PhD Day 2023
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Preliminary research results

Basic medical sciences – preliminary research results

Poster Title: Effects of low dose tetanus toxin injections into the rat basal ganglia on motor performance

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, Ana Knezović, PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Abnormalities in the basal ganglia activity play a central role in the onset of movement disorders such as dystonia and parkinsonism. The basal ganglia and related nuclei are engaged primarily in motor control, together with a wider variety of roles such as motor learning, executive functions, behavior and emotions. Tetanus toxin (TeNT) causes neuronal disinhibition by selectively blocking inhibitory GABA- or glycinergic synapses. The aim of the present study was to examine the behavioral effect of neuronal disinhibition in the basal ganglia induced by low, non-convulsive doses of TeNT.

Materials and methods: Male Wistar rats (N = 9 / group) were unilaterally stereotaxically injected into the caudate putamen (CPu) with 0.8 ng of TeNT, while the smaller globus pallidus internus (GPi) and substantia nigra (SN) regions were injected with 0.4 ng of TeNT. The effects of TeNT were assessed on the 7th, 10th and 14th day after the injection. Motor and behavioral tests such as beam-walking, ladder-walking, gait analysis, open field and swimming test were repeatedly performed to assess the effect of TeNT-induced disinhibition on normal motor performance. On the 10th day after the injection, animals underwent an amphetamine-induced rotation test (D-amphetamine 1 mg/kg i.p.) to intensify ipsilateral circling visible in the open field and swimming tests. An audiogenic seizure test was performed on the last day of the experiment to exclude the possible epileptogenic action of TeNT.

Results: After unilateral TeNT injection into the CPu, plantar misplacement of the hind limb during the beam-walk test was evident on the hind paw contralateral to the injection site. Animals injected into the CPu and GPi showed a tendency to ipsilateral circling behavior in the open field and swimming tests, which was even more emphasized during the amphetamine-induced rotation test. The SN injected group did not develop any observeable deficits.

Discussion: Disinhibition of CPu with TeNT induces visible contralateral motor impairment in the beam-walk test, a relatively complex motor task requiring correct prediction of hind paw placement. On the other hand, disinhibition of CPu and GPi induced an ipsilateral circling tendency. A later result, reminiscent of the effects of experimental parkinsonism-inducing dopaminergic nerotoxins in combination with D-amphetamine, suggests an important interaction of inhibitory and dopaminergic transmission in the basal ganglia for extrapyramidal motor control and impairment.

MeSH/Keywords: Tetanus toxin, basal ganglia, motor impairments

Poster code: R-01-03-016

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 or more times or by at least 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.

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. Diameter 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. DR's 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 linear lamina elastica interna, thinning of tunica media with areas of detachment and leukocyte aggregates in control gorup, whereas treated group showed undular lamina elastica interna, no thinning or detachments in tunica media, and few solitary leukocytes.

Discussion: 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-alpha) 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-01-03-022

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: Syndrome induced in rats by cataleptogenic dose of L-NAME, effect of L-arginine and therapeutic effect of pentadecapeptide BPC-57

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). It is established 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 demonstrate 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 histopathological analysis of the brain, heart, lungs, kidneys, digestive system, posterior caval vein (PCV), 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, thrombosis evaluation and ECG recording. 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 medication application (L-NAME, and L-NAME+ BPC 157). Venography was performed using 1mL of iodine isoosmolar nonionic contrast media via direct PCV cannulation, 5 minutes after medication application.

Results: Control rats exhibited greater brain swelling, in comparison to treated rats. Venography showed PCV 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 PCV was normal in diametar, with much faster blood flow in arterial system and significantly better opacificiation of abdominal parenchymal organs and bowel wall.

Discussion: Preliminary results show BPC 157 counteraction of brain swelling as well as PCV dilatation and congestion before and after L-NAME induced catalepsy. Considering the changes observed and analyzed in previous studies (3-5) , 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 PCV 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 evaluations will be performed within the framework of the proposed doctoral dissertation.

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

Poster code: R-01-03-028

Poster Title: The Toxin Trio and One Antitoxin Combined Together to Understand the Mechanisms of Action of Botulinum Toxin Type A In the Motor System

PhD candidate: Petra Šoštarić Mužić

Part of the thesis: Peripheral and central effects of botulinum toxin type A toxin in the rat motor nervous system

Mentor(s): Ivica Matak, PhD, research associate, Assistant Professor Marco Pirazzini, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Botulinum toxin A is natures most potent presynaptic neurotoxin, which is used as standard therapy in different hyperkinetic movement disorders. Therapeutic effect of botulinum toxin A in hyperactive muscle is classically attributed to neuroparalysis of muscular motor terminals, however, recent research revealed the toxin enzymatic activity in the central nervous system and lasting effects on focal muscle hypertonia, at least in part due to the botulinum toxin A central transcytosis. The aim of the study was to separate the relative contribution of botulinum toxin A peripheral and central effects on normal and spastic muscle by employing subsequent latrotoxin and neutralizing antitoxin to circumvent its muscular or spinal cord enzymatic action, and to challenge its efficacy in muscle hypertonia by tetanus neurotoxin.

Materials and methods: Male Wistar rats were unilaterally injected into gastrocnemius muscle with botulinum toxin A 5 U per kg, each unit corresponding to 48 pg toxin complex. After 24 hours, botulinum toxin type A neutralizing antitoxin 5 international units was administered intrathecally to prevent the toxin central transcytosis. Latrotoxin, 4 g was administered in gastrocnemius on day 3 after botulinum toxin A to abolish the toxins peripheral effects. Motor tests such as gait ability test, digit abduction score and compound muscle action potential measurements were repeatedly performed to assess the motor performance recovery. After the animals recovered from flaccid paralysis, unilateral injection of tetanus toxin 1,5 ng was administered to gastrocnemius to induce spastic paralysis. Recovery of the spastic paralysis was assessed by ankle dorsiflexion resistance test and monosynaptic H reflex measurements.

Results: The botulinum toxin A induced lasting and reversible impairment of examined motor functions, which mostly recovered by day 70 post i.m toxin injection. Destruction and fast regeneration of peripheral motor terminals by latrotoxin accelerated the recovery of motor function and counteracted the lasting effects of botulinum toxin A on CMAP reduction and muscle atrophy. The tetanus toxin increased monosynaptic H reflex was not decreased by latrotoxin or botulinum toxin A treatment.

Discussion: Botulinum toxin A effects on atrophy, gait ability and digit abduction score most likely depend on the peripheral action of the toxin, due to the accelerated recovery enhanced by latrotoxin medited elimination of botulinum toxin A poisoned neuromuscular junctions and their fast subsequent regeneration. Tetanus toxin evoked spastic paralysis was affected by botulinum toxin A central and peripheral effects. This data suggest that combined peripheral and central effects of the peripherally administered botulinum toxin A are responsible for its lasting action in hyperactive muscle, while peripheral toxin effect are more important in motor performance impairment and lasting muscle atrophy.

Acknowledgments: Funding by Croatian Science Foundation project ID UIP 2019 04 8277

MeSH/Keywords: botulinum toxin type A, atrophy, central effects

Poster code: R-01-03-064

Poster Title: Diurnal and nocturnal locomotor patterns during recovery may predict the development of motor impairment in the 6-hydroxydopamine-induced rat model of Parkinsons disease

PhD candidate: Davor Virag

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

Mentor(s): Professor Melita Šalković-Petrišić, MD PhD, Associate Professor Jelena Osmanović Barilar, MD PhD

Affiliation: 1 Laboratory for Molecular Neuropharmacology, Department of Pharmacology, University of Zagreb School of Medicine, 2 Scientific Centre of Excellence for Basic, Clinical and Translational Neuroscience, Croatian Institute for Brain Research

Introduction: The Parkinsons disease rat model produced by intrastriatal injection of low doses of 6-hydroxydopamine (6-OHDA) is an indispensable tool for studying the preclinical stages of the disease, but eventual development of the characteristic phenotype is highly unpredictable. We aimed to examine whether early changes in spontaneous home cage motor activity can be used to predict eventual development of motor dysfunction on the standard rotarod performance test.

Materials and methods: Adult male Wistar rats had 6-OHDA (8 µg) or vehicle (CTR) applied into striata bilaterally (n(6-OHDA)=20, n(CTR)=10) and were subjected to the rotarod test at 14, 28, 35 and 60 days after treatment. Baseline spontaneous motor activity was recorded for 4 days preceding the treatment, and for the following 7 days using MIROSLAV (Multicage InfraRed Open-Source Locomotor Activity eValuator), a novel home cage-mounted system based on passive infrared sensors. Data analysis was performed in R.

Results: Compared to their baseline activity levels, animals that developed a motor deficit at the 60 day rotarod test showed markedly reduced activity during both diurnal and nocturnal phases of the first 3 days, as opposed to the unsuccessful models, which demonstrated an increase in both diurnal and nocturnal activity. These effects largely dissipated at 7 days post-op. The vehicle-treated animals demonstrated increased diurnal, and decreased nocturnal activity.

Discussion: Preliminary MIROSLAV data indicates that 6-hydroxydopamine could have distinct effects on the animals motor patterns during recovery, with opposing effects that seem to predict subsequent development of motor dysfunction.

Acknowledgments: Virag Davor (1,2), Homolak Jan (1,2), Babić Perhoč Ana (1,2), Knezović Ana (1,2), Osmanović Barilar Jelena (1,2), Šalković-Petrišić Melita (1,2) Financiranje: HRZZ projekt IP-2018-01-8938, ZCI GA KK01.1.1.01.0007

MeSH/Keywords: Rats, Parkinson Disease, Locomotion, Circadian Rhythm, Physiologic Monitoring

Poster code: R-01-03-110

Poster Title: The role of checkpoint kinase 1 and bone marrow stromal cells in cytarabine-induced differentiation of acute myeloid leukemia cells

PhD candidate: Tomislav Smoljo

Part of the thesis: Mechanism of cytarabine-induced acute myeloid leukemia cell differentiation

Mentor(s): Professor Dora Višnjić, MD PhD

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

Introduction: Cytarabine (AraC) is the backbone of standard induction therapy for acute myeloid leukemia (AML) because of its cytotoxic effects. Low-dose AraC (LDAC) induces differentiation of AML cells in vitro, but the mechanism is not completely elucidated. We have previously reported that pyrimidine synthesis inhibitors induced differentiation of AML cells by activating checkpoint kinase 1 (Chk1). The aim of this study is to test the role of Chk1 in LDAC-induced differentiation and to determine the effects of bone marrow stromal cells on AML cells treated with AraC.

Materials and methods: Human AML cell lines U937, THP-1 and MOLM-13 were incubated with AraC (10, 50, 100, 1000 nM), AICAr (0.2 mM) or brequinar (0.5 μM) for 72 hours. In some experiments, cells were pretreated with plerixafor (20 μM), anti-TGF- antibodies (0,5 g/mL), mercaptosuccinic acid (100, 600 M), nucleosides (1x) and Torin2 (100 nM). In co-culture experiments, murine stromal cell line MS-5 was seeded 24 hours before human AML cell lines with or without transwell inserts. Hemocytometer and trypan blue exclusion were used to determine the number of viable cells. DNA content for cell cycle analysis, annexin-FITC binding for apoptosis, expression of differentiation markers CD11b and CD64 and dihydrorhodamine 123 staining for reactive oxygen species were analyzed by flow cytometry and FlowJo software. The level of cytokines was measured by LEGENDplex Mouse HSC Myeloid Panel. The level of total and phosphorylated Chk1 and cyclin dependent kinase 1 (Cdk1) were determined by western blot. siRNA transfections were performed using siRNA targeting Chk1 and NeonTM transfection system. Morphology analysis of May-Grünwald-Giemsa stained cells was performed by AxioVert 200 microscope and Axiocam MRc 5 camera. Statistical analysis was performed using Students t-test ($p < 0.05$).

Results: Immunoblotting and flow cytometry analyses revealed that AraC decreased proliferation, induced differentiation and activated Chk1 in AML cell lines. Pharmacological inhibition and down-regulation of Chk1 reduced the effects of AraC on the expression of differentiation markers and cell cycle arrest with a reciprocal increase in AraC-related cell death. The presence of stromal MS-5 cells prevents cell cycle arrest and differentiation of AML cells treated with LDAC independent of mutational status of p53 and FLT3. Transwell experiments demonstrate that the inhibitory effects of MS-5 stromal cells on AML cell differentiation cannot be abolished by lack of cell-to-cell contact. MS-5 cells secrete high levels of CXCL12 and TGF-, but their inhibitory effects on differentiation were not prevented by the addition of plerixafor, a CXCL12 antagonist, and anti-TGF- antibodies. However, the presence of stromal cells reduces the level of reactive oxygen species in leukemia cells treated with AraC and pharmacological inhibition of glutathione peroxidase by mercaptosuccinic acid restores differentiation in response to LDAC.

Discussion: Majority of AML patients are older patients who are unfit for intensive chemotherapy so that an acceptable low-intensity therapy includes LDAC. LDAC triggers AML remissions without toxicity in vivo and induces differentiation of leukemic cells in vitro, but terminal differentiation is rarely described in patients treated with LDAC. The results of our study show that the presence of stroma inhibits LDAC-induced differentiation suggesting that one of the reason for the modest effects of LDAC on AML differentiation in vivo may be due to the impact of BM microenvironment. In addition, our results suggest that LDAC induces differentiation of AML cells by activating Chk1 and thus shares mechanism with pyrimidine synthesis inhibitors. Understanding the mechanism of differentiation effects of LDAC may help to instruct more rationale-based therapeutic approaches in older patients who are ineligible for intensive chemotherapy.

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MeSH/Keywords: acute myeloid leukemia, cytarabine, cell differentiation, Chk1 Kinase, stromal cells

Poster code: R-01-04-103

Poster Title: The role of sphingomyelin synthases (SGSM1 and SGSM2) and sphingomyelin phosphodiesterases (SMPD1, SMPD 2 and SMPD3) in development of calcific aortic valve stenosis

PhD candidate: Matija Miletić

Part of the thesis: The role of sphingomyelin synthases (SGSM1 and SGSM2) and sphingomyelin phosphodiesterases (SMPD1, SMPD 2 and SMPD3) in development of calcific aortic valve stenosis

Mentor(s): Associate Professor Frane Paić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Calcific aortic valve stenosis (CAVS) represents the most prevailing form of degenerative heart valve disease. Morbidity and mortality associated with this disease is constantly growing and with the current trends in the population demographics and increase of elderly population reaches epidemic proportions. Although the recent advances in cardiovascular medicine have provided better insight in molecular background of CAVS development our current picture of this multifactorial disease is still quite partial and elusive. This is primarily noticeable in the lack of useful intervention strategy at the early stage of the disease, the lack of clinically disease specific diagnostic/prognostic biomarkers and the stagnation in available treatment options. The aim of this PhD study is to determine the mRNA and protein expression pattern of sphingomyelin synthases (SGSM1 and SGSM2) and sphingomyelin phosphodiesterases (SMPD1, SMPD2 and SMPD3) in pathologically altered CAVS (calcific aortic valve stenosis) tissue.

