. Perturbations in mitochondrial protein homeostasis trigger the activation of UPRmt, which aims to either repair unfolded or misfolded proteins using repair proteins (chaperones) or eliminate them through proteases. The impact of elevated glucose levels on UPRmt remains unknown.

Materials and methods: Samples were collected from the removed diseased heart of heart transplant patients. Those samples were dived into 2 groups; HFHG (heart failure with hyperglycemia), defined with elevated HbA1c level in the blood (>6.5%, n=31), and HFNG (heart failure with normoglycemia) defined by normal HbA1c levels in the blood (<6.5%, n=34). Six control samples of healthy hearts, without significant cardiac pathology, were obtained from the National Disease Research Interchange (NDRI). Gene expression was conducted using quantitative polymerase chain reaction (qPCR). RNA concentration was determined using the Nano Drop ND-1000 spectrophotometer. The obtained data were used to calculate the reverse transcription reaction so that 1000 ng of RNA would be added to the reaction mixture. Reverse transcription was performed using the High Capacity cDNA Reverse Transcription Kit on the ProFlex PCR System. The synthesized cDNA was utilized for real-time quantitative polymerase chain reaction (qPCR) analysis using TaqMan probes. The analasys of statistically significant differences among individual experimental groups was conducted using one-way (ANOVA) with Tukey post hoc test. P-values less than 0.05 were considered significant.

Results: Our analysis revealed significant differences in the expression of three genes between the two heart failure groups: LONP1, HSPD1, and YME1L. Among these genes, LONP1 exhibited markedly elevated expression in the HFHG group compared to the HFNG group ($p < 0.05$). LONP1, a mitochondrial protease involved in protein quality control, plays a crucial role in maintaining mitochondrial homeostasis. The measured HSPD1 gene, which codes for the HSP60 protein, a molecular chaperone essential for protein folding within the mitochondrial matrix, demonstrated significantly higher expression levels in the HFHG group compared to the HFNG group ($p < 0.05$). Furthermore, YME1L, a mitochondrial protease involved in the processing and turnover of proteins, exhibited elevated expression in the HFHG group relative to the HFNG group ($p < 0.05$). YME1L plays a critical role in maintaining mitochondrial protein quality by degrading damaged or misfolded proteins. We did not find any differences in the expression of OMA1 and HSPA9 between the 2 groups.

Discussion: Suprisngly all 3 statistically significant differences in gene expression were more elevated in in the HFHG group. Since all 3 of these genes suggest a cardioprotective effect of hyperglycemia, one of the possible explanations for this finding is a non-linear, hormetic response. This response, observed through the gene expression suggests a protective mechanism against mitochondrial dysfunction in HF patients with moderately elevated levels of HbA1C. Some studies analyzing data from the ACCORD study showed that patients receiving standard treatment (not aimed at intensive glycemic control) displayed lower risk of all-cause mortality when average HbA1c was slightly greater (7–8%) than the normal level (<6%). Since the avarage HbA1C in the HFHG groups was 7.4%, this could be a potential explenation for these findings.

MeSH/Keywords: UPRmt, hyperglycemia, heart failure, mitochondria

Poster code: R-02-22-123

Poster Title: Expression of inhibitory receptors LAG-3, TIM-3 and TIGIT in renal cell carcinoma

PhD candidate: Borna Vrhovec

Part of the thesis: Expression of inhibitory receptors LAG-3, TIM-3 and TIGIT in renal cell carcinoma

Mentor(s): Assistant Professor Monika Ulamec, MD PhD

Affiliation: University of Zagreb School of Medicine; Ljudevit Jurak Clinical Department of Pathology and Cytology, University Clinical Hospital Center Sestre Milosrdnice

Introduction: Renal cell carcinoma (RCC) is a malignant disease with the highest mortality rate among genitourinary cancers. Because of its immunogenicity, recently developed immune checkpoint inhibitor (ICI) therapy, has led to significant improvements in survival rates of advanced stage RCC patients. The main goal of ICI therapy is the revitalization of the body's immune response to tumour cells that was previously inhibited by the tumour itself. Unfortunately, some patients are resistant to ICI therapy which is partially explained by the existence of other immune checkpoints which include LAG-3, TIM-3 and TIGIT.

Materials and methods: In this cross-sectional, prospective study, inhibitory receptor (LAG-3, TIM-3 and TIGIT) expression in clear cell and non-clear cell groups of RCC is being investigated, in tumour cells, and their microenvironment, mainly in immune cells surrounding the tumour. Using sample size calculating methods we determined that at least 28 cases per group need to be analysed. Main inclusion criteron was RCC while exclusion criteria include: other kidney tumours and small tumour volume (tumours with less than 5 paraffin blocks). Samples of RCC were obtained by nephrectomy or excision of renal tumours between January 1st, 2016. and December 31st, 2021. Histomorphological analysis was performed. After initial analysis, one paraffin block from each patient was chosen for immunohistochemical analysis using antibodies against inhibitory receptors being investigated (LAG-3, TIM-3 and TIGIT). Semi-quantitative immunohistochemistry is used to determine the percentages of positive epithelial tumour cells and immune cells in tumour microenvironment. Prospective element of the study includes the analysis of treatment outcomes and survival after a follow-up period of one year. After data collection, statistical analysis will be performed.

Results: As of April 25th, 2024, 50% of RCC samples have been analysed: 15 samples of clear cell (ccRCC), 10 samples of papillary (pRCC) and 5 samples of chromophobe (chRCC) RCC. Expression of inhibitory receptors in epithelial tumour cells was as follows: TIM-3 was most prominently expressed in ccRCC epithelial tumour cells with 80% of positive samples and high levels of signal intensity (3/3) with >90% of positive epithelial cells in some samples. By contrast, LAG-3 and TIGIT were each positive in only 13,3% of samples. In pRCC, TIM-3 and TIGIT were both expressed in 60% of samples with high levels of signal intensity (3/3) and up to 95% of positive cells. LAG-3 was positive in only 20% of samples. No expression of inhibitory receptors in epithelial tumour cells was found in any of the 5 chRCC samples analysed. Expression of inhibitory receptors in immune cells found in tumour microenvironment was as follows: Both TIM-3 and TIGIT were expressed in 93,3% of ccRCC samples with high levels of signal intensity (2-3/3) and up to 50% of positive cells per sample. LAG-3 was positive in 73,3% of samples in ccRCC, but with low levels of signal intensity (1/3) and low percentage of positive cells (5%). In pRCC TIGIT and TIM-3 were positive in 90% and 80% of immune cells respectively, with high levels of signal intensity (2-3/3) and cell positivity of up to 20%. LAG-3 was positive in 50% of samples, with 2/3 signal intensity and only 5% of positive cells. In chRCC, TIM-3 and TIGIT were positive in all 5 samples with moderate-to-high signal intensity (2-3/3) but with low percentage of positive cells, up to 5%. LAG-3 was positive in only 20% of chRCC samples (1/5) with moderate signal intensity (2) but in only 1% of cells.

Discussion: Expression of TIM-3 in ccRCC, TIM-3 and TIGIT in papillary RCC epithelial tumour cells is proving to be a potentially significant finding. On the other hand, tumour microenvironment immune cells show significantly higher positivity rates in ccRCC with all inhibitory receptors, especially TIM-3 and TIGIT (93,3% postivity for both receptors). Immune cells were also positive in papillary RCC (90% for TIGIT, 80% for TIM-3 and 50% for LAG-3) and chromophobe RCC (100% for both TIM-3 and TIGIT). With respect to the preliminary nature of the results, there is promise that the expression levels of different inhibitory receptors in epithelial tumour cells and immune cells in the tumour microenvironment can be useful in further research and its potential clinical applications.

MeSH/Keywords: Renal cell carcinoma, immunohistochemistry, tumour microenvironment, immune checkpoint inhibitors, LAG-3, TIM-3, TIGIT

Poster code: R-02-23-073

Poster Title: AUTONOMIC TESTING IN CHILDREN WITH INFLAMMATORY BOWEL DISEASE AND IRRITABLE BOWEL SYNDROME: IN SEARCH OF DYSAUTONOMIA

PhD candidate: Paola Ruška

Part of the thesis: The influence of physical activity on the composition of the intestinal microbiota, dysautonomia and symptoms in children with irritable bowel syndrome

Mentor(s): Assistant Professor Iva Hojsak, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Altered brain-gut-microbiome axis (BGM) is considered the main pathophysiological mechanism in functional gastrointestinal disorders, such as irritable bowel syndrome (IBS). In addition, it has been shown that in patients with inflammatory bowel disease (IBD), in the absence of disease activity, symptoms are largely attributable as functional. The autonomic nervous system (ANS) is an important pathway connecting the brain and the gut. Studies on adult patients with IBS and IBD have shown abnormalities in ANS function; however, there are almost no pediatric data.

Materials and methods: Three groups of children were included in this observational study: those with functional gastrointestinal disorder (IBS), organic disorder of the gastrointestinal tract (IBD in remission), and healthy controls (HC). The aim of this study was to investigate the subjective and objective ANS abnormalities in all groups. Moreover, we aimed to compare ANS function in children with IBS and IBD to that in HC. ANS function was assessed using both objective and subjective tests. For objective evaluation heart rate variability (HRV) analysis was performed along with a standard battery of autonomic tests including quantitative sudomotor axon reflex test (QSART) as well as heart rate (HR) and blood pressure (BP) responses to the Valsalva maneuver, deep breathing, and upright tilt. The severity and distribution of ANS function was quantitated using adrenergic, cardiovagal and sudomotor indices of the Composite Autonomic Severity Scale (CASS). For subjective evaluation, the participants completed the Composite Autonomic Symptom Score (COMPASS 31) questionnaire.

Results: A total of 58 children were enrolled in this study. 23 in IBS group (mean age 15 years, male n=7), 18 in IBD (mean age 14.25 years, male n=7) and 17 HC (mean age 14.83 years, male n=8). Analysis has shown that there is mild impairment of sudomotor dysfunction in IBD group while other objective tests showed no statistically significant differences between groups. Furthermore, children with IBS scored highest on the COMPASS 31, followed by patients with IBD and HC (median scores were 10.9, 5 and 1.6, respectively; $p = 0.001$). Moreover, children with IBS scored the highest on questions that involved various gastrointestinal symptoms, followed by children with IBD and HC.

Discussion: Significant subjective ANS abnormalities were found in children with IBS compared with children with IBD and HC. Therefor symptomatic dysautonomia is most frequently observed in children with IBS, indicating important contribution of ANS abnormalities to pathophysiology of IBS.

Acknowledgments: Founding: Croatian science foundation (IP-2019-04-3028)

MeSH/Keywords: functional disorders, pathophysiology, autonomic dysfunction, pediatrics

Poster code: R-02-24-001

Poster Title: Activin A expression in liver and the outcome of biliary atresia treatment

PhD candidate: Petra Džepina

Part of the thesis: Activin A expression in liver and the outcome of biliary atresia treatment

Mentor(s): Professor Jurica Vuković, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Biliary atresia (BA) is a rare liver disease of unknown etiology which, despite timely Kasai hepatoportoenterostomy (HPE), often progresses to liver cirrhosis. It remains the most common indication for liver transplantation in children. Previous research on the correlation between liver histopathological changes at diagnosis and treatment outcomes has yielded conflicting results. Activin A, a member of the transforming growth factor (TGF-β) family, inhibits hepatocyte growth, suppresses liver regeneration, and induces hepatocyte apoptosis. Although it is increased in fibrotic liver tissue, its expression in BA patients' livers has not yet been investigated.

Materials and methods: A retrospective study included patients who underwent HPE at the University Hospital Center Zagreb from 1986 to 2019. Immunohistochemical staining for activin A was performed on liver tissue samples obtained by liver wedge biopsies during HPE. Patients were divided into three groups based on activin A expression levels in cholangiocytes and hepatocytes (weakly positive (+), moderately positive (2+), and strongly positive (3+) reactions).

Results: The study included 37 patients. Jaundice resolution three months after HPE was achieved in 92.3% of patients with weakly positive activin A reaction, compared to 44.4% of patients with moderate and 40% of patients with strongly positive reaction (p=0.008). Two years after HPE, 92.3% patients with weakly positive activin A reaction were alive with native liver, as opposed to 33.3% of patients with moderate and 46.7% of patients with strongly positive reaction (p=0.007).

Discussion: The expression of activin A in the liver serves as a promising histopathological predictor for the treatment outcomes of biliary atresia after hepatoportoenterostomy.

MeSH/Keywords: biliary atresia, activin A, Kasai hepatoportoenterostomy

Poster code: R-02-24-088

Poster Title: The influence of infection on asparaginase activity in the treatment of acute lymphoblastic leukemia in children

PhD candidate: Matej Jelić

Part of the thesis: The influence of infection on asparaginase activity in the treatment of acute lymphoblastic leukemia in children

Mentor(s): Professor Ernest Bilić, MD PhD, Assistant Professor Mila Lovrić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy. ALL is treated according to international protocols in which asparaginase is one of the most important drugs. Currently, in most countries, PEGasparaginase is used as a first-line formulation. Due to immunogenicity, development of hypersensitivity reactions or silent inactivation can limit its use in some patients which implicate necessity of therapeutic drug monitoring of asparaginase to optimize therapy. Because of intensive chemotherapy, patients are immunocompromised which make them susceptible to infections which are the most common cause of treatment related mortality. Furthermore, one study report that infection increase asparaginase clearance for 38%, but there is no data do and how infection influence on asparaginase activity. Many studies report that plasma asparaginase activity levels above 0.1 IU/mL resulted in complete asparagine in plasma and goal is to maintain asparaginase activity above that cut-off 14 days after administration of drug.

Materials and methods: The study will include patients with newly diagnosed acute lymphoblastic leukemia who are treated in University Hospital Centre Zagreb, Department of Pediatrics, Division of Pediatric hematology and oncology. During induction protocol, patients are receiving equally intensive chemotherapy. PEG-asparaginase is administer at the dose of 1500 IU/m2 on the 12th and 26th day. The enzymatic activity is quantified by the measurement of asparagine acid produced after incubation of plasma samples with asparagine, using a gradient fluorescence reverse-phase HPLC assay and derivatization with opthaldialdehyde. Asparaginase activity is going to be measured 7 and 14 days after administration. Participants will be divided in two groups for each time point (19th, 26th, 33th and 40th day of induction protocol) depending on the presence of infection.

Results: So far, 26 patients have met the criteria for this study. On the 26th day of the Induction protocol, there were 20 patients without infection and 6 patients with infection. Average asparaginase activity in the first group was 369 IU/L, while the average asparaginase activity in the second group was 293 IU/L.

Discussion: Although there are not enough patients for statistical analysis, the results showed that asparaginase activity is lower in patients with infection. Further studies with more patients are needed to conclude association between infection and asparaginase activity. We plan to have at least 50 patients at the end of our study.

MeSH/Keywords: acute lymphoblastic leukemia, asparaginase, infection, children

Poster code: R-02-24-089

Poster Title: Is biliary atresia a ciliopathy?

PhD candidate: Matea Kovačić Perica

Part of the thesis: Pathohistological changes of primary cilia of intrahepatic cholangiocytes in patients with biliary arthritis and their relationship with treatment outcomes

Mentor(s): Professor Jurica Vuković, MD PhD, Professor Marijana Ćorić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Biliary atresia is a rare infantile disease of unknown etiology characterized by the obliteration of extrahepatic bile ducts leading to liver cirrhosis. Cholestasis can be present from birth (fetal form) or develop after a jaundice-free interval (perinatal form). Early Kasai portoenterostomy (KPE) is the only available treatment, and most patients require liver transplant in childhood. Age at the time of KPE and resolution of jaundice 3 months after KPE are prognostic factors for survival with native liver (SNL). Several recent studies found that primary cilia are abnormal in intrahepatic and extrahepatic bile ducts in biliary atresia and that these patients commonly have variants in genes involved in ciliogenesis.

Materials and methods: A cohort of patients with biliary atresia who were treated at the Department of Pediatrics of the University Hospital Center Zagreb over 25 years (from 1998 to 2022) was included. The control group consists of infants diagnosed with idiopathic neonatal hepatitis in whom the liver biopsy has been performed. Various patient data has been extracted from the medical records. Liver tissues are stained by the indirect immunofluorescence (IF) method. Two primary antibodies are used: mouse monoclonal antibody to cytokeratin-7 to visualize cholangiocytes and rabbit monoclonal antibody to acetyl tubulin to visualize primary cilia. Secondary antibodies are fluorescently labeled goat antibody to mouse IgG (Alexa Fluor 647), and goat antibody to rabbit IgG (Alexa Fluor 488). The presence and characteristics of intrahepatic bile duct primary cilia (IHBC) will be analyzed visually and by computer-assisted quantification method. The interrelation between changes in IHBC and short-term (the resolution of jaundice and stool color 3 months after KPE), and long-term clinical outcomes (SNL), as well as the form of the disease (perinatal or fetal), will be analyzed.

Results: 44 patients (21 male, 23 female) were included in the study. 30 patients (68,2%) presented with a perinatal form of the disease, while the remaining 14 patients (31,8%) presented with a fetal form. The median age at the time of KPE was 82.5 days in patients with perinatal form, and 48.5 days in patients with fetal form. At 3 months after KPE 63,3% (19/30) of patients with perinatal, and 50% (7/14) of patients with fetal form achieved good jaundice clearance (p=0,515). At the end of the follow-up, 46,7% (14/30) of patients with perinatal form, and 28,6% (4/14) of patients with fetal form survived with native liver (p=0,333). Indirect IF staining of liver tissues is currently ongoing, and the results are pending.

Discussion: In accordance with other studies, most of our patients presented with perinatal disease form. Patients with fetal form had inferior (albeit not statistically significant) short-term and long-term clinical outcomes. At the moment, the rest of the results of our investigation are pending. We expect to find abnormal IHBCs in our patients (especially in those with fetal disease form) and to prove that there is an interrelation between the degree of IHBC disruption and the disease severity and outcomes.

MeSH/Keywords: Biliary atresia; Cholestasis; Primary cilia

Poster code: R-02-24-102

Poster Title: Assessment of breast cancer response to neoadjuvant chemotherapy using shear wave elastography

PhD candidate: Antonio Bulum

Part of the thesis: Assessment of breast cancer response to neoadjuvant chemotherapy using shear wave elastography

Mentor(s): Professor Boris Brkljačić, MD PhD

Affiliation: University of Zagreb School of Medicine, University hospital Dubrava

Introduction: Ultrasound elastography is a newly developed ultrasound technology that detects malignant breast lesions by measuring changes in the elastic properties of breast tissue. There are two types of ultrasound elastography, strain and shear wave elastography (SWE) which is a type of ultrasound elastography by which the elastic properties of breast tissue can be assessed both qualitatively and quantitatively. A significant number of studies have proven the ability of shear wave elastography in the detection of breast cancer and in distinguishing benign and malignant breast lesions. However, only a few preliminary studies have been published that have investigated the contribution of shear wave elastography in assessing the response to neoadjuvant chemotherapy. The assessment of the response to neoadjuvant chemotherapy will be compared with the assessment using magnetic resonance imaging (MRI) which is considered the imaging golden standard.

Materials and methods: This prospective single-centre study is being conducted at our institution and includes female patients diagnosed with breast cancer who are candidates for treatment with neoadjuvant chemotherapy. After pathohistological diagnosis has been confirmed and prior to the start of neoadjuvant chemotherapy treatment the base SWE and MRI examinations are performed. In addition to this, a metal marker is inserted into the tumour bed in order to locate the lesion easier after the treatment has been completed. All patients have been examined using the same MRI and ultrasound machines. Both native and subtraction postcontrast MRI images are obtained. Breast B-mode ultrasound with SWE is obtained during the ultrasound examination. After that, a circular region of interest is placed at the point of the highest measured elastic modulus values of the malignant breast lesion in focus. All patients underwent a surgical resection of the tumour bed after completion of the neoadjuvant treatment after which the residual cancer burden (RCB) is assessed.

Results: So far 21 patients have been included in the study. The multilevel confusion matrix was used to compare the SWE and the MRI findings. In comparison with the golden standard, MRI, SWE showed 100% sensitivity and 90 specificity% in detecting breast cancer response to neoadjuvant chemotherapy. There was a complete agreement between the MRI and SWE findings with the postoperative RCB analysis.

Discussion: According to the results so far, breast ultrasound with the application of SWE is equally effective in assessment of breast cancer response to neoadjuvant therapy in comparison with MRI. SWE has the basis for wider clinical application in the analysis and follow-up of patients with breast cancer undergoing neoadjuvant chemotherapy. In comparison with MRI, breast ultrasound with the application of SWE is more accessible, cheaper and simpler to use in everyday clinical practice and does not require the use of a paramagnetic contrast agent.

Acknowledgments:

MeSH/Keywords: ultrasound, shear wave elastography, magnetic resonance imaging, neoadjuvant chemotherapy, breast cancer

Poster code: R-02-25-008

Poster Title: The effect of low-dose protocol and ultra-low-dose protocol of CT colonography on image quality and polyp detection

PhD candidate: Nikolina Jurjević

Part of the thesis: The effect of low-dose protocol and ultra-low-dose protocol of CT colonography on image quality and polyp detection

Mentor(s): Associate Professor Jelena Popić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: In adhering to the ALARA principle (As Low As Reasonably Achievable), numerous studies have explored methods to minimize radiation exposure in clinical computed tomography (CT) while maintaining image quality and diagnostic accuracy for three-dimensional interpretation.

Materials and methods: A retrospective review was conducted at the University Hospital Merkur, Clinical Department of Diagnostic and Interventional Radiology. The study included 30 clinically indicated standard CT abdomen and pelvis examinations, focusing solely on the single-phase protocol, alongside 30 clinically indicated CTC examinations. The CT colonography (CTC) protocol comprised of Low-Dose CTC (LDCTC) acquisition in the supine position and Ultra-Low-Dose CTC (ULDCTC) acquisition in the prone position. All examinations were performed using a 64-detector CT scanner. Sex, age, height, and weight were collected. Body Mass Index (BMI) was derived by dividing the body mass by the square of the body height and was reported in units of kg/m². Computed Tomography Dose Index Volume (CTDIvol) and Dose-Length Product (DLP)—were collected to quantify the radiation dose to which patients were exposed. CTDIvol represents a standardized measure of the radiation dose output of a particular CT scanner, reported in units of milligray (mGy). DLP, on the other hand, is calculated by multiplying CTDIvol by the scan length and is reported in units of milligray centimeters (mGycm). For each type of examination, the median values of dose quantities—CTDIvol and DLP—were computed, serving as typical dose levels as recommended by the International Atomic Energy Agency (IAEA). To estimate the effective dose, DLP values for each acquisition were converted using a conversion factor (k = 0.015), determined by the patient's size and the scanned region of the body (in this case, the abdomen). The effective dose was reported in units of millisievert (mSv), adhering to the guidelines outlined by the European guidelines on quality criteria.

Results: Among the 60 examinations conducted, 20 involved male patients, while 40 involved female patients. For patients undergoing single-phase CT abdomen and pelvis examinations, BMI averaged (26.7±3.7) kg/m², with a mean body mass of (75±12) kg and an average age of (65±11) years. In contrast, for patients undergoing CTC, the BMI averaged (25.8±3.0) kg/m², with a mean body mass of (74±6) kg and an average age of (69±10) years. During single-phase CT abdomen and pelvis acquisitions, the median CTDIvol was 12.35 mGy, with a corresponding median DLP of 642.1 mGycm. In LDCTC acquisitions performed in the supine position, the median CTDIvol was 5 mGy, accompanied by a median DLP of 258.35 mGycm. Meanwhile, ULDCTC acquisitions in the prone position yielded a median CTDIvol of 3.35 mGy and a median DLP of 180.6 mGycm. The median CTDIvol for LDCTC was 4.7 mGy for women and 5.6 mGy for men. Similarly, the median CTDIvol for ULDCTC was 3.2 mGy for women and 3.75 mGy for men. The mean effective dose for standard single-phase CT abdomen and pelvis acquisitions was 9.3 mSv, which reduced to 4 mSv for LDCTC and further decreased to 2.8 mSv for ULDCTC.

Discussion: To initiate our research, we conducted a retrospective study following the guidelines proposed by a European study on clinical Diagnostic Reference Levels (DRLs), involving a minimum of 30 adult patients (aged > 18 years) with a body mass index between 18.5 and 25 kg/m² or a body mass of 70±15 kg. The aim was to compare dose parameters between LDCTC and ULDCTC protocols with a one-phase CT abdomen and pelvis protocol. We observed that the mean effective dose for a single-phase CT abdomen and pelvis protocol could be as low as 9.3 mSv. In our research, we found the mean effective dose for LDCTC to be 4 mSv. In comparison, our study determined the mean effective dose of the ULDCTC protocol to be 2.8 mSv. Our findings indicate that the effective dose for LDCTC is 2.33 times lower than that of a one-phase CT abdomen and pelvis study, while the dose for ULDCTC is 3.32 times lower. CTDIvol was found to be size-specific and lower in females than males in our LDCTC and ULDCTC acquisitions. Further research will involve a larger patient cohort (N = 100) to strengthen statistical significance.

MeSH/Keywords: Computed tomography colonography; Dose reduction; Low dose protocol; Image quality; Polyp detection

Poster code: R-02-25-157

Poster Title: Cognitive functioning and transcranial magnetic stimulation in patients with negative symptoms of schizophrenia: descriptive data

PhD candidate: Ivana Bahun

Part of the thesis: The impact of 20 days treatment with high-frequency repetitive transcranial magnetic stimulation with H7 coil on cognitive functioning in patients with negative symptoms of schizophrenia

Mentor(s): Professor Igor Filipčić, MD PhD

Affiliation: University of Zagreb School of Medicine, University psychiatric hospital Sveti Ivan

Introduction: Cognitive decline often precedes other symptoms of schizophrenia and persists throughout the entire span of the illness, leading to functional impairment. Previous treatment options have not yielded promising outcomes. This study aims to explore the impact of transcranial magnetic stimulation on cognition in patients suffering from schizophrenia with predominant negative symptoms.

Materials and methods: A total of 59 adult patients diagnosed with schizophrenia with predominant negative symptoms participated in a randomized controlled double-blind study conducted in University psychiatric hospital Sveti Ivan in Zagreb. After giving a written informed consent, patients were enrolled into the study based on PANSS positive and negative subscale results, rated by psychiatrists employed in the hospital. Included patients were allocated into treatment or control group using adaptive randomization by minimization method with 1:1 ratio. Over the course of twenty work days, 28 patients were treated with high frequency repetitive transcranial magnetic stimulation with H7 coil, while 31 patients were exposed to inactive treatment with sham coil. Cognitive functions were assessed at the beginning and end of treatment by three psychologists using Auditive verbal learning test, verbal-logical memory subtest and digit span forward and backward task from Wechsler memory scale, Benton visual retention test, block design subtest and digit symbol substitution subtest from Wechsler adult intelligence scale, Trail making test version B, FAS Verbal fluency test, and Penn emotion recognition test.

Results: Out of 59 patients included in the study, 55 completed the treatment. Two patients withdrew their consent without stating a reason, one patient was excluded from the study due to exacerbation of EEG results, and one due to somatic illness. Four patients lack the results from the second measuring point. Our sample comprised of 36 men and 23 women, age 19 to 54 years (Median = 36). The majority of participants have primary or secondary education level, are unemployed, unmarried and have no children. Median value of disease onset was 21 (IQR = 10). Analysis of results obtained on cognitive tests shows an increase in mean values in the second measurement point, for all subtests apart from digit span forward and backward task and Benton visual retention test. Differences between two measurement points are present in both treatment groups. We recorded a high number of missing values in results on Trail making test. Whereas subjects from the active treatment group had higher average results on Trail making test after treatment, average results of subjects from the sham group on the same test were lower after treatment completion.

Discussion: Sample characteristics correspond to scientific knowledge about patients with negative symptoms of schizophrenia. Illness usually starts in later adolescence or early adulthood, which is consistent with our result. Negative symptoms include a decline in motivation, interests, social engagement, speech and affect expression, influencing one's ability to form and maintain interpersonal relationships, and adhere to work demands. Accordingly, it is not surprising that participants struggled with performing on Trail making test B, a measure of executive functioning. Descriptive results on cognitive tests before and after treatment show a promising, ascending trend, though it remains unclear whether there are any significant effects of transcranial magnetic stimulation treatment. Other factors such as training effect or patients' beliefs about treatment effectiveness could influence their achievement on cognitive tasks. Data collection is still ongoing.

MeSH/Keywords: schizophrenia, cognition, transcranial magnetic stimulation

Poster code: R-02-29-105

Poster Title: Morphological and functional assessment of the vagus nerve in multiple sclerosis

PhD candidate: Ana Abičić

Part of the thesis: Morphological and functional assessment of the vagus nerve in multiple sclerosis

Mentor(s): Associate Professor Mario Habek, MD PhD, Ivan Adamec, PhD, research associate

Affiliation: University of Zagreb School of Medicine, University Hospital Centre Zagreb

Introduction: Autonomic dysfunction (AD) has been linked to vagal nerve atrophy in some neurodegenerative diseases, but atrophy of the vagus nerve in multiple sclerosis (MS) and its association with AD has not been studied. The aim of this study was to determine the relationship between the cross-sectional area (CSA) of the vagus nerve and parasympathetic function in people with MS (pwMS) and healthy controls (HC).

Materials and methods: 40 pwMS (65.0% females, 36.03±9.08 years) and 39 HC (79.5% females, 38.23±7.19 years) were enrolled. The subjects underwent an ultrasound of the vagus nerve and testing of the parasympathetic nervous system was evaluated with the respiratory sinus arrhythmia (RSA), Valsalva ratio (VR) and heart rate variability (HRV).

Results: The mean vagal CSA in pwMS was 1.98±0.44 mm2 on the right and 1.72±0.39 mm2 on the left side. The mean vagal CSA in the HC group was 2.08±0.53 mm2 on the right and 1.73±0.36 mm2 on the left side. There was no significant difference between the two groups in right (p=0.33) or left (p=0.90) vagal CSA. In the HC, there was significant correlation between right vagal CSA and RSA (rp=0.330, p=0.040), and right-left vagal CSA ratio and RSA (rp=0.345, p=0.031). The right-left vagal CSA ratio correlated with the LF/HF (rp=0.322, p=0.049). No correlation was observed in pwMS.

Discussion: The ultrasound characteristics of the vagal nerves correlate with the parasympathetic nervous system measures in HC. This correlation was not observed in pwMS.

MeSH/Keywords: autonomic nervous system, multiple sclerosis, vagus nerve

Poster code: R-02-30-028

Poster Title: Association of endothelial nitric oxide synthase gene polymorphisms with the risk of intracranial aneurysm rupture

PhD candidate: Josip Ljevak

Part of the thesis: Association of genetic polymorphisms of endothelial nitric oxide synthase with the risk of intracranial aneurysm growth and rupture

Mentor(s): Professor Zdravka Poljaković-Skurić, MD PhD

Affiliation: University of Zagreb School of Medicine, University Hospital Centre Zagreb, Department of neurology, University hospital Centre Zagreb, Department of Neuroradiology

Introduction: Due to high mortality and morbidity associated with aneurysmal subarachnoid hemorrhage (aSAH) and the risk of preventive treatment of unruptured intracranial aneurysms (IA), precise estimation of risk of IA rupture is imperative. Results of previous studies of endothelial nitric oxide synthase (eNOS) genetic polymorphisms as a risk factor for IA development and rupture are conflicting and seem to be population dependent.

Materials and methods: The aim of the study was to examine association of polymorphisms (27VNTR, G894T, T786C) of eNOS gene with IA rupture and growth. Patients who were treated for SAH or had either diagnostic workup or preventive occlusion of unruptured IA in the period from June 2013 to December 2014 (N=293) in Department of Neurology UHC Zagreb were included in the study. Subjects had neurological and neuroradiological follow-up according to usual institutional protocol (yearly MRI and clinical examination) and were divided in two groups (aSAH, unruptured IA) during 8-year follow-up period. In the statistical analysis of the collected data results of genetic analysis were analyzed as categorical variables (presence of polymorphism, genotype), while association with IA rupture or growth was tested using chi-square test ($\chi 2$). Regression analysis was used to determine statistical significance of IA growth and rupture predictors, as well as the relative effect of established risk factors for SAH. A priori power analysis was performed, with power set at 0,8 and alpha error probability set at 0,05. Sample size was calculated for testing the polymorphism with the smallest expected effect. Level of statistical significance was set at $p<0,05$.

Results: Preliminary statistical analysis of full cohort initially included in the study was performed, testing for association of IA rupture with eNOS polymorphisms 27VNTR, G894T and T786C. No statistically significant difference in IA rupture incidence between male and female subjects was detected. There was no statistically significant association between 27VNTR polymorphism and IA rupture in full cohort ($p=0,211$) and no significant difference in sex distribution. Also, there was no significant association between G894T polymorphism and IA rupture in neither sex ($p=0,085$). When testing for T786C polymorphism, a signal was found that CC allele predicts IA rupture, more pronounced in female subjects, but statistical significance was not reached ($p=0.061$).

Discussion: Results of our preliminary analysis suggest no significant association of eNOS polymorphisms and IA rupture. Risk signal noted for CC genotype of T786C polymorphism is in accordance with results of several previously published studies. While there is evidence of impairment of NO pathway in experimental SAH and increased NO levels in humans following aSAH, extent of effects of eNOS gene polymorphisms is still not fully established. Additionally, even though epidemiological data suggest genetic component of IA rupture, aSAH most likely has polygenic basis. Our preliminary data also suggests that individual risk from a specific eNOS polymorphism shoud be interpreted in context of established non-genetic risk factors for aSAH.

MeSH/Keywords: intracranial aneurysm, eNOS genetic polymorphism, aneurysmal subarachnoid hemorrhage

Poster code: R-02-30-079

Poster Title: The impact of multimodal forms of art therapy on treatment outcomes and quality of life in Parkinson's disease

PhD candidate: Lucija Čondić Jurjević

Part of the thesis: The impact of multimodal forms of art therapy on treatment outcomes and quality of life in Parkinson's disease

Mentor(s): Professor Srđana Telarović, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: In addition to medical treatment, art therapy plays an extremely important role in Parkinson's disease (PD). Various procedures and methods are used to stimulate compensatory and redundancy mechanisms and neuroplasticity. Based on the results of numerous scientific researches, as well as empirically, the extremely positive effect of various forms of art on the treatment of PD has been confirmed, either by accepting (observing, listening to...) art, or through personal artistic-creative expression.

Materials and methods: Prospective randomized study includes two groups of 30 patients with Parkinson's disease. Inclusion criteria are diagnosis of PD according to relevant guidelines, Hoehn and Yahr disease stage 2–3, MMSE test score ≥23 and age ≤80 years. Exclusion criteria are severe chronic diseases that would prevent the respondents from coming and participating in the research (cardiovascular diseases, diseases of the locomotor system…), stroke and other neurological diseases, previous participation in art therapy workshops, color blindness, hearing disorders, hyperuricemia and age ≥80 years. One group, in addition to drug therapy, undergoes cycles of art therapy (art and graphic expression and music therapy) during a period of three years, while the control group remains exclusively on drug therapy. The uric acid level was determined for all patients at the beginning of the study and will be monitored during and after the study. All participants will be continuously evaluated by completing validated questionnaires and scales (Mini-Mental State Examination (MMSE), Beck Depression Inventory Test II (BDI-II), Unified Parkinson's Disease Rating Scale-part III-Motor Scale (UPDRS-III), Pittsburg Sleep Quality Index (PSQI), Rosenberg Self-Esteem Scale (RSES), SF-36 Questionnaire and The Parkinson's Disease Questionnaire (PDQ-39).

Results: Data collection is still in progress. The first evaluation of the examinee was completed (motor skills, cognition, quality of life, uric acid level, etc.). Over the next year we expect the results of the evaluation of the respondents after completing two cycles of art therapy.

Discussion: Preliminary results show a positive effect of art therapy on the subjects, but it is necessary to conduct more cycles of workshops in order to confirm their effectiveness

MeSH/Keywords: Parkinson's disease, art therapy, quality of life

Poster code: R-02-30-080

Poster Title: The use of evoked potentials in determining the form of the disease and the effects of treatment in adults with spinal muscular atrophy

PhD candidate: Marina Petrović

Part of the thesis: The use of evoked potentials in determining the form of the disease and the effects of treatment in adults with spinal muscular atrophy

Mentor(s): Professor Ervina Bilić, MD PhD, Assistant Professor Ivana Jurjević, MD PhD

Affiliation: University of Zagreb School of Medicine, University Hospital Centre Zagreb

Introduction: Spinal muscular atrophy (SMA) is an inherited motor neuron disease that affects the lower motor neurons. Only one paper has been published that raises the doubt that the sensory pathways in the spinal cord, brainstem and cerebrum are also affected. The work involved children and the research was done before medications for SMA were discovered. The aim of this study is to examine whether there is a difference in evoked potentials in patients suffering from SMA compared to healthy controls, in results within individual types of SMA and in the therapeutic approach and length of treatment.