Materials and methods: Research encompass adult patients undergoing valve replacement (clinical Hospital Dubrava) for severe calcific aortic valve stenosis (n=80) as well as older heart transplant recipients without any visible clinical and morphological signs of aortic valve lesions (n=25). Immediately after surgical removal, all collected aortic valves (CAVS and controls; collected from November 2017 to March 2023) were immersed in RNA Later (Qiagen) and stored at 4°C until transport to the Laboratory for Epigenetic and Molecular Medicine, Department of Medical Biology, University of Zagreb. Two aortic valve cusp were used for macroscopic dissection and subsequent total RNA isolation while the remaining aortic cusp was Formalin-Fixed Paraffin-Embedded for subsequent immunohistological analysis of targeted protein expression. Macroscopic dissection was performed in the RNA Later solution, dividing CAVS tissue into: (1) normal noncalcified, smooth, pliable, and opalescent valve areas; (2) thickened fibrous valve areas; and (3) moderately and (4) heavily calcified areas. Dissected tissues samples were subsequently homogenized with a mortar and pestle and used for RNA isolation. Total RNA isolation was performed by combination of TRIzol Reagent and RNeasy column (Qiagen) separation. Quality and quantity of isolated total RNA was determined by NanoDrop 2000 Spectrophotometer (ThermoFisher scientific). Subsequently, each total RNA samples was reverse transcribed using High-Capacity cDNA Reverse Transcription Kit and obtained cDNA was stored at -800C until further analysis.

Results: We have collected and initially processed (preparation of FFPE tissue blocks; microscopic dissection and homogenization of tissue, total RNA isolation and reverse transcription) all CAVS and control tissue samples that will be analyzed (transcriptional and translational activity of sphingomyelin synthases SGSM1 and SGSM2 and sphingomyelin phosphodiesterases SMPD1, SMPD2 and SMPD3) in this PhD study. FFPE tissue blocks, and cDNA samples were appropriately stored until further analysis.

Discussion: Sphingomyelins represent one of the major tissue and plasma lipoprotein components, and numerous clinical studies lead them to correlation with the occurrence of various cardiovascular diseases such as myocardial infarction, hypertension, coronary blood vessel disease and atherosclerotic lesions in other vascular settings. Their metabolic products play an important role in different cell types of processes and signal pathways such as cell differentiation, proliferation, apoptosis, lipide homeostasis and inflammatory response that are, as previously described, also taking place in the aetiology of CAVS. Some of the enzymes involved in the metabolism of sphingomyelin are also investigated as potential targets for pharmacological therapy of atherosclerosis. But according to currently available information, the role of sphingomyelin cellular/tissue metabolic pathways in the aetiology of CAVS are mostly unknown.

Acknowledgments: I would like to thank the technical staff of the Laboratory for Epigenetics and Molecular Medicine, Department of Medical Biology, School of Medicine, Zagreb for their wholehearted help and understanding during the experimental work.

MeSH/Keywords: CAVS, sphingomyelin synthases, sphingomyelin phosphodiesterases, aortic valve leaflets

Poster code: R-01-05-050

Poster Title: Human cellular modelling and genetic dissection of the trisomy 21 effects on early brain development

PhD candidate: Ana Bekavac

Part of the thesis: Development of cerebral cortex and neurodegenerative changes in Down syndrome on human cerebral organoids model

Mentor(s): Professor Dinko Mitrečić, MD PhD, Assistant Professor Ivan Alić, MD PhD

Affiliation: University of Zagreb School of Medicine, University of Zagreb Faculty of Veterinary Medicine

Introduction: The aim of this research was to describe neuronal differentiation from the disomic (D21) and trisomic (T21) isogenic human induced pluripotent stem cells (iPSCs) and neural stem cells (NSCs) derived from persons with partial and full trisomy 21, and mosaic Down syndrome (DS).

Materials and methods: NSCs were generated from isogenic iPSCs following published protocol. NSCs, between passage 5 and 10, were seeded on the commercially available mouse astrocytes and differentiated to the mature neurons 100 days in vitro (DIV). Moreover, cerebral organoids were generated from the isogenic iPSCs following published protocol with our modification and fixed at 30, 50, 70 and 100 DIV and analysed by immunohistochemistry.

Results: On the DIV0 cells were Nestin and SOX2 positive and TUBB3 negative. There is no morphological difference between T21 and D21 cells. On the DIV14 some cells were TUBB3 positive. Here, T21 neuros were smaller, shorter with reduced number of processes. In terms of mature and synaptic active neurons (DIV108), D21 neurons were branched and equally distributed on coverslip compared to T21. Neurons expressed the same markers but the cell morphology was different and number of neurons was dramatically smaller compared to D21 neurons. The same pattern was observed in cerebral organoids.

Discussion: We find that TUBB3 positive cells show a specific neuronal phenotype. Disomic cells show normal neuronal morphology, whereas trisomic cells show a very specific cellular dysmorphology: neurons were smaller and shorter with thicker processes, and abnormal branching patterns. Both neurons in monolayer, as well as in cerebral organoids expressed all neuronal markers: pan neuronal markers (Dcx, MAP2. TUBB3, SMI, 3R-Tau) and cortical layer specific markers (Reelin, Brn2, Ctip2, SATB2, TBR1 and TBR2). We conclude that trisomic cells could differentiate to mature, synaptic active neurons but morphology of trisomic cells shows a unique and specific dysmorphology pattern.

MeSH/Keywords: induced pluripotent stem cells, neural stem cells, cerebral organoids

Poster code: R-01-08-017

Poster Title: Human oral mucosa stem cells increase survival of neurons affected by in vitro anoxia and improve recovery of mice affected by stroke

PhD candidate: Paula Stančin

Part of the thesis: Regenerative effect of oral mucosa stem cells on hypoxia damaged neurons in vitro

Mentor(s): Professor Dinko Mitrečić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Oral mucosa is a very useful source of stem cells that might be used to treat neural damages.

Materials and methods: Human oral mucosa stem cells were isolated from oral mucosa tissue from volunteers. Tissue was cut into small pieces and then immersed in the mixture of collagenase and dispase overnight to help separate lamina propria from epithelium which was used in further research. Small pieces of lamina propria were plated in 6 well plates with growth medium (LG-DMEM, 10% FBS, 1% Glutamax and antibiotics) and kept in a humidified culture incubator at 37°C and 5% CO2. Cells between the 2nd and the 7th passage were used in experiments. Cells were analysed with immunocytochemical staining and qPCR. Cell viability and damage was quantified by counting of the total number of cells and by an LDH assay. A commercially obtained human IPSC line WTSIi189-A was used in experiments pertaining to anoxia and for obtaining neurons. Cocultures of hOMSC and hIPSC were grown for 28 days, with measurements being obtained on days 0, 14, 28. The induction of ischemic brain stroke was performed on 20 mice (C57Bl/6N) by occluding the middle cerebral artery (MCA). Only those animals which developed clear signs of stroke were used for the transplantation experiments. The transplantation was performed 48 h after the induction of MCAO. One group of animals (n = 5) received one million of hOMSCs in 1 l of LG-DMEM medium, while the control group (n = 5) received 1 l of cell medium. Three tests for assessment of health condition of mice were used; Rotarod, Open field test and Y-maze.

Results: The results showed that hOMSC exhibit high expression of BDNF, NGF, VEGF markers important for regenerative potential, while GDNF was not prominent. When hOMSC were added to human neurons damaged by anoxia, they significantly improved their survival. Interestingly, this capability was gradually decreased in hOMSC exposed to neural cells for 14 and 28 days. Exposure to anoxia revealed that hOMSCs were more resistant than hIPSCs. While no difference in cell survival was observed after 6 hours, significantly more hOMSCs survived 12 and 24 hours after the onset of anoxia. In addition, the beneficial effect of hOMSC were also confirmed in mice affected by stroke. After inducing a stroke the rotarod reveals a dramatic loss in motoric and coordination capability. The difference between treated and placebo-treated group was as well visible in the Open field test.

Discussion: With time it became clear that the source of stem cells remains one of the factors with the highest influence on the success of a clinical trial. As such, the goal of our work was to perform in vitro and in vivo tests on hOMSCs in order to facilitate detailed information on the possible suitability of these cells for treatment of ischemic brain disease. Since their discovery, hOMSCs were successfully tested in several pathological conditions and, among others, they showed positive effects in mitigating spinal cord damage and the symptoms in a Parkinsonian rat model. We exposed hOMSCs to human neurons, obtained from IPSCs and we found that neural cells strongly influence hOMSCs by promoting their differentiation while downregulating expression of genes specific for pluripotency and growth factors. The capability of hOMSCs to face decreased levels of oxygen was confirmed when compared to hIPSCs, where they appeared to be more robust. Improvement of the health condition of experimental animals after ischemic or inflammatory damage of the CNS has been reported with many types of stem cells, so we tested if accelerated or improved recovery can be induced by hOSMCs. To animals in which stroke was induced, 1 million of hOMSCs was transplanted and observed that hOMSC did accelerate and improve the recovery. In conclusion, both in vitro and in vivo results suggest that hOMSC are excellent candidates for clinical trials on patients affected by stroke.

MeSH/Keywords: oral mucosa stem cells, neurons, hypoxia, stroke

Poster code: R-01-08-020

Poster Title: Application and effects of gene modifications mediated by a lentviral vector in the mouse brain

PhD candidate: Laura Skukan

Part of the thesis: Application and effects of gene modifications mediated by a lentviral vector in the mouse brain

Mentor(s): Professor Srećko Gajović, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Lentiviral vectors (LVs) are promising tools for the transfer of genetic material or molecular system to change gene activity. They have excellent safety profile and can be engineered to target specific cell line through pseudotyping and specific promoters. For astrocyte specific expression, glutamine synthetase (GS) promoter and envelope of the Mokola virus were selected while for neuron, cytomegalovirus (CMV) promoter and envelope of the VSV-G virus were implemented. The activation of the immune system is a key factor in assessing the safety of the viral vector in a gene therapy application. Therefore, we evaluated the microglial activation in the mice injected with LVs.

Materials and methods: The LV was produced by HEK293T cells transfected with four plasmid via calcium phosphate transfection method. The vector was concentrated by ultracentrifugation and titers were determined by flow cytometry measuring eGFP positive cells. Fifty male C57BL/6 albino mice aged 1214 weeks were divided in five groups. Each group received 1 µL of LV-shSpry2-neuron, LV-shSpry2-astrocyte, LV-scramble-Spry2 or PBS, both in cortex and striatum. The animals were perfused 7 and 14 days after intracranial injection. Brains were isolated and prepared for immunohistochemical staining. To identify the LV infected cell types, antibodies against cellular markers of neurons (NeuN) and astrocytes (GFAP) were used. For microglial activation sections were stained with Iba-1, a marker for microglial cells.

Results: The images of mouse brain sections, obtained by confocal microscope 7 and 14 days after intracranial injection, showed strong expression of eGFP signal suggesting a successful transduction of the cells by a LV in the mouse brain. Furthermore, eGFP signal of specifically designed LV construct for neurons was colocalized with the NeuN marker while the eGFP signal of the astrocyte construct was colocalized with GFAP labeled cells. Examination of Iba-1 stained sections under lower magnification revealed microglial activation along the needle tract but beyond the needle tract, there was no significant microglial response. Immunohistochemistry for Iba-1 marker indicated higher expression 7 days after intracranial injection in the groups injected with LV-shSpry2 specific for neuron and astrocyte, scramble-control LV and PBS compared to the control group with no intervention. However, Iba-1 expression is significantly reduced 14 days after LV injection in mouse compare to 7 days post LV delivery.

Discussion: Successful colocalization of LV construct marker with markers of neurons or astrocytes indicates the specific transduction of targeted cell lines. Increased activation of microglia was observed only in the area of the needle path in the groups injected with both LV and PBS, while this was not observed in the control group. Consequently, LV injection procedure can cause a short-term activation of microglia only in the needle tract area, which significantly decreased already 14 days after the vector injection, validating the LV as a safe delivery system. Future aim is confirmation of Spry2 downregulation with LV construct by quantifying Spry 2 protein using qPCR and Western blot analysis.

MeSH/Keywords: Lentiviral vector, inflammation, microglial cells

Poster code: R-01-08-036

Poster Title: Regional differences between the frontal, occipital, and cingulate cortex during the subplate formation period of the human fetal brain

PhD candidate: Alisa Junaković

Part of the thesis: Regional differences in expression of molecular markers during formation of the expanded subplate zone in the human fetal cerebral cortex

Mentor(s): Associate Professor Željka Krsnik, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: The human prenatal cortical development is characterized by transient fetal lamination. Subplate (SP) zone, one of the key components of early prenatal neuronal circuitry, is the most prominent fetal cortical compartment and a major site of synaptogenesis and neuronal differentiation. SP is formed between 13 and 15 postconceptional weeks (PCW) from the deep portion of the cortical plate (CP). SP compartment is not uniform across the brain hemisphere and some regional differences, especially between the parietal somatosensory and occipital visual cortex are already known. SP is thicker and more developed in associative brain areas containing more cortico-cortical connections, and it is thinner in the medial and ventral cortical portions.

Materials and methods: Using immunohistochemistry and immunofluorescence on formalin-fixed paraffin-embedded (FFPE) postmortem prenatal human brain tissue, we showed expression patterns of diverse cellular and molecular markers in different brain areas.

Results: We analyzed all transient compartments of the fetal cerebral wall ("compartmental approach"). Our results showed regional differences in the subplate volume, extracellular matrix (ECM) content, and axonal content. In the orbitofrontal cortex, the double cortical plate containing projection neurons was observed. The occipital cortex was characterized by a thinner SP and a sharper border between the SP and the IZ (external capsule). The interhemispheric cingulate cortex shows differences in the CP delamination and SP formation, as well as subventricular zone (SVZ) thickness in the dorsal isocortical and ventral mesocortical cingulate portions.

Discussion: In conclusion, regional differences between the frontal, occipital, and cingulate cortex can be observed early during in utero development, even before 15 PCW, and before the Brodmann-type cortical arealization.

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MeSH/Keywords: cerebral cortex, human brain development, subplate, transcription factors

Poster code: R-01-08-061

Poster Title: FOXP1 and nNOS neuronal populations in the adult human, mouse and rat subthalamic nucleus

PhD candidate: Tila Medenica

Part of the thesis: Comparative analysis of subthalamic nucleus phenotype of adult mouse, rat and human at the level of protein expression

Mentor(s): Associate Professor Goran Sedmak, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: The subthalamic nucleus (STN) is a subcortical structure which is an important part of the basal ganglia circuitry. STN is significantly involved in motor, cognitive and limbic processes in the brain. Despite of that, little is known about its basic cytochemistry and phenotypical profile. The aim of this study was to determine the quantity and spatial distribution of FOXP1 and nNOS protein expressing neurons in the adult human, mouse and rat subthalamic nucleus.

Materials and methods: To investigate the expression of FOXP1 and nNOS proteins in the STN, coronal slices containing the STN, from adult male mouse (strain C57BL/6), rat (strain Wistar Han), and human formalin-fixed paraffin-embedded brains, were used. Immunofluorescent double-labelling was performed by using antibodies for FOXP1 and nNOS proteins with the anti-HuC/HuD antibody (a pan-neuronal marker) on 10 µm thick STN-containing slices. Immunofluorescent slides were imaged using confocal microscopy. Obtained images were used to quantify the colocalization between pairs of proteins (FOXP1 and nNOS, HuC/HuD and FOXP1, HuC/HuD and nNOS). The quantification was performed using the Neurolucida software (MBF Bioscience).

Results: Our results show that FOXP1 and nNOS proteins are present and expressed in the mature STN of all three examined species. Both proteins show almost complete colocalization with anti-HuC/HuD labelling. We show that in mouse, the FOXP1 and nNOS populations are clearly separated and almost non-overlapping. FOXP1 neurons group on the antero-ventral portion of STN, while nNOS neurons group on the postero-dorsal portion. In rat, the populations are still separated but overlapping and colocalizing in the center. In human, the populations are partially colocalizing and they are not spatially separated but rather intermixed and dispersed evenly throughout the STN.

Discussion: FOXP1 is a transcription factor whose deficiency causes FOXP1 syndrome - a neurodevelopmental disorder associated with intellectual disability, language deficits and autism. nNOS is an enzyme which produces nitric oxide, a neurotransmitter implicated in learning, memory, and brain plasticity. Since STN is involved in cognitive processes, it is not surprising that these two proteins seem to be expressed in the STN of all three species. The proteins colocalize greatly with the pan-neuronal marker HuC/HuD which indicates that the proteins are mostly expressed in neuronal cells. Considering spatial distribution of these proteins, we show that in mouse, the FOXP1 and nNOS populations are clearly separated and almost non-overlapping. FOXP1 neurons group on the antero-ventral portion of STN, while nNOS neurons group on the postero-dorsal portion. In rat, the populations are still separated but overlapping and colocalizing in the center. In human, the populations are partially colocalizing and they are not spatially separated but rather intermixed and dispersed evenly throughout the STN. The separation of two neuronal populations in rodents, which is opposite from uniform distribution in humans, remains to be further investigated.

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MeSH/Keywords: Subthalamic nucleus, Basal Ganglia, Immunohistochemistry

Poster code: R-01-08-066

Poster Title: Expression patterns of Wnt signaling pathway participants, LEF1, b-catenin, GSK-3b and DVL1 in human retinoblastomas and normal retinal tissue

PhD candidate: Leon Marković

Part of the thesis: The role of molecular participants of the Wnt signaling pathway, LEF1, b-catenin, GSK-3b and DVL1 in the development and progression of human retinoblastomas

Mentor(s): Professor Nives Pećina-Šlaus, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Retinoblastoma is the most common intraocular malignant neoplasia during childhood and results from the partial or total inactivity of the retinoblastoma protein (pRb). LEF1, b-catenin, GSK-3b and DVL1 are members of the family of proteins related to the Wnt signaling pathway which is proven to be associated with cancer development and progression in certain tumours, but their expression in retinoblastoma has not been studied. The primary objective of our study was to determine the level of expression of Wnt signaling mediators (LEF1, DVL1,b-catenin, GSK-3b) in human retinoblastoma tissue compared to expression in healthy retinal cells and their correlation with expression of tumor suppressor protein Rb1.

Materials and methods: Twenty formalin-fixed paraffin-embedded blocks of retinoblastoma cases and twenty formalin-fixed paraffin-embedded blocks of healthy human retinal tissue from the University Department of Pathology and Cytology, UHC Zagreb, University Department of Pathology and Cytology Ljudevit Jurak, UHC Sestre milosrdnice, and the University Department of Pathology and Cytology, UH Sveti Duh were obtained, classified and immunostained for Rb1, LEF1, b-catenin, GSK-3b and DVL1 using monoclonal antibodies.

Results: Sixteen cases were poorly differentiated retinoblastoma cases. Rb1, LEF1, b-catenin, GSK-3b and DVL1 were expressed in all cases of retinoblastoma although differences in the staining intensity were found between cases. LEF1, b-catenin and DVL1 expression were also observed in retinoblastoma samples with normal retinal tissue but not in samples with only healthy human retinal tissue. A positive correlation between the expression levels of LEF1, b-catenin and DVL1 and the inactivation of Rb1 protein was observed.

Discussion: LEF1, DVL1, b-catenin, GSK-3b are expressed in retinoblastoma, as well as in adjecent retinal tissue. Healthy human retinal tissue doesn't show the same expression patterns as the normal retinal tissue found in retinoblastoma samples. The genetic profile of retinoblastoma is still a big puzzle, and the role of genes and proteins responsible for it is to be discovered the origin and progression of these tumors are of exceptional scientific importance. While precise roles of these proteins must still be determined in retinoblastoma, their expression profiles suggest that further functional studies of LEF1, DVL1, b-catenin, GSK-3b should be pursued in this cancer.

MeSH/Keywords: retinoblastoma, Wnt signaling pathway, Rb1, N-myc, LEF1, b-catenin, DVL1, GSK-3b

Poster code: R-01-08-075

Poster Title: Deep Brain Stimulation and structural brain MRI changes in Parkinson's disease

PhD candidate: Petar Marčinković

Part of the thesis: The influence of deep brain stimulation on structural changes of the central nervous system and the blood-brain barrier in patients with Parkinson's disease

Mentor(s): Associate Professor Darko Chudy, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Deep Brain Stimulation (DBS) is established as an effective therapy for advanced Parkinson's disease (PD). Despite significant symptom improvements with the use of DBS, the exact mechanism of DBS functioning as well as its effect on the central nervous system remains unknown. Furthermore, many issues are unresolved; what is the mechanism of DBS in PD, does DBS induce structural changes in basal ganglia due to stimulation or is it possible to quantify the reorganization of brain structures in PD patients? The aim of this study is to determine the structural changes caused by DBS in patients with PD using volumetric and tractography analysis of magnetic resonance imaging (MRI).

Materials and methods: In this study, 7 patients with PD will perform preoperative and postoperative MRIs, which will, with the use of computer programs for volumetric analysis alongside tractography analysis, provide insight into the DBS effect on CNS structures. Seven PD patients underwent bilateral STN DBS electrode implantation. Brain MRI scans were done before the procedure, a week after the procedure, and approximately two to three years after electrode implantation. In-depth and detailed volumetric analysis was done using automated, observer-independent volumetric software, while tractography analysis was done using the TrackVis program.

Results: Structural changes have been shown using volumetric analysis, while tractography parameters also showed altered data in control MRI scans.

Discussion: The result of this study enables a better understanding of DBS activity in PD patients, provides data on potential structural brain changes in patients with PD, and is a good starting point for further research.

MeSH/Keywords: Parkinson's disease, DBS, MRI, volumetry, tractography

Poster code: R-01-08-095

Poster Title: The role of bradykinin receptor type 2 in murine cerebral glucose metabolism

PhD candidate: Marta Pongrac

Part of the thesis: The role of bradykinin receptor type 2 in murine cerebral glucose metabolism

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

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

Introduction: Bradykinin (BK) is a vasoactive peptide with a prospective role in glucose metabolism. By binding to the constitutively expressed bradykinin receptor type 2 (B2R), BK participates in glucose metabolism in different organs and tissues, modulating glucose transporter expression and activity, as well as increasing the uptake of glucose. However, as its action in cerebral glucose metabolism remains unexplored, the aim of this study was to determine whether B2R participates in murine cerebral glucose metabolism by regulating the expression of glucose transporters and insulin receptors.

Materials and methods: Experiments were conducted on 5 months old male C57BL/6J (WT) and C57BL/6J/Bdkrb2tm1Jfh/SmiJ (B2R-KO) mice. All animal were subjected to basal blood glucose and HbA1c measurements, as well as fasting glucose levels after 6 h fasting period. Then, with at least 7 days gap period, intraperitoneal glucose and insulin tolerance tests were conducted after i.p. administration of 1 g/kg D-glucose and 0,75 IU/kg insulin. Blood glucose levels were measured at 15, 30, 60 and 120 min after injection. Optical in vivo imaging of cerebral fluorescent glucose uptake was performed on the first group of animals after 12-14 fasting period using the IVIS Spectrum imaging system. 50 M fluorescently labeled glucose was given i.v. and cerebral glucose uptake was monitored for 60 min. Following perfusion with phosphate buffered saline, brains underwent ex vivo imaging. From the second experimental group, glucose transporters and insulin receptor expression was determined at the gene level. RNA was isolated with TRIzol-based method and qPCR was performed with TaqMan Gene Expression kit.

Results: B2R-KO mice showed higher basal and fasting glucose levels compared to controls. However, intraperitoneal glucose and insulin tolerance tests showed no significant difference among genotypes. Body weight and glycated hemoglobin showed no significant difference as well. The 60-minute brain glucose imaging in vivo showed significantly higher uptake of fluorescently labeled glucose in the first 10 minutes after injection in B2R-KO mice. Ex vivo imaging 70 minutes after injection showed no significant difference between genotypes. qPCR analysis showed that B2R-KO mice had significantly higher expression of glut8, while glut1, glut3, glut4, insr and irs-1 showed no difference compared to WT mice.

Discussion: Our preliminary results show that B2R causes differences in basal and fasting glucose but has no effect on intraperitoneal tolerance of glucose and insulin. In vivo fluorescence imaging showed that B2R deficiency significantly increases fluorescent glucose uptake compared to controls. Ex vivo imaging showed no significant difference in genotypes, suggesting that B2R has an impact in early kinetics of brain glucose uptake, but has no effect on total brain glucose uptake. B2R-KO mice have higher expression of glut8 compared to WT mice, but there isnt enough evidence to draw conclusions yet, as no research has been published regarding the question.

Acknowledgments: The study is supported by the Croatian Science Foundation project BRADISCHEMIA (UIP-2017-05-8082). The work of doctoral student Marta Pongrac has been fully supported by the "Young researchers' career development project - training of doctoral students" o

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

Poster code: R-01-08-100

Poster Title: MRI evaluation of the filament ischemic stroke animal model (MCAO) and its profound implications

PhD candidate: Rok Ister

Part of the thesis: Neuroradiological properties of the penumbra depending on the time of onset of reperfusion after ischemic lesion of the mouse brain

Mentor(s): Professor Srećko Gajović, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Ischemic stroke remains as one of the main causes of disability and death worldwide despite the breakthrough that recanalization therapy brought almost two decades ago. Recanalization therapy consists of either IV application of tissue plasminogen activator (tPA) or mechanical thrombectomy and is hypothesized that by restoring the blood flow to the border region of infarct lesion (a.k.a. "penumbra"), we can save the tissue at risk if we act quickly enough. The time after the onset of ischemic stroke in which penumbra tissue is still salvageable, remains up to debate. In current clinical practice, recanalization therapy is most often withheld due to uncertainty of exact time of stroke onset, as delayed therapy brings risks that can outweigh the potential benefits. Therefore, its up to preclinical stroke models to elucidate the pathophysiology to help identify said risks and subsequently, help the decision making in clinical practice.

Materials and methods: C57Bl/6-J adult mice (n=30) were subjected to middle cerebral artery occlusion (MCAO) procedure via the access trough the external carotid artery (commonly known as Longa method) in order to minimally influence blood flow to the brain after the procedure. After careful filament positioning and closure of the surgical wound, mice were subjected to an MRI scan in order to assess the anatomy and filament position, as well as to quantitatively measure regional changes in perfusion (perfusion-weighted imaging or PWI) and apparent diffusion coefficients (diffusion-weighted imaging or DWI). After variable ischemia period, the surgical wound was re-opened and the filament was withdrawn, thus completely restoring the blood flow to the infarcted brain region. Following permanent suturing of the wound, the animal was subjected to an identical MRI scan once again, allowing us to ascertain immediate effects of recanalization on the PWI blood flow, apparent DWI lesion and what is to be considered a penumbra region. Mice were left to recover and were subjected to follow-up MRI imaging on the second and seventh day after MCAO, depending on their survival.

Results: To our surprise, complete reperfusion of the infarcted area did not occur in neither of observed experiments after filament withdrawal. During ischemia period, DWI lesion corresponded well with PWI measured ischemia region. After the filament withdrawal, however, the observed outcome appears to have roughly grouped in two distinct scenarios: 1.) Both PWI and DWI did not recover significantly, both in volume and intensity. On the contrary, in significant portion of "no-reperfusion" mice, there was a tendency of ischemia worsening. 2.) DWI lesion has intensified and shrunk to the region of the infarct core and recovered in penumbra region. PWI, on the other hand, has recovered to hyper-physiological levels in the infarct core only, while the penumbra region remained hypoperfused. From survival perspective, second scenario mice fared better than the mice in which no reperfusion were observed on the post-procedure scans.

Discussion: Considering the "non-reperfusion" mice, lack of reperfusion was most probably caused by vessel damage and blood clotting introduced by advancement of the filament. In the second scenario, where reperfusion did occur, the observed results suggest that blood overflows in the necrotic region, possibly due to compromised muscle tone of precapillary arterioles. The interesting part, however, is that after the procedure, penumbra region had not experienced short-term reperfusion but seemed to gradually recover blood perfusion in the following days. This finding, in contrary to the established paradigm, might suggest that isolated penumbra hypoperfusion might be a positive outcome of recanalization therapy or even bring a protective effect on the recovering tissue itself.

Acknowledgments: The study was supported by the Croatian Science Foundation projects RepairStroke (IP-06-2016-1892) and BRADISCHEMIA (UIP-2017-05-8082). The work of doctoral student Rok Ister has been fully supported by the "Young researchers career development project tr

MeSH/Keywords: Ischemic Stroke; Magnetic Resonance Imaging; Middle Cerebral Artery Occlusion; Mice

Poster code: R-01-08-129

Poster Title: A preliminary study on enhancing electrophysiological and clinical guidelines for deep brain stimulation implantation in patients with consciousness disorders

PhD candidate: Gabriela Plosnić

Part of the thesis: Electrophysiological and Clinical Criteria for the Implantation of Deep Brain Stimulation in Patients with Disorders of Consciousness

Mentor(s): Associate Professor Darko Chudy, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Disorders of consciousness are a long-term, serious and therapeutically still unsolved problem of clinical neuroscience. They most commonly result from hypoxic-ischemic and traumatic brain injury. They include outcomes ranging from a persistent coma to recovery to various levels of consciousness, including the unresponsive wakefulness syndrome (UWS) and minimally conscious state (MSS) well as full recovery of consciousness. In the last fifty years, some progress has been made in the treatment of this group of patients by incorporating deep brain stimulation. Our goal is to define new, improved selection criteria for DBS implantation in patients with disorders of consciousness.

Materials and methods: In this study, twenty patients with disorders of consciousness underwent an electrophysiological evaluation to determine the suitability of deep brain stimulation (DBS) implantation under propofol sedation. The analysis included multimodal evoked potentials such as somatosensory evoked potentials (SEP), motor evoked potentials (MEP), brain stem auditory evoked potentials (BAEP), and electroencephalography (EEG), along with high-frequency SEP oscillation thresholds (600 Hz). The patients' preoperative clinical condition was evaluated using the previously utilized Rappaport Disability Rating (RDR) and Coma/Near-Coma Scale (C/NC), as well as the newly introduced Coma Recovery Scale-Revised (CRS-R) scale.

Results: The study revealed that clinical assessment may not always accurately predict the overall outcome, while the presence of evoked potentials showed appropriate cortical response despite pathological parameters. High frequency evoked potential oscillations with amplitudes greater than 70 nV indicated a favorable prognosis, while lower amplitudes suggested severe anoxic encephalopathy that could be incompatible with the return of consciousness.

Discussion: As clinical assessment alone may not always provide sufficient information for this group of patients, we recommend using electrophysiological testing to diagnose and screen individuals with impaired consciousness prior to planning DBS implantation.