Materials and methods: In this study a sample of 50 subjects diagnosed with SMA (type 1,2,3) and a control group of 50 healthy subjects will be analyzed. SMA diagnosis is established based on the clinical picture and molecular genetic analysis. All subjects are registered in the Register of the Centre for Neuromuscular Diseases University Hospital Centre Zagreb and are treated with nusinersen or risdiplam. The following data will be obtained: visual evoked potentials (VEP), brainstem auditory evoked potentials (BAEP) and somatosensory evoked potentials (SSEPm and SSEPt) in 6-month intervals for three years.

Results: The results obtained in the subjects will be compared with a control group matched by gender and age. We will also compare the results of evoked potentials between individual types of SMA, drugs with which the patients are treated, and we will observe the duration of treatment. Out of 50 subjects, 23 are female (46%) and 27 male (54%). The average age of all SMA subjects is 35 years. SMA type 1 has 1 subject (2%), type 2 have 13 subjects (26%) and type 3 have 36 subjects (72%). According to gender SMA type 1 has 1 male (2%), type 2 have 7 males (14%) and 6 females (12%), and type 3 have 19 males (38%) and 17 females (34%). Among them, 13 subjects are treated with nusinersen (26%) that has intrathecal administration and 37 subjects are treated with risdiplam that has systemic administration (74%).

Discussion: Based on preliminary data SMA affects both males and females, with a higher prevalence in males. The majority of subjects suffers from SMA type 3, and most are treated with risdiplam. After 6-month follow-up during 3 years and analyzing all the results of evoked potentials we believe we will prove that in SMA sensory pathways in the spinal cord, brain stem and cerebrum are also affected. We expect that the results of evoked potentials will depend on the type of SMA, therapeutic approach and length of treatment, suggesting that response abnormalities of evoked potentials will be much more frequent in severe form of SMA (type 1 and 2) and that subjects treated with risdiplam will have better results due to systemic administration. Better results are expected also in subjects who are longer in treatment. We strongly believe that results of our research will make a significant scientific contribution and improve the protocols of monitoring, evaluation and assessment of treatment.

MeSH/Keywords: SMA, evoked potentials, nusinersen, risdiplam

Poster code: R-02-30-095

Poster Title: Cognitive outcome in patients with severe COVID-19 disease in different angiotensine converting enzyme gene polymorphisms

PhD candidate: Zrinka Čolak Romić

Part of the thesis: Cognitive outcome in patients with severe COVID-19 disease in different angiotensine converting enzyme gene polymorphisms

Mentor(s): Tereza Gabelić, PhD, research associate, Associate Professor Rok Čivljak, MD PhD

Affiliation: University of Zagreb School of Medicine, Clinical Hospital Dubrava, University Hospital for Infectious Diseases "Dr.Fran Mihaljević"

Introduction: The 2019 coronavirus disease (COVID-19) pandemic has caused high mortality and numerous long-term health consequences in patients, which represents a major public health problem. Neurological symptoms occur in about a third of patients. Involment of the angiotensin converting enzyme (ACE) receptors in the pathophysiology of the disease, as well as polymorphism (DD, ID, II) in the ACE gene, have shown to have effect on severity of the symptoms and mortality rate. The impact of the ACE gene polymorphism on cognitive consequences of the COVID-19 have not been studied so far. The goal of this study is to examine the cognitive outcome in patients with a severe form of the disease of COVID-19 in different polymorphisms of the ACE gene.

Materials and methods: This is a prospective multicentric opservational study conducted in tertiary COVID-19 care centers. Patients hospitalized due to severe form of COVID-19 disease were included, aged from 18 to 65 years. Inclusion criteria were normal levels of vitamin B12, folic acid and TSH, GCS of 15 during examination, O2 saturation ≥93%, axillary temperature ≤37.5^{o}C. Patients with history of prior neurological or psychiatric diseases, head trauma, CNS infection, drug/alcohol abuse, with history of prior cognitive decline (including subjective symptoms), therapy with antidepressants or antiepileptic drugs, acute major depressive episode (evaluated during hospitalisation), severe visual or hearing impairment were excluded from the study. Demographic data, comorbidities, body mass index (BMI), modified Rankin score (mRS), COVID-19 symptoms, laboratory parameters (CRP, feritin, fibrinogen, D-dimeri, prokalcitonin, IL-6, troponin, AST, ALT, GGT), oxygen therapy (days, flow type and duration), duration and complications during hospitalisation were collected and evaluated. ACE polymorphism was studied by real-time polymerase chain reaction (PCR). Clinical examination (including neurological status) and neuropsychological tests Montreal congitive assesment (MoCA) test, Beck depression inventory (BDI) and Fatigue severity scale (FSS) were performed twice during the study period, the first time between the 1st and 28th day of the disease and the second time three months after. The collected data were processed with the usual statistical tests, p value less than 0.05 was taken as significant. For the analysis patients were divided into groups according to ACE gene polymorphism: model with 3 groups (DD, ID, II) and 2 groups (DD, ID vs II; DD vs ID, II).

Results: A total of 157 patients with severe COVID-19 were included in final analysis. 48 female and 109 male, mean age was 55,8. Patients on average had 12 years of education and BMI 30. Most common comorbidity was arterial hypertension (48.4%) and diabetes (22.2%). Only 3% patients were smokers and 7% had history of pulmonary disease (COPB/astma). Average hospitalisation duration was 13,7 days, patients recieved oxygen therapy on average for 10,9 days and in 24.2% of patients treatment required high flow oxygen therapy. Patients carriers of ACE D allele showed lower scores on MoCA test in control period with statistical significance in model with two groups (DD, ID vs II; t−test p=0.006; Wilcox p=0.004) as well as in model with three groups (DD, ID, II; ANOVA p=0.014; Kruskal−Walis p=0.009). They also had lower mRS in the control period and had higher liver tests during the acute phase of the disease. In model with 2 groups (model DD, ID vs II) patients carriers of D alele scored worse on BDI and FSS and were hospitalised for more days than patients with II polymorphism. Patients with DD polymorphism compared with carriers of I allele (ID, II) had lower mRS, lower quality of life, were treated with ramdesivir more often and had altered liver tests.

Discussion: Our results emphasize the role of ACE polymorphism in COVID-19, with patients carriers of D alelle being more susceptible to worse cognitive outcome.

MeSH/Keywords: ACE polymorphism, COVID-19, cognitive outcome

Poster code: R-02-30-132

3. Preliminary research results – public health and healthcare

Poster Title: Breast cancer risk perception in women with family history of breast cancer involved in mammography screening

PhD candidate: Andrea Šupe Parun

Part of the thesis: The role of abbreviated breast magnetic resonance imaging in women with family history of breast cancer involved in National Breast Cancer Screening Program

Mentor(s): Professor Boris Brkljačić, MD PhD, Associate Professor Vanja Tešić, MD PhD

Affiliation: Croatian Institute of Public Health

Introduction: As part of the National Breast Cancer Screening Program, introduced in the Republic of Croatia in 2006, all women aged 50-69 are biannually invited to undergo mammography, regardless of breast cancer risk. This study aims to assess breast cancer risk perception and sources of information about the existence of an increased risk in women with family history of breast cancer involved in mammography screening.

Materials and methods: Research was conducted among women with family history of breast cancer who underwent mammography as part of the 7th cycle of mammography screening (N=178). Data on family history were collected through surveys that participants had filled out when they came in for mammography, and the criteria for inclusion were as follows: one first-degree relative with breast cancer at the age of ≤ 40 years; two or more first-degree relatives with breast cancer, regardless of age; one first-degree relative with breast cancer and one second-degree relative with breast cancer, regardless of age; three or more second-degree relatives with breast cancer, regardless of age. The analysis was carried out using descriptive epidemiology methods.

Results: The results of the conducted research among women with a family history of breast cancer indicate a very high awareness of the increased risk for breast cancer, as 174 out of 178 participants (97%) responded positively to the question of whether they were aware of the elevated risk for breast cancer based on their family history. The research shows that doctors were the primary source (46.6%) in informing the participants about the increased risk for breast cancer, followed by media and internet sources (35.4%). A relatively small percentage (16.3%) of participants got information about the increased risk for breast cancer from family members. All other sources of information in the research had a negligible percentage.

Discussion: Analyzing such a high percentage, it could be concluded that the participants demonstrated a good level of health literacy, indicating their ability to make informed decisions about their health. The very high level of awareness (97.8%) among participants about the existence of an increased risk for breast cancer can be associated with the economic and social characteristics of the participants. Primarily, the participants are from a large urban environment, while, on the other hand, the percentage of participants with the lowest level of education was extremely low (5.6%), Table 2. From this, it can be inferred that women from urban areas with medium to high levels of education are more inclined to actively seek and analyze health information and understand the connection between family history and an increased risk of breast cancer. In general, the mentioned sources of information about the increased risk for breast cancer based on family history reflect the diversity and importance of different communication channels in informing women about this significant health issue.

MeSH/Keywords: Breast cancer, Family history, Health literacy

Poster code: R-03-01-009

Poster Title: Self-reported HPV vaccination status and correlates of HPV vaccination and vaccine hesitancy in a cross-sectional study of emerging adults in Croatia

PhD candidate: Tatjana Nemeth Blažić

Part of the thesis: Knowledge about sexually transmitted infections, sexual behaviour and indicators of sexual health in the Croatian population of young people in the age group 18-25

Mentor(s): Professor Iskra Alexandra Nola, MD PhD, Associate Professor Ivana Božičević, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Human papillomavirus (HPV) vaccination through national immunisation programmes is an important part of cervical cancer and HPV-related diseases prevention. HPV vaccination coverage (HPV-VCR) is a key indicator for evaluation of program implementation. Coverage statistics from administrative data is limited in Croatia. The aim is to present self-reported HPV vaccination status among emerging adults and correlates of HPV vaccination and vaccine hesitancy.

Materials and methods: Data on sexual health were collected in a cross-sectional survey on probabilistic national sample which included Croatian adults 18-25 years old recruited from November 2021 to February 2022 from the commercial online panel. Stratified sampling, by region and settlement size, of targeted age panelists was used, including quota-based sampling with respect to age, gender, and education, to be much the same to target population in Croatia. Stratified random sampling was applied until the planned sample of about 1200 examinees was achieved. Participants completed anonymous online questionnaire on socio-demographics, sexual behaviours, attitudes, knowledge about sexually transmitted infections (STI) and utilisation of sexual and reproductive health services. Participants received an incentive (5 EUR supermarket coupon) for their participation. Analyses included descriptive statistics and multivariate analyses. Data were post-hoc weighted for gender and age and adjusted for clustering effect. All analyses were carried out using the complex samples module in the IBM SPSS 27 software to adjust standard errors for the sampling design.

Results: The sample included 1197 persons aged 18-25 years (Mage = 21.7, 50.6% women). Analyses included 981 participants who correctly answered two “attention trap” questions (52.1% women). The weighted sample and adjusted for clustering effect included 976 participants. Overall, 18.3% of participants (25.0% of women, 11.7% of men) reported that they were HPV vaccinated while 21.9% did not know their HPV vaccination status. Of those vaccinated, 65.6% were women. Most participants (59.1%) knew about the link between HPV and cervical cancer. Of the rest, 36.1% reported that they do not know the answer. The odds of being HPV vaccinated were significantly higher in women (OR=2,46; 95% CI 1.76 – 3.46). The odds of vaccine hesitancy were lower in women (aOR = 0.60), individuals who reported higher perceived STI risk (aOR = 0.89), and those who knew that HPV could result in cervical cancer (aOR = 0.66). Significantly higher odds of reporting vaccine hesitancy were among more religious individuals (aOR = 1.16).

Discussion: The results show low self-reported uptake of HPV vaccination among emerging adults and high level of HPV vaccine hesitancy, especially among men in Croatia. Some countries geographically close to Croatia with a similarly organised health care system achieved by 2017 substantially higher HPV-VCR: Slovenia (46%), Czech Republic (58%), Italy (62%). Low perceived risk of contracting an STI as one of the correlates of HPV vaccine hesitancy could be explained by perceived feelings of invulnerability, which characterise adolescence. The association between religiosity and vaccine hesitancy has been described in the literature and include concerns that vaccination will promote sexual disinhibition among emerging adults. Vast majority of participants who did not know their vaccination status, suggests possible low awareness of vaccination benefits. Study limitations: representativeness of the commercial panel, memory bias, changes in sexual behavior during the COVID-19 pandemic. Findings suggest a need to increase HPV-VCR in Croatia through multidisciplinary approaches for raising awareness about the benefits of HPV vaccine and through implementation of strategies to make vaccination more available. Representative surveys are good data source for programme performance assessment and an additional help in planning targeted interventions for improving HPV vaccination coverage.

Acknowledgments: I would like to express gratitude to Goran Koletić and Ivan Landripet for their help with data collection, and Iskra Alexandra Nola, Ivana Božičević and Aleksandar Štulhofer in providing scientific advice.

MeSH/Keywords: HPV vaccination, self-reported HPV vaccination status, vaccine hesitancy, emerging adults, Croatia

Poster code: R-03-01-144

Poster Title: Incidence and mortality trends of prostate cancer in Croatia, 2006-2016

PhD candidate: Maša Alfirević

Part of the thesis: The association of the regional development indexes and the risk of poverty with epidemiological indicators of prostate cancer in the Republic of Croatia

Mentor(s): Assistant Professor Mario Šekerija, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Prostate cancer is one of the most frequently diagnosed malignancies among males globally. It represents a significant public health concern in Croatia, exhibiting divergent incidence and mortality rates across geographical regions. This study aims to assess prostate cancer incidence and mortality trends in Croatia from 2001 to 2021, with mortality trends extending to 2022. Understanding the changing patterns of prostate cancer epidemiology is essential to provide significant contributions to improving cancer control strategies and the management of population health.

Materials and methods: The incidence data from 2001-2021 were obtained from the Croatian National Cancer Registry. The number of deaths from prostate cancer between 2001 and 2022 was obtained from the Croatian Bureau of Statistics. Data obtained from national cancer registries and mortality databases were used to examine trends in prostate cancer incidence and mortality rates over the specified timeframe. The incidence and mortality rates per 100,000 population were calculated annually, and age-standardised rates were utilised to account for demographic changes over time. Joinpoint regression analysis was conducted to assess the impact on population health.

Results: Over the examined timeframe, the incidence of prostate malignancies totalled 41,855 cases, accompanied by 12,224 fatalities attributed to the disease. Age-standardised incidence rates fluctuated between 54.26 and 130.30 per 100,000 population from 2001 to 2021, while age-standardised mortality rates ranged from 23.14 to 40.85 per 100,000 population from 2001 to 2022. Joinpoint regression analysis delineated two pivotal junctures in incidence trends, revealing a sustained increase with an average annual percentage change (APC) of 1.79% from 2001 to 2014, succeeded by a pronounced surge with an APC of 7.61% from 2014 to 2018, subsequently followed by a marked decline with an APC of -9.12% from 2018 to 2021. Conversely, mortality rates demonstrated a notable decline, with an APC of -0.83% from 2004 to 2022, after an increase in the starting period (2001-2003).

Discussion: The analysis of prostate cancer trends in Croatia in the 21st century reveals significant findings. Factors influencing previously mentioned changes in incidence trends could include the increase in screening practices, advancements in diagnostic modalities, and demographic shifts in the population. The downturn in incidence rates during 2018-2021 could be associated with the impact of the COVID-19 pandemic and lower patient compliance at that time, but it could also be associated with a more conservative approach to detecting prostate cancer. Mortality rates exhibited a consistent decrease (from 2004 to 2022), indicating a steady fall in prostate cancer-related deaths. Those results follow the advancement of treatment in prostate cancer patients. These trends align with global patterns, reflecting an increasing burden of prostate cancer. Comparatively, Croatia's epidemiology trends underscore the need for comprehensive strategies. Efforts to enhance screening, awareness, and treatment options are crucial for mitigating the impact of prostate cancer and improving outcomes. In conclusion, the analysis highlights dynamic patterns in prostate cancer incidence and mortality in Croatia. Urgent action is needed to address rising rates and improve outcomes through evidence-based interventions and collaborative efforts. Further research will evaluate regional variations in association with sociodemographic factors.

MeSH/Keywords: prostate cancer, incidence, mortality, Croatia / epidemiology

Poster code: R-03-01-161

Poster Title: Validation of the Questionnaire to asses nurse's knowledge and attitudes towards vaccination

PhD candidate: Dubravka Pavlović

Part of the thesis: Correlation of geographic and professional characteristics with knowledge and attitudes of nurses about vaccination of children against measles, mumps and rubella

Mentor(s): Assistant Professor Marjeta Majer, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: The results of the mandatory vaccination against measles, mumps and rubella (MMR) in Croatia show continuously lower vaccination coverage in certain counties.The aim of this research is to determine the knowledge and attitudes of primary pediatric and school medicine nurses about MMR vaccination. Questionnaires are commonly used as a data collection tools and ensuring the validity is cruical for quality results.

Materials and methods: Data on the professional and demographic characteristics, knowledge and attitudes, as well as the professional experience and practice of nurses will be collected using the questionnaire method while data on the opinion on the role of nurses, as well as knowledge of the current legislative framework for vaccination qualitative method of in-depth interview. The survey will consist of questions divided into four categories and participants will circle one of a number on a Likert scale. In order to determine the reliability of the measurement scale, Cronbachs alfa coefficient will be determined. The validation of the survey (testing) will be conducted on a sample of nurses. The Principal Components Method (CPA)will be used to examine the functionality of the survey in order to adapt the questions.

Results: The validation of questionnaire started in March 2024. A questionnaire in English was sent for translation to two independent translators, after which discrepancies and need for harmonization of the final version in Croatian will be discussed. Mentor, statistician and psyhologist will be involved in the validation process. A sample of participants (30 nurses) was identified for pilot testing and validation of the questionnaire. Additionally, comprenhensive search of literature databases was conducted in order to identify publications related to implementation and validation of questionnaires about the knowledge and attitudes about vaccines and vaccination. Preliminary review of publications showed implementation of different survey types and methodology and challenges related to comparison of different survey with similar objective.

Discussion: After discussing challenges with mentor it has been decided to dedicate more time to questionnaire validation in order to ensure reliability and the right methodological approach. Validation of the questionnare will be conducted during May 2024 and completed by end June 2024, including consultations with statisticians and preparation of the questionnaire for distribution to targeted research participants. The results of the questionnaire validation and literature review will be analyzed and published by the end of the year in a peer-reviewed journal. Both publications will include collaboration with mentors. The next phase of the research, which is distribution of validated on line survey to intended study participants, is planned from July 2024 onwards. Required approval from ethical comitee has been granted, informed consents have been prepared to ensure smooth implementation of on-line survey when validated and ready for implementation.

MeSH/Keywords: nurse, measles, attitudes, knowledge, vaccination, Croatia

Poster code: R-03-02-048

Poster Title: Risk factors for transitioning from cannabinoids to other substances among young adults

PhD candidate: Maja Valentić

Part of the thesis: Risk factors for transitioning from cannabinoids to other substances among young adults treated for substance abuse

Mentor(s): Professor Mirjana Kujundžić Tiljak, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Although research often highlights cannabis as a gateway substance for other drugs, the factors for transitioning from cannabis to other substances remain mostly unknown.

Materials and methods: This retrospective cohort study will include individuals aged 18 to 25 who were enrolled in the Registry of treated psychoactive substance abusers. Their data will be monitored from January 1, 1995, to December 31, 2023. There will be two groups: individuals whose primary substance remained cannabinoids and those whose primary substance transitioned from cannabinoids to other substances. The subjects will be individually monitored and their data will be anonymized. They will be included in the study at different times based on their treatment start time. Each individual's outcome will be categorized as cannabis as the primary substance or transitioned from cannabis to another substance. The Pompidou questionnaire is a standardized questionnaire used for recording individuals in the Registry. It includes questions regarding their socio-demographic characteristics, drug use habits, psychiatric comorbidities, risky behavior, and legal problems. The properties of the sample will be shown through a frequency distribution. Survival analysis, i.e. the Kaplan-Meier method, will be used to compare the probability of survival between participant groups. The Cox regression model will be used to analyze how the analyzed characteristics affect the probability of survival. We will use multivariate binary logistic regression to analyze the association between the binary outcomes and the characteristics of the participants. The level of statistical significance will be set at $p < 0.05$. IBM SPSS, version 28 (IBM, Armonk, NY, USA) will be used to perform statistical analyses.

Results: A cohort consists of individuals whose data was recorded and followed in the Registry of treated psychoactive substance abusers from January 1, 1995, to December 31, 2023. It includes 14,285 individuals aged 18-25 at entering their first treatment, with cannabinoids being recorded as their first substance. Of the total number of participants in the cohort, 12,075 (84.5%) are young men and 2,210 (15.5%) young women. For 7,144 or 50.0% (M: 44.5%, F: 5.5%) of all the participants in the cohort, cannabinoids remained the primary substance until the end of the follow-up. For 7,141 or 50.0% (M: 40.0%, F: 10.0%) of the participants, other substances became the primary substance, mostly heroin (5,345 or 37.4%, M: 29.5%, F: 7.9%) and cocaine (383 or 2.7%, M: 2.3%, F: 0.4%). The largest number of respondents entered the cohort in 2001 (1,119 or 7.8% of participants) and 2002 (991 or 6.94% of participants), while the smallest number was in 2023 (102 or 0.7% of participants). The average age of the subjects included in the cohort is 20.9 years. The median as well as the mean age of first substance use was 16 for both genders (min. 7, max. 25), whereas the median and mean age of first primary substance use was 17 (min. 7, max. 25), also both genders.

Discussion: The first results show that half of the participants transitioned from cannabinoids to substances such as heroin and cocaine, suggesting a strong potential for cannabis to act as a gateway drug. The significant transition rate to heroin, particularly among young men, shows the need for public health strategies and politics to prevent the escalation of use. The early median and mean ages of first substance use and first primary substance use emphasize the need for preventive measures in early adolescence.

MeSH/Keywords: cannabis, risk factors, substance-related disorders

Poster code: R-03-02-060

Poster Title: The association of socioeconomic and demographic factors with alcohol consumption in the Republic of Croatia before and after the COVID-19 pandemic

PhD candidate: Ana Žegrec

Part of the thesis: The association of socioeconomic and demographic factors with alcohol consumption in the Republic of Croatia before and after the COVID-19 pandemic

Mentor(s): Professor Danijela Štimac Grbić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Alcohol consumption in Croatia exceeds global averages and is associated with numerous negative outcomes. This research aims to investigate the association of socioeconomic and demographic factors with alcohol consumption prevalence and risky alcohol use in Croatia's general population, along with changes in alcohol consumption between 2019 and 2023 during the COVID-19 pandemic. Initial data analysis explores the correlation between age and gender with alcohol consumption patterns in Croatia before the pandemic. Further analyses will include other predefined socioeconomic indicators, as well as data collected in 2023.

Materials and methods: Data from two survey cycles of the Substance Abuse among the General Population in the Republic of Croatia will be used for analysis. General Population Survey collects data on substance use and is developed by the European Monitoring Centre for Drugs and Drug Addiction. It is conducted every four years. The third survey cycle was conducted in 2019 using a representative probabilistic multi-stage stratified sample of 4,994 participants between the ages of 15 and 64. The fourth survey cycle was conducted in 2023. For the analysis, statistical methods of cross-sectional research, descriptive statistics and regression methods for determination of relationship between selected determinants is used.

Results: From September 2019 till December 2019, 4,994 participants were surveyed successfully among which 41.9% were male and 58.1% were female. Data analysis showed that alcohol consumption has been reported by 90.9% of surveyed adults at least once in their lifetime. Alcohol was consumed by most adults (83.3%) in the past year. The age group between 25 and 34 years old had the highest relative prevalence of alcohol consumption in the past year (90%), while the group between 55 and 64 years old had the lowest (71.6%). A higher proportion of men (87.5%) than women (79%) consumed alcohol in the past year. In the month prior to survey, 62.9% of participants consumed alcohol. The highest prevalence of alcohol consumption in the last month was observed among individuals aged 15 to 24 (70%) and the lowest prevalence was found in the age group of 55 to 64 years (51.1%). The highest frequency of alcohol consumption, lasting 20 days or more in the last month, was found in the age group of 55 to 64 years (10.3%) while the lowest was observed in the age group of 15 to 24 years (3.2%). In the last month 12.4% of men and 4.2% of women consumed alcohol for 10 to 19 days, and 10.2% of men and 2.5% of women consumed alcohol for 20 or more days. 52.1% of adults never consumed six or more alcoholic drinks in a row. Daily binge drinking was highest in the age group between 45 and 54 years, at 1.6% and lowest in the age groups between 15 and 24 years and between 35 and 44 years, at 0.6%. In the age group between 15 and 24 years, there were relatively more individuals who binge drank once a month (19.2%) and once a week (13.4%). Average reported age of first alcohol consumption was 16 years and one month. Men engaged in binge drinking significantly more frequently than women.

Discussion: Preliminary analysis found differences between alcohol consumption and gender, with men reporting higher rates of consumption and engaging in more risky drinking behaviours than women. Age was found to influence alcohol consumption patterns, with younger individuals showing higher prevalence rates while older age groups exhibited riskier drinking behaviours. The average reported age of first alcohol consumption was relatively low. Earlier researches show that the age of first exposure to alcohol was found to influence subsequent consumption patterns and that early alcohol consumption may predict frequent drinking and alcohol-related problems later in life, regardless of gender. Further analysis is needed to develop targeted public health interventions aimed at reducing harmful alcohol consumption across different population groups.

MeSH/Keywords: alcohol drinking, demographic factors, Croatia

Poster code: R-03-02-063

Poster Title: A qualitative study on the role and perspectives of informal caregivers in the Republic of Croatia

PhD candidate: Maja Banadinović

Part of the thesis: Informal care as part of comprehensive care for adults in need of long-term care in the Republic of Croatia

Mentor(s): Associate Professor Aleksandar Džakula, MD PhD

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Introduction: Population ageing and the prevalence of multimorbidity represent major challenges for healthcare systems. People who need long-term care have complex conditions requiring both health and social services. Informal caregivers are emerging as an important part of the long-term care ecosystem. This paper aims to explore the position and capacities of informal caregivers in Croatia.

Materials and methods: The research was conducted in two phases. The first phase included the review of bibliographic databases, Web of Science Core Collection, MEDLINE, and Scopus, coupled with document analysis including national legislative documents, policy documents, and statistical reports. In the second phase, two focus groups were conducted with informal caregivers who had cared for a family member. The criteria for the inclusion of participants were that the person is an informal caregiver and not a health professional with at least three months of caregiving experience. Sampling was conducted using purposive sampling through partners and collaborators within the Health Observatory project, complemented by the snowball sampling method. The focus group questions were developed based on the following domains: activities of caregivers in providing care; use of resources in informal care; the relationship between the informal and formal care systems; challenges and opportunities for the development of informal care. The study included 15 participants (13 women and 2 men), representing all four NUTS2 regions of Croatia, with ages ranging from 40 to 68 years. All participants cared for a family member, with their caregiving experience varying, from 3 to 35 years. The focus groups were audiovisually recorded and subsequently transcribed. All participant data were anonymized, with unique identifiers assigned to each individual. Focus groups were conducted using the Zoom platform. Participants received written informed consent via email before participating. The transcripts were analyzed using thematic analysis according to the Braun and Clarke protocol. Initially, authors identified coding patterns and developed initial codes. These codes were then discussed and refined by the authors to align with the research questions, followed by axial coding into themes and subthemes. A final coding scheme was collaboratively agreed upon by all authors. For the presentation of results, selected quotes that best illustrated each theme and subtheme were used.

Results: The analysis of the documents indicates the fragmentation of national policy into health and social policy. Long-term care as a term is recognised only in health policy. However, some components related to long-term care are part of social policy. Caregivers are recognised in social policy, although not in healthcare. Through the thematic analysis of focus groups' transcripts, the codes were identified and grouped into three themes: position and role of the caregivers in the system and society; types of care based on the recipient's need; and support for the caregivers.

Discussion: The research showed that the process of exercising certain rights and services for caregivers is not sufficiently clear and feasible in practice. There is a lack of a clearly defined role of caregivers and relationships towards professional care providers in the system. The key to improving long-term care is connecting community services, including health and social services, both formal and informal, with the process of providing care.

MeSH/Keywords: Informal care, caregiver, long-term care, integrated care

Poster code: R-03-02-139

Poster Title: Patient satisfaction with explanations provided in family medicine practice according to the age and educational attainment level

PhD candidate: Vesna Štefančić Martić

Part of the thesis: Connection of the Socio-Economic, Demographic and Bihevioral Determinants of Health and the Health Status of Elderly Measured by the Self-Perception Method

Mentor(s): Professor Danijela Štimac Grbić, MD PhD, Assistant Professor Ana Ivičević Uhernik, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: One of the important aspects of patients' experience in use of healthcare services is satisfaction with explanations provided by the family doctor in a way that was easy for patient to understand. Aim was to explore if there are differences in percentages of the patients who were dissatisfied with explanations in family medicine practice according to the age and educational attainment level.

Materials and methods: Analysis was done on 3,868 respondents from the entire Croatia aged 15 years and older which participated in European Health Interview Survey 2019. Percentages (with 95% CI) of respondents who were dissatisfied with explanations were calculated and compared among those under age of 65 years and among those aged 65 years and older, in total as well as according to the educational attainment level which was defined as follows: low: completed primary school or less, medium: completed high school, high completed college or more. All data used for calculations were weighted in order to assure representativeness of the results.

Results: Percentage of those dissatisfied with explanations was 5.0 % (4.0% to 6.2%) among those under age of 65 years, while that percentage was 6.2% (4.8% to 8.0%) among those aged 65 years and older. When percentages are additionally analysed according to the educational attainment level, percentage of those dissatisfied under age of 65 years was 7.1% (4.3% to 11.6%) among those with low, 4.7% (3.6% to 6.2%) among those with medium and 4.1% (2.4% to 6.9%) among those with high educational attainment level, while percentage of dissatisfied among those aged 65 years and older was 6.3% (4.5% to 8.6%), among those with low and 7.7% (5.2% to 11.4%) among those with medium which was significantly higher than 0.7% (0.2% to 2.8%) of dissatisfied among those with high educational attainment level.

Discussion: Exploring patients’ expectations is strongly relevant for ensuring delivery of healthcare and the highest quality of care for patient, especially for those at the primary healthcare level. It is important to achieve the satisfactory balance between patients' expectations from the one handside and family doctors' perceptions on the other handside and priorities set by the healthcare administration. Patients who come for medical exam and/or consultation have level of understanding based on their understanding of the illness, cultural background, health beliefs and attitudes, but also on formal education level. Demographics of the patient and visit characteristics can also contribute this. The impact on the successful outcome of the medical exam/consultation also can be influenced by the level of doctors understanding with the patient. The approach to communicate well and to try to develop a trusting relationship is trustworthy. Doctors should strive to ensure patients understand the rationale for treatment and expectations in individual approach e.g. duration of therapy, side effects, costs, etc. The progress in enhancing patient care, safety, satisfaction, and quality, family medicine doctors should be oriented toward listening and responding to patients’ needs and claims without the delays in communication. Patients as individuals, managing their pain, and providing adequate information on treatment are all crucial, among other elements. Despite the lack of time and overload doctors experience, there should always be a room for patients to ask questions, especially if there are concerns not addressed. High educational attainment level is usually associated with lower percentage of those dissatisfied with explanations provided by the family doctor, which is especially visible among those aged 65 years and older. That emphasizes the importance of quality communication and its adjustment to the educational attainment level of the patient. The continuity of care for patients, especially elderly, will continue to evolve and in order to make patient care more efficient, effective and meaningful, the focus should continue on improving patient care and the value of healthcare for patients. This is where and patient centered care that takes into account the value-based system of care become relevant.

MeSH/Keywords: Pateint Satisfaction

Poster code: R-03-02-146

4. Thesis proposals – basic medical sciences

Poster Title: Efficacy of a single dose of Osteogrow (recombinant human bone morphogenetic protein 6 in autologous blood coagulum) in alveolar ridge regeneration following tooth extraction

PhD candidate: Valentina Blažević

Part of the thesis: Efficacy of a single dose of Osteogrow (recombinant human bone morphogenetic protein 6 in autologous blood coagulum) in alveolar ridge regeneration following tooth extraction

Mentor(s): Academician Slobodan Vukičević, Associate Professor Ana Badovinac, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: After the tooth extraction the alveolar process is prone to major resorption in vertical and horizontal dimension and therefore it is necessary to develop treatment options that prevent bone resorption and induces new bone formation in extraction sockets. rhBMP6 in ABC carrier (Osteogrow) has shown osteoinductive activity in various animal models suggesting a potential to induce the bone healing in extraction socket in human, which will be evaluated in this trial.

Hypothesis: The use of the Osteogrow drug will improve the outcome of the treatment by reducing the need for additional surgical interventions and increasing the alveolar ridge for the placement of dental implants.

Aims: The aim of this clinical trial is to evaluate the efficacy of the Osteogrow drug in the regeneration of alveolar ridge bone after tooth extraction.

Materials and methods: This is the first trial of Osteogrow in patients with severe periodontal disease on teeth that need to be extracted. It is a randomized, investigator blinded, single-center controlled clinical trial conducted in Phase II pilot clinical development that will be conducted at one testing center at the Department of Periodontology, at School of Dental Medicine. 36 patients (18 per treatment group) will be randomized in a 1:1 ratio Placebo:Osteogrow. Patients who meet the inclusion criteria and none of the exclusion criteria will be eligible to participate in the trial. The methods of measurement that will be used in this research include: radiological evaluation, clinical measurements, evaluation of the local healing status, monitoring of side effects, filling in questionnaires for oral health index and pain level evaluation, collection of results and information in an electronic test system and statistical data processing. Subjects will be evaluated preoperatively, intraoperatively, and postoperatively on day 1, day 14, month 1, and month 5. Radiological assessment will be performed with CBCT within 24 hours and 5 months after tooth extraction and Osteogrow administration. The success of the treatment will be confirmed based on the data collected during the 5-month follow-up. Clinical success is defined as an increase in vertical bone height and in the width of the alveolar ridge measured by CBCT, and a greater filling of the extraction alveolus with newly formed bone. The safety component of the study will be defined as the absence of serious adverse events. The statistical analysis will cover defined objectives as well as a set of additional related analyses, including demographics, safety and efficacy. Demographic data and clinical variables will be summarized according to descriptive statistics; measures of central tendency and measures of and tabular and graphical. A statistically significant difference between placebo and Osteogrow will be assessed using a two-tailed t-test. For all normally distributed variables, parametric statistical analysis will be applied, while for variables whose distributions deviate significantly from normality, non-parametric statistical analysis will be applied.

Expected scientific contribution: This clinical investigation will significantly contribute to the assessment of Osteogrow's safety and efficacy in treating severe periodontal disease, thereby expanding our clinical understanding of alveolar bone regeneration.

MeSH/Keywords: bmp, clinical trials, implants, periodontal disease, alveolar ridge healing

Poster code: T-01-01-140

Poster Title: The effect of pentadecapeptide BPC 157 on hemodynamic changes in postembolization syndrome caused by intravascular application of hyaluronic acid in rats

PhD candidate: Lucija Gatin

Part of the thesis: The effect of pentadecapeptide BPC 157 on hemodynamic changes in postembolization syndrome caused by intravascular application of hyaluronic acid in rats

Mentor(s): Associate Professor Anita Škrtić, MD PhD, Assistant Professor Sanda Smuđ Orehovec, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: In the last few years, the application of hyaluronic acid has been on the rise in aesthetic medicine and plastic reconstructive surgery. The most dangerous complication is the intravascular application of hyaluronic acid, which leads to vascular occlusion and embolization. Pentadecapeptide BPC 157 is safe to use and with its cytoprotective and organoprotective role it antagonizes "occlusion/occlusion-like syndrome"

Hypothesis: Rats treated with pentadecapeptide BPC 157 will have reduced multiorgan damage induced by vascular dysregulation syndrome after embolism caused by intravascular injection of hyaluronic acid into the inferior vena cava.

Aims: To demonstrate that BPC 157 has an antagonistic effect on vascular occlusion caused by the application of hyaluronic acid and leads to a reduction of secondary multiorgan damage

Materials and methods: Male Wistar rats will be used, and after anesthesia, hyaluronic acids will be applied in the VCI, and saline solution or BPC 157 solution of different concentrations intragastrically. ECG, brain and blood vessel imaging, invasive pressure measurements with five intervals (5, 15, 30, 60 i 180 min), thrombus mass measurements and histological analysis of the brain, thoracic and abdominal visceral organs as well as abdminal large vessels will be performed

Expected scientific contribution: To demonstrate the therapeutic effect of BPC 157 on postembolization syndrome caused by intravascular application of hyaluronic acid in rats and to enable further research into the possible use of BPC 157 in intravascular occlusion, as a complication of hyaluronic acid application in plastic reconstructive surgery and aesthetic medicine.