MeSH/Keywords: minimally conscious state; vegetative state; deep brain stimulation; evoked potentials; somatosensory high-frequency oscillations

Poster code: R-01-08-135

Poster Title: The Role of Ishemic Lesion Localisation in Anterion Brain Circulation in Language Skills in Acute Phase of Stroke

PhD candidate: Lara Pilepić

Part of the thesis: The Role of Ishemic Lesion Localisation in Anterion Brain Circulation in Language Skills in Acute Phase of Stroke

Mentor(s): Professor Marina Roje Bedeković, MD PhD, Professor Miloš Judaš, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: For normal speech and language functioning there are a number of individual but connected brain processes working in the background of a multimodal cognitive function that is language. The knowledge that language is lateralised and localised in specific left hemisphere areas is in Croatia still used as a basis for diagnostics and rehabilitation of language disorders in patients with stroke. Recent neurolinguistic research stress that in healthy individuals both brain hemispheres are responsible for normal language functioning. Latest research even emphasise a more dominant role of right hemisphere in some language activities while examining with functional mangetic resonance. In Croatia not one large neurolinguistic research used a standardised aphasia battery in combination with brain imaging techniques in acute phase of stroke to determine brain ares responsible for speech and language. The aim of this study was to compare language profiles of left and right hemisphere strokes and determine anatomical lesion localisations and lesion clusters responsible for language functioning in patients with acute ishemic stroke in the anterior brain circulation.

Materials and methods: In this research 120 patients (54% male) with ishemic stroke in the anterior brain circulation hospitalised in the intensive care unit of Sestre Milosrdnice University Hospital and 120 control subjects were included. Inclusive criteria the research were: first ischemic stroke in the area of the anterior cerebral blood circulation, normal functioning of corpus callosum, normal psychological status, normal intellectual functioning, completed at least four grades of elementary school, normal state of consciousness, normal vision and hearing. Within a standard neurological and speech-language assessment, an MRI scan and a croatian version of the Comprehensive Aphasia Test (CAT-HR) were performed on each patient during the first 72 hours of stroke onset. A statistical analysis was done in SPSS programme. Kruskal-Wallis test was used to determine differences between left and right hemisphere stroke patients.

Results: We found significant differences between left and right hemisphere stroke patients in language comprehension, significant differences between left hemisphere stroke patients and control subjects, and no differences between right hemisphere stroke patients and control subjects. Furthermore, we also found significant differences between left and right hemisphere stroke patients in language production and significant differences between right hemisphere stroke patients and control subjects on language production. Right hemisphere stroke patients were significantly worse than control subjects in two language variables: naming and auditory sentence to picture matching.

Discussion: Our preliminary results show that left hemisphere stroke patients have most impaired language functioning, both in comprehension and production when compared to right hemisphere stroke patients and control subjects. These findings are in accordance with latest foreign data. Right hemisphere stroke patients were mostly significantly better than left hemisphere stroke patients in all language tasks, but weren't completely normal as control subjects. Naming and auditory sentence to picture matching were significantly impaired in right hemisphere stroke patients which is also in accordance with some recent foreign research. With these findings we conclude that right hemisphere stroke patients aren't intact in language functioning which is why they should always be examined and if needed, rehabilitated during the acute period. Further statistical analysis will focus on lesion localisations responsible for different language tasks.

MeSH/Keywords: language functioning, stroke, lesion localisation

Poster code: R-01-08-136

Clinical medical sciences – preliminary research results

Poster Title: Pericapsular nerve group block vs intrathecal morphine in total hip arthroplasty

PhD candidate: Krešimir Oremuš

Part of the thesis: Randomized comparison between pericapsular nerve group (PENG) block and intrathecal morphine application in patients undergoing total hip arthroplasty

Mentor(s): Associate Professor Slobodan Mihaljević, MD PhD, Professor Miroslav Hašpl, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Pericapsular nerve group (PENG) block has recently been introduced as a motor function and systemic opioid sparing analgesic technique following hip surgery. We hypothesized that in patients undergoing total hip arthroplasty (THA), PENG block was non-inferior to low-dose intrathecal (IT) morphine regarding postoperative analgesia, with no untoward effects on motor function.

Materials and methods: In a randomized, double-blind non-inferiority trial, sixty patients undergoing unilateral THA under spinal anesthesia were randomized to receive a PENG block or low dose IT morphine. Spinal anesthesia was performed in the sitting position at the presumed L3/4 vertebral interspace with 15 mg isobaric 0.5% levobupivacaine using a 27-gauge pencil-point spinal needle. Intrathecal morphine (100 g of preservative-free morphine in 0.5 mL of 0.9% saline) or a matching placebo (0.5 mL of 0.9% saline for patients randomized to PENG block) were delivered along with the spinal anesthesia. For performance of the PENG or sham block patients were in the supine position and a low frequency (1-6 MHz) convex ultrasound probe was placed proximal to the hip joint at the level of the anterior inferior iliac spine and iliopubic eminence. A 22-gauge 120 mm nerve block needle was introduced in-plane from superolateral to posteromedial, and the content was injected with the needle tip positioned on top of the pubic ramus posterior to the iliopsoas tendon. Patients received either 20 mL of 0.5% levobupivacaine with 2 mg of dexamethasone (PENG group) or a matching 20 ml 0.9% saline placebo (IT morphine group). All treatments were administered by anesthesiologists blinded to patient allocation and treatment drugs prepared by a nurse not further involved in patient care. All patients received multimodal postoperative analgesia with paracetamol, etoricoxib and oxycodone, as well as rescue intravenous morphine in the case of breakthrough pain. Three co-primary outcomes were evaluated: maximum patient reported pain at rest and during active 60 degrees hip flexion repeatedly over the first 48 postoperative hours and cumulative opioid consumption over the first 48 postoperative hours. Static and dynamic postoperative pain was evaluated and recorded by a blinded observer using an 11-point numerical rating scale (0-10 NRS). Intergroup opioid consumption was compared by converting per protocol oral oxycodone and on demand intravenous morphine bolus doses into oral morphine milligram equivalents (MME). Secondary outcomes included postoperative quadriceps motor function impairment assessed by a straight leg raise test at 4, 6, 12, 20 and 24 hours. Predefined non-inferiority margins were set at a 0.75 NRS point mean difference for static and dynamic pain and at a 4mg difference in intravenous rescue morphine (equal to 10 MME). The test treatment was to be considered non-inferior only if conditions of non-inferiority were met for all three co-primary outcomes.

Results: Compared to IT morphine, PENG block was non-inferior for all three co-primary outcomes. PENG block vs IT morphine mean difference in time-averaged NRS score at rest was 0.28 (95% -0.15, 0.70), P non-inferiority=0.01; and for active hip flexion it was -0.18 (95%CI 0.82, 0.45), P non-inferiority <0.001. PENG block vs. IT morphine location shift for cumulative MME was 0.00 (95%CI 0.00, 0.00), P non-inferiority <0.001. Proportions of patients with quadriceps motor function impairment were comparable (11.7% vs. 12.8%). Low dose IT morphine did not result in an increased incidence of pruritus, nausea and vomiting but the study was not powered to investigate opioid related side effects.

Discussion: Compared to IT morphine, PENG block provides non-inferior analgesia with no additional compromise of quadriceps motor function in patients undergoing THA under spinal anesthesia.

MeSH/Keywords: Total hip arthroplasty, regional anesthesia, postoperative pain

Poster code: R-02-01-047

Poster Title: Effects of Dexmedetomidine on Cardiopulmonary Bypass Induced Systemic Inflammatory Response

PhD candidate: Zrinka Šafarić Oremuš

Part of the thesis: Investigation of the effect of intraoperative use of dexmedetomidine on the reduction of the inflammatory response induced by the use of a machine for extracorporeal blood flow in the surgical treatment of aortic stenosis

Mentor(s): Assistant Professor Igor Rudež, MD PhD, Associate Professor Vlatka Sotošek, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Aortic stenosis is the most common valvular lesion in patients over 75 years of age. Some of these patients require surgical treatment that mandates the use of cardiopulmonary bypass. Cardiopulmonary bypass and cardiac surgery elicit a systemic inflammatory response and post-perfusion syndrome in the first 24 hours following surgery that can severely disrupt patients recovery. Dexmedetomidine is an alpha 2-adrenergic agonist that beside its sedative effect has proven effective in modulation of systemic inflammatory response.

Materials and methods: Prospective randomized clinical trial will enrol sixty subjects with isolated aortic stenosis presenting for elective aortic valve replacement surgery. Exclusion criteria are BMI >30kg/m2, I, II and III degree AV block, bradycardia (HR<50/min on operating room entry), neurological disorder (Mb. Parkinson, myasthenia gravis, multiple sclerosis, history of brain tumors), recent psychoactive drug use, addiction to alcohol and drugs, diabetes type I with complications. They will be randomized into three groups receiving continuous infusion of dexmedetomidine in dose of 0.5mcg/kg/h, 1mcg/kg/h respectively and the third group will be given continuous infusion of normal saline at the start until the end of the surgery. Indicators of inflammatory response TNF-alpha and IL-6 will be measured perioperatively at multiple intervals. Inflammatory markers CRP and PCT will be measured preoperatively and daily during hospital stay, as well as incidence of infection (wound, pneumonia, urinary). Concentrations of cortisol and adrenocorticotropic hormone will be measured preoperatively and 24h after the operation to determine the level of stress response. Estimation of renal function, incidence of postoperative delirium, amount of analgesic and anesthetic consumption will be measured during the entire hospitalization. Routine daily haematological, biochemical and coagulation analyses will be done according to usual protocol.

Results: All patients admitted for surgical aortic valve replacement at our institution satisfying inclusion criteria agreed to participate in the study. Up to now 48 patients have been randomized in this double blind study. One was excluded due to change in operative plan which included addition of mitral valve repair. Urinary infections occured in 5 patients, while 6 suffered postoperative delirium. Transient elevation of creatinine levels was observed in 5 patients which resulted in increase in dosage of diuretics, none satisfied the criteria for acute renal failure nor required renal replacement therapy. There were no lethal outcomes and all patients were discharged home, except one that was transfered to another medical facility closer to home for further postoperative care. Once all data are collected, statistical analysis will be conducted.

Discussion: With current dynamics of patient enrollment, it is expected for the study to be completed in the following two months. Main limitation of enrollment is exclusion criterion of BMI over 30kg/m2 which tends to be much higher in our population of patients.

MeSH/Keywords: dexmedetomidine, aortic valve stenosis, systemic inflammatory response

Poster code: R-02-01-069

Poster Title: GABA signaling pathway in human endocervical mucosa

PhD candidate: Adam Vrbanić

Part of the thesis: Association of GABA signaling pathway molecules with mucus secretion and inflammation in human endocervical mucosa

Mentor(s): Associate Professor Frane Paić, MD PhD, Associate Professor Držislav Kalafatić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter of the central nervous system. However, its role in non-neural tissues is being studied increasingly. Research made so far have shown the presence of molecules of the GABA signaling pathway in human endocervix, yet have not clarified their role.

Materials and methods: A prospective observational cohort study will be conducted on female patients of the Department of Gynecology and Obstetrics of University Hospital Centre Zagreb. Women with regular menstrual cycles over the past six months will be included in the study, and women with abnormal Pap smear findings, systemic inflammation and urine-positive human chorionic gonadotropin (hCG) will be excluded. Women using hormonal therapy, pregnant women, puerpera, smokers, women with a malignant gynecological disease, or those with a prior surgical procedure on the uterine cervix, will not be involved. Peripheral venous blood and endocervical swabs will be collected. Two sets of the same type of samples will be taken from each patient. The first set of samples will be collected in the early follicular phase (expected low blood estradiol) and the second set will be taken in the pre-ovulational phase (expected high blood estradiol) of the menstrual cycle within 3-6 months. Every woman will be submitted to gynecological ultrasound to confirm an adequate menstrual cycle phase. Also, a Pap smear will be taken. The blood sample will be used to establish estradiol levels and to exclude systemic inflammation (white blood cell count and C-reactive protein level). RNAs and proteins will be isolated from endocervical swabs. Expression of molecules of GABA signaling pathway (3, 1, 3, , sub-units of GABAA receptor, GAD 67), mucins (MUC5B) and markers of inflammation (IL-6, TNF- , TGF-ß) will be quantified at mRNA and protein level by using reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR) and enzyme-linked immunosorbent assay (ELISA).

Results: RT-PCR was performed on 10 swabs of the uterine endocervix. The selection of test subjects was in accordance with the inclusion and exclusion criteria of the study. All subjects were women of reproductive age (median 29 years) without gynecological pathology. Sampling was independent of the menstrual cycle phase 3 patients were in the luteal (secretory) phase, while 7 subjects were in the follicular (proliferative) phase of the menstrual cycle. All test subjects were sampled uniformly, and all of them underwent a PAP smear in the same act. All 10 subjects had normal PAP smears - all samples were negative for intraepithelial lesions or neoplasia. The mRNA molecules of the GABA signaling pathway (mRNA of 3 sub-unit of GABAA receptor and GAD 67) were successfully detected in all samples.

Discussion: With this pilot study, we proved the presence of the GABA signaling pathway mRNA molecules in the cytological swab of the endocervix using the RT-PCR method. Previous studies were performed on histological specimens of the uterine cervix which are difficult to collect. At the same time, the representativeness of such samples is very poor considering that they are primarily postmenopausal women or women with a certain gynecological pathology. The same women in this study are excluded from the research, considering that the idea of this study is to discover the physiological processes in which GABA participates, i.e. to check the connection of the GABA with the process of mucus secretion, but also with inflammation. This minimally invasive sampling method will enable better representativeness of the test group, which is one of the key factors of successful research.

MeSH/Keywords: endocervix, gaba, mucus, mucins, inflammation, estrogen

Poster code: R-02-05-055

Poster Title: Efficiency of the mHealth app in the healthcare for people living with HIV in Croatia

PhD candidate: Ivana Benković

Part of the thesis: Efficiency of the mHealth app in the healthcare for people living with HIV in Croatia

Mentor(s): Professor Josip Begovac, MD PhD

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

Introduction: In the era of highly effective antiretroviral therapy (ART), there has been a significant reduction in the number of HIV related deaths. The life expectancy of people living with HIV (PLHIV) is approximately the same as of the general population. The increased number of health care users is putting a strain on the health system. In order to improve the care of people living with HIV, a mobile health service (mHealth) has been developed that enables the sending of findings and short messages via a mobile application.

Materials and methods: We included more than 630 HIV patients (out of which 309 during EmERGE project under Horizon 2020) who use the mHealth application (intervention group) and 100 patients who do not use the application (control group). Criteria for inclusion in the study are: over 18 years of age, possession of a smartphone and ability to sign an informed consent. All participants are patients at University Hospital for Infectious Diseases "Dr. Fran Mihaljevi". This research is a longitudinal prospective cohort study. Participantss, in addition to regular check-ups, complete the Patient Activation Measure (PAM) questionnaire once a year. PAM measures the self-assessment of activation and the degree of responsibility for our own health. Results are calculated using the answer key, which divides the results into 4 levels of activation. Participants will complete the PAM questionnaire every 12 months. Demographic data and history of major clinical events will be collected from the existing database of patients every 6 months. We will use mixed linear regression model to estimate the effect of time and outcomes.