MeSH/Keywords: BPC 157, hyaluronic acid, embolism, multiorgan vascular syndrome

Poster code: T-01-03-011

Poster Title: Therapeutic and protective effect of the pentadecapeptide BPC 157 on development of abdominal aortic wall damage and vascular failure syndrome caused by ciprofloxacin

PhD candidate: Ivan Škorak

Part of the thesis: Therapeutic and protective effect of the pentadecapeptide BPC 157 on development of abdominal aortic wall damage and vascular failure syndrome caused by ciprofloxacin

Mentor(s): Assistant Professor Tomislav Meštrović, MD PhD, Associate Professor Lovorka Batelja Vuletić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Abdominal aortic aneurysm (AAA) is an enlargement of the abdominal aorta with diameter greater than 3 cm, or enlargement more than 50% of normal diameter. Rupture of AAA is a catastrophic possible outcome with high mortality. Ciprofloxacin is a broad – spectrum fluoroquinolone antibiotic widely used to treat different bacterial infections and has proven association with AAA development, development of aortic dissection and AAA rupture. There are several, mutually different, rodent models (on mice and rats) used to investigate development of aortic disease using ciprofloxacin. As a novel point, we described ciprofloxacin induced aortic wall damage at 5, 15 and 30 minutes after application along with a general multiorgan vascular failure syndrome. Therapy application of pentadecapeptide BPC 157 counteracted development of multiorgan vascular failure syndrome and maintained aortic wall integrity.

Hypothesis: Abdominal aortic wall damage development and multiorgan vascular syndrome induced by the periadventitial administration of ciprofloxacin will be mitigated by intragastric administration of pentadecapeptide BPC 157 therapy.

Aims: To investigate the therapeutic and protective effect of pentadecapeptide BPC 157 in rats in which the abdominal aortic wall damage development and multiorgan vascular syndrome were induced by the administration of ciprofloxacin.

Materials and methods: BPC 157 will be administered intragastrically in male Wistar rats after periadventitial administration of ciprofloxacin (400 mg/kg), with a control group in which 0.9% NaCl will be administered intragastrically. After 5, 15 and 30 min, the ECG, pressures in the aorta, superior sagittal sinus, inferior vena cava and portal vein and the macroscopic and histological appearance of the brain, thoracic and abdominal organs will be investigated.

Expected scientific contribution: Defining the effect of BPC 157 on mitigating the development of abdominal aortic wall damage and multiorgan vascular syndrome after ciprofloxacin administration.

MeSH/Keywords: pentadecapeptide BPC 157, ciprofloxacin, abdominal aorta, multiorgan vascular syndrome, occlusion - like syndrome

Poster code: T-01-03-020

Poster Title: Effect of pentadecapeptide BPC 157 in rats with systemic consequences of reperfusion injury after transient infrarenal occlusion of the abdominal aorta

PhD candidate: Ivan Brižić

Part of the thesis: Effect of pentadecapeptide BPC 157 in rats with systemic consequences of reperfusion injury after transient infrarenal occlusion of the abdominal aorta

Mentor(s): Assistant Professor Predrag Pavić, MD PhD, Associate Professor Lovorka Batelja Vuletić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: In this work, we want to show the effect of pentadecapeptide BPC 157 in rats with reperfusion damage after transient occlusion of the infrarenal abdominal aorta and concomitant multiorgan vascular syndrome, which has not been described so far. During any operation on the arteries, reperfusion damage necessarily occurs due to the transient stoppage of flow through the operated vessel and consequent ischemia distally. Pentadecapeptide BPC 157 has already shown numerous positive effects on the recovery of the organism after ischemia-reperfusion damage of other etiologies, and we expect an analogy in our work as well.

Hypothesis: Administration of the pentadecapeptide BPC 157 immediately after transient infrarenal occlusion of the abdominal aorta in rats will prevent vascular failure and alleviate reperfusion injury in the brain, heart, lung, liver, kidney, stomach, small intestine, and thigh muscle.

Aims: The aim of the study is to demonstrate the effect of pentadecapeptide BPC 157 on reperfusion injury of the brain, heart, lungs, liver, kidneys, stomach, small intestine and thigh muscles in rats following transient occlusion of the infrarenal abdominal aorta.

Materials and methods: Male Wistar rats will receive intraperitoneal administration of BPC 157 following transient occlusion of the infrarenal abdominal aorta, with a control group receiving 0.9% NaCl. Five minutes, 15 minutes, and 1 hour after clamp release, the macroscopic appearance of organs will be investigated using a microcamera, along with ECG, pressures in the aorta, superior sagittal sinus, inferior vena cava and portal vein. Histological analysis will be performed on the brain, lungs, heart, liver, intestines, kidneys, and thigh muscles tissue.

Expected scientific contribution: The positive effect of pentadecapeptide BPC 157 on reperfusion injury could establish BPC 157 as a potential new therapeutic agent for pharmacological treatment in vascular surgeries and emergency situations involving vascular injuries

MeSH/Keywords: BPC 157, reperfusion injury, aorta

Poster code: T-01-03-029

Poster Title: The effect of pentadecapeptide BPC 157 on the expression of VEGFA, VEGFR2, CKIT, BAX proteins in endometriosis autoimplants and the regression of endometriosis autoimplants in rats

PhD candidate: Fran Rašić

Part of the thesis: The effect of pentadecapeptide BPC 157 on the expression of VEGFA, VEGFR2, CKIT, BAX proteins in endometriosis autoimplants and the regression of endometriosis autoimplants in rats

Mentor(s): Associate Professor Vladimir Banović, MD PhD, Assistant Professor Marko Sever, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: The unsatisfactory therapeutic effect of the treatment of endometriosis is the basis of this research in order to find a new approach to the treatment of the disease. The proven cytoprotective and anti-inflammatory effect of BPC 157 in numerous organ systems and tissue types will be tested in an established rat model of endometriosis.

Hypothesis: The use of pentadecapeptide BPC 157 in a rat model of endometriosis leads to a macroscopically and histologically reduced number and size of endometriosis autoimplants, more frequent occurrence of histological signs of endometriosis regression, lower expression of the proteins VEGFA, VEGFR2, CKIT, and higher expression of BAX in the cells of the glandular epithelium, endometrial stroma and blood endothelium vessel of endometriosis.

Aims: GENERAL OBJECTIVE: To demonstrate the effect of oral administration of the pentadecapeptide BPC 157 on the number and size of endometriotic foci, and the expression of VEGFA, VEGFR2, CKIT and BAX proteins in cells of the glandular epithelium, endometrial stroma and endothelium of blood vessels of endometriosis. SPECIFIC OBJECTIVE: To confirm the dose-dependent effect, onset and duration of the effect of oral pentadecapeptide BPC 157 on the number and size of endometriotic foci.

Materials and methods: Three weeks after the endometrial implantation surgery, an exploratory laparotomy will be performed in order to identify endometriotic foci, and the animals will be randomized into treated and control groups. Animals will receive daily BPC 157 dissolved in saline at a dose of 10 µg/kg and 10 ng/kg orally, and control groups will receive an equivalent volume of 0.9% NaCl. In a period of 2, 4, and 6 weeks, the animals will be sacrificed, with macroscopic analysis of the amount of adhesions and implanted endometriotic foci, histologically semiquantitatively analyzed, with immunohistochemical analysis.

Expected scientific contribution: The scientific contribution of the proposed research would be in the discovery of a potential pharmacological treatment for endometriosis by oral administration of pentadecapeptide BPC 157.

MeSH/Keywords: Endometriosis, Rat, Pentadecapeptide BPC 157

Poster code: T-01-03-043

Poster Title: Sequelae after ligation of retrobulbar structures (optic nerve and vascular eye supply) in rats and the therapeutical effect of pentadecapeptide BPC 157

PhD candidate: Kristina Lončarić

Part of the thesis: Consequences of ligation of retrobulbar structures (optic nerve and eye blood supply) in rats and the therapeutic effect of pentadecapeptide BPC 157

Mentor(s): Assistant Professor Sanja Masnec, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Due to the vulnerability to ischemic injury, ischemic damage in the optic nerve and/or retina presents a formidable therapeutic challenge. Currently, there is no proven effective treatment for traumatic and ischemic optic neuropathies. The stable gastric pentadecapeptide BPC 157 has demonstrated notable neuroprotective effects in various experimental models, promoting healing, and restoring function. Research indicates its ischemia-reducing effects across various experimental models by facilitating collateral pathways and reducing local and systemic ischemic effects as well as its cytoprotective, antioxidant, and anti-inflammatory effects. Recent research in a rat experimental model of glaucoma and in a model of retinal ischemia induced by retrobulbar L-NAME application indicate that BPC 157 effectively preserves the thickness of the retina and optic nerve, normalizes intraocular pressure, and mitigates retinal ischemia.

Hypothesis: Ligating the retrobulbar neurovascular bundle will induce an increase in intraocular pressure, ischemic eye injury, and optic neuropathy, whereas administering the pentadecapeptide BPC 157 will mitigate these effects.

Aims: To mitigate optic neuropathy and ocular ischemic injury resulting from retrobulbar neurovascular bundle ligation by employing the pentadecapeptide BPC 157 in two different doses (10μg/kg and 10ng/kg), administered either intraperitoneally or topically.

Materials and methods: Adhering to ethical guidelines, 72 male Wistar rats weighing about 200g will be randomized into treatment (BPC 157) and control (saline) groups. After establishing deep surgical anesthesia with xylazine 5 mg/kg i.m. and ketamine 100 mg/kg, i.p. (confirmed by the paw withdrawal test), local anesthesia with tetracaine 0.5% drops and mydriasis with tropicamide 1% drops, initial measurements of IOP, corneal evaluation, and fundoscopy will be performed. Then, a surgical suture 3-0 Solus-910® will be used to ligate the retrobulbar neurovascular bundle of the right eye. Immediately after ligation, treatment groups will receive BPC 157 10 μg/kg or 10ng/kg either intraperitoneally or topically (2 drops in the right eye), whereas control groups will receive saline in equivalent application mode and volume. IOP will be measured at 5, 10, 20, 30, and 60 minutes post-ligation using Tono-Pen ®(Reichert, New York, USA). Corneal transparency will be graded at 5,10, and 20 minutes post-ligation (0= normal or 1= mild, 2=moderate, 3=severe loss of transparency). Ocular fundus will be assessed by Digital Wide Field® Lens at 5, 10, and 20 minutes post-ligation and graded (0=normal or 1=mild, 2=moderate, 3=severe retinal and choroidal vessel irregularity and optic disc appearance). At 30 and 60 minutes post-ligation, rats will be euthanized, and their right eyeballs explanted for histological analysis to assess signs of tissue damage (edema, vacuolar degeneration, number of pyknotic nuclei) and retinal layers, total retina, and optic nerve thickness. To test for statistical difference, the ANOVA test with post hoc Tukey-Kramer test will be used for quantitative normally distributed variables, and Kruskal-Wallis with post hoc Dunn test for non-normally distributed quantitative variables and qualitative variables.

Expected scientific contribution: We expect to demonstrate the protective effect of BPC 157 in reducing the damage induced by the ligation of the retrobulbar neurovascular bundle. These findings could contribute towards a potential clinical application in treating optic neuropathies and ischemic eye conditions, which is to date limited.

MeSH/Keywords: Pentadecapeptide BPC 157; optic neuropathy, ocular ischemia

Poster code: T-01-03-114

Poster Title: Therapeutic effect of pentadecapeptide BPC157 on pancreatin-induced model of abdominal aorta aneurysm in rats

PhD candidate: Klaudija Hriberski

Part of the thesis: Therapeutic effect of pentadecapeptide BPC157 on pancreatin-induced model of abdominal aorta aneurysm in rats

Mentor(s): Assistant Professor Predrag Pavić, MD PhD, Associate Professor Lovorka Batelja Vuletić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Aneurysms are defined as a fifty percent dilatation of a native blood vessel compared to expected diameter. In this paper we will use novel approach for causing vessel damage that results in forming aortic aneurysm and concomitant vascular failure syndrome. Pentadecapeptide BPC 157, known for its organoprotective and cytoprotective role, antagonizes vascular failure syndrome by opening new vascular collaterals and hinders aneurysm development.

Hypothesis: Pentadecapeptide BPC 157 will prevent development of abdominal aorta aneurysm and linked multisystem syndrome of vascular failure by reversing the toxic effect pancreatin has on vessel wall.

Aims: To induce the development of abdominal aortic aneurysm by intravenous administration of pancreatin and to investigate the therapeutic effect of pentadecapeptide BPC 157 on the development of aneurysm and multisystem vascular syndrome.

Materials and methods: BPC 157 will be administered intragastrically in male Wistar rats after intravenous administration of pancreatin, with a control group in which 0.9% NaCl will be administered. After 15, 30 i 60 min, the ECG, pressures in the aorta, superior sagittal sinus, inferior vena cava and portal vein and the macroscopic and histological appearance of the organs (brain, lungs, heart, liver, intestines and kidneys) will be investigated.

Expected scientific contribution: To demonstrate the therapeutic effect of BPC 157 on pancreatin induced aortic aneurysm development and concomitant vascular failure syndrome. Furthrmore, the goal is to enable further research into the possible use of BPC 157 in treatment of abdominal aorta aneurysms.

MeSH/Keywords: pentadecapeptide BPC 157, pancreatin, AAA, multisystem vascular syndrome

Poster code: T-01-03-125

Poster Title: Correlation between the peripheral fraction of exhaled breath temperature and the core body temperature in healthy subjects

PhD candidate: Kristijan Šipoš

Part of the thesis: Correlation between the temperature of the peripheral fraction of exhaled air and the internal (core) temperature in healthy subjects

Mentor(s): Associate Professor Mirza Žižak, MD PhD, Associate Professor Davor Plavec, MD PhD

Affiliation: University of Zagreb School of Medicine, Health care facility PRIMA NOVA

Introduction: The human body has to maintain body temperature within a narrow range for the smooth development of enzymatic processes. Thermoregulation maintains a stable core body temperature (Tc), regardless of environmental conditions. The gold standard for Tc measurement is the blood temperature from the pulmonary artery. Since exhaled air comes from inside the body, its temperature could be representative of Tc estimation. Fractions of exhaled breath temperature (fEBT) from central airways are lower and more variable than that from peripheral airways. It remains unknown whether there is a correlation between fEBT, especially the fraction from the peripheral airways, and Tc.

Hypothesis: The peripheral fraction of exhaled breath temperature, from the alveoli and respiratory bronchioles, reflects the core body temperature and does not represent the temperature modulated by processes in the lungs themselves.

Aims: To determine the level of correlation between the exhaled breath temperature, especially the peripheral fraction, and the core body temperature in healthy subjects.

Materials and methods: In this prospective cohort study, a random smaple of 30 healthy volunteers from general population will be selected. The subjects will be monitored for 3 non-consecutive days for a total of 7 days. The research will last 4 months. Inclusion criteria: Healthy subjects (who at the time of the examination do not suffer from chronic or acute diseases of the respiratory system or systemic inflammatory diseases) of both sexes, aged 18-65 y. Exclusion criteria: Those who, at the time of inclusion, are suspected or suffer from an acute or chronic disease of the upper or lower respiratory tract and/or lungs, as well as other acute or chronic systemic inflammatory diseases, and if they are unable to perform the diagnostic protocol for any reason. Sample size: With a coefficient of variability of 3% and a confidence interval of 1.8% from the mentioned work, and a statistical power of 99% and alpha = 0.05, the minimum sample size, required to prove the hypothesis, of 43 pairs of measurements was established. As the temperatures described to each subject are measured at 30 time points, for the planned 30 subjects, that is a total of 900 measurements. After signing the informed consent, the subjects will fill out a questionnaire, a detailed history will be taken and a clinical examination will be done. Different fractions of exhaled breath temperature (fEBT) and cumulative exhaled breath temperature (EBT) will be measured simultaneously with continuous monitoring of core body temperature at several time points and in different environmental conditions (before and after physical activity, meals, hot/cold drinks, staying in warm/cold outdoor conditions) for 3 non-consecutive days for a total of 7 days. Statistical methods: The display of measures of central tendency and variability appropriate to the data distribution of the displayed variables (arithmetic mean and standard deviation or median and interquartile range) will be used to display the results. The distribution type will be tested with the Kolmogorov-Smirnov test. Classified data will be displayed as a number and a share (%). The correlation of individual measured temperatures in several points will be carried out by univariate and multivariate regression analysis, using the intraclass correlation coefficient and comparing methods with Bland-Altman analysis and presentation. $P<0.05$ with correction for multiple comparisons will be used as statistically significant. The analysis will be carried out using statistical software packages STATISTICA v12 and MedCalc v22.

Expected scientific contribution: The outcomes should answer important questions from the physiology of breathing and thermoregulation and give the first answer to the question of whether the measurement of exhaled breath temperature could be used for lung status determination.

MeSH/Keywords: temperature, breath, fraction, core, correlation

Poster code: T-01-04-131

Poster Title: Proteomic study of emphysema biomarkers in non-invasively available biological fluids

PhD candidate: Grgur Salai

Part of the thesis: Discovery of potential molecular markers of emphysema in non-invasively available biological fluids by proteomic profiling

Mentor(s): Professor Lovorka Grgurević, MD PhD, Đivo Ljubičić, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Chronic obstructive pulmonary disease (COPD) is a heterogenous disease that presents with permanently present respiratory symptoms accompanied by airflow limitation. COPD is the third cause of death worldwide and cigarette smoking is one of the most common risk factors. Emphysema is a radio-morphological COPD phenotype characterized by an abnormal dilatation of the air spaces distal to the terminal bronchioles and is characterized by permanent enlargement of air spaces with the destruction of their walls and lung parenchyma. Currently, it is not clear which smoking-induced COPD patients will develop emphysema. Furthermore, there are no validated clinically relevant biomarkers which could determine the presence of emphysema from non-invasive biological fluids.

Hypothesis: Proteomic profile of induced sputum and plasma in patients with emphysema are different in comparison to emphysema-free COPD patients and healthy participants.

Aims: To conduct a proteomic analysis of plasma and induced sputum in patients with emphysema and compare it to patients without emphysema and healthy participants (smokers and never-smokers) in order to detect biomarkers of emphysema.

Materials and methods: A cross-sectional translational research will be conducted in which plasma and induced sputum proteomes will be concomitantly analyzed in patients with COPD and emphysema, patients with COPD without emphysema, healthy never-smokers and healthy smokers. All participants will be stratified according to age, sex and body-mass index. COPD patients will be stratified as GOLD 2B and will be taking dual bronchodilator therapy (without inhalational corticosteroids). Exclusion criteria will be known alpha-1-antitrypsin deficiency, peripheral eosinophilia, concomitant asthma, autoimmune or malignant disease, uncontrolled comorbidities or acute COPD exacerbation in the previous 6 months. Plasma samples will be obtained by standard venipuncture; sputum will be induced by inhalation of normal and/or hypertonic saline. Proteomic analysis will be performed by employing liquid chromatography and mass spectrometry (LC-MS), following bioinformatical data analysis which will identify differentially expressed proteins (DEPs) between research groups. Furthermore, gene enrichment analysis will be conducted. Literature search will be performed for each identified DEP. Based on the previously mentioned steps, a proposal of potential biomarkers of emphysema will be made.

Expected scientific contribution: By comparing plasma and induced sputum proteome profiles of emphysema patients with emphysema-free COPD patients and healthy controls, we would identify potential biomarker candidates, which could, in the future, after additional testing, become clinically relevant.

MeSH/Keywords: proteomics, emphysema, chronic obstructive pulmonary disease, sputum, plasma

Poster code: T-01-05-068

Poster Title: Proteomic analysis of blood plasma and extracellular vesicles of patients with pulmonary disease manifestations of systemic sclerosis

PhD candidate: Stela Hrkač

Part of the thesis: Proteomic analysis of blood plasma and extracellular vesicles of patients with pulmonary disease manifestations of systemic sclerosis

Mentor(s): Professor Lovorka Grgurević, MD PhD, Assistant Professor Joško Mitrović, MD PhD

Affiliation: Center for Translational and Clinical Research, Department of Proteomics, School of Medicine, University of Zagreb; University Hospital Dubrava

Introduction: Systemic sclerosis (SSc) is a rare, chronic autoimmune disease characterised by diffuse fibrosis of the skin and internal organs. The leading cause of death in SSc patients is SSc - associated interstitial lung disease (SSc-ILD) and/or pulmonary hypertension. Pulmonary manifestations of SSc are considered the most common and important internal organ manifestation of the disease. Although prior research has made significant progress in this field, the pathophysiology of SSc is still not fully understood and there is still a need for a reliable, non-invasive biomarker of SSc-ILD. Prior proteomic research has shown promise and yielded results in this field.

Hypothesis: The proteomic profile of blood plasma and extracellular vesicles of patients with SSc-ILD is different compared to patients without SSc-ILD and healthy subjects.

Aims: The aim of this study is to perform a proteomic blood plasma and plasma extracellular vesicle (EV) analysis of patients with SSc in order to analyse underlying pathophysiological processes and to possibly identify a candidate biomarker for SSc-ILD.

Materials and methods: This observational, cross-sectional study will include 60 subjects: 40 SSc patients divided into two groups (w/ or w/o SSc-ILD), as well as a control group consisting of 20 healthy subjects. All included patients have to fulfil the American college of rheumatology (ACR)/European league against rheumatism (EULAR) classification criteria for SSc. Presence of SSc-ILD will be determined by using multi-slice computed tomography (MSCT). MSCT images will additionally be analysed by an experienced radiologist in order to quantify SSc-ILD presence using the Goh score which stratifies SSc-ILD into subcategories based on its extensiveness. Results of pulmonary function tests (spirometry and diffusing capacity for carbon monoxide – DLCO) will also be analysed. Blood samples of the included subject will be collected and centrifuged to obtain blood plasma. EVs will be isolated from the blood plasma samples by centrifugation, which is followed by sonication in order to lyse the EV membrane and release its protein content, which will undergo further purification methods. EV protein isolates, as well as blood plasma samples will undergo quantification of their protein concentration by a commercially available method (BioRad RC DC Proteins Assay). The protein samples will undergo further purification methods, followed by digestion into peptides using overnight incubation with a trypsin solution. The obtained peptides will further be purified and concentrated using C18 Stage tips. The peptides will be separated by high-performance liquid chromatography (HPLC) and sequenced using mass spectrometry (MS). Data obtained by MS will undergo bioinformatic analysis using the MaxQuant v2.4.13.0 programme. Analysis of differentially expressed proteins among the different groups will also be performed, as well as analysis of proteins of potential pathophysiological significance. Gene enrichment analysis of the identified proteins will also be performed using the Funrich 3.1.3. analytical tool. Individual protein analysis will also be performed by searching the available scientific literature. According to the performed analyses, a protein will be selected as a potential SSc-ILD biomarker for further analysis which will consist of quantification by Enzyme-Linked Immunosorbent Assay – ELISA.

Expected scientific contribution: This study will aim to further elucidate the pathophysiological implications of the blood proteome and EVs in SSc and potentially identify a biomarker for SSc-ILD to aid precision in the diagnosis and treatment of SSc.

MeSH/Keywords: proteomics, systemic sclerosis, interstitial lung disease

Poster code: T-01-05-083

Poster Title: The impact of DNA repair mechanisms, MGMT, MMR and BER on the response to temozolomide treatment and occurrence of neoantigens in glioblastoma patients

PhD candidate: Niko Njirić

Part of the thesis: The impact of DNA repair mechanisms, MGMT, MMR and BER on the response to temozolomide treatment and occurrence of neoantigens in glioblastoma patients

Mentor(s): Assistant Professor Anja Kafka, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Glioblastoma is the most common primary malignant brain tumor. Temozolomide is an alkylating agent which activates at least three DNA repair mechanisms: O6-metyhlguanine-DNA-methyltransferase (MGMT), mismatch repair and base excision repair. Their interplay has not been sufficiently analyzed in regards to treatment outcomes. Inactive DNA repair leads to a hypermutator phenotype, neoantigen accumulation and increased tumor immunogenicity.

Hypothesis: The efficiency of DNA repair mechanisms has an impact on the clinical response to temozolomide in patients with glioblastoma and results in the occurrence of specific neoantigens in glioblastoma cells.

Aims: The general aim is to analyse the differences in activity of select DNA repair mechanisms (BER, MMR and MGMT) in GBM patients receiving temozolomide, and to determine their impact on neoantigen occurrence and survival. Specific aims are: 1) To analyse the expression levels of MSH2, MSH6, MLH1, PMS2, MGMT and polymerase beta in paired specimens of the same patient (first specimen - after surgery, prior to receiving TMZ, second specimen - after second surgery and after receiving TMZ); 2) To analyse MGMT promoter methylation status in paired specimens; 3) To determine the association between neoantigen occurence and expression of MSH2, MSH6, MLH1, PMS2, MGMT and polymerase beta; 4) To analyze the impact of MGMT promoter methylation status on the survival of patients; 5) To analyse the impact of changes in expression of MSH2, MSH6, MLH1, PMS2, MGMT and polymerase beta on the survival of patients; 6) To correlate the results with select clinical parameters

Materials and methods: Brain tumor specimens from 50 patients with newly diagnosed glioblastoma will be collected, with 13 paired recurrent tumors. The expression of proteins involved in DNA repair mechanisms will be determined via immunohistochemistry. The occurrence of neoantigens will be examined via whole exome sequencing. Their occurrence in regards to the activity of DNA repair mechanisms will be analysed. The results will be interpreted in the context of clinical and radiological parameters.

Expected scientific contribution: Our results could serve to stratify patients into groups based on DNA repair mechanism activity, and could thereby add to the current body of knowledge regarding diagnostic and prognostic molecular markers in patients with glioblastoma.

MeSH/Keywords: glioblastoma; DNA mismatch repair; MGMT; excision repair; DNA repair

Poster code: T-01-05-151

Poster Title: Influence of dural sinuses anatomy on hemodynamics in different body positions of patients and phantom

PhD candidate: Filip Njavro

Part of the thesis: Influence of dural sinuses anatomy on hemodynamics in different body positions of patients and phantom

Mentor(s): Professor Marijan Klarica, MD PhD

Affiliation: University of Zagreb School of Medicine, Department of Diagnostic and Interventional Neuroradiology (UHC Zagreb), Polyclinic Neuron

Introduction: The mechanisms of regulation of blood volume and pressure, interstitial fluid and cerebrospinal fluid are not fully understood and are the subject of ongoing research.

Hypothesis: Inside the dural sinuses, venous blood pressure behaves according to the law of fluid mechanics and becomes subatmospheric in the upright position, with values corresponding to the hydrostatic difference between the point of measurement and the opening at the bottom of the sinus.

Aims: The general aim of the research is to test the hypothesis that the venous pressure within the dural sinuses is actually a hydrostatic pressure dependent on the anatomical and biophysical characteristics of the sinus, which changes according to the law of fluid mechanics when the position of the sinus changes.

Materials and methods: Through retrospective analysis of MR venography of the brain using post-contrast MP-RAGE sequences at the Department of Diagnostic and Interventional Neuroradiology (UHC Zagreb) and Polyclinic Neuron, we will determine the anatomical and biophysical characteristics of large dural venous sinuses. Using an advanced search in the Radiological Information System (RIS), we will select patients between the ages of 18 and 65 who have undergone MR venography of the brain (20 male and 20 female) and who have completely normal MR findings (including dural venous sinuses and CSF spaces), and for each individual patient we will specify the indication for which the image was taken, age and sex. We will also select twenty patients of both sexes, aged between 18 and 65 years, with a radiological description of hypoplasia of one of the dural venous sinuses and a normal CSF space. Three-dimensional reconstructions of the major dural venous sinuses (upper sagittal sinus, both transverse and both sigmoid sinuses) are then made by manual segmentation using a volumetric software package (ITK-SNAP). From these reconstructions, we will determine the individual and total volumes of the large dural venous sinuses of all enrolled patients, as well as the biophysical characteristics and hydrostatic relationships between individual dural sinuses. According to the three-dimensional reconstructions of the dural venous sinuses in healthy patients and in patients with hypoplasia of one of the dural sinuses, we will use a 3D printer to produce experimental phantoms that correspond to human sinuses in appearance and anatomical dimensions, consisting of a superior sagittal sinus, both transverse sinuses and both sigmoid sinuses. The phantoms will be made of materials that best represent the dural sinuses due to their properties (elasticity and plasticity), with the internal lumen of the phantom being a "cast" of three-dimensional reconstructions of the dural sinuses filled with saline. During the experiment, we will continuously measure the pressure through 3 cannulas, which will be placed in the right transverse sinus, above the superior sagittal sinus and in front of the confluence of the superior sagittal sinus with the transverse sinuses. In the first series, we will measure the pressures in a vertical and horizontal position (as on the left side) and at an angle of 45° without any additional manipulation. In the second series, we will create an artificial stenosis with metal braces in three different places and then measure the pressures in the positions mentioned above.

Expected scientific contribution: We expect that this research will show the volume of blood in the DVS and the normal oscillations of this volume, and confirm the hypothesis that there is hydrostatic pressure in the DVS, in accordance with the law of fluid mechanics. We will also try to determine how the existence of a negative hydrostatic pressure in the DVS represents an evolutionary adaptation for brain perfusion during upright walking, where from a biophysical point of view the DVS cannot be the main site of CSF absorption.

MeSH/Keywords: cranial sinuses, hydrostatic pressure

Poster code: T-01-08-044

Poster Title: Defining neuroradiological biomarkers of normal and abnormal brain development in preterm infants using structural and diffusion magnetic resonance imaging

PhD candidate: Domagoj Lasić

Part of the thesis: Defining neuroradiological biomarkers of normal and abnormal brain development in preterm infants using structural and diffusion magnetic resonance imaging

Mentor(s): Professor Milan Radoš, MD PhD, Finn Lennartsson, PhD

Affiliation: University of Zagreb School of Medicine

Introduction: According to data from the Croatian Institute of Public Health, preterm births constitute approximately 6% of the total number of births in the Republic of Croatia, which corresponds to a total of about 2,000 prematurely born children annually Advances in perinatal care over the last two decades have improved survival rates significantly; however, these infants still face high risks of perinatal cerebral lesions, leading to considerable developmental challenges. Early therapeutic interventions, leveraging the brain's early-life plasticity, can mitigate these effects significantly. Despite its accuracy, brain MRI at corrected term age in preterms is not fully predictive of future neurodevelopmental outcomes. Newer techniques like diffusion MRI and advanced HARDI protocols provide more detailed insights into brain microstructures, potentially improving diagnostic accuracy and aiding in early intervention.

Hypothesis: By analyzing thalamocortical projections in preterm infants using standard and diffusion magnetic resonance imaging with the method of constrained spherical deconvolution, neuroradiological biomarkers of normal and abnormal brain development can be defined, which are predictors of later neurodevelopmental outcomes.

Aims: To demonstrate how the characteristics of thalamocortical projections on standard and diffusion MR imaging in preterm infants at corrected term age correlate with neurodevelopmental outcomes, and to define diffusion and structural neuroradiological biomarkers of normal and abnormal brain development.

Materials and methods: This study plans to include 90 preterm infants who will undergo brain imaging with magnetic resonance (MR) at the term equivalent age using the most advanced structural and diffusion sequences. Using the method of constrained spherical deconvolution, thalamocortical projections will be reconstructed and their microstructural characteristics will be quantified. Neuroradiological findings will be correlated with neuropediatric tests that will be conducted during the first two years of life of the preterm infants.

Expected scientific contribution: Our research can assist in the more reliable identification of perinatal lesions in preterm infants, which is a prerequisite for earlier therapeutic intervention that improves the overall neurodevelopmental outcome.

MeSH/Keywords: thalamocortical projections, preterm infants, constrained spherical deconvolution, neurodevelopmental outcome.

Poster code: T-01-08-061

Poster Title: Povezanosti razina kolina u limbičkom sustavu mjerenih 1H-MRS s depresivnom simptomatikom nakon preboljenja COVID-a

PhD candidate: Tamara Foro

Part of the thesis: Choline Levels in the Limbic System and their Association with Depressive Symptoms Post COVID-19 Recovery: A Proton Magnetic Resonance Spectroscopy Study

Mentor(s): Professor Neven Henigsberg, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: After recovering from the acute phase of COVID-19, the risk of developing depressive symptoms, accompanied by changes in cognitive and general functioning, is increased.

Hypothesis: Choline levels determined by proton magnetic resonance spectroscopy (1H-MRS) in regions of interest (ROIs) in the limbic system will differ between groups of patients with the same degree of depressive symptoms (those who have recovered from COVID-19 and control groups) and could point to a pattern of changes associated with depressive symptoms after recovering from the disease of COVID-19.

Aims: The etiology of the onset of depressive symptoms has not been clarified. Recognition of the specificity of the neurochemical basis and etiological reasons of depression would contribute to its understanding.

Materials and methods: In 58 subjects (29 in the COVID-19 group and 29 in the control group), choline levels will be determined by proton magnetic resonance spectroscopy in 4 regions of interest in the limbic system: dorsomedial prefrontal lobe, insula, amygdala and hippocampus. The primary variable for assessing depressive symptoms is the MADRS scale. The evaluation will be carried out at the beginning of the research and after one year. Differences in choline levels between groups will be analyzed, their association with the degree of depression, and changes in choline levels and depressive symptoms within the groups.

Expected scientific contribution: Understanding the etiology of depressive symptoms after recovering from COVID-19.

MeSH/Keywords: Depression, Magnetic Resonance Spectroscopy, COVID-19

Poster code: T-01-08-106

Poster Title: Vision functioning and joint attention

PhD candidate: Renata Peharec Ramov

Part of the thesis: Vision functioning and joint attention

Mentor(s): Professor Zdravko Petanjek, MD PhD, Associate Professor Sonja Alimović, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Early vision experinces play fundamental role for social communication and joint attention development. Numerous studies confirm the correlation of certain visual functions with development of social communication development. However, there are limited research that measure vision functioning in children with delayed development of joint attention.

Hypothesis: Characteristics of visual functioning in children with typical development of joint attention differ from characteristics of visual functioning in children with delayed development of joint attention.

Aims: Aim of this research is to determine the characteristics of visual functions and functional vision in children with delayed development of joint attention. Specific aims are: 1.Determine the specificities of visual functions in children with delayed development of joint attention. 2.Determine the specificities of functional vision in children with delayed development of joint attention. 3.Compare the differences in visual functioning between children with typical development and children with delayed development of joint attention.

Materials and methods: The study will include 25 participants with delayed development of joint attention ages 18 to 30 months and 25 tipically development participants age matched. Exclusion criteria will be presence of hearing impairment, vision impairment, severe motor disabilities, bilingualism. Vision functioning will be measured by following variables: 1) Vision functions: pupil reaction, fixation, eye alignment, eye movements, vision acuity, contrast sensitivity, vision field, stereovision, motion perception. Vision functions will be coded based on age related norms. 2) Functional vision: visual communication, orientation and mobility, near vision tasks, activities of daily living, vision attention. Functional vision assessment will be videotaped and analysed. For each variable behaviors will be coded on Likert scale: 1 = excellent, 2 = very good, 3 = good, 4 = poor, 5 = very poor. Joint attention will be measured by Early Social Communication Scales - ESCS (Mundy i sur., 2003). ESCS is a videotaped structured observation measure of social communication for children between 8 and 30 months of mental age. It involves 17 tasks and time length of examination is from 15 to 30 minutes for each participant. Participant get point for every purposeful bid and response. The lowest score is 0 and the upper score is not limited. Differences between groups will be tested based on distribution. Distribution will be calculated using Shapiro-Wilk test. Statistical significant will be P-value < 0,05. The research will be conducted at Special hospital for Orthopedics and Rehabilitation „Martin Horvat" Rovinj – Rovigno after approval of The Ethics committe of the hospital and The Ethics committe School of Medicine, University of Zagreb. Participant will be enrolled in the research after singed parental consent.

Expected scientific contribution: The analysis of the visual functioning in children with joint attention development delays would enable a better understanding of the early development and joint attention developmet. The identification of specificities of visual functioning in children with delayed development of joint attention would enable a better understanding of the factors influencing the development of joint attention.

MeSH/Keywords: vision, social behavior, development

Poster code: T-01-08-118

5. Thesis proposals – clinical medical sciences

Poster Title: The effect of total intravenous anesthesia and balanced anesthesia on concentration of N terminal brain natriuretic propeptide in head and neck cancer patients

PhD candidate: Anita Visković

Part of the thesis: The influence of the type of anesthesia on the level of N terminal brain natriuretic propetide in patients undergoing head and neck cancer surgery

Mentor(s): Professor Dinko Tonković, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: N-terminal brain natriuretic propeptide (NT-proBNP) as a biomarker is used in cardiac risk assessment since elevated values are associated with cardiovascular events after non-cardiac surgery. Head and neck surgery are estimated as surgery of intermediate risk.Types of anesthesia have different effects on cardiac function, hemodynamic changes, reduced stress and consequently on the release of cardiac biomarkers. Inhalation and total intravenous anesthesia (TIVA) have a cardioprotective effect through different mechanisms. Cardioprotective effect of inhalation anesthetics in non-cardiac surgery has not been proven.

Hypothesis: Use of total intravenous anesthesia in compared to balanced anesthesia leads to a smaller increase in concentration of N terminal brain natriuretic propeptide in head and neck cancer patients.

Aims: The aim of the research is to compare the influence of total intravenous anesthesia in relation to balanced anesthesia on the change in the concentration of N terminal brain natriuretic propeptide in head and neck cancer patients. Specific aims: 1. Show the impact of total intravenous anesthesia on hemodynamic stability during surgery. 2.Show the impact of balanced anesthesia on hemodynamic stability during surgery. 3.To assess the relationship of changes in N terminal brain natriuretic propeptide concentration with cardiovascular events within 30 days of the surgery.