Results: Statistical methods used were descriptive statistics. A total of 630 participants were included in intervention group, out of which 600 (95.2%) are men and 30 are women (4.8%). In control group, there are 136 (97.1%) men and 4 (2.9%) women. Age distribution in intervention group shows that 16.5% participants are older than 50 years and 83.5% younger than 50 years of age, while in control group there are 41.4% participants over age of 50. Almost half of all participants are living in Zagreb (45%), following rural areas (35%), other big cities (17%) and other countries (3%). Results of PAM questionnaire results at inclusion indicate levels 3 and 4 for the vast majority of respondents in both groups (83.4%). Detailed results are shown on a graph.

Discussion: Gender distribution in this research is very similar to distribution among people who live with HIV in Croatia as well as in other Western European countries (i.e. United Kingdom, Belgium, Portugal, Spain) and reflects the most common mode of transmission in those countries (men sleeping with men MSM). As seen from the demographic data, majority participants using mHealth application are younger than 50 years, which can be explained by reluctance of older people towards new means of communication and digitalization. With this research, we wanted to determine whether the use of the mHealth application affects the active role in self-care of HIV infection. Baseline data shows that at least 83.4% participants are fully or partially responsible for engaging in personal health. Whether the introduction of mHealth will further influence self-management and responsibility towards health remains to be seen.

Acknowledgments: This research is a part of project "Molecular epidemiology, clinical features and care for HIV infection in Croatia" funded by Croatian Science Foundation.

MeSH/Keywords: HIV, mHealth, telemedicine, patient activation

Poster code: R-02-07-013

Poster Title: Cytokine profile in patients with hepatic steatosis and severe COVID-19.

PhD candidate: Lara Šamadan

Part of the thesis: Cytokine profile in patients with hepatic steatosis and severe COVID-19.

Mentor(s): Assistant Professor Neven Papić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Liver steatosis is considered an independent risk factor for the development of more severe forms of COVID-19. Activation of the immune system plays a key role in the pathogenesis of COVID-19, which is also important in the development and progression of fatty liver disease.

Materials and methods: Study is prospective, observational study that took place in University hospital for infectious diseases "Dr. Fran Mihaljevic" Zagreb for 1 year on 160 subjects (80 with hepatic steatosis and 80 without hepatic steatosis). The study included adult patients with severe COVID-19 requiring hospital treatment. Exclusion criteria were age over 75 years, chronic viral hepatitis, history of significant alcohol consumption, hepatotoxic drugs intake, malignancies, immunocompromised, palliative care, pregnant women, need of oxygen for less than 24 hours, admitted to the intensive care unit within the first 24 hours of admission, died within 48 hours of admission, corticosteroid therapy intake before admission. All patients were screened for steatosis by ultrasound during hospitalization. Routine demographic and clinical parameters were collected collected as well as laboratory findings. Patients were monitored daily during hospitalization and clinical parameters were collected. Blood sample were taken on admission to determine serum cytokine profile using multiplex technology. Data is analyzed using descriptive and analytical methods.

Results: The cohort of 94 adult hospitalized patients were included in the study. Of them, 51 were diagnosed with NAFLD. There were no differences in age, sex, and comorbidities between the groups, except for higher BMI and waist-hip ratio in patients with NAFLD. The median time interval from the onset of disease to admission was similar between groups. Patients with NAFLD had higher C-reactive protein, procalcitonin, alanine aminotransferase, lactate dehydrogenase and fibrinogen on admission. There were no differences in other routine laboratory parameters, except for platelets, which were lower in the NAFLD group.There were no differences in the choice of treatment between the groups. As for panel of cytokines and chemokines associated with antiviral response in patients with and without NAFLD, interleukins 6 and 10 were significantly higher in patients with NAFLD as well as chemokines IL-8 (CXCL8) and IP-10 (CXCL10). Serum concentrations of IFN-gamma were significantly lower in patients with NAFLD. There were no differences in IFN-alpha2, IFN-beta, GM-CSF, and TNF-alpha levels.

Discussion: In this study, COVID19 patients with NAFLD have distinct serum cytokine profiles than patients without NAFLD. This includes higher levels of IL-6, IL-8, IL-10, and IP-10, and lower IFN-gamma. Serum IL-6 is natively elevated in NAFLD patients because of immunologically activated adipose tissue so increased levels in COVID-19 are not an unexpected finding. Furthermore, there are some pathological similarities between the progression of NAFLD and the development of ARDS in COVID-19. Briefly, in both NAFLD and COVID-19 activated neutrophils contribute to disease progression where they synthesize IL-8, a chemotactic cytokine that recruits and activates additional neutrophils. In severe COVID-19, an early increase in IL-10 concentration was observed. This could be explained as a failed attempt to suppress the hyperinflammatory response and tissue damage since IL-10 concentrations strongly correlated with IL-6 and other inflammatory markers. There is also a possibility of IL-10 resistance in which activated immune cells escape the antiinflammatory IL-10 signaling, thereby enhancing the hyperinflammatory response. The decreased IFN concentrations are most likely associated with the functional impairment of NK cells (main cellular sources of IFN-gamma) that were previously described in NAFLD and elevated levels of IP-10 were reported to be associated with COVID-19 severity and mortality. This suggests that patients with NAFLD and COVID-19 might have different regulations of IP-10 secretion.

MeSH/Keywords: cytokines; COVID-19; steatosis; NAFLD;

Poster code: R-02-07-014

Poster Title: Bacterial sexually transmitted diseases in the first two years of pre-exposure prophylaxis use for HIV in Croatia

PhD candidate: Nikolina Bogdanić

Part of the thesis: Bacterial sexually transmitted diseases in the first two years of pre-exposure prophylaxis use for HIV in Croatia

Mentor(s): Professor Josip Begovac, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Pre-exposure prophylaxis (PrEP) is an HIV prevention method which uses antiretroviral drugs.

Materials and methods: This was a retrospective and partially prospective cohort study in the period from September 2018 to December 2022 at University Hospital for Infectious Diseases Dr. Fran Mihaljevi in Zagreb where all PrEP users in Croatia are followed-up. Included were men who have sex with men (MSM) 18 years old who use PrEP. The primary aim was to compare incidences of sexually transmitted diseases (STDs) in the first and the second year of PrEP use. The secondary aims were to assess prevalence of HIV and viral hepatitis A, B, C, syphilis, gonorrhea and chlamydia at the initial evaluation for PrEP use, estimate loss to follow-up and risk factors associated with loss to follow up. Here we present preliminary results of the study which includes 129 participants. We assessed the results of HIV test, viral hepatitis A, B, C serology, syphilis serology, Neisseria gonorrhoeae and Chlamydia trachomatis detection from urine, rectal and pharyngeal sample at the initial evaluation for PrEP use.

Results: All 129 participants were men, 106 (82.2%) were Croatians, with median age of 34.0 (IQR: 30.4-40.5) years. The initial evaluation for PrEP use was in 2018 in 43 (33.9%) participants and in 2019 in 84 (66.1%) participants. Overall 60 (46.5%) participants took PrEP after the initial evaluation. C. trachomatis was detected in 7 (5.4%) rectal samples, 5 (3.9%) pharyngeal samples and two (1.5%) urine samples. N. gonorrhoeae was detected in 15 (11.6%) rectal samples, 15 (11.6%) pharyngeal samples and three (2.3%) urine samples. Overall 24 (18.6%) participants had evidence of past syphilis and 4 (3.1%) had current syphilis episode of which one had secondary syphilis, one had early latent and two had late latent syphilis. All of them received treatment for syphilis at the UHID. All participants had negative serology for HCV, 63 (48.8%) were immune to HBV due to vaccination, 18 (13.9%) had serologic evidence of resolved HBV infection, and one had chronic HBV infection. Two (1.5%) participants were diagnosed with acute HAV infection, while 22 (17.0%) had evidence of past HAV infection or immunization. Five (3.9%) participants were HIV positive and were excluded from the further study.

Discussion: This study will assess impact of PrEP on STDs incidence during the first two years of follow-up. Understanding STDs and their relationship to PrEP use, and considering the future directions in STD prevention among PrEP users will be a way to improve sexual health. The preliminary results of this study showed low rates of immunization due to HAV and HBV, relatively high number of participants with past or present syphilis and somewhat lower numbers of other bacterial STDs among which rectal and pharyngeal gonorrhea was most common.

MeSH/Keywords: Pre-Exposure Prophylaxis; HIV; Men Who Have Sex With Men; Sexually Transmitted Diseases

Poster code: R-02-07-025

Poster Title: Patient safety culture in two intensive care units within the same hospital

PhD candidate: Vanja Vončina

Part of the thesis: Evaluation of impact of the introduction of a checklist for assessing the patient's readiness for discharge from the intensive care unit on patient safety

Mentor(s): Assistant Professor Hana Brborović, MD PhD, Assistant Professor Jadranka Pavičić Šarić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Even though checklists of patient readiness for discharge from intensive care units (ICU) have long been proposed as a tool to increase patient safety during the discharge process, the first comprehensive checklist has been published in 2022. Whether such a checklist would influence patient safety culture (PSC) is yet to be determined. A positive change in PSC could lead to an overall improvement in both quality of care and treatment outcomes.

Materials and methods: A prospective, controlled intervention study will assess PSC prior to and following checklist implementation. Two groups of subjects, intervention and control, are healthcare professionals working in two distinct ICUs within the same hospital (surgical ICU intervention unit and medical ICU control unit). A validated Croatian translation of the Hospital Survey on Patient Safety Culture is used to test PSC. PSC was first tested in December of 2022., right before introducing the checklist to the intervention ICU through a period of one year. The testing will be repeated in January 2024.

Results: We tested a total of 56 participants from the intervention unit and 18 participants from the control unit. The Shapiro-Wilk test for normality demonstrated a normal distribution for the majority of variables. The results revealed poor values for the dimension Staffing in both departments and the highest values for dimension Teamwork Within Units and Overall Perception of Patient Safety. Furthermore, statistically significant difference was found for four dimensions in total using Student t-test for independent samples. The differences were in favour of the control unit. Teamwork Within Units revealed statistically significantly higher values in the control unit (X=3.96, SD=0.6) than in the intervention unit (3.54, SD=0.75), t(79)=-2,17, p=0,034. Overall Perception of Patient Safety also revealed statistically significantly higher values in the control unit (X=3.97, SD=0.69) than in the intervention unit (X=3.43,SD=0.77), t(79)=-2,47, p=0,015. Frequency of Events Reported also revealed statistically significantly higher values in the control unit (X=3.7, SD=1.12) than in the intervention unit (X=3.02, SD=1.12), t(79)=-2,24, p=0,028; as well as results for the Nonpunitive Response to Errors, control unit (X=3.42,SD=1.02) and intervention unit (X=2.93, SD=0.94), t(79)=-1,98, p=0,051. Single-statement dimension Patient Safety Grade revealed both units graded it as very good and excellent. Number of Events Reported dimension revealed statistically significantly higher number of participant from the intervention unit did not report any events in the last year.

Discussion: PSC is defined as a pattern of individual and organisational behaviour, based upon shared beliefs and values that continuously seeks to minimise patient harm, which may result from the process of care delivery. Our results clearly indicate a need for improvement in both the intervention and control units for two, out of the 12 tested, dimensions: Organisational learning and Staffing, as both groups scored 3 or under in those dimensions. Furthermore, the intervention group scored under 3 for dimensions Nonpunitive response to errors and Frequency of events reported. Even though both groups scored adequately for dimensions Overall perception of patient safety and Teamwork within the units, there was a statistically significant difference between the two groups. The overall difference between the two groups can easily be attributed to organisational differences between them, in spite of both ICUs being within the same hospital. Patient handovers are moments when patient safety is most at risk, especially within the context of ICU discharge as the sickest patients are being moved from departments with most resources (monitors, staffing). Discharge readiness checklists have long been proposed as tool to improve safety but first such evidence-based checklist has been published only in 2022. As checklists in general have been used in medicine for nearly three decades, we do know that whether or not they truly affect patient safety or are just a tickbox exercise, mainly depends on the ability to affect the overall patient safety culture. Hence, this

doctoral thesis will assess the influence of applying a newly published set of criteria to evaluate discharge readiness for adult ICU patients on PSC.

MeSH/Keywords: patient safety culture, patient safety, discharge readiness, checklist, patient handover, intensive care unit

Poster code: R-02-08-034

Poster Title: Arterial stiffness and R wave in aVL lead on electrocardiogram as cardiovascular risk factors in arterial hypertension

PhD candidate: Juraj Jug

Part of the thesis: Arterial stiffness and R wave in aVL lead on electrocardiogram as cardiovascular risk factors in arterial hypertension

Mentor(s): Associate Professor Ingrid Prkačin, MD PhD, Assistant Professor Valerija Bralić Lang, MD PhD

Affiliation: University of Zagreb School of Medicine, Health Center Zagreb - West

Introduction: In many studies, pulse wave velocity (PWV), and R in aVL amplitude show a significant correlation with cardiovascular (CV) risk and mortality. Despite this, CV risk is still most often calculated using SCORE 2 in clinical practice. We aimed to determine if patients with uncontrolled hypertension (UH) have higher PWV and R wave amplitude in the aVL ECG lead compared to hypertensive patients and healthy individuals and link them to the SCORE 2 risk for the same subjects.

Materials and methods: The cross-sectional study was conducted in 2022 on 326 subjects divided into three groups: A (93 healthy participants), B (140 hypertensive patients), and C (90 patients with UH) using 24-hour ambulatory blood pressure monitor (ABPM) findings. SCORE 2 (Systematic coronary risk evaluation) risk was calculated, and PWV was measured (Agedio® B900 oscillometric device) in all participants. The ABPM and ECG devices used were from BTL Cardiopoint®. The R wave in the aVL ECG lead amplitude was measured digitally. Student t-test, MANOVA, and multivariate regression were used in Statistica v.12.0 to analyze the data.

Results: The mean age of all participants was 56.1±7.9 years (group A was the youngest, 54.7±7.7 vs. 57.2±7.6 years, $p<0.01$). Group C had the highest values of all three groups in heart rate (71.4±7.7 vs. 73.4±9.5 vs. 76.6±9.1 bpm; $p<0.001$), fasting plasma glucose (5.4±0.6 vs. 5.5±0.7 vs. 6.4±2.0 mmol/L; $p<0.001$), uric acid (295.6±71.7 vs. 338.5±84.0 vs. 369.8±96.1; $p<0.001$), body mass index (24.9±3.6 vs. 28.2±4.8 ± 30.3±4.7 kg/m2; $p<0.001$), SCORE2 risk (4.3±3.0 vs. 6.7±3.6 vs. 10.1±5.3%; $p<0.001$), R in aVL lead amplitude (0.19±0.26 vs. 0.49±0.27 vs. 0.78±0.23; $p<0.001$), and PWV (7.81±1.10 vs. 8.45±1.01 vs. 9.52±1.36 m/s, $p<0.001$). In the first regression model, age (B=0.668), fasting plasma glucose (B=0.125), mean arterial pressure (B=0.160), R in aVL (B=0.228), and SCORE 2 (B=0.094) showed as independent factors for higher PWV (Adj R2=0.735, F=176.04, standard error of estimate=0.672). In the second model, age (B=-0.289), mean arterial pressure (B=0.223), body mass index (B=0.191), SCORE2 (B=0.213), and PWV (B=0.465) showed as independent factors for higher R in aVL lead amplitude (Adj R2=0.467, F=56.39, standard error of estimate=0.248). According to SCORE 2, high CV risk was found in groups A, B, and C in 5.4%, 20.0%, and 53.3% of patients, respectively. When measuring PWV, high CV risk (PWV > referent for patient's age and gender) had 16.1%, 29.3%, and 65.6% of patients in groups A, B, and C. Finally, R in aVL amplitude 0.7mV (assumed high CV risk) was found in 3.2%, 27.9%, 71.1% of patients in these three groups, respectively.