Materials and methods: Randomized prospective study on patients older than 18 years, scheduled for an elective surgical procedure lasting more than 3 hours. After a standard induction of anesthesia, maintenance of anesthesia in one group by inhalation of sevoflurane, and in other group by continuous intravenous infusion of propofol. In both groups, the concentratio of N terminal brain natriuretic propeptide will be measured the evening before the procedure, immediately after the procedure, 24 hours after the procedure, and the level of change will be monitored. Intraoperative measurement of MAP (mean arterial pressure), CI (cardiac index), SVV (stroke volume variations). Follow-up of the patient for 30 days for evaluation cardiovascular events (monitoring during hospitalization, and after discharge by phone).

Expected scientific contribution: Research results could enable application types of anesthesia that better maintain heart function, hemodynamic stability and thus contributes fewer cardiovascular events

MeSH/Keywords: N-terminal brain natriuretic propeptide, total intravenous anesthesia, balanced anesthesia, head and neck cancer surgery

Poster code: T-02-01-104

Poster Title: Observational study of the application of the ClinFit measuring instrument in relation to the functionality of patients with chronic non-specific low back pain

PhD candidate: Ivan Ljudevit Caktaš

Part of the thesis: Observational study of the application of the ClinFit measuring instrument in relation to the functionality of patients with chronic non-specific low back pain

Mentor(s): Assistant Professor Frane Grubišić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Low back pain is one of the main causes of disability. In order to determine with certainty, the functionality of our patients, we use various questionnaires that they fill out.

Hypothesis: The ClinFIT questionnaire is effective and applicable in patients with chronic non-specific low back pain.

Aims: Validation of the ClinFIT questionnaire with methods used in daily clinical work, determining whether there is a statistically significant correlation between the results of the ClinFIT questionnaire and the results of the Roland Morris questionnaire and whether there is a statistically significant correlation between the results of the ClinFIT questionnaire and the functionality of the respondents.

Materials and methods: In this observational study, a suitable sample of 120 subjects of both sexes, aged 25-65, with clinically diagnosed chronic non-specific low back pain will be selected, where pain and spine functionality will be analyzed as the main research measure. At the first examination, a detailed medical history will be taken for each patient and socio-demographic data will be recorded. Also, each patient will indicate the intensity of their own symptoms on the VAS scale. A detailed clinical examination will then be performed to determine the patient's functional status and to assess the severity of the clinical picture. The patient's functional status will be measured in several ways: Thomayer's measure, Schober's measure and the index of sagittal mobility. After the medical history and initial clinical examination, all subjects will fill out the Roland Morris questionnaire for self-assessment of disability and the ClinFit (Clinical Functioning Information Tool) instrument for self-assessment of functioning, disability and health, after which they will be randomly assigned to the physical therapy. Therapeutic ultrasound along with a standard exercise program as well as low-intensity electromagnetic therapy along with a standard exercise program will be conducted for 15 consecutive days. Immediately after the end of physical therapy and 3 months after it, all patients will undergo a detailed clinical examination, as was done initially before physical therapy. Also, upon completion of the clinical examination, all subjects will fill out the previously mentioned questionnaires that they filled out before the therapy and the VAS pain scale as a self-assessment of the intensity of their own symptoms.

Expected scientific contribution: Chronic non-specific low back pain represents a significant public health problem and is associated with numerous medical costs. Assessment of the effect on the individual's functionality is carried out using a series of questionnaires such as Rolland-Morris. The ClinFit questionnaire has not been used to assess the functionality of patients after rehabilitation. By mutual comparison of both questionnaires, we would conclude which questionnaire better predicts outcomes related to pain and functionality.

MeSH/Keywords: Low back pain, ClinFit (Clinical Functioning Information Tool)

Poster code: T-02-03-012

Poster Title: Postprostatectomy urinary continence after functional magnetic pelvic stimulation

PhD candidate: Helena Kolar Mitrović

Part of the thesis: Postprostatektomijska urinarna kontinencija nakon funkcionalne magnetske stimulacije zdjelice

Mentor(s): Associate Professor Porin Perić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Although radical prostatectomy (RP) is considered the gold standard for optimal treatment of localized prostate cancer (PCa), it carries a significant risk of urinary incontinence (UI).

Hypothesis: Functional magnetic stimulation after radical prostatectomy reduces the duration and intensity of postoperative urinary incontinence.

Aims: To evaluate the role of functional magnetic stimulation (FMS) in the treatment of UI after robotic RP.

Materials and methods: This prospective, double-blind, randomized study will include a minimum of 45 patients who underwent robotic RP at the KBC Zagreb Urology Clinic. After surgery, patients will have FMS, a total of 8 stimulations a month. UI will be assessed by the number of medical diapers/templates that patients will use in 24 hours and based on the value of internationally validated questionnaires of urinary incontinence “The International Consultation on Incontinence Questionnaire (ICIQ-UI SF)” and symptoms of lower urinary system “International Prostate Symptoms Score (IPSS)”.

Expected scientific contribution: We would like to receive information about the role of FMS in postprostatectomy UI and, in case of positive results, we could recommend FMS for its treatment.

MeSH/Keywords: prostate cancer, radical prostatectomy, urinary incontinence, erectile dysfunction, functional magnetic stimulation, pelvic muscles

Poster code: T-02-03-026

Poster Title: Determining the value of laboratory parameters of antiphospholipid syndrome in healthy pregnant women

PhD candidate: Gordana Horvat

Part of the thesis: Determining the value of laboratory parameters of antiphospholipid syndrome in healthy pregnant women

Mentor(s): Vladimir Blagaić, Assistant Professor Vanja Radišić Bijak, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: The diagnosis of antiphospholipid syndrome (APS) is based on a specific clinical presentation and accompanying laboratory test results. According to current diagnostic criteria, it is necessary to determine lupus anticoagulant, antiphospholipid antibodies of IgG and IgM isotypes, and antibodies to beta2-glycoprotein I of IgG and IgM isotypes. Current recommendations do not advise on determining individual coagulation tests that are components of APS during pregnancy, although identifying pregnant women with APS is essential for prevention and treatment.

Hypothesis: Antiphospholipid syndrome laboratory parameters levels differ between healthy pregnant women and healthy non-pregnant women.

Aims: The aim of this research is to determine the levels of laboratory parameters of APS in healthy pregnant women. Additionally, we will ascertain the dynamics of changes in these parameters during the course of pregnancy and postpartum in healthy women.

Materials and methods: This is an observational study that will include 140 healthy pregnant women with uncomplicated singleton pregnancies. Blood sampling will be conducted once in each trimester and after delivery during the postpartum period. Anamnestic indicators will be collected through a questionnaire, and indicators about pregnancy outcomes will be obtained from the hospital information system. In the statistical data analysis, ANOVA or the Kruskal-Wallis test will be used to compare numerical variables between groups. The $\chi 2$ test or Fisher's exact test will be used to compare categorical variables between groups.

Expected scientific contribution: This study will determine levels of laboratory parameters of antiphospholipid syndrome in healthy pregnant women and the dynamics of changes in these parameters during pregnancy and puerperium in healthy women.

MeSH/Keywords: antiphospholipid syndrome, levels of laboratory parameters, healthy pregnant women

Poster code: T-02-05-002

Poster Title: The human endocervical ionocytes, cells possibly responsible for cyclical changes in cervical mucus.

PhD candidate: Egon Kruezi

Part of the thesis: The human endocervical ionocytes, cells possibly responsible for cyclical changes in cervical mucus.

Mentor(s): Associate Professor Vesna Elveđi Gašparović, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Cervical mucus is a glycoprotein gel whose biochemical and biophysical properties vary depending on the menstrual cycle. One of the proposed mechanisms of mucus secretion regulation involves the molecule CFTR (cystic fibrosis transmembrane conductance regulator). CFTR is a transmembrane transporter of chloride and bicarbonate ions that is associated with mucus changes. Mutations of this ion channel cause cystic fibrosis, the most common autosomal recessive inherited disease.

Hypothesis: CFTR impacts cervical mucus with the same mechanism as it affects mucus in the respiratory and digestive systems, but does it under the influence of sex hormones.

Aims: Demonstrate there are FOXI1-positive cells (ionocytes) in the endocervical epithelium that express CFTR (cystic fibrosis transmembrane conductance regulator) in healthy subjects, but do not express CFTR in subjects with cystic fibrosis. Also to determine the relationship between the concentration of estradiol in the serum, expression of CFTR in the cervix and the properties of mucus in both groups of.

Materials and methods: The research will include healthy controls and patients suffering from cystic fibrosis. A minimum of 35 participants per group will be included in the research. Given that one group consists of patients with a rare disease, the number of subjects in the group with cystic fibrosis will be smaller, statistical methodology will be adjusted depending on the real strength of the effect. Chemical and biochemical procedures will determine the concentration of hormones in the blood, as well as the physical and chemical properties of mucus. The concentration of mucin and CFTR in the mucus will be determined by the ELISA procedure, and the expression of the FOXI1 and CFTR proteins will be determined immunocytochemically, thereby proving the presence of FOXI1-positive ionocyte cells.

Expected scientific contribution: These findings will be the basis for future research into the mechanism of hormonal regulation of mucus via ionocytes and CFTR, which will contribute to the understanding of subfertility in women with cystic fibrosis.

MeSH/Keywords: ionocytes, cystic fibrosis, cystic fibrosis transmembrane conductance regulator, cervical mucus

Poster code: T-02-05-003

Poster Title: Association between pregnancy course, perinatal outcomes and quality of life in pregnant women with diabetes mellitus type 1 and their children.

PhD candidate: Iva Miličić Pašalić

Part of the thesis: Association between pregnancy course, perinatal outcomes and quality of life in pregnant women with diabetes mellitus type 1 and their children.

Mentor(s): Professor Marina Ivanišević, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Epidemiologic importance of diabetes mellitus is seen in the data of Croatian national diabetes mellitus (diabetes mellitus - DM) register which shows that 1 of 10 people in Croatia has DM. Similar facts are shown in numerous international registers as well as in scientific paper reviews - according to which, in six years the global estimate of DM prevalence will be around 700 million people. The vast prevalence of DM in elderly population is well known, however, nowadays it is not rarely present in younger population, as well as in pregnant women. The impact of DM on clinical outcomes such as mortality and occurrence of various comorbidities, better known as complications of diabetes mellitus, is especially well researched on the middle aged and elderly population. Because such outcomes in young population are extreme rarity, quality of life is imposed as an infallible factor in estimating healthcare quality of these patients.

Hypothesis: Quality of life of pregnant women with diabetes mellitus type 1 is worse than in healthy pregnant women.

Aims: The aim of this study is to assess the quality of life of women (QOL) with T1DM during two time periods (during and after pregnancy) as well as QOL of their children. Also, the correlation between QOL, breastfeeding and laboratory biomarkers will be assessed respectively.

Materials and methods: The study will include women with T1DM (n=76) and their children (n=76); depending of the mother's consent for including the child in the study. QOL will be assessed via international standardized questionnaire of World Health Organisation (WHOQOL-BREF) and the pediatric version of the questionnaire (WHO-PedsQOL).

Expected scientific contribution: This is a first prospective study conducting research about T1DM pregnancies and children of these mothers which could open further possibilities for scientific study of this field. Quality of life is a necessary and indispensable indicator of healthcare assessment considering the low mortality of pregnant women. This study is conducted on a highly selected cohort of pregnant women and mothers with T1DM and their children which could help in designing an algorithm of clinical guidelines for T1DM pregnancies. Hence, we expect scientific results that might be important for clinical management of pregnancies with T1DM, especially considering the insight into the health conditions of children born from T1DM mothers. Last but not least, we hope to improve clinical and scientific interworking of pediatricians and obstetricians.

MeSH/Keywords: Pregnancy, diabetes mellitus type 1, quality of life, WHOQOL-BREF, WHO-PedsQOL

Poster code: T-02-05-023

Poster Title: Comparison of the effect of lateral and mediolateral episiotomy on development of postpartum anal incontinence in primiparous women

PhD candidate: Ivan Brlečić

Part of the thesis: Comparison of the effect of lateral and mediolateral episiotomy on development of postpartum anal incontinence in primiparous women

Mentor(s): Associate Professor Goran Augustin, MD PhD, Associate Professor Držislav Kalafatić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Obstetric anal sphincter injuries (OASIS) are a significant risk factor for development of anal incontinence. Episiotomy is a surgical incision of the perineum, a controlled injury for prevention of OASIS and consequent postpartum anal incontinence. Although lateral and mediolateral episiotomy have a similar effect on the incidence of postpartum anal incontinence, current guidelines from international professional societies are not consistent regarding the use of one or the other form of episiotomy.

Hypothesis: The use of mediolateral episiotomy in primiparous women is more effective in preventing the occurrence of postpartum anal incontinence compared to the use of lateral episiotomy.

Aims: The aim of this study is to assess efficiency and directly compare lateral and mediolateral episiotomy in prevention of development of postpartum anal incontinency.

Materials and methods: 120 primiparas will be included, with labour contractions or ruptured amniotic membrane within 18 hours, gestation age 37+0 or more. At initial examination, the strength of pelvic floor muscles and anal sphincter will be measured with perineometer device (Peritron TM, Laborie, Canada), and consecutive measurements will be conducted in defined periods after birth.

Expected scientific contribution: The results of this study could contribute to better and more objective evaluation of two most used forms of episiotomy and help establish an algorithm of prevention and early recognition of anal sphincter injury.

MeSH/Keywords: episiotomy, fecal incontinence, pelvic floor, perineometry

Poster code: T-02-05-042

Poster Title: The influence of the parturient's temperament, character and shame on the satisfaction with childbirth

PhD candidate: Branimir Krištofić

Part of the thesis: The influence of the parturient's temperament, character and shame on the satisfaction with childbirth

Mentor(s): Assistant Professor Josip Juras, MD PhD, Professor Darko Marčinko, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: The assumption is that many and varied factors influence the satisfaction of the mother with childbirth, and that not all factors have been identified to date. Pain relief and the way the pregnancy is completed are commonly researched factors of satisfaction with childbirth. Perception of the course of childbirth and skin-to-skin contact immediately after childbirth proved to be significant predictors of women's satisfaction with childbirth. Satisfaction with childbirth was proven to be influenced by previous satisfaction with antenatal care, understanding of information provided by health professionals, not experiencing disrespect and abuse during childbirth. The question remains, can some traits of character, temperament and even shame have an impact on the satisfaction with childbirth? And does the above have an impact on satisfaction regardless of the perception of social support and satisfaction with one's own life, or is it related? If so, to what extent is it related? Is it possible to suggest measures for the health care staff to increase the satisfaction of the parturient with childbirth and reduce their traumatic experience?

Hypothesis: The degree of satisfaction with childbirth is related to the temperament, character and shame of the mother, and greater satisfaction will contribute to greater life satisfaction and social support of the mother.

Aims: GENERAL OBJECTIVE: To show the connection between the degree of satisfaction with childbirth and the temperament, character and shame of the mother. SPECIFIC OBJECTIVES: to examine the connection between certain characteristics of the mother's character, temperament and shame with satisfaction with childbirth, to examine the risk factors related to the characteristics of temperament, character and shame and satisfaction with life and social support of the mother on childbirth satisfaction. Furthermore, to examine the connection between satisfaction with childbirth and the way the pregnancy was completed, and to propose preventive measures to reduce the mother's dissatisfaction with childbirth.

Materials and methods: The subject research will be interdisciplinary, cohort, prospective, survey based and multicenter, with a convenient sample. In one year, it is planned to conduct a survey in three maternity hospitals in the Republic of Croatia (KBC Zagreb, ŽB Čakovec, OB Nova Gradiška). With the study power conditions of 80%, $\alpha = 0.05$ and $\beta = 0.20$, it is necessary to collect about 200 respondents, but a larger sample is planned. The City-BiTS scale will be used to examine the satisfaction with childbirth, the BSS-R scale to examine the conditions during childbirth. Character traits and temperament traits will be tested with the TCI-R scale, shame with the EISS scale, and life satisfaction of the mother with the SWLS scale. The PSS scale will be used to assess the mother's perception of social support. Beck's Depression Inventory will be used to determine the possible presence of depression. Surveys will be handed to the respondents on the first day after giving birth, with a request to fill them out on the second day after giving birth. Inclusion criteria include adulthood, legal capacity, previously signed informed consent, delivery at term, test subjects without diagnosed psychiatric diseases or in therapy for them, absence of severe fetal diseases. All relevant data related to the state of health of the mother, fetus/newborn, data related to the course of pregnancy, week and method of termination of pregnancy, perinatal outcome, anthropometric data of both mother and newborn, and data related to possible therapy during pregnancy will be collected.

Expected scientific contribution: This research could contribute to elucidating the connection between the character traits, temperament and shame of the mother giving birth and her satisfaction with childbirth. The research could contribute to the possibility of proposing preventive measures to reduce dissatisfaction with childbirth.

MeSH/Keywords: Childbirth, parturient, satisfaction, character, temperament, shame, social support

Poster code: T-02-05-154

Poster Title: The profile of immune semaphorins in patients with severe community-acquired pneumonia and liver steatosis

PhD candidate: Branimir Gjurašin

Part of the thesis: The profile of immune semaphorins in patients with severe community-acquired pneumonia and liver steatosis

Mentor(s): Assistant Professor Neven Papić, MD PhD

Affiliation: University of Zagreb School of Medicine, University Hospital for Infectious Diseases Zagreb

Introduction: Metabolic dysfunction-associated steatotic liver disease (MASLD) is a known risk factor for severe community-acquired pneumonia (sCAP) outcome. Although semaphorins (SEMA) are important regulators of immune response, their role in MASLD and sCAP has not been analyzed.

Hypothesis: Patients with sCAP have different concentrations of immune semaphorins on the first and fifth day of hospitalization, dependent on the presence of liver steatosis, which correlate with clinical outcomes.

Aims: To analyze differences in concentrations and kinetics of SEMA and cytokines in sCAP patients with and without MASLD, and their correlation with clinical outcomes.

Materials and methods: This prospective cohort observation study will include 120 adult patients with sCAP which will be screened for MASLD. sCAP will be defined as community-acquired pneumonia diagnosed within 48 hours of hospital admission with either at least one major criterion (invasive OR noninvasive mechanical ventilation OR high-flow nasal oxygen with ≥50% + PaO2/FiO2 ≤300 OR septic shock with a need for vasopressors OR blood pH <7.30) or at least three minor criteria (respiratory rate ≥30 breaths/min, PaO2/FiO2 ≤ 250 mmHg, multilobar infiltrates, confusion/disorientation, elevated blood urea nitrogen, WBC count ≤4x109/L, platelet count ≤ 100x109/L or > 400x109/L, body temperature <36 °C, hypotension requiring aggressive fluid resuscitation). Exclusion criteria will be any of the following: patients transferred from other hospitals after >48 hours of hospitalization, immunocompromise, pregnancy, palliative care, other chronic liver diseases, active neoplastic disease, autoimmune diseases, daily consumption of alcohol ≥20 g for women and ≥30 g for men, use of medications associated with liver steatosis, clinical history suggesting aspiration of gastric content, SARS-CoV-2 infection in the last 90 days or tuberculosis. Liver steatosis presence and degree will be determined by controlled attenuation parameter and liver stiffness by transient fibroelastography. Routine clinical and demographic data will be collected: gender, age, comorbidities, chronic medication use, days from symptom onset on admission, severity of pneumonia (SOFA, PSI, CURB-65, SMART-COP scores), body-mass index, waist-to-hip ratio, waist-to-height ratio, routine laboratory blood studies on admission and on the fifth day of hospitalization and routine microbiologic work-up. An additional serum specimen will be collected on admission and on the fifth day of hospitalization, which will be stored at -80°C for analysis of SEMA and cytokine concentrations. Patients treated with invasive mechanical ventilation will also have their bronchoalveolar lavage fluid collected for cytology, biochemistry, microbiology studies as well as SEMA and cytokine concentration analysis. All patients will be monitored daily until discharge and their clinical course and outcomes will be noted (in-hospital mortality, 28-day mortality, hospitalization duration, ICU admission and days of ICU stay, development of complications). The cohort will be divided into two groups, depending on presence of MASLD determined in patients with liver steatosis without other liver conditions mentioned in exclusion criteria and at least one of metabolic risk factors: BMI ≥ 25 kg/m2 or waist circumference >94 cm for men and >80 cm for women; glucose intolerance or type 2 diabetes; arterial hypertension; dyslipidemia. Serum SEMA and cytokine concentrations will be measured on the 1st and 5th day of hospitalization in all patients, as well as their concentration in bronchoalveolar lavage fluid in selected patients. The impact of MASLD and concentrations of SEMA and cytokines with clinical outcome will be analyzed.

Expected scientific contribution: This research will describe the role of SEMA in patients with sCAP and MASLD for the first time. This could lead to acknowledging SEMA as novel biomarkers of sCAP outcome.

MeSH/Keywords: pneumonia, non-alcoholic fatty liver disease, liver steatosis, semaphorins

Poster code: T-02-07-025

Poster Title: Comparison of depression and anxiety between people living with HIV and users of pre-exposure prophylaxis for HIV prevention in Croatia

PhD candidate: Sanja Belak Škugor

Part of the thesis: Prevalence and factors associated with depression and anxiety in people living with HIV in Croatia

Mentor(s): Associate Professor Marija Santini, MD PhD

Affiliation: University of Zagreb School of Medicine, University Hospital for Infectious Diseases "Dr. Fran Mihaljevic"

Introduction: People living with HIV (human immunodeficiency virus) (PWH) experience higher prevalence of mental health conditions then general population. Similar situation with high percentage of psychological difficulties is described among men who have sex with men (MSM) and who use pre-exposure prophylaxis (PrEP) for preventing HIV belong to vulnerable groups because they are at increased risk for HIV infection and are exposed to minority stress. Psychiatric comorbidities have an aggravating impact on the entire continuum of HIV care.

Hypothesis: The prevalence of depression and anxiety in people living with HIV is higher compared to users of pre-exposure prophylaxis for HIV prevention.

Aims: The aim of the study is to compare the prevalence of depression and anxiety in men living with HIV who have sex with men (MSM) with a comparison group of HIV-negative MSM using PrEP. Also, we will examine the association of stigma and other sociodemographic, economic, clinical, and psychosocial factors related to HIV on depression and anxiety in people with HIV.

Materials and methods: It is an observational cross-sectional study that will be conducted for one year starting in May 2024 at the University Hospital for Infectious Diseases in Zagreb where all PWH in Croatia are treated. The research will include PWH above 18 years of age and a control sample of men who have sex with men (MSM) and who use pre-exposure prophylaxis (PrEP) for preventing HIV. Consecutive sampling method will be used to select 800 PWH and 300 PrEP users. Data will be collected using standardized structured questionnaires.

Expected scientific contribution: We expect to establish so far unexplored differences in the frequency of psychological disorders (depression and anxiety) between people living with HIV and PrEP users and to contribute to a better understanding of potential risk factors for mental disorders that have not been investigated in Croatia so far. The results of the research could be applied in clinical practice through targeted preventive public health measures, evaluation of the need and availability of mental health care services.

MeSH/Keywords: HIV, depression, anxiety, mental health, pre-exposure prophylaxis

Poster code: T-02-07-038

Poster Title: Distinct cytokine profiles in patients with steatotic liver disease associated with metabolic dysfunction in sepsis

PhD candidate: Nina Vrsaljko

Part of the thesis: Distinct cytokine profiles in patients with steatotic liver disease associated with metabolic dysfunction in sepsis

Mentor(s): Assistant Professor Neven Papić, MD PhD

Affiliation: University Hospital for Infectious Diseases 'Dr. Fran Mihaljević', Zagreb, Croatia

Introduction: Sepsis is a life-threatening organ dysfunction resulting from the host's unregulated immune response to infection. Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most common chronic liver disease and is associated with a state of low-grade chronic inflammation and an altered immune response, which may have potential consequences for the course of infectious diseases. Cytokines and chemokines belong to the group of glycoproteins and are key mediators of the immune response, also recognized in the immunopathogenesis of MASLD. So far, the cytokine profile in patients with MASLD and sepsis has not been described, and the specificities and changes in the inflammatory response in these patients have not been investigated.

Hypothesis: Patients with steatotic liver disease associated with metabolic dysfunction (MASLD) in sepsis have different serum concentrations of cytokines and chemokines on the first and fifth days of hospitalization compared to patients without MASLD.

Aims: The aim of this research is to compare the cytokine and chemokine profile in patients with sepsis, between groups with and without MASLD, and to analyze the association of these biomediators with disease outcomes.

Materials and methods: 120 adult patients diagnosed with sepsis will be included in the study. In all patients, the presence and degree of liver steatosis will be determined using the controlled attenuation parameter (CAP), based on which the patients will be divided into MASLD and non-MASLD groups. The levels of individual cytokines in the serum and their correlation with the severity of the clinical picture will be determined.

Expected scientific contribution: The research results could help better understand the specificity of the immune response in patients with MASLD and identify new biomarkers of the disease.

Acknowledgments:

MeSH/Keywords: sepsis; non-alcoholic fatty liver disease; NAFLD; metabolic dysfunction associated steatotic liver disease ; MASLD; cytokines; immune response

Poster code: T-02-07-054

Poster Title: The effect of semaglutide on eating behaviour disorders in type 2 diabetic patients

PhD candidate: Jelena Marinković Radošević

Part of the thesis: The effect of semaglutide on eating behaviour disorders in type 2 diabetic patients

Mentor(s): Associate Professor Jelena Osmanović Barilar, MD PhD, Assistant Professor Velimir Altabas, MD PhD

Affiliation: University of Zagreb School of Medicine, Clinical Hospital Centre Sestre milosrdnice

Introduction: Eating behaviour disorder often occurs in patients with type 2 diabetes (T2D) and can potentiate the development of numerous complications of T2D as well as glycaemic variability. The effect of semaglutide on eating behaviour disorder in patients with overweight and T2D, or its influence on glycaemic variability and the level of incretin hormones in said patients, has not been examined.

Hypothesis: The use of the GLP-1 receptor agonist semaglutide reduces the intensity of eating behaviour disorder in patients with overweight and type 2 diabetes.

Aims: General aim is to examine the effect of semaglutide on the intensity of eating behaviour disorder as quantified by the EAT-26 questionnaire in patients with overweight and type 2 diabetes. Specific aims are: (1) to assess the effect of 12-week semaglutide therapy on the EAT-26 scores, (2) to assess the effect of 12-week semaglutide therapy on parameters derived from the procedure of continuous interstitial glycemia measurement (CGM): a) mean daily glycemia; b) indicator of glucose management and c) glycaemic variability (coefficient of variability), (3) to assess the interrelationship of EAT-26 score >20 (versus ≤20) and cross-sectional concentrations of incretins GLP-1 and GIP in the blood, and (4) to assess the relationship between EAT-26 score >20 and the dynamics of GLP-1 and GIP concentrations in the blood in the mixed meal test.

Materials and methods: This prospective clinical research will be conducted for the duration of 1/2/2024 to 31/12/2024. We aim to recruit 68 patients with T2D and BMI≥28 kg/m2 and randomize them based on the presence of an eating behaviour disorder diagnosed by a Eating Attitude Test (EAT-26) (1:1). Patients with eating behaviour disorder will further be randomized (1:1) to receive semaglutide. EAT-26 score above 20 points indicates the existence of symptomatology of eating behaviour disorder. The inclusion criteria will be subjects of both sexes with T2D, BMI≥28 kg/m2, age 18 - 65 years, HbA1c>7%, GLP1-RA naïve. Exclusion criteria will be impaired liver function (Child-Pugh C) and kidney function (GFR<30 ml/min), use of drugs that affect food intake, pathological conditions that can affect food intake, the existence of a contraindication for the use of semaglutide. At baseline, we will evaluate serum glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) levels. At baseline and after 12 weeks we will determine parameters derived from a continuous glucose monitoring device (CGM) over 10 days. All subjects will keep a food diary for 12 weeks with analysis on energy intake, macronutrient and micronutrient intake. Power analysis of the test (with %CV 0.27-0.30, alpha=0.05) showed that a total of 60 subjects are required. Due to the possibility of subjects withdrawing from the study, the required number of subjects will be increased to a total of 68.A factor analysis of EAT-26 scores will be conducted to form 3 (expected) latent variables. To assess the effect of semaglutide on eating habits, the difference in EAT-26 total score and subscales between treated and control subjects will be evaluated after 3 months of treatment. Data will be analysed in a generalized linear model, with basal covariates: age, gender, BMI and score at the beginning of treatment. GLP1 and GIP concentrations will be compared between subjects with EAT-26 score >20 and EAT-26 score ≤20, in a generalized linear model for repeated measurements (GLP1 concentration before and during the MMT test) with fixed covariates: age, sex, BMI, time (before or after MMT) and exposure*time interactions. Type 1 error level=0.05.

Expected scientific contribution: The proposed research could contribute to understanding the effect of GLP-1RA, especially semaglutide, on eating behaviour disorder in patients with overweight and type 2 diabetes and to clarifying the role of incretin hormone levels and glycaemic variability in eating behaviour disorder in these patients.

MeSH/Keywords: Eating behaviour, Glucagon-like peptide-1, Glucose-dependent insulinotropic polypeptide, Type 2 diabetes

Poster code: T-02-09-027

Poster Title: The significance of mucoid impactions identified by Computed Tomography of the Lungs in the evaluation of the clinical efficacy of benralizumab and mepolizumab in patients with severe eosinophilic asthma

PhD candidate: Mirna Vergles

Part of the thesis: The significance of mucoid impactions identified by Computed Tomography of the Lungs in the evaluation of the clinical efficacy of benralizumab and mepolizumab in patients with severe eosinophilic asthma

Mentor(s): Assistant Professor Andrea Vukić Dugac, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Pathophysiological features of severe eosinophilic asthma (SEA) are eosinophilic infiltration, goblet cell hyperplasia, mucoid impactions (MI) and smooth muscle hypertrophy. An increased number of MI correlates with the level of T2 inflammation, airway obstruction and exacerbation rate. The finding of MI can be objectified by CT. In patients with SEA, biological anti-eosinophilic therapy relieves T2 inflammation in the airways, reduces mucus production, and the number of eosinophils.

Hypothesis: Biological anti-eosinophilic drugs, mepolizumab and benralizumab, reduce the number of mucoid impactions in patients with severe eosinophilic asthma, thereby improving treatment outcomes

Aims: Primary objective: To assess the effect of anti-eosinophilic drugs on the occurrence of mucoid impactions by comparing CT scans of the lungs before and after at least 12 months of their use, utilizing the mucoid impaction score (MPS). Secondary objectives: To investigate the association of the effects of therapy on MPS with basic demographic characteristics, as well as characteristics important for severe asthma such as asthma duration, allergic status, pulmonary function findings, and asthma-related comorbidities. Explore the correlation of the effects of therapy on the MPS in subgroups of subjects according to treatment outcome (complete response, partial response, or no clinical response to therapy). Investigate whether the MPS can be a predictor of therapeutic response (complete response to therapy, partial response, or no clinical response to therapy). Examine the impact of the degree of eosinophil depletion (complete or incomplete) on the reduction of the MPS.

Materials and methods: This is a prospective study conducted from December 9, 2019, to October 27, 2023, at KB Dubrava with the approval of the Ethics Committee. The study included subjects of both sexes aged over 18 years who have been treated with anti-eosinophilic drugs (mepolizumab and benralizumab) at KB Dubrava for severe eosinophilic asthma for at least 12 months. All subjects as inclusion criteria must have two CT scans of the lungs, the first before the introduction of biological anti-eosinophilic therapy, and the second after at least 12 months of treatment. All CT findings will be analyzed by one radiologist at two different times with a minimum interval of one month between readings. The analysis of the CT scans will determine the total number of mucoid impactions (Mucus plug score - MPS). Segments of each lobe are systematically analyzed for the presence or absence of mucus plugs, scored as 1 or 0. A segment will receive a score of 1 only if it is scored 1 in both readings, indicating the obstruction of the lumen by mucus plugs; otherwise, it is scored as 0. The sum of the findings in all segments results in a total score ranging from 0 to a maximum of 20 (20 lung segments). The MPS difference before and after anti-eosinophilic treatment will be compared with basic demographic characteristics of patients (gender, age) and all important SEA determinants. To calculate the necessary sample size (number of subjects) for conducting the described research, a retrospective pilot study was conducted on a sample of 17 subjects, which already confirmed the hypothesis. We have increased the sample to 60 subjects to be able to compare the effects of two anti-eosinophilic drugs. Statistical analysis will be performed using descriptive and inferential methods.

Expected scientific contribution: Based on the results of this research, mucoid impactions described by computed tomography of the lungs could become a predictive factor in the selection of anti-eosinophilic drugs, as well as a predictor of treatment outcomes with anti-eosinophilic therapy. Timely identification of patients with unchanged mucoid impaction findings during treatment with anti-eosinophilic therapy could be an indication for changing biological therapy and simultaneously a marker of poorer disease outcome.

MeSH/Keywords: severe asthma, mucoid impactions, computed tomography, eosinophils, biological therapy

Poster code: T-02-09-033

Poster Title: Galectin-3 and intestinal ultrasound in assessing the effects of a modified mediterranean diet on ulcerative colitis disease activity

PhD candidate: Dominik Kralj

Part of the thesis: The effects of a modified mediterranean diet on ulcerative colitis patients

Mentor(s): Assistant Professor Alen Bišćanin, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: previous studies have demonstrated the impact of specific diets on disease prevention and symptom regulation in Ulcerative colitis (UC) but current guidelines do not specifically recommend any dietary pattern for inducing or maintaining disease remission. Intestinal ultrasound (IUS) is an emerging method for evaluating disease activity as well as extent and complications of inflammatory bowel disease. Galectin-3 is a soluble lectin that plays a role in adaptive immunity and has shown correlation with rheumatoid arthritis disease activity and may be used to assess UC activity.

Hypothesis: implementing a modified mediterranean diet with low carbohydrate content (MMD) in mild to moderately active UC patients for 12 weeks adjunct to standard medication will result in a higher clinical response rate (reduction in Simple Clinical Colitis Activity Index (SCCAI) to 3 or SCCAI <= 2) as well as reduced galectin-3 levels compared to the control group receiving standard nutritional recommendations.

Aims: to establish galectin-3 as a serum biomarker for assessing disease activity and demonstrate that following a MMD in conjunction with standard therapy, has a favorable impact on symptoms and objective markers of inflammation in UC patients compared to standard nutritional guidance.

Materials and methods: a prospective randomized study will enroll 62 participants divided into a control and an intervention group. Over the course of 12 weeks, they will adhere to MMD under nutritionist guidance or follow standard dietary recommendations. The sample size has been estimated based on the expected rate of clinical response, using data from previous studies. Statistical analysis will involve checking the normality of distribution, comparing groups, and analyzing associations. A significance level of $P \leq 0.05$ will be considered statistically significant. Randomization will be stratified based on corticosteroid use. Adult patients with mild to moderate UC will be enrolled in the study based on inclusion and exclusion criteria including endoscopically confirmed ulcerative colitis within 16 weeks, SCCAI score, calprotectin levels and/or IUS criteria. The patient will need to be on a stable dose of medications. Relevant information regarding the disease course, treatment history, comorbidities, and extraintestinal manifestations will be recorded. Anthropometric measurements and body composition assessment using bioelectrical impedance will be performed. Stool samples will be collected for fecal calprotectin analysis, and blood samples will be taken for galectin-3 levels, complete blood count and biochemical tests. Dietary habits will be assessed using the Mediterranean Diet Questionnaire. Quality of life will be evaluated using visual analog scales and quality-of-life and fatigue questionnaires (IBDQ32, SF-36, EQ-5D-5L / EQ VAS, FACIT-Fatigue). Participants will receive individualized counseling from nutritionists based on questionnaire results and anthropometry. Counseling will include education and personalized weekly meal planning. Assessment of bowel wall thickness and other ultrasonographic parameters will be performed using a high-frequency linear ultrasound probe by an experienced sonographer trained in IUS.

Expected scientific contribution: UC is a chronic disease with a significant impact on quality of life where a substantial proportion of patients fail to achieve disease remission and biomarkers of activity are lacking. It is a multifactorial disease, and the influence of diet on its onset and course remains underexplored. Favorable effects of the mediterranean diet on numerous diseases have been confirmed in clinical research, but in the case of ulcerative colitis, there is a lack of well-designed studies.

MeSH/Keywords: ulcerative colitis; Mediterranean diet; diagnostic ultrasound

Poster code: T-02-09-034

Poster Title: The significance of eosinophils in the development of emphysema in patients with severe asthma

PhD candidate: Dina Rnjak

Part of the thesis: The significance of eosinophils in the development of emphysema in patients with severe asthma

Mentor(s): Professor Sanja Popović-Grle, MD PhD, Assistant Professor Maja Hrabak Paar, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Asthma is a heterogeneous pulmonary disease and a serious public health problem. The pathophysiological characteristics of asthma are chronic inflammation and variable airway obstruction. The severity of asthma is determined after a minimum of three months of treatment, and the assessment is based on the therapy required to control symptoms and exacerbations. Categorizing the severity of the disease is most important for the group of patients with severe asthma, which is defined as asthma refractory to conventional therapy with high doses of inhaled corticosteroids and bronchodilators. Severe asthma is divided into two groups, severe asthma with type 2 (T2) inflammation and severe asthma without elements of T2 inflammation. Patients with type 2 inflammation are further divided into a group of patients with severe eosinophilic asthma and a group of patients with severe allergic asthma. Emphysema is a pathological term defined by an abnormal and permanent increase in the diameter of the airways distal to the terminal bronchioles. The relationship between asthma and emphysema has not been sufficiently investigated, the factors causing emphysema in asthma have not been identified, and the clinical course of asthma with the presence of emphysema has not been clarified.

Hypothesis: The prevalence of emphysema is higher in the group of patients with severe eosinophilic asthma compared to patients with other phenotypes of severe asthma.

Aims: GENERAL AIM: to compare the prevalence and characteristics of emphysema among subjects with severe eosinophilic asthma with the frequency and characteristics of emphysema among subjects with other phenotypes of severe asthma. SPECIFIC AIMS: to compare the prevalence and characteristics of emphysema with: (1.1) the number of eosinophils in the peripheral blood, (1.2) the number of eosinophils in the induced sputum, (1.3) anthropometric characteristics of the respondents, (1.4) smoking status, (1.5) to the results of an allergy skin test, (1.6) according to the results of alpha-1-antitrypsin analysis, (1.7) presence of Aspergillus spp. in sputum, and (1.8) results of lung function tests.

Materials and methods: The subjects will be equally divided into three groups corresponding to the defined phenotypes of severe asthma: severe eosinophilic, severe allergic, and severe non-T2 type asthma. Among certain groups, a comparison of the prevalence, type, and percentage of lung parenchyma affected by emphysema on chest CT with anthropometric characteristics, smoking status, number of eosinophils in peripheral blood, number of eosinophils in induced sputum, results of microbiological analysis sputum, laboratory test results, and lung function test results will be performed. The radiation dose will be cited. The SyngoVia Pulmo 3D software will be used for the automatic quantification of emphysema. The data will be statistically processed using the computer program R (The R Project for Statistical Computing; www.r-project.org). Differences between categorical variables will be tested using $\chi 2$, or Fisher's exact test. Differences between numerical variables will be examined by analysis of variance with Tukey's post hoc test or Student's t-test in the case of two independent variables, in the case of normally distributed data. The relationship between numerical variables that are normally distributed will be examined using the Pearson correlation test, and between variables that are not normally distributed using the Kendall tau test. All p-values will be two-tailed and the level of statistical significance is set at $\alpha=0.05$. The a priori estimated minimum sample size is 108 respondents, based on the following characteristics, calculated using the G*power computer program: type 1 error ($\alpha=0.05$), power ($1-\beta=0.8$), and size effect 0.3.

Expected scientific contribution: Clarification of the relationship between asthma and emphysema, the possibility of adjusting the therapeutic approach in severe asthma.

MeSH/Keywords: severe asthma, emphysema

Poster code: T-02-09-041

Poster Title: Prognostic factors of survival in patients with metastatic adrenocortical cancer

PhD candidate: Anja Barač Nekić

Part of the thesis: Prognostic factors of survival in patients with metastatic adrenocortical cancer

Mentor(s): Professor Darko Kaštelan, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Adrenocortical carcinoma (ACC) is an aggressive tumour with a poor prognosis, especially in metastatic disease. Although the management of ACC has improved over the years, its prognosis remains unfavorable.

Hypothesis: Hypercortisolism and the presence of liver metastases are predictors of a unfavorable outcome in patients with metastatic ACC.

Aims: General aim: To determine the prognostic factors for survival in patients with metastatic ACC. Specific aims: (1) to investigate the clinical outcomes, overall survival (OS) and progression free survival (PFS) in patients with metastatic ACC, (2) to investigate the types of systemic therapy (mitotane, chemotherapy) and their impact on treatment, outcomes in patients with metastatic ACC, (3) to investigate the influence of surgical treatment on the outcome of patients with metastatic ACC, and (4) to investigate the influence of locoregional therapy on outcome in patients with metastatic ACC.

Materials and methods: All patients diagnosed with metastatic ACC and treated at the Department of Endocrinology, UHC Zagreb, will be included in the study. Medical history data, hormonal and radiological evaluations and pathohistological analyses of tumour tissue will be recorded in the electronic medical records. Analysis will include patients age, sex, modality of tumor diagnosis, tumor characteristics (tumor side, size, Ki67 index, Weiss score), presence of thrombus in the inferior vena cava or renal vein, number of organs affected by the disease, presence of liver metastasis, type of therapy (mitotane, chemotherapy, locoregional therapy), surgical resection of the primary tumor and metastases. Survival will be analysed in relation to the pathohistological and hormonal characteristics of the tumour, the number of organs affected by metastases, liver involvement and type of therapy.

Expected scientific contribution: The results we expect to obtain will allow a better stratification of patient outcomes depending on the parameters studied, and it will be possible to make an appropriate decision on further therapeutic procedures.

MeSH/Keywords: adrenocortical carcinoma, survival, metastases

Poster code: T-02-09-045

Poster Title: Factors affecting use of guideline-recommended drugs in patients with heart failure with reduced left ventricular ejection fraction

PhD candidate: Jelena Kursar

Part of the thesis: Factors affecting use of guideline-recommended drugs in patients with heart failure with reduced left ventricular ejection fraction

Mentor(s): Associate Professor Tomislav Letilović, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Clinical guidelines for the treatment of heart failure recommend the use of drugs in doses that are proven to reduce mortality and morbidity. Treatment is started with low doses of drugs that are well tolerated by most patients, and then the doses are gradually increased to doses that have been proven effective in large randomized studies (or to maximally tolerated doses) (1). Previous clinical investigations have documented a gap between guidelines and practice, although there were no real contraindications or intolerances. The use of drugs for the treatment of heart failure in contemporary Croatian practice is unknown.

Hypothesis: Clinician inertia is a leading factor affecting drug prescribing and guideline-based drug dosing recommendations in patients with heart failure with reduced left ventricular ejection fraction

Aims: The aim of the study is to determine, based on the type and dose of drugs used to treat the subjects, whether there are differences compared to the type and dose of drugs recommended by the guidelines, and to identify factors that influence the prescription of drugs and cause deviations. General objective is to determine factors that influence adherence, discontinuation of therapy and achievement of recommended drug doses in the treatment of heart failure with reduced ejection fraction Specific goals are to determine the factors that influence the achievement of recommended doses or discontinuation of ACE-i/ARB/ARNI therapy, to determine the factors that influence the achievement of the recommended doses or the discontinuation of beta blocker therapy, to determine the factors that influence the achievement of recommended doses or the discontinuation of MRA therapy and to determine the factors that influence the discontinuation of SGLT2 inhibitor therapy

Materials and methods: The subjects of the study are patients with newly diagnosed heart failure with reduced ejection fraction treated in Clinical Hospitals Dubrava and Merkur and Clinical Hospital Center Osijek. The study is the part of the registry "Short-term and long-term outcomes of treatment with SGLT2 inhibitors in patients with heart failure" which was founded at Clinical Hospital Dubrava in March 2022. It is cohort observational study. The study will monitor whether subjects take ACE inhibitors (ACE-I), angiotensin receptor and neprilysin inhibitors (ARNI), angiotensin receptor blockers (ARB), beta blockers, mineralocorticoid receptor antagonists (MRA) and SGLT2 inhibitors and in which doses they take them in three different time points: at the beginning of treatment, after 6 months and after 12 months. Based on a conversation with the subject, an insight into the medical history through the hospital information system, blood pressure and pulse values, laboratory findings, it will be determined whether the failure to reach the target doses of the recommended drugs or the discontinuation of therapy is a consequence of : -objective medical reasons such as hemodynamic intolerance (bradycardia, hypotension), hyperkalemia, renal dysfunction, drug intolerance for other reasons, worsening of heart failure symptoms - limitations/availability of medicines through health insurance - patient decisions/adherence - decisions/inertia of the doctor-clinician treating the subject

Expected scientific contribution: The results of this research will potentially determine the type and doses of drugs used to treat heart failure patients with reduced left ventricular ejection fraction at Clinical Hospitals Dubrava and Merkur and the Clinical Hospital Center Osijek and the patterns of introduction of different types of drugs, whether there are deviations in relation to clinical guidelines, what are the reasons for possible deviations from the guidelines, which factors can potentially be influenced in order to improve the application of the guidelines in clinical practice in Croatia

MeSH/Keywords: heart failure, drugs, evidence-based doses, adherence, clinical inertia

Poster code: T-02-09-051

Poster Title: The influence of pre-transplant coronary angiography on major cardiovascular events (MACE) in kidney and pancreas transplant recipients in the Republic of Croatia from 2003 to 2023.

PhD candidate: Ena Kurtić

Part of the thesis: The influence of pre-transplant coronary angiography on major cardiovascular events (MACE) in kidney and pancreas transplant recipients in the Republic of Croatia from 2003 to 2023.

Mentor(s): Associate Professor Tomislav Letilović, MD PhD, Bojana Šimunov, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Type 1 diabetes mellitus (T1DM) is associated with a significantly increased risk of atherosclerosis, particularly coronary artery disease (CAD), often asymptomatic. Meta-analysis results indicate a twofold increased risk of CAD in individuals with T1DM compared to those without. The duration of T1DM, along with standard risk factors, is a significant predictor of CAD. Simultaneous pancreas-kidney transplantation (SPKT) is a complex surgical procedure for patients with T1DM and advanced chronic kidney disease, necessitating targeted cardiological evaluation to minimize potential adverse events and improve patient outcomes.

Hypothesis: Recipients of pancreas and kidney transplants who underwent pre-transplantation coronary angiography experience fewer major adverse cardiovascular events (MACE) compared to those without the procedure.

Aims: General Objective: To determine the association of cardiovascular outcomes in pancreas and kidney transplant recipients based on whether they underwent pre-transplantation coronary angiography. Specific Objectives: To determine the long-term survival of SPKT recipients at 1, 5, and 10 years post-transplantation. To ascertain the proportion of cardiovascular mortality and MACE. To assess the impact of graft function on cardiovascular mortality. To examine the influence of dialysis modality on post-transplant cardiovascular outcomes. To evaluate the predictive role of pre-transplantation SYNTAX score on long-term cardiovascular risk.

Materials and methods: This study includes SPKT recipients with T1DM and chronic kidney disease between 2003 and 2023. Patients will be recruited from Clinical Hospital Merkur's transplant registry. Baseline assessment will include demographic data, medical history, physical examination, and laboratory tests. Patients will be divided into two groups based on pre-transplantation coronary angiography, with further stratification based on revascularization. Clinical and laboratory parameters will be assessed at baseline, 1 year, and 5 years post-transplantation. The primary outcome is MACE occurrence post-transplantation. Statistical analysis will employ Kaplan-Meier survival analysis and log-rank tests. Continuous variables will be presented with means or medians, while categorical variables will be summarized with frequencies and percentages.

Expected scientific contribution: This research may provide new insights into diagnostic methods and reducing mortality in pancreas and kidney transplant recipients. Being conducted at the Clinical Hospital Merkur, a leading center for solid organ transplantation in the region, this study holds the potential for significant scientific contribution.

MeSH/Keywords: transplantation, SPKT, type 1 diabetes, CV mortality, coronary angiography

Poster code: T-02-09-055

Poster Title: Correlation of serum sphingosine-1-phosphate concentration with posttransplantation diabetes mellitus in kidney transplant patients

PhD candidate: Zrinka Šakić

Part of the thesis: Correlation of serum sphingosine-1-phosphate concentration with posttransplantation diabetes mellitus in kidney transplant patients

Mentor(s): Associate Professor Nikolina Bašić Jukić, MD PhD, Associate Professor Slavica Potočki, MD PhD

Affiliation: Zagreb University Hospital Center, University of Zagreb School of Medicine

Introduction: Posttransplantation diabetes mellitus (PTDM) denotes the manifestation of diabetes following transplantation, irrespective of the timing of diagnosis or whether it was latent before transplantation. Various modifiable and nonmodifiable factors contribute to the increased risk of PTDM among kidney transplant recipients, including immunosuppressive agents, preoperative impaired glucose tolerance, perioperative hyperglycemia, obesity, and infections. Sphingolipids, a diverse group of lipids with structural and signaling roles in cellular processes, are categorized into three main groups, with their disorders linked to several cardiometabolic comorbidities. Previous research on animal and human models has indicated associations between sphingolipid metabolism disorders, diabetes, and kidney disease, involving complex molecular mechanisms such as differential expression of sphingosine-1-phosphate (S1P) receptors and phosphorylases associated with increased insulin resistance in podocytes. Studies on serum S1P concentration variability have demonstrated decreased ApoM/S1P complex concentration in advanced diabetic kidney disease (DKD), whereas S1P concentration is elevated in individuals with type 2 diabetes (T2D), positively correlating with glycated hemoglobin levels. Despite lacking research on kidney transplant recipients, studies have linked decreased podocyte expression of the sphingolipid enzyme SMPDL3b with the recurrence of focal segmental glomerulosclerosis (FSGS) in kidney grafts. Moreover, the nonselective S1P receptor modulator fingolimod has been explored as a potential treatment for graft rejection, with the fingolimod-cyclosporine combination significantly enhancing graft survival, suggesting a possible involvement of sphingolipid concentration disorders in kidney transplant recipients. However, associations between serum sphingolipids and PTDM remain unexplored.

Hypothesis: Increased serum S1P concentration correlates with the presence of PTDM in kidney transplant recipients.

Aims: The main aim is to determine the association between serum S1P concentration and PTDM in kidney transplant recipients. Specific aims include comparing serum S1P concentration among observed groups, assessing S1P association with renal function and diabetes regulation indicators, identifying parameters influencing serum S1P concentration in PTDM patients with transplanted kidneys, and determining the correlation between serum S1P concentration and lipid profiles in all observed groups.

Materials and methods: A cross-sectional study will be conducted at Zagreb University Hospital Center, dividing participants into three groups: those with transplanted kidneys and PTDM, those with transplanted kidneys and T2D, and those with transplanted kidneys without diabetes (control group). Inclusion criteria encompass adult patients with transplanted kidneys for over 12 months, aged over 18, diagnosed with PTDM or T2D, and exhibiting stable graft function in the past year. Exclusion criteria include age over 65, malignancy, liver disease, and other comorbidities (coronary artery disease, peripheral artery disease, autoimmune diseases) affecting serum S1P levels. Blood sampling will be performed during routine clinic visits and will not require additional visits. For sample size calculation a power analysis was performed using the independent samples t-test for a difference in serum S1P concentration among the studied groups, with an expected moderate Cohen effect size, d=0.5, significance level α=0.05, and power 80%. Therefore, each group must include a minimum of 64 participants. The study will enroll 192 participants in total, and its duration is until the planned sample size is achieved.

Expected scientific contribution: The study may elucidate S1P metabolism disorders' role in PTDM pathogenesis and highlight differences between T2D and PTDM in kidney transplant recipients. Additionally, insights into S1P in these recipients may inform PTDM diagnosis, prognosis, and treatment research.

MeSH/Keywords: sphingosine-1-phosphate, posttransplantation diabetes mellitus, type 2 diabetes

Poster code: T-02-09-058

Poster Title: Relationship of clinical and pathological findings in non-specific hepatitis after liver transplantation

PhD candidate: Diana Ilić

Part of the thesis: Relationship of clinical and pathological findings in non-specific hepatitis after liver transplantation

Mentor(s): Professor Tajana Filipec Kanižaj, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Objective: Non-specific hepatitis after liver transplantation includes a wide spectrum of immunopathohistological changes in the structure of the liver parenchyma that can lead to late liver graft dysfunction. It is an alloimmune reaction beyond classic post-transplantation complications, which became relevant due to the frequent subclinical course. Despite the positive aspects and the growing number of non-invasive post-transplantation monitoring techniques, an increasing number of authors emphasize the need for protocol biopsies. The aim of the work is to determine the relationship between standard non-invasive monitoring parameters and timely insight into the pathohistological changes of the liver parenchyma, which could provide information on the severity of structural damage and the potential for progression to clinically significant hepatological morbidity. Subjects and methods: In a prospective study, we will investigate the association of clinical, biochemical and non-invasive parameters with pathohistological findings of liver graft from protocol biopsies. Expected contribution to the field: The above could contribute to better identification of high-risk patients, individualized therapeutic and diagnostic approach, optimization of immunosuppressive therapy and attitude towards protocol liver biopsy

Hypothesis: By combining clinical, biochemical and non-invasive markers, it is possible to determine the existence of histological changes associated with non-specific hepatitis in protocol graft biopsies after liver transplantation

Aims: To determine clinical, biochemical and/or non-invasive markers that are associated with the finding of non-specific hepatitis in the liver graft biopsy after liver transplantation

Materials and methods: Minimum of 70 subjects will be included in the prospective study. They will be monitored in the post-transplantation period through regular visits control in the hepatology transplant clinics of the Institute of Gastroenterology, KB Merkur. All patients who underwent cadaveric orthotopic liver transplantation will be included in the study. It is planned to monitor the following parameters: 1. Patient characteristics 2. Characteristics of the donor 3. Laboratory findings 4. Transient elastography (FibroScan®) 5. Pathohistological findings of liver biopsies (in zero liver biopsy and protocol biopsies in the 6th and 12th months after liver transplants) 6. Immunosuppressive therapy 7. Post-transplantation complications 8. Outcome. For all liver recipients, the follow-up period after the liver transplant surgical procedure will be 12 months. Patients will be divided into two groups. Group 1 will consist of patients with normal or borderline pathohistological findings. Group 2 will consist of patients with pathological PHD finding. At 6 months and 12 months after TJ, clinical, laboratory and elastographic data will be collected and it will be done needle biopsy of the liver. The mutual correlation of the mentioned parameters and with the PHD finding of the basic biopsy will be examined. The data will be presented in tabular and graphical form. An analysis of the normality of the distribution of numerical data will be made (Smirnov- Kolmogorov's test) and corresponding parametric and/or non-parametric statistics will be applied according to the obtained results analysis and ways of displaying data. Quantitative data will be presented through ranges, arithmetic means and standard deviations, that is, medians and interquartile ranges in cases of non-parametric distribution. Survival analysis will be analyzed by Kaplan-Meier curve corresponding log-rank test. All P values less than 0.05 will be considered significant.

Expected scientific contribution: We expect that the proposed research should contribute to a better estimation of the frequency of non-specific hepatitis in post-transplantation follow-up of the patient and a deeper understanding of the correlation of clinical and pathohistological findings. The focus would shift to an individualized approach, optimization of immunosuppressive therapy with the aim of improving the quality of life and long-term survival of the graft and recipient.

MeSH/Keywords: hepatitis, liver transplantation, liver biopsy

Poster code: T-02-09-062

Poster Title: The role of continuous glucose monitoring in assessing beta cell function and clinical manifestations in patients with cystic fibrosis

PhD candidate: Lora Stanka Kirigin Biloš

Part of the thesis: The role of continuous glucose monitoring in assessing beta cell function and clinical manifestations in patients with cystic fibrosis

Mentor(s): Assistant Professor Maja Baretić, MD PhD, Assistant Professor Velimir Altabas, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Early dysglycemia detected using continuous glucose monitoring (CGM) has been associated with worse clinical outcomes in patients with cystic fibrosis (CF), but the relationship between CGM parameters and beta cell dysfunction have not been fully characterized. Furthermore, prospective data evaluating CGM as a tool to predict CF-specific outcomes are lacking.

Hypothesis: CGM measures of hyperglycemia and glucose variability are associated with beta cell dysfunction, body composition, and pulmonary function changes in patients with CF.

Aims: General aim: Determine which CGM parameters are the most associated with changes in beta cell function. Specific aims: determine which CGM parameters are the most associated with the development of glucose intolerance and cystic fibrosis-related diabetes, determine which CGM parameters are the most associated with pulmonary function changes, and determine whether CGM parameters are associated with weight and body composition changes.

Materials and methods: Participants: This prospective observational study will recruit 53 patients from the Cystic fibrosis center for children and adults, University Hospital Centre Zagreb. Inclusion criteria: patients with genetically and laboratory-confirmed CF, age ≥16 years, patients/caregivers capable of understanding informed consent, patients receiving at least 2 years of cystic fibrosis transmembrane conductance regulator (CFTR) modulator therapy. Exclusion criteria: patients with cystic fibrosis-related diabetes (CFRD) treated with insulin, type 1 diabetes, pregnancy, transplant recipients. Methods: Baseline assessment will include anthropometric and anamnestic data including CFTR genotype, medications, pulmonary exacerbations over the past year, chronic infection with microorganisms in the sputum, pancreatic insufficiency, %predicted forced expiratory volume in 1 second (FEV1), and forced vital capacity (FVC). Body composition will be assessed using bioelectrical impedance and report %fat-mass (FM) and fat-free mass (FFM). Laboratory testing will include: complete blood count and biochemistry profile, C-peptide, hemoglobin A1c (HbA1c) and a 2 h 75 g oral glucose tolerance test (OGTT). The OGTT results will be used to classify patients as: normal glucose tolerance (NGT), abnormal glucose tolerance (AGT), and CFRD according to American Diabetes Association criteria. The Homeostasis model assessment indices of beta cell function (HOMA-%beta) and insulin resistance (HOMA-IR) will be calculated. HOMA-%beta <100 will indicate insulin secretion deficiency. HOMA-IR >2 will indicate insulin resistance. All patients will have CGM for up to 2 weeks using the FreeStyle Libre2 (Abbott). CGM parameters analyzed: %time when the sensor was active, mean glucose, glucose management indicator, %time in range of 3.9-10 mmol/L, %time >10 mmol/L, %time >13.9 mmol/L, %time <3.9 mmol/L, %time <3 mmol/L, standard deviation, and coefficient of variation. Data will only be analyzed if there is at least 70% data captured. Protocol: The study includes 2 visits. Visit 1 (T0): baseline clinical and laboratory assessments. Patients with CFRD that do not require insulin, and those with AGT and NGT will be followed until visit 2 (T0+12 months), when repeat assessments will be made. A repeat OGTT will be performed in patients with NGT and AGT. Data will only be analyzed during stable disease. Changes in beta cell function will be defined as >10% change in HOMA-%beta from baseline, changes in pulmonary function as >2% change in FEV1, changes in body composition as >5% change in FM and/or FFM, and weight loss/gain >5%. We will assess which CGM parameters are the most strongly correlated with the aforementioned outcomes using multivariate analysis of variance.

Expected scientific contribution: Identifying CGM parameters associated with beta cell dysfunction and clinical manifestations could help risk-stratify patients with CF. Intermittent CGM could be a complementary risk-assessment tool in patients with CF to implement early therapeutic measures, potentially improving CF complications.

MeSH/Keywords: cystic fibrosis, cystic-fibrosis-related diabetes, beta cell dysfunction, continuous glucose monitoring, body composition, pulmonary function

Poster code: T-02-09-070

Poster Title: Development of a model for non-invasive diagnosis of portal hypertension in advanced chronic liver disease by using time-intensity curves from contrast-enhanced liver ultrasound

PhD candidate: Mislav Barišić-Jaman

Part of the thesis: Development of a model for non-invasive diagnosis of portal hypertension in advanced chronic liver disease by using time-intensity curves from contrast-enhanced liver ultrasound

Mentor(s): Associate Professor Ivica Grgurević, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Portal hypertension (PH) is a clinical syndrome characterized by a pathological increase in pressure in the portal vein, commonly due to advanced chronic liver disease (ACLD). PH severity, assessed by hepatic venous pressure gradient (HVPG) which is a golden standard for PH stratification, significantly impacts prognosis and treatment decisions. Complications of PH occur only when HVPG is≥10 mmHg, when PH is considered clinically significant (CSPH), and the introduction of non-selective beta-blockers (NSBB) is recommended to prevent decompensation and variceal bleeding. However, the invasiveness and limited availability of HVPG measurements have imposed the need for noninvasive methods for assessing PH severity. Contrast-enhanced ultrasound (CEUS) and time-intensity curve (TIC) analysis offer potential for assessing PH severity as CEUS can detect hemodynamic (HD) changes of blood flow through the liver, which is changed in patients ACLD and PH.

Hypothesis: In this study, our hypothesis is that the analysis of the TIC curves obtained from liver blood vessels upon administration of ultrasound contrast can differentiate the severity of PH in patients with ACLD.

Aims: The main objective of this study is to assess the correlation between liver TIC parameters and numerical values of HVPG across the spectrum of PH severity. Specific aims are: (a) to determine if the analysis of TIC curves of liver circulation can differentiate the severity of portal hypertension in patients with ACLD, such as the presence of CSPH (HVPG ≥10 mmHg), and severe portal hypertension (SPH, HVPG≥16 mmHg), and (b) to determine if parameters of TIC analysis of liver circulation change in relation to the presence of hemodynamic response to NSBB, by which it could be potentially possible to identify hemodynamic non-responders in non-invasive manner.

Materials and methods: In this prospective study, patients with ACLD will be included and separated into three groups: patients with cACLD without CSPH, patients with cACLD and CSPH, and patients with decompensated cirrhosis (DC). Patients will be considered of having cACLD based on Liver Stiffness Measurement ≥10 kPa by using transient elastography (TE), and confirmed histologically by liver biopsy, whereas the presence of DC will be established on the ground of typical clinical, imaging and endoscopic criteria. Every patient will undergo laboratory tests, upper gastrointestinal endoscopy, and HVPG measurement. Patients with DC by definition have CSPH. Correlation between the numerical parameters from TIC analyses and the numerical values of HVPG will be investigated, followed by the assessment of the diagnostic performances of TIC analyses to separate the patients according to the presence of CSPH and SPH. The correlation between the changes in TIC analysis parameters with the haemodynamic response to NSBB as assessed by HVPG, will be assessed as well. Prior to HVPG measurement, every patient will undergo CEUS of the liver, followed by TIC analysis to calculate contrast HD parameters including peak intensity, 10% arrival time, time to peak intensity, time to maximum intensity, and intrahepatic transit time. Propranolol will be intravenously administered peri-procedurally to patients with CSPH at a dose of 0.15 mg/kg, and HD response will be assessed by the repeated HVPG measurement. Patients with CSPH and HD response to propranolol will undergo repeated liver CEUS with TIC analysis, immediately after the HVPG measurement. Based on previous studies, a power analysis was conducted, indicating that a total of 72 patients (24 patients per group) are needed to ensure statistical significance, with a recruitment planned over a period of three years.

Expected scientific contribution: We expect to develop a new, noninvasive method for the assessment of severity of PH in patients with ACLD, as well as for monitoring the HD response to NSBB, which is unmet need. This approach might enable more precise risk stratification and precise therapy guidance to patients with ACLD.

MeSH/Keywords: Contrast enhanced ultrasound, Time-intensity curves, Cirrhosis

Poster code: T-02-09-075

Poster Title: Expression levels of circulating miRNAs as biomarkers during treatment of multiple myeloma

PhD candidate: Martina Bogeljić Patekar

Part of the thesis: Dynamics of circulating microRNAs (miR-15a, miR-16-1, miR-21, miR-34a) in patients with multiple myeloma

Mentor(s): Inga Mandac Smoljanović, PhD, research associate

Affiliation: University of Zagreb School of Medicine; University Hospital Merkur Zagreb

Introduction: Multiple myeloma (MM) is a malignant hematological disease of mature B lymphocytes characterized by a proliferation of malignant plasma cells. MicroRNAs (miR) are small non-coding fragments of RNA molecules that regulate post-transcriptional gene expression, and they can play the role of oncogenes and tumor suppressor genes. Some miRNAs are recognized as possible diagnostic and prognostic biomarkers in malignant diseases.

Hypothesis: The expression levels of circulating miR-15a, miR-16-1, miR-34a in patients with multiple myeloma increase, while the level of miR-21 decreases during treatment and can represent a potentially non-invasive marker in the assessment of response to autologous stem cell transplantation.

Aims: General aim is to investigate dynamics in expression of circulating microRNAs (miR-15a, miR-16-1, miR-21, miR-34a) in patients with multiple myeloma at diagnosis and during treatment. The specific aims are: 1. To determine microRNAs expression levels at three time points: at diagnosis, after 4 cycles of induction therapy and after autologous stem cell transplantation. 2. To examine whether there is a correlation between the expression of these microRNAs determined at diagnosis and clinical factors: age, gender, ECOG status, disease stage based on disease staging and risk stratification systems for multiple myeloma (ISS, R-ISS, R2-ISS). 3. To examine whether there is a correlation between the expression of individual microRNAs determined at diagnosis with the type of multiple myeloma and with the percentage of plasma cells (CD138+) determined on histological bone marrow films at diagnosis. 4. To analyze the possible association of the expression of individual microRNAs with laboratory parameters in all three monitoring points (Hb, Trc, B2M, total protein, albumin, creatinine, urea, LDH, Ca). 5. To compare the expression of selected microRNAs with the response to the implemented therapy.

Materials and methods: This prospective study will successively include about 50 newly diagnosed transplant eligible multiple myeloma patients over a period of three years to determine the level of selected miRNAs in the plasma of subjects. First measurement point is at diagnosis, before starting antitumor treatment, second measurement point is after 4 cycles of induction therapy and a third measurement point is +50 days after reinfusion of autologous peripheral stem cells. MicroRNA levels will be determined from the subjects' plasma using the RT-qPCR method on a 7300 Real Time PCR System measuring instrument (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. A total of 6 ml of whole blood is taken in one tube with anticoagulant EDTA repeated in three measurements. Plasma will be extracted from whole blood samples by centrifugation and ultracentrifugation and then stored at -80˚C until miRNA isolation. Isolation and purification of extracellular microRNA from plasma will be done with commercial kits according to the manufacturer's protocol. Quantitative analysis of the expression of certain miRNAs (miR-15a, miR-16-1, miR-21, miR-34a) will be determined by quantitative real time polymerase chain reaction (RT-qPCR). Adequate commercial kits will be used for detection of poor-quality samples and normalization of possible variability in miRNA isolation, monitoring of reverse transcription efficiency (miRNA to cDNA) and as an endogenous control for determination of miRNA expression level. The final step is normalization and analysis of data.

Expected scientific contribution: The results of this research could contribute to a better understanding of the pathophysiology of multiple myeloma. Determining the expression of selected microRNAs at diagnosis could become a potential marker for the prognostic stratification of patients with multiple myeloma. The analysis of the dynamics of the expression of selected microRNAs in multiple myeloma could be of clinical significance in monitoring the response to the applied therapy as well as in the possible individualization of the therapeutic approach in this group of patients.

MeSH/Keywords: multiple myeloma, microRNA

Poster code: T-02-09-103

Poster Title: Effect of SGLT2 inhibitors on epicardial adipose tissue in patients with type 2 diabetes without current or previous symptoms of heart failure

PhD candidate: Ana Đuzel Čokljat

Part of the thesis: Effect of SGLT2 inhibitors on epicardial adipose tissue in patients with type 2 diabetes without current or previous symptoms of heart failure

Mentor(s): Associate Professor Zdravko Babić, MD PhD

Affiliation: University of Zagreb School of Medicine, General Hospital Dubrovnik

Introduction: Epicardial adipose tissue (EAT) is an active participant in metabolism that can affect myocardial function due to shared circulation. Given its rapid metabolism, EAT is one of the possible targets for medications modulating fat, such as SGLT2 inhibitors.

Hypothesis: SGLT2 inhibitors reduce epicardial adipose tissue thickness in patients with type 2 diabetes mellitus.

Aims: To evaluate the effect of SGLT2 inhibitors on the thickness of epicardial adipose tissue measured at three different sites in patients with type 2 diabetes on metformin monotherapy and preserved ejection fraction.

Materials and methods: We plan to conduct a prospective, monocentric, observational study in 75 patients with diabetes mellitus type 2 on metformin monotherapy. Patients will be screened among those routinely reffered to the Division of Cardiology outpatient clinic, General Hospital Dubrovnik. Enrollement will be based on the following inclusion criteria: age ≥ 18, type 2 diabetes mellitus, prior therapy with metformin only, no signs/symptoms of heart failure, left ventricular ejection fraction ≥ 50%, and the following exclusion criteria: angina pectoris, myocardial infarction or cardiac revascularization one year prior to enrollement, moderate or severe valvular heart disease, infiltrative heart disease, primary hypertrophic heart disease, congenital heart disease, patients who are pregnant, who are breastfeeding, current use of DPP-4 inhibitors, GLP-1 agonists, insulin, thiazolidinediones, patients with acute infection, chronic inflammatory systemic disease, active malignant disease, elevated ASTand/or ALT >3x URL, eGFR<30 ml/min/1,73 m2, HbA1c level>9%. Once patient eligibility and written consent are obtained, the study participants will be scheduled for the baseline clinic visit. Each patient will undergo a transthoracic echocardiographic study and a full physical examination, and fasting blood will be drawn for laboratory measurements. Study patients will then be scheduled for follow-up visits at 6 months and 12 months. EAT thickness will be measured as the echo-free space between the outer wall of the myocardium and the visceral layer of the pericardium at the point on the free wall of the right ventricle along the midline of the ultrasound beam, perpendicular to the aortic annulus. The average value of 3 cardiac cycles will be calculated and used for analysis. The second measurement will take place at midventricular parasternal short axis on the right ventricular free wall along the midline of the ultrasound beam perpendicular to the interventricular septum at midchordal and tip of the papillary muscle level. The third EAT measure will be with performed with a high-frequency linear probe in the anterior interventricular groove. The primary outcome of the study is to evaluate EAT thickness changes during treatment with SGLT2 inhibitors. We also want to evaluate whether changes in EAT could be significant after 6 months. Taking into account that the main analysis is ANOVA with repeated measures (in order to assess EAT thickness delta in time), the number of participants was calculated as follows: one group, EAT thickness measures during three visits, moderate effect size (d=0.25), alpha 0.05, beta=1-0.80. Gpower 3.1 for apriori test power calculated number of individuals N=75 for the expected power >0.90. The statistical analysis will be performed by using SPSS Statistics version 25 (IBM Corp., NY, USA).

Expected scientific contribution: Assessment of cardiovascular benefit of epicardial adipose tissue lipolysis in the context of SGLT2 inhibitor treatment, better definition of the cardiovascular disease continuum risk profiles.

MeSH/Keywords: epicardial adipose tissue, SGLT2 inhibitors, type 2 diabetes, heart failure, echocardiography

Poster code: T-02-09-117

Poster Title: Effects of intravitreal bevacizuab on laboratory markers and occurrence of systemic adverse vascular events in patients with neovascular eye diseases

PhD candidate: Karolina Majstorović Barać

Part of the thesis: Intravitreal bevacizumab causes changes in clinical and laboratory parameters that indicate an increased risk of systemic adverse vascular events and diseases in patients with neovascular eye diseases.

Mentor(s): Ivana Mikačić, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Intravitreally (IVT) administered anti-VEGF drugs are standard of treatments for the ocular neovascular diseases. Bevacizumab is an anti-VEGF drug approved for the treatment of malignant diseases. When administered systemically, it has adverse cardiovascular effects. The off-label IVT use of bevacizumab for the treatment of ocular neovascularization is rather common. When administered IVT; it reaches the systemic circulation and reduces the circulating VEGF levels. However, since this practise is off-label, data on cardiovascular safety of IVT bevacizumab are limited and inconclusive.

Hypothesis: Intravitreal bevacizumab causes changes in clinical and laboratory parameters that indicate an increased risk of systemic adverse vascular events and diseases in patients with neovascular eye diseases.

Aims: The proposed study aims to estimate the effect of IVT bevacizumab on laboratory markers and clinical events in patients with neovascular eye diseases.

Materials and methods: During a five-year period, patients who were treated for neovascular eye diseases were enrolled in a registry specifically structured to closely monitor their associated cardiovascular risk.

Expected scientific contribution: Safety assessment of the IVT bevacizumab application concept could indirectly help us for the other anti-VEGF drugs.

Acknowledgments: Clinical Hospital Sveti Duh, Zagreb

MeSH/Keywords: bevacizumab, ocular neovascularization, cardiovascular incidents.

Poster code: T-02-09-120

Poster Title: Long-Term Follow-Up of Lipid-Lowering Therapy Adherence and Cardiovascular Outcomes Post-Acute Myocardial Infarction

PhD candidate: Anđela Jurišić

Part of the thesis: The Association of Sociodemographic Factors and Adherence to Lipid-Lowering Therapy with Long-Term Outcomes in Patients After Acute Myocardial Infarction

Mentor(s): Assistant Professor Igor Rudež, MD PhD, Ivana Jurin, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Despite advancements in modern medicine, acute myocardial infarction (AMI) remains a significant cause of mortality worldwide. Coronary heart disease (CHD) accounts for a substantial portion of global deaths annually, with ST-elevation myocardial infarction (STEMI) presenting notable mortality risks. Recent improvements in treatment modalities have led to decreased short-term and long-term mortality post-STEMI. However, challenges persist in ensuring adherence to secondary prevention measures, particularly in sustaining lifestyle changes and medication regimens.

Hypothesis: We hypothesize that the failure to achieve target LDL-C levels post-AMI is due to non-adherence, poor adherence, and therapeutic inertia in intensifying lipid-lowering therapy (LLT).