Discussion: Although very practical and low time-consuming, PWV and R wave in aVL lead measurements are neglected in clinical practice. Many studies show that these two measures are crucial for a more accurate CV risk assessment as SCORE 2 predicts the same CV risk for all patients with systolic blood pressure >160 mmHg depending on non-HDL cholesterol, age, and smoking status. Reclassification of the patient's CV risk in this way, i.e. from moderate to high risk, would mean an indication for drug introduction. In contrast, according to SCORE 2, the same patient only needs a lifestyle change. This study shows new possibilities and emphasizes the importance of correct and timely CV risk assessment, already in a family medicine setting, to achieve better treatment outcomes in all patients. Patients with uncontrolled hypertension had significantly higher PWV and R wave amplitude in aVL ECG lead than patients with controlled hypertension and healthy individuals. If CV risk was assessed using PWV, or R in aVL amplitude, rather than SCORE 2, already high CV risk was found in a higher ratio of patients with uncontrolled hypertension.

MeSH/Keywords: arterial hypertension; cardiovascular risk; electrocardiography; hypertensive urgency; vascular stiffness

Poster code: R-02-09-021

Poster Title: Clinical relevance, risk factors and treatment outcomes of M.xenopi and other non tuberculous mycobacterial pulmonary disease patients

PhD candidate: Goran Glodić

Part of the thesis: Genotyping and clinical significance of Mycobacterium xenopi subtypes in the development of pulmonary mycobacteriosis

Mentor(s): Mateja Janković Makek, PhD, research associate, Professor Ivana Mareković, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Pulmonary disease caused by M.xenopi (MX-PD) is associated with an increased morbidity and mortality compared to other non-tuberculous mycobacteria (NTM) in cohorts from Western Europe.1,2 Preliminary data suggested a lower clinical relevance and mortality rate of patients with MX-PD in Croatia.3 We aimed to determine the clinical relevance of NTM, M.xenopi and different M.xenopi subtypes, and describe the clinical characteristics, treatment outcomes and mortality of MX-PD patients. Subsequently we will perform whole genome sequencing (WGS) of the available M.xenopi isolates.

Materials and methods: All Croatian residents with NTM isolates from 2006 to 2022 were included in the study, with planned future follow up until 31.12.2023. ATS/ERS/ESCMID/IDSA clinical guidelines (fig. 1) were used to establish NTM-PD diagnosis, determine the clinical relevance of isolates, and score the adequacy of the used treatment regime and its duration.4 Treatment outcomes, recurrence and reinfection were defined according to the NTM-NET consensus statement.5 Risk analysis calculations were made on the 439 isolation episodes that were classified as definitive NTM-PD (n= 137) or no disease (n= 302). After determining the clinical relevance we will perform WGS on a minimum of 36 M.xenopi isolates 12 patients with MX-PD and 24 patients with clinically insignificant colonization based on previous sequencing studies of NTM. WGS and sequencing data analysis will be performed in collaboration with Radboud University Medical Center, Nijmegen, the Netherlands.

Results: NTM-PD was mostly caused by M. xenopi and M.avium complex (MAC), and the overall 5-year mortality amounted to 37.6% in our cohort. Female gender, presence of bronchiectasis, low BMI and long term systemic corticosteroid treatment were independent risk factors associated with NTM-PD (table 1). High dose ICS treatment was a significant risk factor for developing NTM-PD (aOR = 4.73, CI 1.69-13.23 p= 0.003). Adequate NTM treatment was administered in 35/137 patients with definite NTM-PD. Cure results stratified according to the species and treatment protocol are shown in table 2. Irrespective of the given treatment, 24.1% patients with MX-PD and 17% with MAC-PD died within the first 12 months. In contrast to MAC-PD, for MX-PD, high cure rates (>80%) were achieved both with adequate NTM treatment and TB treatment (p=0.572). Compared to MAC, higher cure rates in MX-PD were achieved irrespective of radiological manifestation or administered treatment (table 2).

Discussion: Adequate NTM treatment was administered in only one third of the treated cases and resulted in a four-time higher chance of being cured. While adequate NTM treatment was important for the outcomes of patients with MAC-PD, FC and smear positive disease, we observed no clear impact of treatment regimens on cure rates of patients with MX-PD and in AFB smear negative patients. Looking into the impact of NTM species on the overall results, we confirmed our preliminary data that M. xenopi, the second most frequently isolated NTM species and the most common cause of NTM-PD in Croatia, seemingly has a lover clinical significance and pathogenicity in comparison to data from the Netherlands and France.1-3 Only about one third of all evaluated M. xenopi isolates truly represented disease. Furthermore, although one-year mortality of M. xenopi and MAC-PD was comparable (24.1 vs. 17%), microbiological cure seemed to be easier to achieve in MX-PD. In contrast to MAC-PD, treatment of MX-PD resulted in high cure rates with all treatment protocols. This observation warrants further investigations into bacterial virulence factors as well as into bacterial factors underlying the different response to treatment, both of which could be explained by the existence of different M.xenopi subtypes using WGS.

MeSH/Keywords: NTM-PD, M.xenopi, treatment outcomes, whole genome sequencing

Poster code: R-02-09-023

Poster Title: Prevalence of posttraumatic stress disorder following acute coronary syndrome and clinical characteristics of patients referred to cardiac rehabilitation

PhD candidate: Ivana Sopek Merkaš

Part of the thesis: The association of posttraumatic stress disorder after acute coronary syndrome in patients undergoing cardiac rehabilitation with subsequent adverse cardiovascular events

Mentor(s): Professor Zdenko Sonicki, MD PhD, Associate Professor Nenad Lakušić, MD PhD

Affiliation: University of Zagreb School of Medicine; Special Hospital for Medical Rehabilitation Krapinske Toplice

Introduction: Studies have demonstrated that patients who have experienced acute coronary syndrome (ACS) have an increased risk of developing posttraumatic stress disorder (PTSD) and experiencing worse survival outcomes than those who do not develop PTSD. Nevertheless, the prevalence rates of PTSD following ACS vary widely across studies, and it is noteworthy that in most cases, the diagnosis of PTSD was based on self-report symptom questionnaires, rather than being established by psychiatrists. Additionally, the individual characteristics of patients who develop PTSD after ACS can differ widely, making it difficult to identify any consistent patterns or predictors of the disorder.

Materials and methods: The participants of this study are patients who have experienced ACS with or without undergoing percutaneous coronary intervention and are enrolled in a 3-week cardiac rehabilitation (CR) program at the largest CR center in Croatia, the Special Hospital for Medical Rehabilitation Krapinske Toplice. Patient recruitment for the study took place over the course of one year, from January 1, 2022, to December 31, 2022, with a total of 504 participants. The expected average follow-up period for patients included in the study is about 18 months, and currently ongoing. Using self-assessment questionnaire for PTSD criteria and clinical psychiatric interview, a group of patients with a PTSD diagnosis was identified. From the participants who do not have a PTSD diagnosis, patients who would match those with a PTSD diagnosis in terms of relevant clinical and medical stratification variables and during the same rehabilitation period were selected to enable comparability of the two groups.

Results: A total of 507 patients who were enrolled in the CR program were approached to participate in the study. Three patients declined to participate in the study. The screening PCL-C questionnaire was completed by 504 patients. Out of the total sample of 504 patients, 74.2% were men (N=374) and 25.8% were women (N=130). The mean age of all participants was 56.7 years (55.8 for men and 59.1 for women). Among the 504 participants who completed the screening questionnaire, 80 met the cutoff criteria for the PTSD and qualified for further evaluation (15.9%). All 80 patients agreed to psychiatric interview. Among them, 51 patients (10.1%) were diagnosed with clinical PTSD by a psychiatrist according to DSM-5 criteria. Among the variables analyzed, there was a noticeable difference in the percentage of theoretical maximum achieved on exercise testing between the PTSD and non-PTSD groups. Non-PTSD group achieved a significantly higher percentage of their maximum compared to the PTSD group (p 0.035).

Discussion: The preliminary results of the study indicate that a significant proportion of patients with PTSD induced by ACS are not receiving adequate treatment. Furthermore, the data suggest that these patients may exhibit reduced physical activity levels, which could be one of the possible underlying mechanisms in observed poor cardiovascular outcomes in this population. Identifying cardiac biomarkers is crucial for identifying patients at risk of developing PTSD and may derive benefits from personalized interventions based on the principles of precision medicine in multidisciplinary CR programs.

MeSH/Keywords: Cardiac rehabilitation; Acute coronary syndrome; Posttraumatic stress disorder; Psychiatric interview; Multidisciplinary team; Cardiac biomarkers

Poster code: R-02-09-033

Poster Title: Red cell distribution width as a predictor of outcome in hospitalized COVID-19 patients

PhD candidate: Ana Jordan

Part of the thesis: The coefficient of variability of erythrocyte volume distribution as a predictor of thirty-day outcomes in hospitalized patients with COVID-19

Mentor(s): Associate Professor Šime Manola, MD PhD, Marko Lucijanić, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Anisocytosis, ie, unequal red blood cells (RBC) size, is a sensitive marker of distress in erythropoiesis or RBC destruction. It can be induced by various metabolic and inflammatory stimuli, nutrient deficiencies, infections, spleen disorders, and specific drugs interfering with RBC production.More severe clinical presentation and higher mortality rates were also found in COVID-19 patients with higher RDW levels. However, an association of RDW with other clinical outcomes in hospitalized COVID-19 patients, as well as the relationship with increased mortality in the context of other established prognostic scores, are not well defined. Thus, we aimed to investigate the clinical and prognostic significance of RDW in a large cohort of hospitalized COVID-19 patients from our institution

Materials and methods: We retrospectively analyzed the records of 3941 patients admitted to University Hospital Dubrava because of acute COVID-19 infection from March 2020 to March 2021. We included only patients who had available data on RDW on admission. Patients were treated according to contemporary guidelines. Only index hospital admissions for acute COVID-19 were investigated. Data were obtained from the hospital registry through an analysis of electronic and written medical records of 4014 COVID-19 patients. Seventy-three patients were excluded due to a lack of available RDW data on admission. RDW on admission was expressed as a coefficient of variation (%) of mean corpuscular volume (MCV) as reported by Advia 2120i automated cell counter (Siemens Medical Solutions Diagnostics Pte Ltd, Swords, Ireland). COVID-19 severity at admission was graded according to the World Health Organization (WHO) recommendations and national guidelines (17,18) as mild, moderate, severe, and critical. Comorbidities, assessed as individual entities, were summarized by using the Charlson comorbidity index. Mortality and other clinical outcomes were assessed from the start of the hospital stay. Consortium 4C mortality score; COVID-gram; and Veterans Health Administration COVID-19 (VACO) index were used as prognostic risk scores. The chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula was used for the calculation of the estimated glomerular filtration rate (eGFR) .

Results: The median age was 74 years. The median Charlson comorbidity index (CCI) was 4. The majority of patients(84.1%) on admission presented with severe or critical COVID-19. Patients with higher RDW were significantly more likely to be older and female, to present earlier during infection, and to have higher comorbidity burden, worse functional status, and critical presentation of COVID-19 on admission. RDW was not significantly associated with C-reactive protein, the occurrence of pneumonia, or need for oxygen supplementation on admission. During the hospital stay, patients with higher RDW were significantly more likely to require high-flow oxygen therapy, mechanical ventilation, and intensive care unit, and to experience prolonged immobilization, venous thromboembolism, bleeding, and bacterial sepsis. Thirty-day and post-hospital discharge mortality gradually increased with each rising RDW percent-point. In a series of multivariate Cox-regression models, RDW demonstrated robust prognostic properties at >14% cut-off level. This cut-off was associated with inferior 30-day and postdischarge survival independently of COVID-19 severity, age, and CCI; and with 30-day survival independently of COVID severity and established prognostic scores.

Discussion: Our study, the largest single-institution cohort of hospitalized COVID-19 patients investigated so far, showed a gradual worsening of 30-day and post-hospital discharge survival with each rising percent point of RDW on admission. In addition, our study was first to investigate the associations of RDW with various clinical outcomes and to assess the prognostic role of RDW in the context of established COVID-19 prognostic scores. We demonstrated that RDW possessed independent prognostic properties and had a good potential for improvement of prognostication of hospitalized COVID-19 patients.

MeSH/Keywords: COVID-19, RDW, prognostic models, outcome

Poster code: R-02-09-039

Poster Title: Association of gene polymorphism MTHFR C677T and MTHFR A1298C with atrial fibrillation

PhD candidate: Rea Levicki

Part of the thesis: Association of gene polymorphism MTHFR C677T and MTHFR A1298C with atrial fibrillation in overweight patients

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

Affiliation: University of Zagreb School of Medicine

Introduction: MTHFR C677T and MTHFR A1298C polymorphisms are associated with hiperhomocysteinemia that results in prothrombogenic and atherogenic effect and could influence atrial fibrillation onset.

Materials and methods: We included 55 patients (31M, 23W) with atrial fibrillation. To all patients MTHFR C677T and MTHFR A1298C polymorphisms were determined, routine laboratory tests were done, transthoracic echocardiography was performed, body mass index was determined.

Results: In analysis of MTHFR C677T polymorphisms, there were 20 patients (36,4%) with healthy genotipe (without present mutation) CC, 29 patients (52,7%) heterozygous CT, and 6 patients (10,9%) with homozygous mutation TT. In analysis of MTHFR A1298C there were 24 patients (43,6%) with healthy genotipe AA, 23 patients (41,8%) heterozygous AC, and 8 patients (14,5%) with homozygous mutation CC. There were no differences in left atrium diameter in different genotype groups of patients.

Discussion: While incidence of MTHFR C677T homozygus mutation TT was similar in our group of patients with atrial fibrillation as in general population in our geographic region, the incidence of MTHFR C677T heterozygous mutation (52,7%) was significantly higher than the incidence of general population; approximately 20-40% of Caucasian. (2) MTHFR A1298C homozygous mutation CC incidence in our group of patients with atrial fibrillation (14,5%) was higher than in European population (7-12%). Pathological MTHFR C677T and MTHFR A1298C poymorphism distribution in our group of patients with atrial fibrillation, that includes high incidence of heterozygous mutation and higher incidence of homozygous mutation than in general population, could indicate association of pathological MTHFR polymorphisms with atrial fibrillation onset.

MeSH/Keywords: MTHFR polymorphisms, atrial fibrillation

Poster code: R-02-09-048

Poster Title: Serum proteomic profile in the diagnostics of portal hypertension in patients with compensated advanced chronic liver disease

PhD candidate: Frane Paštrović

Part of the thesis: Serum proteomic profile in the diagnostics of portal hypertension in patients with compensated advanced chronic liver disease

Mentor(s): Assistant Professor Ivica Grgurević, MD PhD, Ruđer Novak, PhD, senior research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Portal hypertension (PH) drives the progression of liver cirrhosis to decompensation and death. The standard of PH quantification is hepatic venous pressure gradient (HVPG) measurement and clinically significant PH (CSPH) is defined as HVPG10 mmHg. Timely intervention in patients with compensated advanced chronic liver disease (cACLD) with CSPH can alter the course of their disease, making an early diagnosis of CSPH crucial. However, HVPG measurement is invasive, expensive, and not widely available, so there is a need for simple, non-invasive and reliable substitute for portal pressure assessment. We aimed to identify potential candidate biomarkers of CSPH by serum proteomic profiling of patients with cACLD.