Aims: This study aims to comprehensively assess LLT adherence and outcomes post-AMI, with a specific focus on demographic indicators, education level, clinical follow-up frequency, and medication regimen complexity.

Materials and methods: The study cohort comprises 2500 AMI patients who underwent treatment with percutaneous coronary intervention (PCI) or coronary artery bypass grafting at Dubrava Clinical Hospital between January 1, 2017, and December 31, 2023. Patients will be longitudinally followed up for a duration of 5 years through telephone calls or clinical visits to monitor LLT adherence, LDL-C levels, and cardiovascular events. Instances of non-adherence or therapeutic inertia will prompt randomization into intervention or control groups.

Expected scientific contribution: This study endeavors to offer valuable insights into LLT adherence post-AMI, identify key factors influencing adherence, and evaluate the impact of therapeutic inertia on cardiovascular outcomes. The findings hold potential to inform the development of targeted strategies aimed at enhancing adherence and reducing cardiovascular risk post-AMI.

MeSH/Keywords: Acute Myocardial Infarction, Secondary Prevention, Adherence, Lipid-Lowering Therapy, Therapeutic Inertia.

Poster code: T-02-09-134

Poster Title: Comparison of pharmacotherapeutic approaches in the treatment of left ventricular assist device carriers

PhD candidate: Filip Puškarić

Part of the thesis: Comparison of pharmacotherapeutic approaches in the treatment of left ventricular assist device carriers

Mentor(s): Associate Professor Maja Čikeš, MD PhD, Associate Professor Jasper Brugts, MD PhD

Affiliation: University of Zagreb School of Medicine, University Hospital Centre Zagreb, University Hospital Dubrava, Erasmus University Medical Centre, University Medical Centre Utrecht, John Paul II Hospital, Institute for Clinical and Experimental Medicine

Introduction: Heart failure is a heterogeneous group of cardiovascular diseases, including patients with heart failure with reduced ejection fraction (HFrEF) and within it, patients who have advanced heart failure. They are often treated by implanting a left ventricular assist device (LVAD), commonly the HeartMate 3 (HM3) LVAD. This PhD will be based on data collected as part of a multicenter, randomized, open-label, phase IV, parallel-group pilot study to assess the safety and tolerability of sacubitril/valsartan in HM3 LVAD carriers - ENVAD-HF, sponsored by the University of Zagreb School of Medicine.

Hypothesis: Sacubitril/valsartan is equally safe and tolerable in the HM3 LVAD device carriers as the standard of care for the treatment of hypertension in respect to overall mortality, deterioration of renal function, hyperkalemia or symptomatic hypotension leading to drug withdrawal.

Aims: GENERAL AIM: The aim of this research is to compare pharmacotherapeutic approaches in the treatment of LVAD carriers. SPECIFIC AIMS: The primary objective of the trial is to evaluate the safety and tolerability of sacubitril/valsartan in HM3 LVAD device carriers, compared to the standard treatment of hypertension in terms of: 1. reduction of total mortality; 2. deterioration of renal function (defined as end-stage renal disease, renal death or a permanent drop in eGFR of 50%); 3. hyperkalemia; 4. symptomatic hypotension that leads to drug withdrawal. Additionally, there are multiple secondary and exploratory objectives.

Materials and methods: A total of 60 clinically stable HM3 LVAD patients who have either recently (within one year) been implanted with an HM3 LVAD device, are stable and considered ready for hospital discharge, or are chronic, stable, ambulatory HM3 patients with an LVAD will be included in this trial. The study protocol defines a total of eight study visits. After the screening visit, a subject is randomly (1:1) assigned either to sacubitril/valsartan or to the standard of care for hypertension treatment. For both groups of subjects, the therapeutic goal of blood pressure treatment will be achieving and maintaining mean blood pressure between 75 and 90 mmHg. Except for the questionnaires on the quality of life, study protocol states standard examinations undertaken as part of outpatient follow ups of patients with an LVAD. The data will be collected in the secure, online platform REDCap. Each involved trial participant will receive a unique participant code under which their de-identified data will be collected. Summary statistics will be available by treatment group for demographic and baseline clinical characteristics, in mean values and standard deviations for normally distributed continuous variables, i.e. in medians and interquartile ranges for continuous variables whose distribution deviates from normal or in frequency and percentages for categorical variables.Time-to-event will be computed as the number of days from randomization to the start date of the primary endpoint event (first occurrence of any of the primary endpoint components). Kaplan-Meier and Cox regression analyses will be used for the assessment of the primary outcome, and based on the Safety population. Survival data will be presented using the Kaplan-Meier method. A one-tailed level will be used to demonstrate statistical significance level of 0.025 (or two-sided of 0.05).

Expected scientific contribution: We expect that the results will provide new information about pharmacotherapeutic approaches in LVAD carriers. We expect the results of the ENVAD-HF trial to demonstrate whether blood pressure treatment in HM3 LVAD carriers with sacubitril/valsartan is safe. Furthermore, we believe that it will provide a strong hypothesis-generating basis for future trials that will be able to achieve sufficient statistical power to draw conclusions about the efficacy of sacubitril/valsartan.

Acknowledgments: I would like to acknowledge all the help in making this PhD thesis proposal possible, especially from Professor Maja Čikeš, MD, PhD (University of Zagreb School of Medicine, Department of Cardiovascular Diseases, University Hospital Center Zagreb).

MeSH/Keywords: sacubitril/valsartan, LVAD, pharmacotherapy, hypertension

Poster code: T-02-09-135

Poster Title: Comparison of the effects of mTOR and calcineurin inhibitors on nutrition and metabolic outcomes in kidney transplant recipients

PhD candidate: Armin Atić

Part of the thesis: Comparison of the effects of mTOR and calcineurin inhibitors on nutrition and metabolic outcomes in kidney transplant recipients

Mentor(s): Associate Professor Nikolina Bašić Jukić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Kidney transplantation is currently the best treatment option for end-stage kidney disease. Organ transplantation, however, necessitates the use of chronic immunosuppressive therapy. Current immunosuppression protocols mainly consist of calcineurin inhibitors (CNI) or mammalian target of rapamycin inhibitors (mTORi) in combination with mycophenolate and corticosteroids. These drugs, however, carry a sizeable metabolic burden that manifests with impairments in glucose and lipid metabolism, weight, and body composition. The introduction of CNI and mTORi has significantly improved short-term outcomes in kidney transplant recipients (KTRs), however, long-term outcomes have remained largely unchanged in the past two decades. The nutritional and metabolic status of KTRs is associated with significant changes in both short and long-term outcomes. Having a worse nutritional state (e.g. obesity) at the time of transplantation is associated with a higher prevalence of delayed graft function and wound healing, surgical complications, acute graft rejection, and prolonged hospital stay. In the long term, obese patients are more likely to develop cardiovascular disease, and post-transplantation diabetes mellitus and have a higher overall mortality. Both mTORi and CNI are diabetogenic, but this effect is more pronounced in CNI, particularly with tacrolimus. The effects of CNI on the nutritional states have been studied, however, the long-term effects of mTORi on the nutritional status are unknown. A complete understanding of chronic immunosuppressive therapy's effects is imperative for improving long-term outcomes in this patient population. The complex interaction of earlier chronic kidney disease, renal replacement therapy, and existing nutritional and metabolic disorders coupled with the effects of chronic immunosuppressive therapy form an incomplete mosaic of optimal management of KTRs.

Hypothesis: Patients treated with CNI have worse nutritional and metabolic outcomes compared to patients treated with mTORi.

Aims: The aim is to determine the association of the type of chronic immunosuppression with the occurrence of nutritional and metabolic disorders in kidney transplant recipients.

Materials and methods: The study is designed as a cross-sectional study including adult kidney transplant recipients (age > 18 years) who were transplanted over a year ago, with stable graft function and without signs of acute disease or other states requiring hospital admission or work-up. All participants will have laboratory examinations, anthropometric examinations (weight, height, waist, arm, and hip circumference, triceps and scapular skin fold), validated nutritional status questionnaires (Malnutrition-inflammation score, Subjective Global Assessment), functional tests, and body composition measurement using bioelectrical impedance. Selected outcomes based on the tests are sarcopenia, obesity, and protein-energy wasting. Participants are split into two groups based on the chronic immunosuppressive used (CNI or mTORi). The two groups are split into subgroups depending on the time since transplantation (1-3 years, 3-5 years, and more than 5 years). To reduce confounding, participants will be matched using propensity score matching. A power analysis for the strength of 80% has determined a total study sample of 192 participants, with equal numbers in each subgroup. Standard statistical tests will be used to compare the groups, including a correlation and regression analysis to create a multivariate regression model.

Expected scientific contribution: The findings of this study may examine the association of the type of immunosuppressive therapy with nutritional and metabolic disorders. This study may determine the association of long-term mTORi use with nutritional disorders in KTRs. The results may add information for individualizing immunosuppressive therapy protocol after kidney transplantation.

MeSH/Keywords: kidney transplantation, nutritional disorders, metabolic disease, immunosuppression

Poster code: T-02-09-138

Poster Title: Correlation between diastolic function and atrioventricular interval length in cardiac conduction system stimulation

PhD candidate: Mislav Nedić

Part of the thesis: Correlation between diastolic function and atrioventricular interval length in cardiac conduction system stimulation

Mentor(s): Assistant Professor Vjekoslav Radeljić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Cardiac pacing represents a crucial therapeutic intervention in the management of bradycardic rhythm disorders, with precise determination of the atrioventricular (AV) interval playing a pivotal role in optimizing pacemaker function. This parameter is vital for ensuring synchronized activation of the atria and ventricles, which is fundamental for maintaining effective cardiac function. However, conventional right ventricular apex pacing, while widely accepted, carries certain limitations, including the risk of worsening cardiac hemodynamics, including systolic and diastolic function, due to asynchronous myocardial contractions. In this context, contemporary research highlights conduction system pacing as a potentially superior option, as it allows for physiologically favorable cardiac stimulation resulting in coordinated activation of myocardium in both ventricles. In previous studies, this innovative method has shown benefits in patients with heart failure with preserved ejection fraction (HFpEF), where diastolic dysfunction is a key pathophysiological mechanism. Despite evident progress, there is currently a lack of firmly established scientific guidelines that unequivocally define optimal atrioventricular interval settings in the context of conduction system pacing. Therefore, further research and clinical experience are necessary to better understand the most effective strategies for managing cardiac rhythm and function.

Hypothesis: In patients with conduction system pacing and longer programmed atrioventricular interval than in right ventricular apex pacing, left ventricular filling pressures measured by E/e' will be lower.

Aims: The general aim of the study is to determine the optimal value of the atrioventricular interval in relation to left ventricular filling pressures measured by E/e'. Specific objectives include assessing the impact of atrioventricular interval length on other echocardiographic parameters of cardiac function, N-terminal pro-B-type natriuretic peptide (NTproBNP) levels, and patient quality of life.

Materials and methods: A prospective cohort study is planned at the Department of Cardiology, University Hospital Sisters of Charity, involving patients aged 50 to 80 with second or third-degree atrioventricular block admitted for pre-syncope or syncope. Patients without a history or clinical signs of cardiac or systemic diseases will be enrolled. All patients will undergo dual-chamber cardiac pacemaker implantation with conduction system stimulation. The AV interval will be initially set to 200ms and subsequently adjusted to 150ms and 250ms at one-month intervals. Diastolic function parameters, NTproBNP levels, and functional capacity will be assessed at each visit. Echocardiography and NTproBNP measurement will be performed. Patients with <90% ventricular pacing on the first visit will be excluded. Statistical analysis will categorize data based on programmed AV interval. Participants will be followed for 3 months with echocardiographic monitoring every 30 days. The analysis will include at least 46 subjects to assess the association between atrioventricular interval and E/e' ratio, as well as various echocardiographic parameters, NTproBNP levels, and quality of life, to determine the impact of atrioventricular interval length on outcomes.

Expected scientific contribution: This research represents a pivotal endeavor, aiming to bridge the connection between physiological cardiac electrostimulation and cardiac hemodynamics. By identifying the optimal atrioventricular interval during the procedure, it offers insights into its hemodynamic effects and potential enhancements to patients' quality of life.

MeSH/Keywords: Conduction system pacing; Pacemaker optimization; Cardiac Pacing; Atrioventricular Interval; Conduction System of Heart; Diastolic Dysfunction; Hemodynamics

Poster code: T-02-09-147

Poster Title: Expression and prognostic significance of HER 2, estrogen and progesterone receptors in gallbladder carcinoma

PhD candidate: Suzana Janković

Part of the thesis: Expression and prognostic significance of HER 2, estrogen and progesterone receptors in gallbladder carcinoma

Mentor(s): Assistant Professor Mario Zovak, MD PhD, Melita Perić Balja, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Gallbladder carcinoma is the most common biliary tract malignancy. Its aggressive malignancy is usually diagnosed in advanced stages of disease. Therapeutic options are limited and 5-year survival of this disease is poor.

Hypothesis: Increased expression of the HER 2 receptor is associated with a worse prognosis in patients with gallbladder cancer. Increased expression of estrogen and progesterone receptors is associated with a better prognosis in patients with gallbladder cancer.

Aims: To examine the expression of HER 2, estrogen and progesterone receptors in gallbladder carcinoma, their mutual connection as well as the relation to other clinical-pathological features in patients operated due to gallbladder carcinoma.

Materials and methods: A retrospective research will be conducted in which the archival materials of the „Ljudevit Jurak“ Clinical department for pathology (UHC Sestre Milosrdnice) will be used together with all relevant patient clinical dana obtained from BIS (hospital information system) in at least 60 patients who underwent surgery due to gallbladder carcinoma. Immunohistochemical analysis using HER 2, estrogen and progesterone receptor antibodies will be performed on the slides from representative paraffin-embedded tissue block. The results will be compared to other clinicopathological features. Descriptive and analytical statistical methods will be used in the analysis of the results.

Expected scientific contribution: The existence of a possible connection between the expression of HER 2 and estrogen and progesterone receptors could have a prognostic value and lead to the development of new treatment modalities for gallbladder carcinoma.

MeSH/Keywords: gallblader carcinoma, HER 2, estrogen, progesterone, prognosis

Poster code: T-02-10-072

Poster Title: Assessing the clinical significance of using fascia lata versus temporalis muscle fascia for skull base reconstruction in sellar region

PhD candidate: Marcel Marjanović Kavanagh

Part of the thesis: Assessing the clinical significance of using fascia lata versus temporalis muscle fascia for skull base reconstruction in sellar region

Mentor(s): Associate Professor Goran Mrak, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Postoperative cerebrospinal fluid leaks (CSFL) are a significant problem following endoscopic resection of tumors in the sellar region. The reconstruction of dural defects remains a challenge. In recent research, the use of temporal muscle fascia for dural reconstruction has been shown to reduce the frequency of postoperative complications and has shown superior biomechanical properties compared to fascia lata.

Hypothesis: Utilizing the temporalis fascia compared to fascia lata for the dural defects reconstruction following endoscopic tumor resections in the sella turcica region decreases the incidence of postoperative complications.

Aims: GENERAL OBJECTIVE: Determine and compare the level of donor site pain and assess the clinical value of using fascia lata and temporal muscle fascia in the reconstruction of defects in the dura mater following endoscopic resections of tumors in the sellar region. SPECIFIC OBJECTIVES: * Determine the incidence of complications among the groups: meningitis, postoperative CSF leak, donor site dehiscence, donor site infection, donor site seroma, facial nerve paresis, hypoesthesia of donor site skin, diabetes insipidus (temporary/permanent); * Analyze the quality of life in the study groups after surgery (EQ-5D-5L); * Compare the duration of surgical procedures; * Compare the length of hospitalization between the groups.

Materials and methods: The study, approved by the Ethical Committee of the Clinical Hospital Center Zagreb, will start in August 2023 and aims to last 2-3 years, focusing on reconstructing the sella turcica floor using fascia lata and temporal fascia in 68 adult patients undergoing endoscopic transnasal surgery for sellar pathology. Participants will be randomized into two groups of 34, using either fascia lata or temporal fascia, selected via Research Randomizer software. The study excludes patients with donor site pathology, absence of intraoperative leaks, or previous radiation. All surgical procedures will be standardized and performed by a dedicated team to minimize biases. Additionally, sociodemographic data will be collected and a strict anonymization protocol will be employed to protect participant privacy.

Expected scientific contribution: The Expected Contribution of the Research is to identify the optimal method and materials for skull base reconstruction with the lowest frequency of complications, in order to reduce perioperative morbidity and enable earlier rehabilitation of patients.

MeSH/Keywords: cerebrospinal fluid leak, fascia lata, skull base, tissue transplants, quality of life

Poster code: T-02-10-091

Poster Title: Use of S100B biomarker for determination of severity, prognosis and need for brain neuroradiological assessment in paediatric patients with traumatic brain injury

PhD candidate: Hrvoje Krpina

Part of the thesis: Use of S100B biomarker for determination of severity, prognosis and need for brain neuroradiological assessment in paediatric patients with traumatic brain injury

Mentor(s): Assistant Professor Ivana Jurjević, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Protein 100B is a part of astrocytic signaling system. In case of traumatic brain injury, disruption of brain tissue and blood-brain barrier could elevate of S100B concentration in blood serum. Thus, it is considered a promising biomarker regarding traumatic brain injury. According to accepted guidelines, its elevated serum levels can help clinicians in determining the severity, prognosis, and the need for neuroradiological assessment of adult head injury patients. To date, it is not clear if it is possible to do the same in paediatric population.

Hypothesis: Measurement of S100B protein concentration in the blood could predict severity and prognosis of traumatic brain injury in paediatric population, as well as reduce the need for neuroradiological assessment.

Aims: To investigate the S100B protein potential for reducing the use of radiological methods (especially computed tomography) in paediatric population, to investigate its ability to discriminate between children with mild, moderate and severe traumatic brain injury, and to explore its prognostic potential.

Materials and methods: Investigation will take place at Zadar General Hospital and Children's Hospital Zagreb. Patient will be divided according to Glasgow Coma Scale and Head Injury Severity Score in groups of mild (GCS13-15), moderate (GCS 9-13) and severe (GCS 3-9). Each group will contain a minimum of 30 participants, as well as a healthy control group. S100B protein will be extracted from blood serum upon arrival (and not more than 6 hours after the trauma) for all participants, and for neurotrauma patients again 24 and 72 h after the injury. Patients will also be clinically assessed in the same time intervals. Cerebrospinal fluid S100B protein will be sampled together with serum samples in severe injury patient group if external ventricular drainage is placed. Other clinical and laboratory findings will be considered in the study (isolated trauma, polytraumatic patient, hipo or hypervolemic patients). Patients will be divided into age-appropriate subgroups, and according to the literature, different cut-off levels of S100B protein will be applied.

Expected scientific contribution: This study will contribute to a more reliable and efficient use of S100B protein as a biomarker, as well as reduce the need for x-ray exposure in children, concomitantly reducing the cost of paediatric neurotrauma management.

MeSH/Keywords: traumatic brain injury, TBI, protein S100B, biomarker, computerizes tomography, CT, magnetic resonance imaging, MRI, blood serum, cerebrospinal fluid, CSF

Poster code: T-02-10-150

Poster Title: Correlation of non-small cell lung cancer metabolic characteristics on 18F FDG PET/CT with expression of immune ligands PD-L1 and TIGIT on tumor cells

PhD candidate: Anita Tabain

Part of the thesis: Correlation of non-small cell lung cancer metabolic characteristics on 18F fluorodeoxyglucose positron emission tomography/computed tomography with expression of immune ligands PD-L1 and TIGIT on tumor cells

Mentor(s): Assistant Professor Tihana Regović Džombeta, MD PhD, Assistant Professor Marija Gomerčić Palčić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Lung cancer is the leading cause of cancer-related mortality globally, but recently the mortality rate has declined primarily due to advances in tumor immunotherapy. The therapeutic approach to non-small cell lung cancer (NSCLC) is determined by the tumor histopathological characteristics and the extent of the disease. Pathohistological immune ligand PD-L1 represents the gold standard in the selection of patients who will benefit from immunotherapy, while researches on drugs that target another immune ligands, like TIGIT, are still ongoing. Positron emission tomography/computed tomography with 18F-fluorodeoxyglucose (18F-FDG PET/CT) is a non-invasive imaging procedure used in oncology for staging of the disease and evaluating treatment efficacy. Standardized uptake value (SUV) derived from 18F-FDG PET/CT is a significant metabolic prognostic imaging biomarker which correspond with multiple NSCLC characteristics. Correlation of imaging and pathohistological biomarkers in NSCLC processing could have impact on treatment optimization and also novel therapeutic development.

Hypothesis: The standardized uptake value of 18F-fluorodeoxyglucose on positron emission tomography/computed tomography will be higher with increased expression of immunological ligands PD-L1 and TIGIT on tumor cells and/or in the tumor microenvironment.

Aims: Primary aim is to assess PD-L1 and TIGIT ligand expression in non-small cell lung cancer subtypes (adenocarcinoma and squamous cell carcinoma) and the tumor microenvironment, comparing these results with tumor metabolic characteristics obtained by 18F-fluorodeoxyglucose PET/CT scans. Additional aim is to correlate PD-L1 and TIGIT expression with clinical factors (age, gender, smoking, stage, survival) and also compare PET/CT imaging findings with same clinical-pathological characteristics.

Materials and methods: The study will include patients managed within the Multidisciplinary Thoracic Tumor Board, who underwent 18F-FDG PET/CT to assess the extent of potentially operable NSCLC. Standard oncological PET/CT procedure will be performed, with semiquantitative analysis determining standardized uptake value (SUV), as a measure of tumor metabolic activity. Histological tissue samples obtained by biopsies or surgical excisions will be used for the analysis of PD-L1 and TIGIT immune ligand expression with anti-TIGIT in vivo gen and PD-L1 Ventana (SP142) clone, categorized into negative, weakly positive, and positive results. Predominantly retrospective, and partly prospective, non-interventional study will be conducted. A minimum of 23 patients, each in the adenocarcinoma and squamous cell carcinoma groups (total of 46 patients), is required for statistical significance, determined through sample size calculations based on statistical power. Data distribution normality will be assessed using the Smirnov-Kolmogorov test. Parametric or non-parametric tests will be used accordingly, along with appropriate presentation of continuous values (mean, standard deviation, or median). Given multiple variables, tests compensating for patient-to-variable ratios will be applied. Analysis methods would include binary logistic regression, Kruskal-Wallis or ANOVA tests, multivariate Cox regression analysis, and Kaplan-Meier curves for survival analysis.

Expected scientific contribution: Previous scientific studies have not addressed the relationship between the expression of new immunohistochemical ligand (TIGIT) in non-small cell lung carcinoma and the metabolic characteristics of tumors on 18F FDG PET/CT. We expect that the findings will deepen our understanding of tumor behaviour, aiming for further development of personalized treatment approaches.

MeSH/Keywords: NSCLC, 18F FDG PET/CT, immunotherapy, PD-L1, TIGIT

Poster code: T-02-17-069

Poster Title: Correlation between thyroid function indicators and biometric eye components in children with Down syndrome

PhD candidate: Eva Kos

Part of the thesis: Correlation between thyroid function indicators and biometric eye components in children with Down syndrome

Mentor(s): Assistant Professor Nevena Krnić, MD PhD, Associate Professor Mirjana Bjeloš, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Down syndrome, the most prevalent chromosomal disorder among live births, presents with various physical and developmental characteristics. Among these are ophthalmic abnormalities and a predisposition to autoimmune thyroid dysfunction. Ophthalmic findings associated with Down syndrome include upslanting palpebral fissures, epicanthus, refractive errors, strabismus, nystagmus, Brushfield's spots, cataract, glaucoma, and corneal abnormalities, namely keratoconus. Thyroid hormones play an important role in the growth, development and maturation of somatic and neural tissues, and therefore in the development of eye structures. Understanding the correlation between thyroid function and ocular biometrics in Down syndrome children can improve our knowledge of relationship between thyroid and eye conditions and can facilitate the management of patients with Down syndrome.

Hypothesis: Thyroid function indicators correlate with biometric eye components in children with Down syndrome.

Aims: To determine correlation between thyroid function indicators and biometric eye components in children with Down syndrome.

Materials and methods: Children with Down syndrome aged 0-17 are included in the research. Biometric eye measurements include axial length of the eye, white-to-white corneal diameter, lens thickness, anterior chamber depth, intraocular lens (IOL) power (Acrysoft IQ monofocal SN60WF (Alcon Laboratories Inc., Fort Worth, Texas, USA) calculated by the SRK-T formula for the refractive value of emmetropia. Additionally, corneal tomography parameters, integral to the diagnosis of keratoconus, including corneal thickness, anterior, and posterior curvature are analyzed. The measurements will be obtained using corneal tomogram Pentacam® and the optical biometer IOL Master®. As part of a multidisciplinary monitoring, all patients will be analyzed for thyroid function parameters: free thyroxine concentration (fT4), thyroid-stimulating hormone (TSH), thyroglobulin antibody (TgAb), and tissue peroxidase antibody (TPOAb). Height, weight and head circumference will be measured for all patients.

Expected scientific contribution: The research will expand the current knowledge regarding the interplay between thyroid function and ocular biometrics in Down syndrome children. By establishing normative ocular biometrics databases for this population, and the assessment of disease prevalence characteristics, we aim to facilitate the early identification of individuals at risk of developing or progressing keratoconus. Additionally, our findings may inform clinical strategies for the timely intervention and monitoring of these patients, thereby improving their long-term ocular health outcomes.

Acknowledgments: Prof. Vladimir Trkulja, MD PhD, Prof. Zdenko Sonicki, MD PhD

MeSH/Keywords: Down syndrome, biometric eye components, thyroid, keratoconus

Poster code: T-02-18-040

Poster Title: Microvascular changes in macula analyzed by optical coherence tomography angiography after clear lens extraction

PhD candidate: Ranka Motušić Aras

Part of the thesis: Microvascular changes in macula analyzed by optical coherence tomography angiography after clear lens extraction

Mentor(s): Professor Nenad Vukojević, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: The aim of this study is to investigate microcirculation changes in the macular area after clear lens extraction (CLE) using optical coherence tomography angiography (OCTA).

Hypothesis: Clear lens extraction does not lead to significant changes in the macular circulation.

Aims: The overall objective of this study is to investigate in detail changes in the vascular density of the macular area before and after CLE using optical coherent tomography angiography (OCTA). Specific objectives: 1. Assess vascular density: Analyze vascular density in superficial vascular complex (SVC) and deep vascular complex (DVC) of the retina before and after CLE; 2. Consider the clinical implications of the results, especially in relation to the optimal selection of patients who have undergone CLE; 3. Consider whether changes in retinal microcirculation affect postoperative recovery.

Materials and methods: The aim of this prospective study is to investigate the change in vascular parameters of the macular area after clear lens extraction (refractive lens replacement). The study will be conducted at The Bilic Vision Polyclinic on a total of 52 patients with clear lens. All patients will undergo microincision phacoemulsification with intraocular lens implantation under topical anesthesia. Criteria for inclusion in the study: 1. clear lens, 2. axial length 20-26 mm, 3. intraocular pressure (IOT) measured by Goldmann applanation tonometry 10-21 mmHg, 4. age up to 60 years. The exclusion criteria for the study are: glaucoma, other pathology of the anterior or posterior segment of the eye (corneal changes, diabetic retinopathy, senile macular degeneration, retinal vascular diseases...), history of eye trauma, systemic diseases such as diabetes mellitus, unregulated arterial hypertension, inflammatory or cardiovascular diseases that could affect macular circulation, drugs affecting circulation, intraoperative and/or postoperative complications, poor quality of OCTA images. Prior to the surgery, all patients will have detailed ophthalmic examination that will include: best corrected visual acuity, auto-refractokeratometry, endothelial speculative biomicroscopy, optical biometry, IOT measurement with Goldmann applanation tonometry, optical coherence tomography (OCT) of the macula and the optic disc, anterior segment examination on a biomicroscope and posterior segment examination in mydriasis and OCTA imaging. Also, all patients will have their blood pressure measured before surgery. OCT and OCTA will be recorded with DRI OCT Triton Swept source OCT. Microvascular parameters will be measured using OCTA before surgery, 1 week, 1 month, 3 and 6 months after surgery obtaining images in size 3x3 mm. Vascular density will be measured in the superficial and deep vascular complex of the retina. All parameters will be measured automatically. Statistical analysis will be performed using MedCalc statistical software. Variables will be presented as median and interquartile ranges or mean values and standard deviations. Comparison of the values obtained before and after surgery will be performed using the nonparametric Friedman ANOVA test. The significance level is set at $p < 0.001$.

Expected scientific contribution: The scientific contribution of this study lies in the investigation of the impact of clear lens extraction on the microcirculation of the macular area via optical coherent tomography angiography (OCTA). This study focuses on the analysis of vascular density changes after CLE and provides insight into the dynamics of the microvascular network in the macula.

MeSH/Keywords: optical coherence tomography angiography, clear lens extraction, refractive lens replacement, vascular density

Poster code: T-02-18-152

Poster Title: The relationship between phase angle values and the neutrophil-lymphocyte ratio as a measure of reduced muscle mass and their value as potential prognostic and predictive factors for treatment outcomes in patients with metastatic gastric cancer

PhD candidate: Irma Goršić

Part of the thesis: The relationship between phase angle values and the neutrophil-lymphocyte ratio as a measure of reduced muscle mass and their value as potential prognostic and predictive factors for treatment outcomes in patients with metastatic gastric cancer

Mentor(s): Professor Stjepko Pleština, MD PhD, Juraj Prejac, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Phase angle (PA) and neutrophil-to-lymphocyte ratio (NLR) are potential new biomarkers for prediction of treatment outcomes in oncology patients. To date, performed studies are mostly retrospective, patients with metastatic gastric cancer (mGC) treated with chemotherapy were not included, nor was the association of PA and NLR with treatment outcomes investigated.

Hypothesis: Patients with metastatic gastric cancer who have a lower phase angle measured have worse treatment outcomes.

Aims: GENERAL OBJECTIVE: To determine the relationship between the size of the phase angle and NLR as new biological indicators and predictors of a worse therapeutic outcome of treatment with the outcomes of first-line treatment of patients with metastatic gastric adenocarcinoma SPECIFIC GOALS: 1. to measure the NLR of the subjects before the start and at the end of the first-line treatment and to determine the association with sarcopenia and tolerance of chemotherapy 2. to determine the correlation of the size of PA and NLR with treatment outcomes 3. determine the relationship between the size of PA and NRL with previous biochemical markers of sarcopenia and clinical indicators of the general condition during chemotherapy and to detect the need for earlier reevaluation of treatment in the case of worsening of the above-mentioned parameters

Materials and methods: Patients with mGC who are starting with chemotherapy will have NLR calculated and PA values measured using a BIA scale. Measurements will be perforemd once a month. The association of PA and NLR values with treatment outcomes, primerly time until disease progression to first-line treatment, association with tumor biological characteristics, previous treatment and patient characteristics will be monitored.

Expected scientific contribution: To prove the value of PA and NLR as potential new predictive and prognostic biomarkers of the treatment outcome of patients with mGC obtained by non-invasive measurement and to potentially detect disease progression earlier based on the obtained measurements. If this proves to be the case, it would lower the costs of applying unefficient chemterapy as well as unnecessary exposure of patients to ineffective therapy and toxicity from it.

MeSH/Keywords: Phase angle, neutrophil-to-lymphocyte ratio, reduced muscle mass, metastatic gastric cancer

Poster code: T-02-19-014

Poster Title: Contribution of bevacizumab to improving treatment outcomes in ovarian cancer patients in Croatia

PhD candidate: Kristina Katić

Part of the thesis: Contribution of bevacizumab to improving treatment outcomes in ovarian cancer patients in Croatia

Mentor(s): Associate Professor Marija Milković Periša, MD PhD, Professor Eduard Vrdoljak, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Ovarian cancer (OC) is the leading cause of death in women with gynecological cancers. Most patients are present with advanced disease at diagnosis and have poor prognosis. The standard of care is a combination of cytoreductive surgery and platinum-based chemotherapy Despite the initial therapy, the majority of patients develop progression of the disease within three years The addition of bevacizumab prolongs progression-free survival, both in the first line of treatment and in patients with a first recurrence of the disease. The impact of bevacizumab on real-world clinical outcomes, particularly in transitional and single-country healthcare systems, has not been studied.

Hypothesis: The addition of bevacizumab to first-line chemotherapy in patients with OC prolongs progression free survival (PFS) compared to patients not treated with bevacizumab.

Aims: To examine the efficacy of bevacizumab in first- and second-line treatment compared to the patients with similar characteristics who did not receive bevacizumab.

Materials and methods: This retrospective cohort trial will include patients with ovarian, fallopian tube or primary peritoneal cancer treated with chemotherapy with bevacizumab at UHC (University Hospital Center) Zagreb, UHC Split, UHC Sestre Milosrdnice and UHC Osijek in the period from March 20117 to August 2020, as well as patients with the same characteristics treated only with chemotherapy during the period when bevacizumab was not approved (from January 2013 to March 2017). We estimate that we will enroll 200 patients treated with chemotherapy and bevacizumab and 100 patients treated with chemotherapy alone. Demographic, clinical and treatment data will be collected from the hospital information system. The primary endpoint is PFS in the first-line treatment, and secondary endpoints are overall survival (OS) in the first-line and PFS and OS in the second-line treatment. We will stratify PFS and OS data in newly diagnosed patients by residual disease after surgery and pathological type of disease, and in second-line treatment by time to disease progression and type of chemotherapy. We will examine hematological and nonhematological adverse events during treatment (graded according to the Common Terminology Criteria for Adverse Events, version 5.0). The hypothesis will be tested by a multivariable Cox proportional hazard regression analysis with adjustment for age, performance status, histological type of tumor, previous chemotherapy treatment, and PFS and OS using the Kaplan-Maier method. The two-tailed level of statistical significance will be set at p<0-05 and the confidence interval at 95%.

Expected scientific contribution: To define the role of bevacizumab in the treatment of patients with EOC.

MeSH/Keywords: ovarian cancer, maintenance therapy, bevacizumab

Poster code: T-02-19-096

Poster Title: The significance of time to next treatment and time on treatment for the overall survival of patients with metastatic melanoma treated with systemic therapy

PhD candidate: Krešimir Blažičević

Part of the thesis: The significance of time to next treatment and time on treatment for the overall survival of patients with metastatic melanoma treated with systemic therapy

Mentor(s): Assistant Professor Daška Štulhofer Buzina, MD PhD, Assistant Professor Davorin Herceg, MD PhD

Affiliation: University Hospital Center Zagreb

Introduction: The treatment of metastatic melanoma has been significantly improved with the introduction of new treatments - targeted therapy and immunotherapy. Efficacy has been confirmed in randomized controlled trials, but real-world data is increasingly being explored. Treatment efficacy is evaluated by clinical outcomes and overall survival is the gold standard. Due to the need for earlier assessment of treatment effects, surrogate outcomes are increasingly being used. Progression-free survival and response rates did not appear to be optimal, while time to next treatment and time on treatment may be better surrogate outcomes for overall survival.

Hypothesis: The time to next treatment and time on treatment are related to the overall survival of patients with metastatic melanoma treated with systemic therapy.

Aims: The aim of research is to determine the relation of new surrogate outcomes with overall survival in patients with metastatic melanoma treated with systemic therapy (immunotherapy and targeted therapy). Secondary aims include comparison of the outcomes for first-line treatment (immunotherapy versus targeted therapy) and also determining the association of prognostic factors with the overall survival of patients with metastatic melanoma treated with systemic therapy.

Materials and methods: In this observational retrospective cohort study, patients treated with systemic therapy for metastatic melanoma will be analyzed. Data will be analyzed from the Central South Eastern Europe Registry (CSEEREG) as part of the European Melanoma Registry (EUMelaReg). Patients who started first-line treatment in the period from January 1, 2017 to June 30, 2023 will be analyzed, and the planned number of subjects is about 500. In the first line of therapy, there are two cohorts of patients, one received immunotherapy (pembrolizumab or nivolumab), while second group received targeted therapy (BRAF and MEK inihibitors - dabrafenib and trametinib or vemurafenib and cobimetinib). In all cohorts, progression-free survival, overall survival, time to next treatment and time on treatment will be analyzed. Correlation between time to next treatment and time on treatment with overall survival for both therapeutic options (immunotherapy and targeted therapy) will be analyzed. Statistical analysis will be based on correlation coefficients (Pearson's coefficient and Spearman's correlation coefficient). The analysis of survival, regardless of whether it is overall survival or surrogate survival outcomes, will be analyzed using the Kaplan-Meier method with the log-rank test and the Cox hazard ratio model.

Expected scientific contribution: In this research, we will examine the relation of surrogate outcomes with the overall survival of patients with metastatic melanoma treated with targeted therapy and immunotherapy. So far, only two papers have examined the relation between surrogate outcomes and overall survival, but only in those treated with immunotherapy. We will also analyze the association of prognostic factors with the survival outcomes. All of the above could provide new insights into the survival outcomes of patients with metastatic melanoma and contribute to faster and easier clinical judgment in systemic treatment of these patients.

MeSH/Keywords: Melanoma, immunotherapy, targeted therapy, survival outcomes

Poster code: T-02-19-159

Poster Title: Investigation of the proteomic profile of anthrochoanal polyps in children

PhD candidate: Filip Bacan

Part of the thesis: Investigation of the proteomic profile of anthrochoanal polyps in children

Mentor(s): Associate Professor Jakov Ajduk, MD PhD, Ruđer Novak, PhD, senior research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Antrochoanal polyps (AP) are rare solitary masses that originate in the maxillary sinus and pass through its ostium reaching the choana. Considering that AP is the most common type of polyposis in children, there is an assumption of a connection between the etiopathogenesis and the child's age of the patient.

Hypothesis: The proteomic profile of antrochoanal polyps in children differs from the proteomic profile of nasal polyps in adult patients with chronic rhinosinusitis.

Aims: GENERAL OBJECTIVE: To investigate the proteomic profile of antrochoanal polyps in children and adults. SPECIFIC GOALS: 1. To compare the expression of individual proteins of the proteomic profile of antrochoanal polyps in children with the profile of antrochoanal polyps in adults and bilateral polyps in adult patients with chronic sinusitis; 2. To identify those proteins that are differentially expressed in the tissue as possible etiopathogenetic hubs that indicate the differences between these diseases; 3.To compare the expression of the identified proteins with the histopathological features of the preparation.

Materials and methods: This research is retrospective, non-interventional, non-randomized and cohort. The proteomic profile of formalin-fixed and paraffin-embedded (FFPE) tissue from surgically removed nasal polyps will be analyzed. One group of subjects will consist of children up to 14 years of age with a histopathological diagnosis of antrochoanal polyps (30 subjects), the second group will be adult patients aged >30 years with unilateral antrochoanal polyps (20 patients), and the third, the control group, will be adult patients with bilateral nasal polyposis in as part of histopathologically confirmed chronic rhinosinusitis (20 subjects).The number of samples is comparable to previous research, and a "post hoc" power analysis of the required sample size will be conducted. The inclusion criteria for this study are all patients with clinically and radiologically determined antrochoanal polyps, which were then confirmed by pathohistological analysis. The control group consists of a group of patients with bilateral polyposis as part of chronic rhinosinusitis. All patients were treated surgically. Patients who had previously been operated on due to nasal polyposis were excluded from the study. The research is interdisciplinary and consists of a clinical part, histopathological analysis and proteome analysis and will be carried out at the Clinic for Otorhinolaryngology and Head and Neck Surgery and the Department of Pathology "Ljudevit Jurak" (KBC Sestre milosrdnice) and the Proteomics Department of the Center for Translational and clinical research at the Faculty of Medicine, University of Zagreb. All intraoperative material was taken with informed consent and with the approval of the Ethics Committee of the KBC Sisters of Charity. The material was processed by a standard histological method, which includes fixation of the tissue in 10% buffered formalin and embedding in paraffin blocks, sectioning at a thickness of 5-40 μm, deparaffinization and staining with the standard hemalaun eosin (HE) method. The proteomic profile of nasal polyps tissue will be made using an SDS-PAGE electrophoresis system (Invitrogen, XCell tub; NuPAGE gels), nano liquid chromatogram Easy-nLC (Proxeona) and mass spectrometer LTQ Orbitrap Discovery (Thermo). Statistical analysis of relative protein expression levels will be performed with the help of IPython interactive Python shell, NumPy and Matplotlib database.

Expected scientific contribution: This scientific research would identify the hitherto unknown tissue proteomic profile of pediatric antrochoanal polyps and link the etiopathogenesis of the disease to age-related changes.

Acknowledgments: I would like to express my deepest gratitude to my two mentors, Jakov Ajduk and Novak Ruđer who my project would be impossible without. I would like to extend my sincere thanks to all of my colleagues and family for professional and moral support.

MeSH/Keywords: Nasal polyposis, antrochoanal polyps, proteomics, children, etiology

Poster code: T-02-21-093

Poster Title: Objective and perceptual characteristics of the voice after endotracheal intubation in head and neck surgery

PhD candidate: Ivana Šimić Prgomet

Part of the thesis: Objective and perceptual characteristics of the voice after endotracheal intubation in head and neck surgery

Mentor(s): Professor Drago Prgomet, MD PhD, Associate Professor Slobodan Mihaljević, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Possible consequences of endotracheal intubation are post-intubation voice changes.

Hypothesis: More significant voice disorders are expected after endotracheal intubation in head and neck surgery compared to abdominal procedures.

Aims: The overall goal is to investigate the short-term and long-term effects of endotracheal intubation on voice quality in head and neck surgery. Specific goals: 1. To determine whether there is an influence of endotracheal intubation on the appearance of voice disorders registered by videostroboscopy and objective, perceptive and subjective methods of voice assessment 2. To compare the voice disorder in thyroidectomies in relation to other operations on the head and neck. 3. To compare the voice disorder in head and neck operations compared to abdominal operations. 4. To determine whether there are differences in the speed of recovery of recorded voice disorders after thyroid surgery compared to other head and neck surgeries. 5. To determine whether there are differences in the speed of recovery of recorded voice disorders after head and neck operations compared to abdominal operations

Materials and methods: Material, subjects, methodology and research plan: The prospective observational cohort study that will include patients operated with endotracheal intubation lasting up to 3 hours. The research will include 200 patients divided into three groups: thyroid surgery, parotid gland surgery and abdominal surgery control group. Videostroboscopy, perceptual and objective acoustic voice analysis will be recorded before surgery, on the second postoperative day, two weeks and 1 month after surgery.

Expected scientific contribution: The scientific contribution of this research would be an understanding of the risk factors and the connection of voice disorders after endotracheal intubation, as well as the ability to determine differences in this risk in patients undergoing different operations. This would determine whether specific operations (thyroid, head and neck) pose a greater risk for the development of voice disorders. This kind of prospective research has not been done before and could become a fundamental source of data for future research.

MeSH/Keywords: endotracheal intubation, surgery, head and neck, voice disorder

Poster code: T-02-21-121

Poster Title: The effect of hyperglycemia on the expression of IL-17, IL-22, ROS and SOD in human heart in chronic heart failure

PhD candidate: Dubravka Vrljić Borojević

Part of the thesis: The effect of hyperglycemia on the expression of IL-17, IL-22, ROS and SOD in human heart in chronic heart failure

Mentor(s): Assistant Professor Ana Šepac, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Chronic inflammation is important in the pathogenesis of chronic heart failure and complications of diabetes mellitus. Reactive oxygen species (ROS) and cytokines IL-17 and IL-22 play an important role in pathological cardiac remodeling.

Hypothesis: Hyperglycemia increases the expression of IL-17 and increases the formation of ROS and decreases the expression of IL-22 and superoxide dismutase (SOD) in human heart samples from patients with chronic heart failure.

Aims: To investigate the influence of hyperglycemia on the expression of IL-17, IL-22, ROS and SOD in human heart in chronic heart failure.

Materials and methods: The effect of hyperglycemia on the expression of IL-17, IL-22, ROS and SOD in samples of human heart in patients with chronic heart failure and normal and elevated levels of HbA1c will be examined.

Expected scientific contribution: Better understanding of the role of Th-17 cytokines, oxidative stress and antioxidants in the development of chronic heart failure in diabetics may lead to improvement of diagnostics and development of targeted therapy in the future.

MeSH/Keywords: IL-17, IL-22, ROS, hyperglycemia, heart failure

Poster code: T-02-23-137

Poster Title: Clinical, biochemical, radiological and molecular characteristics of patients with congenital panhypopituitarism

PhD candidate: Duje Braovac

Part of the thesis: Clinical, biochemical, radiological and molecular characteristics of patients with congenital panhypopituitarism

Mentor(s): Assistant Professor Katja Dumić Kubat, MD PhD

Affiliation: University Hospital Centre Zagreb, University of Zagreb School of Medicine

Introduction: Congenital panhypopituitarism (CP) is a rare, potentially life-threatening disease that requires lifelong treatment and monitoring.

Hypothesis: By integrating the clinical, hormonal and neuroradiological phenotype with the results of genetic analysis, which will be performed in patients with CP using two molecular methods, new clinical-neuroradiological-molecular entities will be identified and the old ones will be confirmed. The most common causes of CP in the examined group will be determined which will contribute to a better understanding of the etiology and pathogenesis of this rare disease.

Aims: To link the clinical, hormonal, neuroradiological phenotype and genotype in children suffering from CP.

Materials and methods: The study will include a cohort of patients with CP treated in the Division of Pediatric Endocrinology and Diabetes, Department of Pediatrics, University Hospital Centre Zagreb over a period of 20 years. Clinical, biochemical and neuroradiological data from the medical records of all patients will be collected and molecular analysis will be performed using array-based comparative genomic hybridisation (aCGH) as well as clinical exome analysis using next generation sequencing (NGS). The relationship between clinical and hormonal phenotype, neuroradiological findings and genotype will be investigated, analyzed and compared.

Expected scientific contribution: This research might contribute to a better understanding of the etiopathogenesis of this rare disease and to the development of new diagnostic, monitoring and treatment strategies.

MeSH/Keywords: congenital panhypopituitarism, phenotype, genotype, aCGH, NGS

Poster code: T-02-24-030

Poster Title: Consequences of altered methylation on the pathogenesis and clinical presentation of the disease due to S-adenosylhomocysteine hydrolase deficiency

PhD candidate: Katarina Šikić

Part of the thesis: Consequences of altered methylation on the pathogenesis and clinical presentation of the disease due to S-adenosylhomocysteine hydrolase deficiency

Mentor(s): Professor Ivo Barić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: S-adenosylhomocysteine hydrolase deficiency (SAHH) is a rare methylation disorder that we described for the first time in the world, and today we know of 33 patients. In this disease, the activity of SAHH is reduced, whose main task is the hydrolysis of S-adenosylhomocysteine (AdoHcy) into adenosine and homocysteine. AdoHcy is formed by transmethylation reactions from S-adenosylmethionine, the most important methyl group donor during methylation reactions. An elevated concentration of AdoHcy inhibits transmethylation processes, which is considered one of the main pathogenetic mechanisms of this disease. The clinical picture is variable, and therapeutic options are limited.

Hypothesis: Altered methylation in patients suffering from S-adenosylhomocysteine hydrolase deficiency in different ways impacts the pathogenesis of the disease and it's clinical picture.

Aims: Clarifying the pathogenesis of this disease and its long-term course.

Materials and methods: The research will be on at least five patients who were treated for this disease in our Clinic. Different methods will be used for different parts of the research: next-generation metabolic screening for metabolome research, liquid chromatography with mass spectrometry for proteomic research, EpiSign for genome methylation research, and data collection through a questionnaire on the natural course of the disease.

Expected scientific contribution: The results will contribute to a better understanding of the pathogenesis of this disease.

MeSH/Keywords: S-adenosylhomocysteine hydrolase, homocysteine, methylation, methyltransferase

Poster code: T-02-24-037

Poster Title: Serum vitamin D and urine cathelicidin levels as predictors of urinary tract infection recurrence in children

PhD candidate: Iva Sorić Hosman

Part of the thesis: Association of serum vitamin D and urine cathelicidin levels with the risk of developing recurrent urinary tract infections in children

Mentor(s): Lovro Lamot, PhD, research associate, Professor Andrea Cvitković Roić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Within one year after the first urinary tract infection (UTI), up to 30% of children experience recurrent UTI (rUTI), even in the absence of well-known risk factors, such as vesicoureteral reflux. In addition to the lack of predictive markers of UTI recurrence, there is no clear benefit of any currently available intervention for prevention of rUTI and its long-term consequences such as renal scarring, hypertension and renal insufficiency. Consequently, rUTI represent a great health care and economic burden. Continuous antibiotic prophylaxis (CAP) has become standard of care for prevention of rUTI, irrespective of minimal or no effect in reducing the recurrence of UTI, while increasing the risk of microbial resistance and host gut dysbiosis predisposing patients for future development of various autoimmune diseases. New biomarkers of the increased risk of rUTI could therefore assist in avoiding such outcomes by revealing more specific patient population which could benefit from additional interventions such as CAP. In this light, the recent findings suggesting a crucial role of vitamin D induced urothelial expression of antimicrobial peptide called cathelicidin in protection of urinary tract from invading uropathogens might offer new opportunities for predicting UTI recurrence.

Hypothesis: Low serum vitamin D and urine cathelicidin levels are predictors of UTI recurrence within one year from the first UTI in children aged one month to two years.

Aims: The aim of this thesis is to determine the association of serum vitamin D and urine cathelicidin levels with the risk of developing rUTI within one year after the first UTI in children aged one month to two years.

Materials and methods: One hundred and fifty consecutive patients aged one month to two years admitted at the Division of Nephrology, Department of Pediatrics, University Hospital Centre Zagreb due to the first UTI will be enrolled in this prospective cohort research. Serum vitamin D and urine cathelicidin levels, along with the standard laboratory examination (complete blood count, C-reactive protein, urea, creatinine, biochemical analysis of urine and urine culture), will be measured in all subjects during the first UTI. Urinary tract ultrasound will be performed on all the enrolled children to assess size of the kidneys, bladder wall thickness and presence and grade of urinary tract dilatation. In those with detected urinary tract abnormalities, additional imaging diagnostic methods will be made according to the current clinical guidelines. All patients will be followed up for 12 months. Depending on the development of rUTI during the follow-up period, participants will be divided into a group of those who have developed rUTI and those who have not. Eventually, all the obtained values (results of the standard laboratory examination, serum vitamin D, urine cathelicidin, results of the imaging diagnostic methods) will be analyzed and compared between the two groups. The predictive value of serum vitamin D and urine cathelicidin level as well as the cumulative predictive value of all the measured parameters (laboratory and imaging) for developing UTI recurrence will be statistically calculated.

Expected scientific contribution: The findings of this study will enhance our ability to identify children who are at an increased risk of developing recurrent urinary tract infections (rUTIs) following their initial UTI. This, in turn, has the potential to reduce unnecessary exposure to antibiotic prophylaxis and invasive imaging diagnostic procedures. As a result, it could alleviate trauma experienced by children and yield substantial healthcare cost savings.

MeSH/Keywords: vitamin D, cathelicidin, antimicrobial peptides, urinary tract infection, recurrent urinary tract infections

Poster code: T-02-24-053

Poster Title: Determining the frequency of symptomatic and asymptomatic signs of the central nervous system in children with newly diagnosed coeliac disease and evaluating the impact of gluten-free diet on these signs

PhD candidate: Lana Lončar

Part of the thesis: Determining the frequency of symptomatic and asymptomatic signs of the central nervous system in children with newly diagnosed coeliac disease and evaluating the impact of gluten-free diet on these signs

Mentor(s): Assistant Professor Zrinjka Mišak, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Coeliac disease is an immune-mediated disease caused by gluten. Neurologic manifestations occur in 1/5 patients. Assuming that structural and electrophysiological changes develop before clinical signs, early diagnostic evaluation is important. We want to establish the frequency and type of neurologic symptoms in children with newly diagnosed coeliac disease, the presence of structural and electrophysiologic changes, and the influence of a gluten-free diet on neurologic symptoms.

Hypothesis: Abnormalities of the central nervous system are common in children with coeliac disease, and adherence to a gluten-free diet affects the reduction of these abnormalities.

Aims: General aim To determine the frequency and type of neurological symptoms in children with newly diagnosed celiac disease and to determine the influence of a gluten-free diet on these signs Specific aims 1. to determine the existence of pathological changes in the brain and changes in the volume of the cerebellum and basal ganglia in children with newly diagnosed coeliac disease 2. to determine the existence of electrophysiological changes in the central nervous system in children with newly diagnosed coeliac disease 3. repeat the clinical, radiological and electrophysiological treatment after a correctly implemented gluten-free diet in children in whom changes were initially detected

Materials and methods: This is a prospective study. It will last a minimum of 42 months, of which 36 months will include respondents, and the follow-up time of each respondent will be a minimum of 6 months. We will include 44 respondents in the research. The sample size was calculated with a test power of 80%. In the research, we will include subjects aged 6-18 who were diagnosed with coeliac disease at the Zagreb Children's Disease Clinic. All test subjects must be in an adequate psychophysical condition that enables diagnostic methods to be performed without the use of any form of sedation, and must not have a known neurological disease. Each subject will be evaluated clinically, radiologically and electrophysiologically after the diagnosis of coeliac disease, and before the introduction of a gluten-free diet. At the earliest, 6 months after starting a gluten-free diet, we will check whether subjects adhere to the diet based on the Biagi questionnaire and antibody titer check. In subjects who adhere to a gluten-free diet, we will re-evaluate the existence of neurological symptoms, their intensity and the subject's neurological status. For those in whom changes were determined by neuroradiological and electrophysiological methods at the time of diagnosis, we will repeat the tests at the earliest 6 months after a properly implemented gluten-free diet.

Expected scientific contribution: Through research, we would assess the presence of signs of central nervous system abnormalities in children as a result of coeliac disease. If we determine that there is a significant frequency of asymptomatic and symptomatic signs, we would also determine the need to include the entire neurological treatment in the basic care of patients with coeliac disease. If we estimate that a gluten-free diet causes a reduction in these signs, we would confirm the need for strict adherence to a gluten-free diet also due to neurological abnormalities.

MeSH/Keywords: coeliac disease, neurologic symptoms, gluten-free diet

Poster code: T-02-24-136

Poster Title: The Influence of Premature Birth on Subcortico-Cortical Functional Connectivity

PhD candidate: Karlo Stemberger

Part of the thesis: The impact of premature birth on the development of brain neural networks analysed with resting state functional magnetic resonance imaging

Mentor(s): Assistant Professor David Ozretić, MD PhD, Assistant Professor Jelena Božek, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Preterm birth, before 37 weeks of gestation, can impact neurodevelopment due to potential disruptions in subcortico-cortical brain connectivity during the third trimester. Although structural MRI has limitations in predicting outcomes for preterm infants without significant brain lesions, resting-state functional MRI (rs-fMRI) offers a deeper look into these disruptions. This research will focus on developing preprocessing pipeline for rs-fMRI to analyze and compare brain connectivity between preterm and term-born children and examine how these connections affect neurodevelopmental outcomes in preterm infants.

Hypothesis: We hypothesize that preterm-born children will show altered subcortico-cortical connectivity patterns compared to term-born children, and that these connectivity patterns within the preterm group will correlate with performance on neuropediatric assessments.

Aims: • Develop a robust preprocessing pipeline for resting-state functional MRI data. • Examine the connections between specific subcortical areas (thalamus, hippocampus, caudate nucleus, amygdala, lentiform nucleus, and subthalamic nucleus) and cortical regions in children born preterm compared to those born at term, utilizing seed-based analysis techniques. • Investigate the relationship between brain connectivity and neurodevelopmental scores in preterm children.

Materials and methods: This study will include 100 preterm infants from the Pediatric Clinic of UHC Zagreb and 100 term-born controls obtained in "Developing Human Connectome Project", all scanned at the same post-menstrual age. Preterm infants will undergo MRI at the term-equivalent age at "Neuron" polyclinic, excluding any with significant brain damage or scan artifacts. We will compare their scans to the dHCP's scans of healthy, term-born infants. Preterm infant MRIs will be performed on a 3T Siemens Prisma Fit with a 64-channel coil during sleep, covering structural, diffusion, and functional scans. Functional imaging will use an 8-minute EPI sequence (TR=900ms, TE=37ms, 2mm voxels). Control data was collected on a 3T Philips Achieva with similar settings (TR=392ms, TE=38ms, 2mm voxels). Preterm children will receive neuropediatric assessments until age 2, including early visual and movement tests, IMP motor tests (3-18 months), and the Beery-Buktenica and Bayley-III scales at 2 years. Results will categorize development as normal, mildly, or clearly abnormal for analysis. Data will be preprocessed using FSL and SPM for brain extraction, motion correction, and outlier removal. Procedures include band-pass filtering, anatomical segmentation, spatial normalization to a standard template, and spatial smoothing. Quality assurance measures like visual inspections will ensure preprocessing accuracy. Functional connectivity will be analyzed using the CONN toolbox in MATLAB, focusing on 47 ROIs defined by the Kuklisova-Murgasova atlas. Analyses will include ROI-to-ROI and seed-to-voxel methods, employing the General Linear Model and Pearson's correlation. Second-level analyses will compare preterm vs. term connectivity and relate it to neuropediatric test results. Statistical significance will be controlled using combined FDR/FWE correction. Results will be presented through matrices, network graphs, and parametric maps.

Expected scientific contribution: This research aims to enhance our understanding of the impact of preterm birth on brain networks by comparing connectivity patterns in preterm and term-born children and linking these patterns to neurodevelopmental outcomes. Identifying vulnerable networks and potential biomarkers could lead to better early interventions and tailored support, ultimately improving the long-term outcomes for preterm infants.

MeSH/Keywords: functional connectivity, resting-state fMRI, CONN toolbox, seed-based analysis, preterm birth, pediatric neurodevelopment, subcortico-cortical connectivity, brain development

Poster code: T-02-25-015

Poster Title: Comparison of the degree of occlusion of unruptured internal carotid artery aneurysms using coated and uncoated endovascular flow diverter devices

PhD candidate: Vladimir Kalousek

Part of the thesis: Comparison of the degree of occlusion of unruptured internal carotid artery aneurysms using coated and uncoated endovascular flow diverter devices

Mentor(s): Associate Professor Krešimir Rotim, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: The use of flow diverter stents represents a significant advance in endovascular treatment intracranial aneurysms. Such stents are specially designed to divert blood flow away from the aneurysm and towards the main flow of blood. However, the use of stents for flow diversion also carries certain risks and challenges. Patients must take drugs that prevent blood clotting (antiplatelet agents) to reduce the risk of thrombosis within the stent. A promising approach to the problem of balancing the risk of thromboembolic complications and the risk of hemorrhagic complications associated with flow diverters is the application of surface coating technologies on the same.In this way, the platelets do not stick to the foreign material of the stent and physiological coagulation homeostasis is maintained.

Hypothesis: The degree of occlusion of unruptured internal carotid artery aneurysms will be higher after endovascular procedure using coated flow diversion stents, versus uncoated flow diversion stents

Aims: To show the difference in the degree of occlusion of unruptured aneurysms of the internal carotid artery after endovascular intervention using coated and uncoated flow diverters. To show the morphological differences of unruptured aneurysms of the internal carotid artery before the procedure and compare them with the degree of postprocedural occlusion. To present the clinical picture of the patient before and after the procedure and post-procedural complications after the use of coated and uncoated flow diverters.

Materials and methods: A minimum of 130 consecutively diagnosed patients with unruptured aneurysm of internal carotid artery will be included in the proposed prospective study, regardless of age and gender, within the time period from September 1, 2024 to September 1, 2026. Subjects included in the study will be randomly divided into two groups, depending on the flow diversion device used for endovascular procedures: group treated using coated flow diverter and a group treated using uncoated flow diverter. The applied dual antiplatelet therapy will be identical for both group, which begins at least one day before endovascular therapy and will lasts 6 months until control MR angiography using 3D TOF technique. All included subjects will undergo CT/MR angiography and DSA as a method of determining hemodynamic changes before the endovascular procedure of occlusion of unruptured aneurysms of the internal carotid artery. Preprocedural measurement of the width of the neck and fundus of unruptured internal carotid artery aneurysms on images of brain blood vessels will be expressed in millimeters and will serve to determine the relationship of the neck to the fundus of the aneurysm. The analysis of the degree of occlusion of the aneurysm will be done using the Raymond-Roy scale on MR (TOF and FLAIR methods) six months after the procedure, as well as a clinical examination using the modified Rakin Scale (mRS). Postprocedural complications will be divided into two groups, ischemic and hemorrhagic, within the group with ischemic complications we will have two subgroups; first with clinically significant symptoms, while in the second group there will be patients with so-called silent strokes that are not clinically significant symptoms, we will analyze the above using T2 and FLAIR measured sequence on MR examination.

Expected scientific contribution: The expected scientific contribution of the proposed study would be to determine the existence of a difference in the degree of occlusion of unruptured internal aneurysms carotid arteries using coated and uncoated flow diverters. The proposed research would be significant contribution to a more precise assessment of the success of the use of endovascular flow diverting stents, and too remove any doubts about the success of the treatment, but also to set a model for further evaluation of the use of flow diverting stents.

MeSH/Keywords: aneurysm, flow diversion, internal carotid artery, coated, uncoated

Poster code: T-02-25-016

Poster Title: Predictive value of modified Brixia score on the initial chest X-ray images in hospitalised patients with COVID-19 pneumonia for developing acute respiratory distress syndrome

PhD candidate: Armin Mehmedović

Part of the thesis: Predictive value of modified Brixia score on the initial chest X-ray images in hospitalised patients with COVID-19 pneumonia for developing acute respiratory distress syndrome

Mentor(s): Assistant Professor Maja Hrabak Paar, MD PhD

Affiliation: University of Zagreb School of Medicine; University Hospital for Infectious Diseases "Dr. Fran Mihaljević"

Introduction: Development of acute respiratory distress syndrome (ARDS) in patients with COVID-19 pneumonia is linked to poor clinical outcomes. Modified Brixia score (MBS) is a grading score which quantifies extent and intensity of changes in lung parenchyma in COVID-19 pneumonia on chest X-ray images (CXR).

Hypothesis: Hospitalized patients with COVID-19 pneumonia who have higher values according to the MBS on the initial CXR are at a higher risk of developing ARDS compared to patients with lower values according to the MBS.

Aims: Our aims are; to analyse the initial CXR in hospitalized patients with PCR-proven COVID-19 pneumonia, determine the value of the MBS, and compare the values in patients who developed ARDS and those who didn't; to examine the relationship between MBS and the outcome of patients with COVID-19 pneumonia and to determine the threshold value of MBS for predicting the fatal outcome of patients with COVID-19 pneumonia; to determine differences in complications (pulmonary embolism, DIC, acute renal failure, septic shock, pneumothorax) in patients with high and low MBS; to compare the duration of mechanical ventilation and hospitalization in patients depending on MBS value; to determine inter-examiner variability in MBS assessment; to examine the association of other factors that may be associated with the occurrence of ARDS in patients with COVID-19 pneumonia; and to stratify patients according to the day of illness upon admission and analyse the dependence of MBS on the stage of illness.

Materials and methods: An observational retrospective study including patients (initially 1308) with PCR-proven COVID-19 infection hospitalized in the University Hospital for Infectious Diseases "Dr. Fran Mihaljević" from July 1, 2021. until December 31,2021. Inclusion criteria are age 18 and over and PCR-proven COVID-19 infection, and in patients with ARDS - diagnosis according to the Berlin definition. Exclusion criteria: absence of initial CXR, initial CXR performed in another institution, preexisting pathology preventing adequate scoring, applied antibiotic and/or corticosteroid therapy before hospitalization, transfer from another institution, age under 18, pregnancy and breastfeeding. All relevant clinical and demographic data will be collected from the medical records of the participants. Each CXR will be analysed and scored by 3 radiologists with 25, 6 and 3 years of experience in thoracic radiology. Each lung on the CXR will be divided into 3 zones (upper, middle, and lower zone with points of division being in craniocaudal order: pulmonary apex, cranial contour of the aortic arch, lower border of the left hilum, diaphragm). Each zone will be scored from 0 to 3 depending on changes in the lung parenchyma (0: no infiltrates; 1: only interstitial infiltrates; 2: presence of consolidation up to 50%; 3: presence of consolidation ≥50%; maximum value 18). The level of significance will be defined as $p<0.05$. Normality of the distribution will be tested by visual inspection and the Shapiro-Wilk test. Independent t-test or Mann-Whitney U test will be used to compare different numerical variables of two groups. The dependence of categorical variables will be tested with the chi-square test or Fisher's exact test. Predictive value of the MBS for complications and outcomes of COVID-19 pneumonia will be tested using a binary logistic regression model which will be characterized by the receiver operating characteristic curve with the calculation of the area under the curve value. Intraobserver and interobserver variability will be assessed by percentage of agreement and Cohen's coefficient.

Expected scientific contribution: The research could determine incompletely investigated differences between patients with COVID-19 pneumonia who developed ARDS and those who did not, as well as the importance of assessing the degree and extent of lung involvement for the purpose of timely treatment opening the possibility of applying the MBS in potential other viral respiratory infections in the future.

MeSH/Keywords: chest X-ray, COVID-19, scoring system, ARDS

Poster code: T-02-25-047

Poster Title: Predictive value of leptomeningeal collateral score on single-phase CT angiography in determining long-term outcome after endovascular treatment of anterior circulation large vessel occlusion

PhD candidate: Tomislav Herega

Part of the thesis: The value of leptomeningeal collateral circulation on single-phase CT angiography as a predictor of long-term functional outcome after endovascular treatment of anterior circulation artery occlusion

Mentor(s): Professor Marko Radoš, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Mechanical thrombectomy (MT) is an endovascular procedure for the treatment of acute ischemic stroke (AIS) caused by large vessel occlusion (LVO). Despite the high recanalization rates, the benefits of treatment are not the same for all patients. The presence or absence of a robust network of leptomeningeal collaterals (LMC) is increasingly recognized as an important prognostic factor. Several classification systems for the assessment of LMC on the CT angiography (CTA) have been described in the literature. The first and most commonly used is the Tan collateral score (CS).

Hypothesis: A higher Tan collateral score (CS) is a predictor of favorable long-term functional outcome (LTFO), as measured by the modified Rankin Scale (mRS), in patients treated with MT for LVO in the anterior circulation.

Aims: The aim of this retrospective study is to determine the value of LMC status in predicting LTFO after MT. Secondary objectives are as follows: (1) to investigate the association of Tan 2 and 3 CS with favorable LTFO (mRS 0 – 2) after MT, (2) to analyze the value of the Tan CS in predicting LTFO after MT before and after accounting for confounding factors identified in univariate analyzes, and (3) to determine the cut-off value of the Tan CS that distinguishes favorable and unfavorable LTFO with high positive and negative predictive value.

Materials and methods: We will conduct a retrospective review of medical records and radiologic imaging data of patients treated with MT for LVO in the anterior circulation between January 1, 2019 and December 31, 2023. Patients with an occlusion in the intracranial segment of the internal carotid artery (ICA) or in the M1/M2 segments of the middle cerebral artery (MCA) will be included in the study. Patients with occlusions in the posterior circulation, without available initial neuroradiologic imaging data, with a CTA that does not meet the defined minimum standards, with a technically unsuccessful MT or lost follow-up will be excluded. Data extracted will include patient age, gender, comorbidities, onset and duration of symptoms, severity of symptoms quantified with the with the National Institute of Health Stroke Scale, administration of intravenous thrombolysis, and mRS LTFO based on 3-month neurologic follow-up (F/U). Neuroradiologic imaging data will be reviewed and the following data will be extracted. Initial non-contrast CT of the head will be assessed for early ischemia size quantified using the Alberta Stroke Programme Early CT score. CTA will be assessed for location of occlusion and LMC status, quantified using the Tan CS. DSA examination will be assessed for the presence of first-pass recanalization, the timing of recanalization, the duration between symptoms and recanalization, and the degree of recanalization quantified by the modified Treatment in Cerebral Ischemia scale. We will divide the patients into two groups: Patients with favorable LTFO (mRS 0-2 at 3-month F/U) and patients with unfavorable LTFO (mRS 3-6 at 3-month F/U). The extracted data will be analyzed to determine possible statistically significant differences between the two groups using appropriate statistical tests. Variables will be considered for multivariable analysis (stepwise logistic regression). Results with $p < .05$ will be considered statistically significant.

Expected scientific contribution: Determining the predictive value of LMC status at presentation of patients with acute stroke could enable an individualized approach and support the early post-recanalization treatment.

MeSH/Keywords: collateral circulation, ischemic stroke, mechanical thrombectomy, endovascular treatment

Poster code: T-02-25-074

Poster Title: Association of Neuroradiological Findings with Disease Severity and Outcomes in Patients with Neuroinvasive Arboviral Infections

PhD candidate: Thomas Ferenc

Part of the thesis: Association of Neuroradiological Findings with Disease Severity and Outcomes in Patients with Neuroinvasive Arboviral Infections

Mentor(s): Associate Professor Marija Santini, MD PhD, Associate Professor Tatjana Vilibić Čavlek, MD PhD

Affiliation: University of Zagreb, School of Medicine; Department for Infections in Immunocompromised Patients, University Hospital for Infectious Diseases Dr. Fran Mihaljevic, Zagreb; Department of Virology, Croatian Institute of Public Health, Zagreb

Introduction: Arboviruses are a heterogeneous group of viruses that may cause central nervous system infections. Magnetic resonance imaging (MRI) is the most sensitive modality in the neuroradiological workup of patients with neuroinvasive arboviral infections.

Hypothesis: MRI imaging patterns of the brain and/or spine of patients with neuroinvasive arboviral infections are associated with the severity of their clinical symptoms, duration of the hospitalization, and treatment outcome.

Aims: To determine the prevalence of pathological MRI findings of the brain and/or spine in patients with neuroinvasive arboviral infection, describe brain and/or spine MRI features of these patients, correlate findings with demographic parameters and blood and cerebrospinal fluid laboratory parameters, determine the association of MRI findings with the severity of clinical symptoms, duration of the hospitalization and treatment outcome and to determine the association of MRI findings with specific arboviruses.

Materials and methods: Multicentric (13 hospitals), cross-sectional study will include a cohort of 45 patients of both genders with confirmed neuroinvasive arboviral infection (West Nile virus, tick-borne encephalitis virus, Toscana virus, and Usutu virus) and initial MRI examination performed in the period from 2017 to 2025. The analysis will be conducted retrospectively (2017-2022) and prospectively (2023-2025). The study included patients with confirmed cases of arboviral infection according to the ECDC and the diagnosis of meningitis, meningoencephalitis, encephalitis, and/or myelitis. Their imaging studies performed on MRI scanners of 1,5 and/or 3,0 T field strength will be analyzed. MRI imaging was or will be conducted according to the standardized protocols for suspicious neuroinvasive viral disease (T1 and T2 weighted images, FLAIR sequences, contrast-enhanced images, and diffusion-weighted images with apparent diffusion coefficients). Clinical data (clinical signs and symptoms, duration of hospitalization), blood and cerebrospinal fluid laboratory parameters (blood leukocyte count, CRP levels, CSF cell count, differential CSF leukocyte count, CSF protein, and glucose levels), and severity of clinical symptoms will also be evaluated. MRI of the brain and/or spine will be interpreted as normal or pathological with a) signs of isolated restriction in diffusion, b) T2 and FLAIR hyperintensities, c) signs of meningeal inflammation, or d) altered MRI signal solely in the spine. The severity of clinical symptoms will be quantified according to previously set criteria and categorized as mild (0-8 points), moderate (9-22 points), and severe (>22 points). The outcome will be defined at the end of the hospitalization (survival/death) and according to a modified Rankin scale (mRS) which measures the degree of disability or dependence in the daily activities of patients.

Expected scientific contribution: The results of this study could be of scientific interest due to defining morphological changes for certain arboviruses and their association with clinical features and disease outcomes, however, they could also be used for the assessment of the clinical course and outcome of hospital treatment.

MeSH/Keywords: Arboviruses, neuroinvasive disease, magnetic resonance imaging

Poster code: T-02-25-076

Poster Title: BIOMEDICAL IMAGING OF BREAST CANCER LIVER METASTASIS

PhD candidate: Ružica Galunić Čičak

Part of the thesis: Biomedical imaging of breast cancer metastasis in the liver

Mentor(s): Associate Professor Maja Prutki, MD PhD, Veronika Pedić Tomić, PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Radiomics include feature extraction from clinical images, these features are related to tumor size, shape, intensity, and texture, collectively providing comprehensive tumor characterization, the so-called radiomics signature of the tumor. Radiomics is based on the hypothesis that extracted quantitative data derives from mechanisms occurring at genetic and molecular levels. In this research, the purpose is to evaluate radiomic features from computed tomography of breast cancer liver metastasis for prediction of receptor subtype of metastasis.

Hypothesis: We hypothesize that radiomics signature of the breast cancer liver metastasis imaged on computed tomography could predict receptor subtype of liver metastasis.

Aims: Examine whether radiomic phenotypes of liver cancer metastases obtained from CT can predict receptor status determined by immunohistochemical analysis (ER, PR and Her2). Develop a machine learning model to recognize the receptor status of liver metastasis

Materials and methods: This retrospective study will include at least 100 patients with breast cancer liver metastasis who underwent computed tomography (CT) of the liver. In this study, an analysis of liver lesions that, using a core needle biopsy, are pathohistologically verified as metastases of breast cancer. For segmentation of these lesions, ITK-SNAP, free open source cross-platform software, will be used. After segmentation, the radiomic features of these lesions will be calculated using Pyradiomics, an open source library for analysis data in Python programming language. During the analysis, radiomic features obtained from liver CT will serve as the basis for development of machine learning models.

Expected scientific contribution: This is the first study to predict the receptor status of breast cancer metastases in the liver using radiomics.