Materials and methods: A total of 76 consecutive patients with mixed etiology chronic liver disease and a suspicion of cACLD, based on the results of imaging and/or liver stiffness measurement (LSM) 10 kPa by transient elastography, were included in the initial cohort. A standardized work-up was performed including HVPG measurement, transjugular liver biopsy and serum sampling for proteomic analysis. cACLD was histologically confirmed (presence of advanced fibrosis or cirrhosis) in 48/76 patients, and they were included in the further analysis. Serum samples of patients with cACLD were pooled based on the HVPG value (group 1 patients with subclinical PH, HVPG<10 mmHg (N=18); group 2 patients with CSPH, HVPG10 mmHg (N=30)). Following affinity purification of the pooled sera on a heparin column, the samples were analyzed by liquid chromatography-mass spectrometry (LC-MS). Gene enrichment analysis of the acquired data was performed, and a comprehensive literature review for proteins with the most striking differences in expression levels between the groups.

Results: From a total of 389 proteins identified by LC-MS, 337 proteins were identified in both groups, 31 proteins were identified solely in the CSPH group and 21 only in the subclinical PH group. Among the proteins expressed solely in the CPSH group we found an abundance of CD44, a protein known to play role in hepatic inflammation and the progression of liver diseases. Also, in the CSPH group vascular endothelial growth factor C (VEGF-C) and lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1) were identified, both involved in the formation of lymphatic vessels. Regarding the proteins present both in the CSPH and non-CSPH group, an abundance of proteins involved in neutrophil extracellular traps (NET) formation such as myeloperoxidase, proteins S100-A8 and S100-A9, histones H2B and H4 and bactericidal permeability-increasing protein was found in patients with CSPH. Tenascin C, autotaxin and nephronectin which have earlier been reported to take role in chronic liver inflammation and hepatic stellate cell contraction were also abundant in the CSPH group.

Discussion: Abundance of proteins involved in NET formation in the CSPH group, as well as presence of CD44 solely in the CSPH group, strengthens the case for the emerging role of inflammation in the progression of PH. NETs role is to kill pathogens in the circulating blood stream, which goes in hand with a presumed role of the increased gut permeability in PH progression. Furthermore, NETs also bind platelets, activate the coagulation cascade and prolong the inflammatory response, which damages adjacent tissues and leads to microthrombus formation, perpetuating increased intrahepatic vascular resistance. Secondly, VEGF-C and LYVE-1 were identified only in the CSPH group, which can be explained by an increase in lymph production and flow seen with the increase of portal pressure which stimulates hepatic lymphangiogensis. Our work provides novel insight in the biological processes involved in PH development and, pending future validation, several of the aforementioned proteins might serve as potential candidate biomarkers of CSPH.

MeSH/Keywords: compensated advanced chronic liver disease, clinically significant portal hypertension, proteomics, biomarkers, neutrophile extracellular traps formation, lymphangiogenesis

Poster code: R-02-09-051

Poster Title: Inflammatory bowel disease is associated with an increased risk for COVID-19-related hospitalization, but not with mortality: Croatian nationwide cohort study

PhD candidate: Ivan Kodvanj

Part of the thesis: Inflammatory bowel diseases as risk factors for illness and more severe forms of COVID-19

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

Affiliation: University of Zagreb School of Medicine

Introduction: The impact of COVID-19 has been of great concern in patients with inflammatory bowel disease (IBD) due to the possibility of increased risk of severe outcomes, which can result in increased hospital admission or increased mortality. This study aims to estimate the effect of IBD on mortality and the risk of hospitalizations in patients diagnosed with COVID-19.

Materials and methods: This study included all COVID-19 patients in Croatia from the begging of the pandemic till the 15th of August 2021 and compared the COVID-19-related mortality and hospitalization risk in IBD patients vs general population and ulcerative colitis (UC) vs Crohns disease (CD) patients. In all comparisons, patients were exactly matched on age, sex, vaccination status, time period of the pandemic, Charlson comorbidity index, important pharmacological therapy and relevant comorbidities. For sensitivity analysis, UC and CD patients were also matched using the optimal full + exact matching algorithm due to a smaller number of patients with the same set of covariates (except age was supplied as a continuous variable; and vaccination status, Charlson comorbidity index, and age binned to 10 years were supplied as exact covariates). Log-binomial regression with a robust sandwich variance estimator was used to calculate relative risk and 95% confidence intervals (RR and 95%CI) for outcomes (mortality and hospitalization).

Results: We identified 3067 IBD patients among 433609 COVID-19 patients. Crude proportions of COVID-19-related mortality in unmatched data and calculated relative risk after matching indicated no difference in mortality of IBD and non-IBD patients [2.8% vs 2.7%, and RR = 0.85 (95%CI 0.60 1.19)]. On the other hand, the hospitalization rate was higher in IBD population both prior to matching (6.1% vs 4.5%) and after matching [RR = 1.43 (95%CI 1.17 1.75)]. Among 3067 IBD patients, we identified 2061 UC and 797 CD patients (for 209 patients ibd was unspecified). Unmatched comparison indicated an increased risk for COVID-19-related death (3.2% vs 1.8%) and hospitalization (6.1% and 5.8%) in patients suffering from UC. However, after matching using two different matching algorithms, we found no difference in mortality risk [RRprimary = 0.55 (95%CI 0.18 1.61), and RRsensitivity = 1.14 (95%CI 0.57 2.30)] nor hospitalization risk [RRprimary = 1.14 (95%CI 0.72 1.80) and RRsensitivity = 0.92 (95%CI 0.62 1.35)] between UC and CD.

Discussion: Our results indicate that IBD patients have a greater risk for hospitalization related to COVID-19 than the general population. On the other hand, COVID-19-related mortality risk is not increased in IBD patients, and no difference in COVID-19-related mortality and hospitalization was observed between UC and CD patients.

MeSH/Keywords: IBD; Crohn's disease; ulcerative colitis; COVID-19

Poster code: R-02-09-054

Poster Title: The effect of Ad26.CoV2.S coronavirus vaccine on the parameters of hemostatis and inflammation

PhD candidate: Iva Ivanko

Part of the thesis: The effect of the BNT162b2 and Ad26.CoV2.S coronavirus vaccines on pro-inflammatory and coagulation indicators

Mentor(s): Associate Professor Petar Gaćina, MD PhD, Josipa Josipović, PhD

Affiliation: University of Zagreb School of Medicine

Introduction: For the last three years COVID-19 disease has become a worldwide healthcare and socioeconomic issue. As a result of strenuous effort to control the pandemic crisis, vaccination was introduced as a secure and highly effective instrument against the disease. Soon after the vaccine releasement individual cases of thromboembolic events and changes in the blood pressure have been assigned to the vaccination raising concerns on the vaccine safety. The primary aim of our study is to investigate differences between various haemostasis and inflammatory markers in the subjects vaccinated with two different SARS-CoV-2 vaccines; mRNA BNT162b2 and vector Ad26.CoV2.S vaccine

Materials and methods: This prospective observational clinical study included 87 adult subjects who were vaccinated with mRNA vaccine and 84 subjects vaccinated with a vector vaccine. Several exclusion criteria were applied: recent surgery and arterial or vein thrombosis, pregnancy and puerperium, use of an anticoagulant therapy, malignant disease, history of haemophilic disorders etc. Blood samples were collected from each subject in the mRNA group at 5 different time points: before 1st vaccination, 7 and 14 days after 1st, 7 and 14 days after 2nd vaccination. The period between two mRNA vaccinations was 21 days. In the case of the vector vaccine, blood was drawn at 3 different time points: before vaccination (within 24 hours), 7 and 14 days after. At each time point, total of 15 mL of vein blood was collected in test tubes: one with K2EDTA anticoagulant (full blood count), two with 3.2 % sodium citrate (PV, APTV, fibrinogen, D-dimers, TAT, F 1+2, VWF:Ac, big endothelin-1) and one without anticoagulant (CRP, IL-6, PF4 antibodies). Laboratory investigation of full blood count and CRP were done immediately, while the rest of the laboratory parameters were measured from the specimens of the serum and plasma stored at -80°C. All the laboratory markers were measured by a well-established laboratory method. Distribution of the data was tested by using Kolmogorov-Smirnov test. For the data which were not followed normal distribution, results are presented as median and interquartile range (IQR). Non-parametric Mann-Whitney test was used to test the difference between mRNA BNT162b and Ad26.CoV.S vaccine groups. Differences between results obtained at different time points for each tested parameter in the same vaccine group were tested using Friedman test. The value of $P<0.05$ was considered statistically significant.

Results: In the group of Ad26.CoV.S vaccinated subjects, differences between three time points were demonstrated for CRP ($P=0.006$); D-dimers ($P=0.004$); VWF:Ac ($P<0.001$); and platelets ($P=0.021$). In the subgroup of subjects with arterial hypertension statistically different changes for CRP ($P=0.019$); fibrinogen ($P=0.021$); D-dimers ($P=0.042$) and VWF:Ac ($P=0.023$) were noted when compared to the subjects without hypertension

Discussion: Subtle rise of D-dimers, CRP and VWF activity can be explained by a mild proinflammatory reaction to the vaccination which was acknowledged by the vaccine manufacturer. Several studies with adenovirus SARS-CoV-2 vaccines showed a rise in D-dimer levels postvaccination. However, these studies have serious downsides in insufficient number of subjects and time points of the venepuncture. In our study none of the participants experienced thromboembolic event. Subjects with AH had a subtle rise in a several haemostatic and proinflammatory markers, however, due to a smaller number of subjects with AH these results should be taken cautiously. None of the subjects reported changes in the blood pressure by the time this abstract was written.

Acknowledgments: The study was supported by the Foundation of Croatian cooperative group for hematologic diseases KROHEM.

MeSH/Keywords: COVID-19, vaccine, haemostasis, inflammation

Poster code: R-02-09-057

Poster Title: Relation between major adverse cardiovascular events and intracranial artery calcifications in patients on hemodialysis

PhD candidate: Danilo Gardijan

Part of the thesis: Relation between major adverse cardiovascular events and intracranial artery calcifications in patients on hemodialysis

Mentor(s): Professor Zdravka Poljaković-Skurić, MD PhD, Vedran Premužić, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Hemodialysis patients suffer from accelerated atherosclerosis which increases CV mortality. Currently, arterial stiffness and pulse wave velocity are standard in cardiovascular incidents prognosis. Intracranial artery calcification is diagnosed with computed tomography and is more often in patients with chronic kidney disease. Still, there is no consensus on its relation with cardiovascular incidents in patients on hemodialysis.

Materials and methods: All patients for the study are currently identified. After all exclusion criteria are implemented, there are 100 patients from the hemodialysis unit of the Internal medicine department and 110 stroke patients from the Neurology department, University Hospital Zagreb. Data is collected In a prospective manner. All radiological data(number and place of calcification of arteries, IAC and CAC score) for patients is collected in Excel spreadsheets, separately by two independent radiologists, and analyzed in SPSS software. Calcium scoring is done using a semiquantitative method where 0 points is given to arteries with no calcification and 1 point to arteries without calcification. For analysis we are using two scores; IAC(intracranial artery score) score with a maximum of 11 points for intracranial artery calcification and CAC(cerebral artery score) with a maximum of 7 points. CAC doesn't include intracranial segments of the internal carotid and vertebral arteries. Medical history and medication data are still being collected in spreadsheets and analyzed. Two cohorts of patients will be age and sex-matched. 10 percent of patients are still in their 48-month follow-up period so we don't expect there will be a dropout big enough to interfere with our initial plan of 80 patients in each of two cohorts. Demographic data, anthropometric measures, habits, history of the disease, medication data, and complete blood count with differential are also being currently collected in sheets Additional data for HD patients(including arterial pressure, pulse wave velocity as arterial stiffness measure, CD ultrasound of carotids and vertebral arteries, anckle-brachial index, and continuous measure of arterial pressure) is also currently being collected and evaluated. Statistical analysis will be performed using SPSS software. A p-value <0.05 will be considered significant.

Results: Preliminary statistical analysis of radiological data between two cohorts of patients currently shows there are no differences in IAC scores for the two groups of patients. There is a trend toward higher number of calcified MCA in a diabetic group of patients and higher CAC scores in HD patients. No current demographic, clinical and diagnostic data is available for definitive statistical analysis.

Discussion: All patients are identified and radiological data with preliminary results is collected and statistically analyzed. Currently, there is ongoing input for all other patient's data in Excel spreadsheets. Around 10 percent of patients are still unavailable for definitive analysis since they didn't fulfill all conditions.

MeSH/Keywords: hemodialysis, intracranial artery calcification, computed tomography, major adverse cardiovascular events, arterial stiffness.

Poster code: R-02-09-067

Poster Title: Diagnostic performance of transient elastography and two-dimensional shear wave elastography for non-invasive staging of liver fibrosis in patients with compensated chronic liver disease

PhD candidate: Anita Madir

Part of the thesis: Diagnosis of clinically significant portal hypertension by two-dimensional ultrasound elastography

Mentor(s): Assistant Professor Ivica Grgurević, MD PhD

Affiliation: University of Zagreb School of Medicine; Department of Gastroenterology, Hepatology and Clinical Nutrition, University Hospital Dubrava

Introduction: Liver biopsy (LB) is considered the gold standard method in assessing the stage of liver fibrosis in chronic liver diseases (CLD). However, LB is invasive, expensive, and not acceptable to all patients. Therefore, alternative non-invasive methods are needed. We aimed to evaluate diagnostic performance of transient elastography (TE) and two-dimensional shear wave elastography (2DSWE) as ultrasound based non-invasive methods for staging of liver fibrosis, and to test their accuracy in the cohort of patients with compensated CLD.

Materials and methods: Consecutive patients with compensated CLD scheduled for LB to stage liver fibrosis and/or to reveal the underlying etiology were prospectively included in this study. All patients underwent LSM by TE and 2DSWE on the same day, prior to LB. Liver biopsy was performed via the transjugular route, as all patients were suspected of having advanced CLD, based on previous laboratory or imaging methods during their work-up. The cohort also underwent measurements of hepatic vein pressure gradient (HVPG) in order to assess the presence and grade of portal hypertension. Liver biopsy samples were paraffin-embedded, sliced, stained by Hematoxylin & Eosin and Masson trichrome, and evaluated by an experienced pathologist. Liver fibrosis was staged according to the Ishak classification, where stage 0 represents no fibrosis and stage 6 developed cirrhosis. Compensated advanced chronic liver disease (cACLD) was considered in patients with bridging fibrosis or cirrhosis (Ishak stages F3-F6).