MeSH/Keywords: radiomics, breast cancer, liver metastasis, computed tomography

Poster code: T-02-25-092

Poster Title: The effectiveness of stereotactic ablative radiotherapy for stage I non-small cell lung carcinoma compared to standard surgical treatment methods

PhD candidate: Hrvoje Feljan

Part of the thesis: The effectiveness of stereotactic ablative radiotherapy for stage I non-small cell lung carcinoma compared to standard surgical treatment methods

Mentor(s): Assistant Professor Marija Gomerčić Palčić, MD PhD

Affiliation: Special hospital Radiochirurgia Zagreb, University hospital Dubrava Zagreb and University of Zagreb School of Medicine

Introduction: The treatment of non-small cell lung cancer (NSCLC) depends on the pathohistological subtype of the tumor, the stage of the disease, the localization of the tumor and the general condition of the patient. Surgery, radiotherapy and systemic therapy are standard treatments and can be used individually or in combination. The current standard of care for stage I non-small cell lung cancer (NSCLC) is radical surgical resection. However, for older patients and those with multiple comorbidities, surgery is not a treatment option due to possible complications and mortality. An alternative for these patients is precisely ablative stereotactic radiotherapy (SBRT), which applies a high local dose of radiation in fewer fractions. Several years ago, SBRT showed better rates of local disease control and less toxicity to surrounding structures compared to standard fractionated radiotherapy (CFRT).

Hypothesis: Overall survival (OS) and progression free survival (PFS) in period of 3 years is better for ablative stereotactic radiotherapy compared to surgical treatment in operable patients for stage I non-small cell lung cancer .

Aims: The aim of the work is to investigate the effectiveness of stereotactic ablative radiotherapy (stereotactic body radiation therapy, SBRT) in operable patients (who do not agree to surgical treatment) in relation to surgical treatment on overall survival (OS) and survival without disease progression (progression free survival, PFS) after 3 years in patients with non-small cell lung cancer (NSCLC) in the first stage of the disease.

Materials and methods: The research will be conducted as a retrospective study to monitor treatment outcomes. The subjects are patients with NSCLC of the first stage of the disease who will be divided into two groups depending on the method of treatment (SBRT and surgical treatment) with defined exclusion criteria. Over a period of 3 years, OS and PFS will be determined and compared, and the influence of demographic characteristics and pathohistological type of tumor will be additionally investigated.

Expected scientific contribution: The contribution of this research could show the better efficacy and safety of SBRT in patients with NSCLC of the first stage of the disease who are operable compared to surgical methods of treatment. The results could provide a possibility of selection for patients with first-stage NSCLC regardless of the clinical condition of the patient and contribute to the improvement and at the same time minimally invasive treatment of patients with NSCLC regardless of age, sex, pathohistological subtype and health status of the patient.

MeSH/Keywords: SBRT, NSCLC, surgery, survival

Poster code: T-02-26-129

Poster Title: Calcium kidney stones and early signs of increased cardiovascular risk

PhD candidate: Krešimir Đapić

Part of the thesis: Povezanost kalcijske urolitijaze i ranih znakova povišenog kardiovaskularnog rizika

Mentor(s): Associate Professor Živka Dika, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: There is increasing evidence of correlation between urolithiasis and cardiovascular diseases.

Hypothesis: Patients with calcium urolithiasis have greater arterial stiffness and higher total cardiovascular risk.

Aims: The aim of the study is to clarify whether apparently healthy people presenting with calcium urolithiasis have an increased cardiovascular risk.

Materials and methods: This observational study will include 50 patients aged 18 to 50 years with calcium urolithiasis and same amount of age and sex matched controls. Stones will be collected by spontaneous elimination or endoscopic procedure. Calcium lithiasis will be confirmed by Fourier-transform infrared spectroscopy (FTIR). Early indicators of increased cardiovascular risk will be compared (arterial stiffness, cardiac calcifications, carotid intima media thickness and albuminuria) between the groups. Total cardiovascular risk will be determined. Patients with treated chronic disease, myocardial infarction, cerebrovascular disease, chronic kidney disease, pregnancy and mental illness will be excluded from the study.

Expected scientific contribution: The data and results from this unique study will provide information about cardiovascular status and target organ damage in patients with calcium urolithiasis and low cardiovascular risk. This study will present that apparently healthy people with urolithiasis, are in a need of a comprehensive approach, and not just a stone treatment.

MeSH/Keywords: Calcium urolithiasis, kidney stones, cardiovascular risk, arterial stifness

Poster code: T-02-28-059

Poster Title: The influence of CYP2C19 and ABCB1 gene polymorphisms on sertraline bioavailability and treatment outcomes in patients with major depressive disorder

PhD candidate: Paula Marinović

Part of the thesis: The influence of CYP2C19 and ABCB1 gene polymorphisms on sertraline bioavailability and treatment outcomes in patients with major depressive disorder

Mentor(s): Maja Živković, PhD

Affiliation: University of Zagreb School of Medicine, UHC Zagreb, Department of psychiatry and psychological medicine, Department of Laboratory diagnostics

Introduction: It is estimated 30-60% of patients with major depressive disorder don't respond adequately to antidepressants. Selective serotonin reuptake inhibitors (SSRI) are the first-choice medication in treating major depressive disorder (MDD). Sertraline, antidepressant belonging to SSRI class, is a CYP2C19, CYP3A4, CYP2B6, CYP2D6 substrate with CYP2C19 shown to have the most important role in vivo. Sertraline is shown to be a P-glycoprotein substrate, which is a transport protein encoded by ABCB1/MDR1 gene. The patients taking SSRIs can be susceptible to adverse effects or inadequate therapeutic response due the effect of gene polymorphisms on SSRI biotransformation.

Hypothesis: CYP2C19 gene polymorphisms, specifically CYP2C19 enzyme phenotype and the three most commonly occurring ABCB1 gene polymorphisms affect sertraline bioavailability and therapeutic efficacy in adults with major depressive disorder.

Aims: The main aim of the study is to determine CYP2C19 and ABCB1 gene variants that, with concomitant benzodiazepine use, can serve as a sertraline bioavailability predictor impacting therapeutic efficiency in treating MDD.

Materials and methods: This prospective observational study will enroll up to 200 participants, both sexes,18-65 years old, with a confirmed major depressive disorder diagnosis according to the Diagnostic and statistical manual of mental disorders, DSM-V, taking sertraline and benzodiazepines (diazepam or alprazolam). The study will be conducted at the UHC Zagreb,Department of Psychiatry and Psychological Medicine and Department of Laboratory Diagnostics. The severity of depressive and anxious symptoms will be evaluated by Hamilton depression rating scale (HAM-D17) and anxiety (HAM-A); Montgomery-Asberg depression rating scale (MADRS). Four weeks after treatment initiation blood samples will be drawn; first one before taking the medication to determine sertraline trough concentration along with the sample for pharmacogenetic analysis and then 2,4 and 6 hours after the first dose to determine sertraline plasma concentration.

Expected scientific contribution: This study could help better understanding CYP2C19 and ABCB1 pharmacogenetic variations role in sertraline bioavailability and therapeutic efficacy, used concomitantly with benzodiazepines, which could contribute to developing personalized treatment methods for depression and achieving better patient compliance.

MeSH/Keywords: major depressive disorder, CYP2C19, CYP2B6, ABCB1, sertraline

Poster code: T-02-29-022

Poster Title: The relationship between defense mechanisms, shame, pathological narcissism and quality of life in people with multiple sclerosis

PhD candidate: Marina Milošević

Part of the thesis: The relationship between defense mechanisms, shame, pathological narcissism and quality of life in people with multiple sclerosis

Mentor(s): Professor Darko Marčinko, MD PhD, Josip Sremec, PhD, research associate

Affiliation: University of Zagreb School of Medicine, Clinical Hospital Sveti Duh

Introduction: Multiple sclerosis is a chronic, autoimmune, inflammatory demyelinating disease of the central nervous system characterized by damage to myelin and axons. Psychological traits and defense mechanisms are an important variable in assessing individual differences in acceptance of the disease, coping style with stressful situations, and psychological stability.

Hypothesis: People with multiple sclerosis with maladaptive defense mechanisms, a higher level of shame and pathological narcissism have a worse quality of life and more pronounced fatigue compared to those with adaptive defense mechanisms.

Aims: The aim of the proposed study is to determine whether there is a relationship between defense mechanisms, shame, and pathological narcissism with quality of life and fatigue in people with multiple sclerosis.

Materials and methods: The study will include people with multiple sclerosis who will complete psychological questionnaires, a quality-of-life questionnaire, and a modified fatigue impact scale, and their level of disability will be measured using standardized scales.

Expected scientific contribution: The potential relationship between these factors and quality of life could contribute to the development of individualized interventions alongside standard therapeutic procedures and empowerment of patients in their daily coping with the disease.

Acknowledgments: .

MeSH/Keywords: multiple sclerosis, defense mechanisms, shame, pathological narcissism, quality of life

Poster code: T-02-29-024

Poster Title: Association of Symptomatic COVID-19 with Psychiatric Symptoms in Alcohol-Dependent Patients

PhD candidate: Lea Kozina

Part of the thesis: Association of COVID-19 Disease with Psychiatric and Cognitive Symptoms in Alcohol-Dependent Patients

Mentor(s): Associate Professor Zrnka Kovačić Petrović, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: COVID-19 has been associated with numerous acute and chronic psychiatric symptoms which occur during acute infection or after recovery. Research in the general population indicate that the most common psychiatric acute and long COVID symptoms are fatigue, depression and anxiety symptoms, sleep disorders, and cognitive symptoms. Alcohol-dependent patients are a risk group for SARS-CoV-2 infection, COVID-19 complications, hospitalization, and death due to COVID-19. Furthermore, alcohol-dependent patients are at increased risk for developing at least one psychiatric symptom associated with acute COVID-19 infection. Research in the general population suggest that problematic alcohol drinking may be one of the symptoms of long COVID syndrome.

Hypothesis: Alcohol-dependent patients who have experienced symptomatic COVID-19 have more severe symptoms of anxiety, depression, insomnia, fatigue, and more severe cognitive symptoms compared to those symptoms in alcohol-dependent patients who have not experienced symptomatic COVID-19 at the beginning of the study and after a six months follow-up.

Aims: To examine the association between symptomatic COVID-19 and psychiatric symptoms in alcohol-dependent patients during the six months follow-up.

Materials and methods: 39 alcohol-dependent patients who have experienced symptomatic COVID-19 and 39 alcohol-dependent patients who have not experienced symptomatic COVID-19 are included in the study. Symptomatic COVID-19 refers to having a positive PCR test for SARS-CoV-2 at any time since the beginning of COVID-19 pandemic. Participants are undergoing hospital, outpatient, or day hospital treatment for alcohol dependence and are consecutively included in the study. The alcohol dependence diagnosis is determined according to the 10th revision of the International Classification of Diseases and Related Health Problems (ICD-10) and according to the Croatian version 5.0.0. of the Mini International Neuropsychiatric Interview (M.I.N.I.). The study includes alcohol-dependent patients who have not abstained from alcohol for the past 12 months. Other inclusion criteria are: negative alcohol test (0.00‰) and negative urine test for illicit psychoactive substances on the day of assessment; no psychopharmacotherapy or prescribed anxiolytics, antidepressants, anti-alcoholics, or a combination; age 18-65 years; having legal capacity; signed informed consent. Exclusion criteria are: lifetime use of any other psychoactive substance; history of severe mental illness; prescribed psychopharmacotherapy beside anxiolytics, antidepressants, and anti-alcoholics; positive family history of psychosis; severe neurological disorder; acute severe physical illness requiring treatment in a somatic department; pregnancy and breastfeeding; severe alcohol withdrawal symptoms; hearing and/or vision impairment; intellectual disabilities or dementia; patient withdrawal from the study. The assessment is conducted at the time of enrollment in the study and again after 6 months. The initial assessment is conducted at least 10 days after achieving the initial abstinence. The planned duration of the study is from 20th March 2023 to 21st March 2025. Sociodemographic and medical data, alcohol consumption, and symptomatic COVID-19 recovery information are collected using psychiatric interviews. Alcohol dependence severity, craving for alcohol, anxiety and depression symptoms, sleep quality, fatigue, and cognitive symptoms are assessed by rating and self-report scales. The minimum sample size was determined using the G Power program, which is 26 participants in each group.

Expected scientific contribution: The study could increase scientific knowledge about the association of symptomatic COVID-19 with psychiatric symptoms in alcohol-dependent patients.

MeSH/Keywords: alcoholism, cognition, COVID-19, psychiatry, prospective study

Poster code: T-02-29-049

Poster Title: IL-6, IL-15, CRP, albumin and zinc in children and adolescents age from 12 to 18 years old suffering from anorexia nervosa

PhD candidate: Petra Lederer

Part of the thesis: IL-6, IL-15, CRP, albumin and zinc in children and adolescents age from 12 to 18 years old suffering from anorexia nervosa

Mentor(s): Professor Alma Mihaljević-Peleš, MD PhD

Affiliation: University of Zagreb School of Medicine, University hospital centre Zagreb, Clinic for child and adolescent psychiatry

Introduction: Previous research has shown that changes in the biological systems of immune regulation can be related to the pathophysiology of eating disorders. However, the number of studies on children and adolescents in the literature is limited.

Hypothesis: There is a difference in the values of IL-6, IL-15, CRP, zinc and albumin between patients aged 12 to 18 years with anorexia nervosa (AN) and healthy controls.

Aims: The goal of this cross-sectional study is to investigate changes in selected parameters: IL-6, IL-15, CRP, albumin and zinc in a group of children and adolescents aged 12 to 18 and a group of healthy subjects, and to compare their values and variations with statistical processing. To show whether the indicated indicators are changed in patients with anorexia nervosa, how they affect the clinical picture, whether they can be potential biomarkers and how they can affect the diagnosis and treatment of eating disorders at a younger age.

Materials and methods: The study would include diagnostics based on the ICD-11, EDE-Q questionnaire, sociodemographic questionnaire, DASS-21 self-assessment scale, and blood sampling to determine the above indicators.

Expected scientific contribution: The results of the research could contribute to a better understanding of the pathophysiology of eating disorders, better and more objective diagnostics, and better treatment of various forms of eating disorders in children and adolescents.

MeSH/Keywords: anorexia nervosa, child and adolescent psychiatry, cytokines, CRP, albumin, cinc, IL-15, IL-6, eating disorders

Poster code: T-02-29-158

Poster Title: Role of CYP2C19 pharmacogenetics in clopidogrel-treated patients undergoing unruptured intracranial aneurysm stenting

PhD candidate: Katarina Starčević

Part of the thesis: Significance of pharmacogenetically defined CYP2C19 metabolic phenotypes in predicting intracranial aneurysm stenting complications in clopidogrel-treated patients.

Mentor(s): Professor Zdravka Poljaković-Skurić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Stents are commonly used in the endovascular treatment of UIA (unruptured intracranial aneurysms). Clopidogrel is still a widely used antiplatelet P2Y12 inhibitor in these patients. Since it's a prodrug, it must be activated via the CYP2C19 pathway thereby being influenced by its polymorphysms. Depending on the genotype, patients can be NM (normal), PM (poor), IM (intermediate), RM (rapid) or UM (ultrarapid metabolizers).

Hypothesis: Our null hypothesis is that there is no difference in thrombotic cerebral events between two groups of clopidogrel-taking patients undergoing stent-assisted UIA embolization, those defined as NMs on one, and IMs and PMs on the other.

Aims: Defining the relevance of CYP2C19 testing in clopidogrel-treated UIA patients. Improving safety and efficacy of stenting of UIA by tracking not only thrombotic but hemorrhagic events as secondary outcome measures. Looking for the association between CYP2C19 pharmacogenetics and LTA (light transmission aggregometry) as the current golden standard

Materials and methods: This study is a single-center retrospective study of 266 prospectively collected patients between 2015 and 2020 undergoing UIA endovascular stenting procedures and taking clopidogrel as part of the dual antiplatelet therapy. Excluded are patients on triple antithrombotic therapy, renal insufficiency with GFR<30ml/min/1,73m, and hepatic failure. Patients had been premedicated with clopidogrel 75mg once daily for at least five days before the intervention. As a part of procedural preparation, each patient had one sample of full EDTA blood drawn and analyzed using real-time PCR (polymerase chain reaction) TaqMan DME assays and LTA (light transmission aggregometry). According to allele CYP2C19 genotypes are classified as 1*/*1, *1/*17, *17/*17, *1/*2, *2/*17, *2/*2 which are further classified into clopidogrel metabolic phenotypes: *1/*1 as NM, *1/*17 as RM, *17/*17 as UM, *1/*2 and *2/*17 as IM and *2/*2 as PM (poor metabolizers). Those with high residual platelet reactivity defined as PRU results on LTA equal to or higher than 208, are excluded from the main hypothesis testing because of being assigned to an alternative P2Y12 inhibitor for obvious ethical reasons. Those with PRU results below 208 continued taking clopidogrel for at least three months. The first follow-up was in three months. We analyzed clinical data (history suggestive of transitory ischaemic attack, stroke, retinal ischemia) and early neuroradiologic follow-up imaging – brain MR with TOF MRA or dynaCT or DSA (digital subtraction angiography) looking for signs of ischemia and signs indicative of stent thrombosis. Main outcome events are defined as ischemic stroke, in-stent thrombosis, transitory ischemic attack, and retinal ischemia, all of these related to the stented vessel. Secondary outcomes are hemorrhagic events classified as either intracranial or extracranial. Using medical records, we collected other variables as well: demographic (age and gender), history of hypertension, diabetes, smoking, type of stent used and concomitant chronic therapy that could potentially interact via the CYP2C19 mechanism. In descriptive statistics, we'll present data as absolute numbers and frequencies for categorical variables, and mean values with a range for numerical variables in tables and graphically in plots. In detecting correlation we'll be using Pearson and Spearman correlation coefficient. Statistical tests that will be used are t-test and ANOVA, in case non-parametric tests are needed, chi-square, Fisher exact, Mann-Whitney U, or Kruskal Wallis will be used. Level of significance will be defined at 5%, all measured p-values will be two-sided.

Expected scientific contribution: Contribution to the existing knowledge about the role of pharmacogenetics in personalized antithrombotic treatment of patients undergoing stent-assisted endovascular procedures for intracranial aneurysms.

MeSH/Keywords: Clopidogrel, CYP2C19 pharmacogenetics, Unruptured intracranial aneurysm, Stent-assisted embolization, Aggregometry

Poster code: T-02-30-032

Poster Title: The effect of continuous positive airway pressure therapy on brain-derived neurotrophic factor concentrations in patients suffering from obstructive sleep apnea syndrome and post-traumatic stress disorder

PhD candidate: Hrvoje Grbavac

Part of the thesis: The effect of continuous positive airway pressure therapy on brain-derived neurotrophic factor concentrations in patients suffering from obstructive sleep apnea syndrome and post-traumatic stress disorder

Mentor(s): Assistant Professor Petrana Brečić, MD PhD, Assistant Professor Domagoj Vidović, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Obstructive sleep apnea syndrome (OSA) and post-traumatic stress disorder (PTSD) are chronic conditions, often co-occurring and presenting complex clinical challenges. Both disorders are independently associated with altered concentrations of brain-derived neurotrophic factor (BDNF), a key neurotrophin for neuronal growth and survival. The aim of the study is to investigate the potential modulating effects of continuous positive airway pressure (CPAP) therapy on BDNF concentrations in individuals suffering from OSA and PTSD. The hypothesis is that CPAP therapy can affect BDNF concentrations, providing insight into its potential therapeutic mechanisms.

Hypothesis: CPAP therapy will have a modulating effect on BDNF concentrations in patients suffering from OSA and PTSD, reflecting a potential mechanism through which CPAP may manifest its therapeutic effects. The hypothesis is based on the understanding that OSA and PTSD are independently associated with changes in BDNF levels, and the interaction of CPAP therapy with these neurobiological pathways may contribute to significant changes in BDNF concentrations.

Aims: To improve the understanding of the interplay between OSA, PTSD, BDNF, and CPAP therapy. By investigating the potential modulating effects of CPAP on BDNF concentration in patients suffering from OSA and PTSD, the research aims to contribute to the advancement of knowledge in sleep medicine and psychiatry.

Materials and methods: The study will prospectively include a total of 60 patients with previously diagnosed PTSD who are under outpatient supervision at the Institute for Psychophysiology and Organically Conditioned Mental Disorders with the Center for Wakefulness and Sleep Disorders, Vrapče Psychiatry Clinic. Research timeline: 1. clinical evaluation, filling in questionnaires for the subjective evaluation of night sleep (Pittsburgh Sleep Quality Index; PSQI), daytime sleepiness (Epworth Sleepiness Scale; ESS), OSA screening questionnaire (STOP-BANG) and PTSD symptom self-assessment scales, PTSD checklist for DSM 5 (PCL-5), and then they will undergo a standardized clinical interview Clinician-Administered PTSD Scale (CAPS); 2. determination of anthropometric measurements (height, weight, neck circumference); 3. polysomnographic recording; 4. after polysomnography, blood samples will be taken from all subjects to determine BDNF concentrations in serum (ELISA); 5. Introduction of CPAP in the treatment of patients with an AHI higher than 15; 6. control blood sampling to determine BDNF concentrations in the serum will be performed 3 months after initiation of CPAP therapy; The criteria for inclusion in both groups will be age over 18 and up to 65 and previously diagnosed PTSD. The exclusive criteria for both groups will be an effective sleep time shorter than 240 min and the presence of other significant conditions and diseases (dementia and other neurodegenerative diseases, history of severe head trauma, acute infectious events, active neoplastic processes, etc.).

Expected scientific contribution: Investigating the potential effects of CPAP on BDNF concentrations may reveal the impact of CPAP treatment on BDNF dynamics. If the hypothesis is confirmed, it would suggest that CPAP therapy affects not only respiratory symptoms, but also neurotrophic factors involved in cognitive function and emotional regulation. The expected scientific contribution is the improvement of the understanding of the effect of CPAP therapy on BDNF concentrations in patients with PTSD and OSA.

MeSH/Keywords: Obstructive sleep apnea syndrome, post-traumatic stress disorder, brain-derived neurotrophic factor, continuous positive airway pressure therapy, neurotrophin

Poster code: T-02-30-110

Poster Title: Changes of cerebrospinal fluid volume and biomarker concentration in patients with normal pressure hydrocephalus before and after external drainage

PhD candidate: Ivan Koprek

Part of the thesis: Changes of cerebrospinal fluid volume and biomarker concentration in patients with normal pressure hydrocephalus before and after external drainage

Mentor(s): Professor Marijan Klarica, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Idiopathic normotensive hydrocephalus (iNPH) is a progressive neurodegenerative disease characterized by dementia, gait disturbances and urinary incontinence (Hakim's triad). The prevalence of iNPH in the population over 65 years old is about 3.7%. The pathophysiology of iNPH is unknown. CSF diversion procedures are now considered the therapy of choice, with clinical improvement in up to 85% of patients. Through our research so far, we have established that many physiological phenomena related to the CSF system have not been (properly) investigated, such as the total volume of CSF in the cranial and spinal space and it's impact on CSF pressure. Pathophysiological changes in the brain tissue in various CNS diseases, including iNPH and AD, are reflected in the CSF. Biomarkers that have so far been most investigated in iNPH and AD are: peptides amyloid beta (Aß1-42) and (Aß1-40), total tau protein (t-tau) and phosphorylated tau (f-tau) protein. Within the CSF there are concentration gradients of various substances which is contrary to the classical understanding of the physiology of cerebrospinal fluid.

Hypothesis: Patients with NPH who would respond positively clinically to external drainage have an increased volume of spinal fluid before drainage and significant changes in the concentration of biomarkers for neurodegenerative diseases during drainage.

Aims: GENERAL AIM: To examine the values of cranial and spinal volume of cerebrospinal fluid (CSF), and the concentration of biomarkers (Aβ1-42, Aβ1-40, t-tau and p-tau) in cerebrospinal fluid before, during and after extended external drainage in patients with normotensive hydrocephalus. SPECIFIC AIMS: 1. to measure anatomical dimensions of the cranial and spinal space, and CSF pressure by lumbar puncture in patients with iNPH; 2. to create a correlation between the measured values of CSF volume, CSF pressure and the outcome of the operation; 3. measure biomarker concentrations changes in cerebrospinal fluid (Aβ1-42, Aβ1-40, t-tau and p-tau) before, during and after drainage; 4. to correlate the volume of cerebrospinal fluid in the cranial and spinal space with the concentration of biomarkers.

Materials and methods: The subjects are patients older than 60 years with clinical signs of dementia and neuroradiological criteria for the existence of iNPH. Patients will undergo the usual preoperative workup and screening (clinical testing, MR imaging, lumbar puncture), with volumetric analysis of the cerebrospinal fluid by segment, determination of the changes in concentration of biomarkers (Aβ1-42, Aβ1-40 t-tau and p-tau in the drained cerebrospinal fluid, and clinical and radiological follow up.

Expected scientific contribution: We expect that the results of our research will show the values of the CSF volume in the cranium and spinal cord. Thus, in the procedures for testing and treating patients with iNPH, we would know what volume of cerebrospinal fluid we should maintain, how much cerebrospinal fluid should be drained (it is possible that we drain too little or too much in many cases). In addition, for the first time, they could determine the predictive value of parameters such as the volume of cerebrospinal fluid in certain segments and the concentration of certain constituents of cerebrospinal fluid. The results of this research could more precisely show the relationship between the volume and pressure of the cerebrospinal fluid in normal and different pathological conditions. We expect that the results will be in accordance with the new hypothesis of the physiology of cerebrospinal fluid, according to which cerebrospinal fluid is formed and disappears at the level of the capillary network of the brain and spinal cord depending on hydrostatic and osmotic forces, and does not depend on the secretion, circulation and absorption of cerebrospinal fluid as assumed by the classic hypothesis.

MeSH/Keywords: cerebrospinal fluid, normal pressure hydrocephalus, CSF volumetry, biomarkers fo neurodegenerativne deseases, intracranial pressure, lumbar puncture

Poster code: T-02-30-142

Poster Title: Development and validation of a competency evaluation index system for nurses caring for patients with respiratory disease

PhD candidate: Andreja Šajnić

Part of the thesis: Development and validation of a competency evaluation index system for nurses caring for patients with respiratory disease

Mentor(s): Professor Marko Jakopović, MD PhD, Georgia L. Narsavage, PhD, professor emeritus

Affiliation: University of Zagreb School of Medicine / Department for Respiratory Diseases Jordanovac, University Hospital Center, Zagreb, Croatia

Introduction: The Study aims to construct and validate a competency evaluation index system for nurses caring for patients with respiratory disease.

Hypothesis: The competency evaluation index system will clarify educational areas in respiratory nursing that need development.

Aims: GENERAL AIM: Construct a competency evaluation index system for nurses caring for patients with respiratory diseases. SPECIFIC AIMS: 1. Provide an index list of competencies that are needed by a nurse related to caring for patients with respiratory diseases; 2. Provide the competency evaluation index system for nurses caring for patients with respiratory disease as a scientific foundation for nursing managers to accurately understand, describe, analyze, and evaluate the competency level of nurses caring for patients with respiratory diseases. 3. Validate the competency evaluation index system for nurses caring for patients with respiratory disease in the United Kingdom.

Materials and methods: Based on the results from the Scoping Review Protocol we were not able to identify any available respiratory nursing competency frameworks in databases (MEDLINE, EMBASE, CINAHL, PsycINFO, ERIC, Cochrane database of systematic reviews, and Web of Science). By using a snowball technique, existing respiratory nursing care frameworks and lists of competencies will be obtained from multiple sources. A first draft of the index system Delphi questionnaire will consist of extracted competency items (knowledge, skills and attitudes) from available frameworks related to nursing and the respiratory field developed and published by respiratory organizations, respiratory nursing associations and organizations or nursing associations related to the respiratory field. Modified Delphi technique is planned with two rounds using an Expert Panel. Purposive sampling will be used to select 10-12 respiratory nursing experts from (n=10) countries. The first Delphi round with listed items will be distributed to the identified expert panellists that consent to participate. The experts will complete the questionnaire independently without consultation with each other and will complete their self-assessment survey for calculation of the expert competence index (K). The items included in the second draft of the index system Delphi questionnaire will be based on the findings of the first Delphi questionnaire. The second Delphi round will include all members of the International Coalition of Respiratory Nurses (ICRN). After the second Delphi round, the analyzed results obtained from first Delphi (expert panel) and second Delphi (ICRN members) round will be discussed with the Expert Panel. The expert panel discussion and consensus will be used to prepare the final competency evaluation index system for nurses caring for patients with respiratory diseases. To validate the competency evaluation index system for nurses caring for patients with chronic respiratory diseases, a sample of nurses and nurse managers from the United Kingdom caring for patients with respiratory diseases will complete the index as a self-assessment as well as an evaluation of the index usefulness and ease of completion.

Expected scientific contribution: The acquisition of data from the competency evaluation index system for nurses caring for patients with respiratory diseases will be targeted and comprehensive, which can clarify weak areas of nurses' competencies in respiratory patient care. The index system will provide a scientific basis for nursing managers to accurately understand, describe, analyze, and evaluate the competency level of nurses caring for patients with respiratory diseases with direction for their clinical education and training.

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MeSH/Keywords: Competencies, respiratory nursing, evaluation index system

Poster code: T-02-31-122

6. Thesis proposals – public health and healthcare

Poster Title: Longitudinal Research about Psychological Outcomes of Family Medicine Doctors During COVID-19 Pandemic

PhD candidate: Sunčana Vlah Tomičević

Part of the thesis: Longitudinal Research about Psychological Outcomes of Family Medicine Doctors During COVID-19 Pandemic

Mentor(s): Assistant Professor Valerija Bralić Lang, MD PhD, Associate Professor Marina Šagud, MD PhD

Affiliation: University of Zagreb, School of Medicine

Introduction: Doctors working in family medicine in Croatia face significant daily burdens. The COVID-19 pandemic largely exacerbated workload and altered approach in everyday work with additional administrative tasks, numerous non-medical and unexpected stressors, as well as negative public comments so adverse consequences on doctors' mental health are expected.

Hypothesis: Among family medicine doctors, the proportion of symptoms of stress, anxiety, depression, and post-traumatic stress disorder (PTSD) is increasing with the progression of the COVID-19 pandemic and is influenced by sociodemographic factors.

Aims: To determine changes in the proportion of symptoms of stress, anxiety, depression, and PTSD among doctors working in family medicine during the COVID-19 pandemic and to define protective and risk factors contributing to mental burden, to explore the association between sociodemographic factors of participants and changes in the proportion of symptoms and the association between participants' comorbidities and changes in the proportion of symptoms of stress, anxiety, depression, and PTSD.

Materials and methods: This is a longitudinal study conducted during May 2020, May 2021, and concluding in May 2023, carried out across Croatia among doctors working in family medicine. Data is collected using an anonymous electronic survey administered via Google Forms® and is distributed via email to the electronic addresses of professional societies in family medicine. Participation in the study is voluntary. The survey is designed for research purposes and consists of the following sections: sociodemographic factors, occupation, years of practice, comorbidities, specific stressors, Depression, Anxiety, and Stress Scale-21 (DASS-21), and Impact of Event Scale-Revised (IES-R). Both DASS-21 and IES-R instruments have been translated, validated, and widely available for use. Descriptive statistics will be performed for all analyzed variables. The Kolmogorov-Smirnov test will be used to determine the sample distribution. Sociodemographic data will represent independent variables, while the results of the DASS-21 and IES-R instruments will be dependent variables. Mann-Whitney U test will be used for comparing two groups, and two or three-way analysis of variance with interactions will be conducted for comparing three or more groups. A significance level of 5% will be considered statistically significant for all statistical tests. Predictions of the association between stress, anxiety, depression, and PTSD as dichotomous variables and individual risk factors for each of the investigated years will be further analyzed using univariate and multivariate logistic regression analysis, defining odds ratios (OR) with a 95% confidence interval (CI) for each variable. Available software solutions will be used for data processing.

Expected scientific contribution: The research would contribute to understanding the mental health difficulties that family medicine doctors may experience as a result of the COVID-19 pandemic. Scientifically analyzing factors that could affect mental health, those contributing most to the mental burden would be identified. This would open up opportunities for new research and suggestions for improving mental health.

MeSH/Keywords: mental health, psychological difficulties, family medicine, doctors, COVID-19 pandemic

Poster code: T-03-04-019

Poster Title: Utilizing Artificial Intelligence for follow up of patients with arterial hypertension

PhD candidate: Renata Romić

Part of the thesis: Utilizing Artificial Intelligence for follow up of patients with arterial hypertension

Mentor(s): Associate Professor Venija Cerovečki Nekić, MD PhD, Assistant Professor Andrija Štajduhar, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Arterial hypertension is one of the leading health issues both in the world and Croatia, mainly due to its high prevalence, but also due to insufficient awareness among patients about the disease, poor self-control, poor adherence to therapy, and followed by unsatisfactory clinical outcomes.

Hypothesis: Patients with arterial hypertension usig digital assistant via smartphone app have more success in blood pressure control, adhere better on prescribed medication therapy, also their consultation with family medicine doctor regarding blood pressure control lasts shorter.

Aims: Our aim is to investigate the benefits of a digital assistant related to monitoring of the clinical outcomes of patients with arterial hypertension and improvement of adherence to therapy, and to evaluate the impact of such digital tool on family medicine specialist's time consumption during the examination and care for these patients.

Materials and methods: By random selection of suitable patients (with inclusion criteria) in family medicine offices, we plan to include 180 patients, and 90 of them would form the study group, while 90 would form the control group. They will alternately be assigned to record blood pressure logs at home with a digital assistant (Study group) or on paper (Controls). Four medical consultations will be performed during the 16-month study period. During the study, monitored parameters are arterial blood pressure (systolic and diastolic), adherence to therapy according to the Čulig-Scale and doctor’s consultation time.

Expected scientific contribution: Current clinical outcomes in the management of arterial hypertension are unsatisfactory, there is global lack of doctors and most of them have limited time available for patient consultation, therefore, it is necessary to look for solutions that will save valuable time and thus create time but also help us in specific health processes with management of arterial hypertension.

MeSH/Keywords: AI, hypertension, digital assistant, medical adherence, blood pressure control, family medicine

Poster code: T-03-04-108

Poster Title: Changes in the scope and work structure in family medicine and the experiences of family physicians during the COVID-19 pandemic

PhD candidate: Asja Ćosić Divjak

Part of the thesis: Changes in the scope and work structure in family medicine and the experiences of family physicians during the COVID-19 pandemic

Mentor(s): Associate Professor Goranka Petriček, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: COVID-19, declared a pandemic by the World Health Organization in March 2020, significantly impacted healthcare. Most patients with mild to moderate symptoms were treated at home, primarily by family medicine in outpatient settings. European primary care physicians played a crucial role in COVID-19 patient care and routine services. However, limitations in personal interaction and remote care posed challenges for family doctors, diverting attention from early disease detection and chronic patient treatment.

Hypothesis: During the COVID-19 pandemic, family doctors faced an increase in workload due to pandemic-related activities and changes in clinical assessment methods, struggled to maintain continuity of care and accessibility, conducted fewer activities related to chronic diseases, and also reported lower job satisfaction.

Aims: The general research aim is to describe changes in the scope and structure of work in family medicine related to the most common chronic diseases and COVID-19, as well as to explore family physicians' experiences with work changes during the COVID-19 pandemic. Specific aims of this study are to present the scope and structure of work in family medicine for the most common chronic diseases and COVID-19 through selected diagnostic and therapeutic procedures in 2019 and pandemic years (2020 and 2021), to compare said scope and structure of work for the most common chronic diseases and COVID-19 using selected diagnostic and therapeutic procedures between 2019 and pandemic years (2020 and 2021) and also to investigate family physicians' experiences with work changes during the COVID-19 pandemic, specifically related to continuity of care, home visits, new forms of consultations, the therapeutic relationship between physicians and patients, and workload impact on job satisfaction.

Materials and methods: This study combines quantitative and qualitative research using a triangulation design. Data will be collected from June 1st to December 1st, 2024. The quantitative component involves a cross-sectional study. The sample includes all family medicine practices in health centers and private practices in the Republic of Croatia. We'll compare family medicine practices' work scope and structure in Croatia for most common chronic diseases and COVID-19 during April and September in 2019, 2020, and 2021. using diagnostic and therapeutic procedures, categorized into six groups. The data will be presented using contingency tables and descriptive measures. Appropriate statistical tests (parametric or non-parametric) will be applied, with results interpreted at a 5% significance level. Data processing will be performed using statistical software SPSS, version 21. In the qualitative phase, we'll explore how family doctors adapted their daily work during the COVID-19 pandemic. This includes continuity of care, home visits, new consultation methods, doctor-patient relationships, and work impact on job satisfaction. We'll collect data from at least 24 family doctors employed across three Health Centers in Zagreb. The sample will be obtained through purposive sampling and the 'snowball' method. If data saturation isn't achieved, we'll increase the sample size. Semi-structured individual interviews, guided by a research questionnaire designed for research purposes, will be conducted. To ensure study quality, researcher triangulation will be employed. Subsequently, we'll compare qualitative and quantitative findings to determine if they converge in understanding the researched phenomenon.

Expected scientific contribution: This research could contribute by investigating how family physicians in Croatia adapted their clinical assessment approach during the COVID-19 pandemic. It examines changes in continuity, care availability, and job satisfaction. Additionally, it would explore coping strategies employed by family physicians with findings enhancing knowledge for improving family medicine care quality and mitigating negative impacts during pandemics through education and preparedness.

MeSH/Keywords: COVID-19, family medicine, mixed method research, workload

Poster code: T-03-04-111