Results: Seventy-six (76) patients underwent LB (78.9% males, median age 62 (34-76); 36.8% alcohol-related liver disease, 30.3% non-alcoholic fatty liver disease; 14.5% chronic hepatitis B or C, and 9.2% other etiology). cACLD was histologically confirmed in 61 (80,3%) patients, whereas 15 (19.7%) patients had mild-moderate fibrosis (Ishak stages F1-2). Liver stiffness progressively increased by both methods (TE and 2DSWE) with the increasing stage of liver fibrosis. Some overlapping in LSM values existed especially between Ishak stages F3-4 and F5-6. LSM by TE had very good discriminative capability to differentiate F0-2 vs. F3-6 stages of liver fibrosis, with AUROC 0.92 at LSM cut-off 15.9 kPa (sensitivity (Se) 70.5% (confidence interval, CI 57-81.5); specificity (Sp) 100% (CI 78-100); positive predictive value (PPV) 100% (CI 92-100) and negative predictive value (NPV) 45% (CI 28-64)). Similar performance for distinguishing F0-2 vs F3-6 was obtained by 2DSWE, with AUROC 0.94 at LSM cut-off 9.4 kPa; Se 100% (CI 94-100); Sp 73.3% (CI 44.9-92.2); PPV 94% (CI 85 98%) and NPV 100% (CI 72-100). The correlation between LSM values as measured by two elastographic methods (TE and 2DSWE) was high (Pearsons r=0.854), but the agreement between them was not good. LSM by 2DSWE produced a 2.8 kPa lower values mean, as demonstrated in Bland-Altman analysis. Among the patients with LSM 10 kPa measured by TE (criterion for the suspicion of cACLD according to Baveno consensus, N=65/76) bridging fibrosis or cirrhosis (i.e. presence of cACLD) was histologically confirmed in 59 (90.8%). Of the remaining 11/76 (14%) patients with LSM<10 kPa, 2/11 (18%) had bridging fibrosis or cirrhosis.

Discussion: Results of this study reveal a good correlation between LSM as measured by TE and 2DSWE, and the histological stage of liver fibrosis. Both elastographic methods demonstrated high diagnostic accuracy in distinguishing between patients with advanced and earlier stages of liver fibrosis in the investigated cohort, with a somewhat better overall performance of 2DSWE. 2DSWE gives lower LSM values for the same stage of liver fibrosis in comparison to TE, and thus, the LSM results of these two methods cannot be used interchangeably. At the proposed LSM cut-off value of 10 kPa, TE can predict the presence of cACLD with 90% accuracy.

Acknowledgments: Special thanks goes to Professor Vladimir Trkulja, who did the statistical analysis of the data and helped in their interpretation.

MeSH/Keywords: chronic liver disease; transient elastography; two-dimensional shear wave elastography; liver biopsy; liver histopathology.

Poster code: R-02-09-074

Poster Title: Glycemic Variability in Type 1 Diabetes Mellitus PregnanciesNovel Parameters in Predicting Large-for-Gestational-Age Neonates: A Prospective Cohort Study

PhD candidate: Gloria Lekšić

Part of the thesis: The association of glycemic variability and incidence of large-for-gestational-age neonates in pregnant patients with type 1 diabetes mellitus who are using continuos glucose monitoring

Mentor(s): Assistant Professor Maja Baretić, MD PhD, Professor Marina Ivanišević, MD PhD

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

Introduction: Pregnancies with type 1 diabetes mellitus (T1DM) have a high incidence of large-for-gestational-age neonates (LGA) despite optimal glycemic control. In recent years, glycemic variability (GV) has emerged as a possible risk factor for LGA, but the results of the conducted studies are unclear. This study analyzed the association between GV and LGA development in pregnancies with T1DM.

Materials and methods: This was a prospective cohort study of patients with T1DM who used continuous glucose monitoring (CGM) during pregnancy. Patients were followed from the first trimester to birth. GV parameters were calculated for every trimester using the EasyGV calculator. The main outcomes were LGA or no-LGA. Logistic regression analysis was used to assess the association between GV parameters and LGA. In total, 66 patients were included

Results: The incidence of LGA was 36%. The analysis extracted several GV parameters that were significantly associated with the risk of LGA. The J-index was the only significant parameter in every trimester of pregnancy (odds ratios with confidence intervals were 1.33(1.02,1.73),3.18(1.12,9.07),and1.37(1.03,1.82),respectively.

Discussion: Increased GV is a risk factor for development of LGA. The J-index is a possible novel GV parameter that may be assessed in all three trimesters of pregnancy together with glycated hemoglobin and time-in-range.

MeSH/Keywords: diabetes mellitus type 1, pregnancy, large-for-gestational-age neonates, glycemic variability, continuous glucose monitoring, J-index

Poster code: R-02-09-077

Poster Title: Estimated arterial stiffness and cardiovascular risk in chronic kidney disease - a study protocol

PhD candidate: Marija Domislović

Part of the thesis: Estimated arterial stiffness and cardiovascular risk in chronic kidney disease

Mentor(s): Academician Bojan Jelaković

Affiliation: University of Zagreb, School of Medicine; University Hospital Centre Zagreb, Department of Nephrology, Hypertension, Dialysis and Transplantation

Introduction: The prevalence of chronic kidney disease (CKD) in the general population is 9.1%. Current guidelines recommend a cut-off GFR value of 60 mL/min/1.73 m2 for diagnosis of CKD, without considering the physiological decline of GFR with aging, or the association with cardiovascular (CV), cerebrovascular or renal outcomes. There is also an increase in arterial stiffness with aging, which is estimated by pulse wave velocity (ePWV). ePWV predicts CV incidents independent of CV risk assessment using SCORE tables and traditional risk factors defined by the Framingham study. Although CKD is known to be an independent factor of CV risk, the question is how much and in what way ePWV on age-adjusted definition of CKD contributes to the increase in CV, cerebrovascular and renal risk. Arterial stiffness is an independent predictor of cardiovascular, cerebrovascular, and renal outcomes regardless of age-adjusted definition of chronic kidney disease. The aim of this study is to determine whether the predictivity of estimated arterial stiffness (ePWV) for fatal and nonfatal CV, cerebrovascular, and renal outcomes is independent of guideline-defined chronic kidney disease (CKD) that is defined as eGFR <60 mL/min/1.73 m2 or age-adjusted definition of CKD. Also, this study will analyse risk factors for ePWV depending on the stage of CKD determined according to the guidelines and using an age-adjusted definition of CKD. We will show predictability of all traditional CV risk factors and ePWV for total and CV mortality, and for certain fatal and non-fatal outcomes and analyse causes of death and frequency of morbidity depending on ePWV in subjects classified as persons with CKD defined in both ways, and in persons without CKD.

Materials and methods: This will be a prospective, observational study that will include 2,058 adults, subjects of the scientific research project Endemic nephropathy in Croatia - epidemiology, diagnostics and etiopathogenesis (Ministry of Science, Education and Sports 108-0000000329). Subjects will be monitored for an average of 12 years (2005-2021). At the initial follow-up point, all subjects signed a written informed consent and filled out an extensive questionnaire containing information on personal and family history and were clinically examined, and blood and first urine sample was taken. In final follow-up point we will obtain the data on fatal and non-fatal CV, cerebrovascular and renal outcomes from the archives of general practitioners and from the register of deaths of the Croatian Institute of Public Health. Causes of fatal and non-fatal outcomes will be classified using ICD 10 codes. We will calculate ePWV values using the validated equation: ePWV = 9.587 0.402 × age + 4.560 × 103 × age2 2.621 × 105 × age2 × mean arterial pressure (MBP) + 3.176 × 103 × age × MBP 1.832 × 102 × MBP. The total risk will be calculated using accepted risk sums (Heart Score and Framingham risk score).

Results: Out of 2,232 subjects who were part of the project, 2,058 were followed. 491 of them did not had information on eGFR and ePWV at the starting point of the study. 1,741 of them had information on eGFR and ePWV at the starting point of the study, and for 1,175 of them, data on survival was found. For 566 subjects there was no information of survival. For 377 the information on survival was not found though CEZIH system, and 189 were subjects whose general practitioners refused to participate in the study. Out of 1,175 subjects for whom survival data was known, 263 of them died. In 240 subjects, the exact cause of death was known, and for 23 there was unknown cause of death.

Discussion: The expected scientific contribution of this study is that, based on the obtained results, the contribution of the ePWV, independent of CKD, to the total CV, cerebrovascular and renal risk could be confirmed, which was not done in previous studies. Secondly, our results would contribute to the introduction of an age-adjusted definition of CKD in clinical practice.

MeSH/Keywords: chronic kidney disease, ePWV, cardiovascular risk, arterial stiffness

Poster code: R-02-09-079

Poster Title: Association between the single nucleotide polymorphisms for PNPLA3 (rs738409) and inflammatory cytokines in patients with alcoholic liver cirrhosis

PhD candidate: Ivan Budimir Bekan

Part of the thesis: Association between the single nucleotide polymorphisms for PNPLA3 (rs738409) and inflammatory cytokines in patients with alcoholic liver cirrhosis

Mentor(s): Associate Professor Anna Mrzljak, MD PhD, Associate Professor Tomislav Kelava, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: PNPLA3 polymorphism is associated with the development of fatty liver disease and hepatoceluular carcinoma. Carriers of the G allele (CG i GG) have a more severe clinical progression compared to wild type (CC). General aim of this research is to investigate the association between PNPLA3 (rs738409) polymorphism and inflammatory cytokines in patients with alcoholic liver cirrhosis.

Materials and methods: This is a prospective observational study on patients with alcoholic liver cirrhosis without hepatocellular carcinoma (n = 90), patients with hepatocellular carcinoma in the setting of liver cirrhosis (N = 40), and control patients without liver disease, without acute and chronic inflammation (n = 40). The control group consists of patients without liver disease and without acute or chronic inflammatory disease, who have a blood sample taken during mole excision or extirpation of atheroma, fibroma or lipoma at the Department of Surgery, University Hospital Merkur. If the PHD finding is malignant, the specified control patient is excluded from the study. Inclusion criteria are: patients over 18 years of age, signed informed consent, alcoholic cirrhosis. For the control group, the included criteria are: over 18 years of age and signed informed consent. Exclusion criteria are a liver disease of etiology other than alcoholic. Exclusion criteria for the control group of patients: liver disease, acute or chronic inflammation, malignant tumor. The study includes patients with alcoholic cirrhosis, who are on the list for liver transplantation. Liver disease as well as HCC are histologically verified during the transplant procedure. All patients in the study are previously informed about the research and have signed an informed consent. Sample storage, DNA isolation and determination of gene polymorphism is performed in the Laboratory for Molecular Immunology at the Croatian Institute for Brain Research. Based on this analysis, subjects are divided into 3 groups based on different genotypes (CC, CG and GG alleles). Flow cytometric analysis (ThermoFisher Scientific Attune flow cytometer) is used to determine the concentration of cytokine markers of inflammation (interleukin (IL) IL- 1, IL - 6, IL-- 8, IL- 10, IL - 12p70, IFN , MCP1, TNF-a) using a commercially available kit (LEGENDplex Human Inflammation Panel 1 (13 - plex) with Filter Plate).

Results: In the past year, database was completed. More precisely, all blood samples were collected from the patients who make up the study population: patients with alcoholic cirrhosis of the liver without HCC, patients with cirrhosis and HCC, and a control group , a total of 170 subjects. All patients had a blood sample taken once as part of a routine examination. Part of the sample was stored until DNA isolation and gene polymorphism analysis. Based on this analysis, the subjects were divided into 3 groups based on different genotypes (CC, CG and GG alleles). The remaining sample was used for flow cytometric analysis, which determined the concentration of cytokines. Preliminary results, after processing part of the data, are promising. We found an increased concentration of cytokines in carriers of the G allele of the PNPLA3 polymorphism. Given that we analyzed only part of the data, it is still not possible to provide clear research results.

Discussion: As previously stated, a small part of the data was processed and analyzed. Although the preliminary results are promising, we cannot draw clear research conclusions based on them. By reviewing the available papers, it is clear that carriers of the G allele of the PNPLA3 polymorphism (rs738409) have a faster progression of alcoholic liver disease and a shorter life on the transplant list. Although in attempts to explain the faster progression of the disease in patients with the G allele of PNPLA3, the increased inflammatory activity in these patients is most often mentioned, the mechanism by which the polymorphism of the gene and the presence of the G allele leads to the progression of alcoholic liver disease to cirrhosis and, eventually, the development of HCC is unknown.

MeSH/Keywords: PNPLA3, liver cirrhosis, inflammatory cytokines

Poster code: R-02-09-080

Poster Title: Intra-procedural three-dimensional rotational angiography in cryoballoon ablation of atrial fibrillation - preliminary data from a randomised clinical trial

PhD candidate: Ivan Prepolec

Part of the thesis: Intra-procedural three-dimensional rotational angiography in cryoballoon ablation of atrial fibrillation

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

Affiliation: University of Zagreb School of Medicine

Introduction: To facilitate atrial fibrillation (AF) ablation procedures there is a variety of approaches for pre-procedural imaging of pulmonary veins (PV) and left atrium (LA), including CT angiography and cardiac MRI. Intra-procedural three-dimensional rotational angiography (3DRA) is the least employed method and it can be advantageous regarding efficiency and procedural logistics. Data supporting different practices is still evolving and there is an open question whether LA imaging could improve safety and outcomes of CB ablation.

Materials and methods: The aim of this study is to evaluate possible benefits of 3DRA for procedural success, safety and long-term outcomes of CB ablation. We designed a single-centre unblinded randomised clinical trial and recruited patients with paroxysmal and early-persistent AF. Patients were randomised to no imaging or 3DRA which was performed intra-procedurally after trans-septal puncture. Angiographic images were segmented and overlaid to the fluoroscopy screen to guide the ablation procedure. All patients are followed for 1 year and data concerning procedural characteristics, safety outcomes and AF recurrence are collected.

Results: We previously reported acute outcomes of this study during the period of patient recruitment. Here we present data regarding the first 40 patients from each group who completed follow-up (80 patients in total). Most patients in both groups were male (65.0% of the control group and 34.4% of the 3DRA group) and there was no significant age difference between the groups (60.0±12.0 vs. 58.0±12.8 years in the control and 3DRA group respectively). Only minor complications were reported in both groups. One patient in each group developed a large haematoma. One impending or transient phrenic nerve palsy was observed in both groups. The average follow-up was 20.6±6.5 months and didn't differ significantly between the groups. Clinical success of ablation was defined as freedom from atrial arrhythmia. Regular follow-up visits and 24-hour ECG Holter monitors were planned 3, 6 and 12 months after ablation. It is worth noting that due to COVID-19 pandemic this could not always be followed. In the control group 34 patients (85.0%) were free from atrial arrhythmia compared with 35 (87.5%) patients in the 3DRA group (OR 1.24, 95% CI 0.34-4.43, p=0.75). Patients who experienced atrial fibrillation recurrence often underwent redo procedures (4 and 3 patients in the control and 3DRA group respectively).

Discussion: Although 3DRA is a safe and efficient intra-procedural imaging method to guide CB ablation for AF, this limited data suggests that it might not have influence on arrhythmia recurrence. Follow up data of all enrolled patients is still being collected and will subsequently be presented.

MeSH/Keywords: atrial fibrillation, catheter ablation, arrhythmia ablation, rotational angiography

Poster code: R-02-09-084

Poster Title: The effect of physical activity on vascular stiffness in endurance and strenght sports

PhD candidate: Petra Vitlov

Part of the thesis: The effect of physical activity on vascular stiffness in endurance and strenght sports

Mentor(s): Associate Professor Milan Milošević, MD PhD, Associate Professor Šime Manola, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Arterial stiffness is an independent predictor of cardiovascular events. Arterial stiffening occurs with age and is closely associated with the progression of cardiovascular disease. Physical activity reduces oxidative stress, plasma noradrenaline and endotelin 1 (potent vasoconstrictor peptide) concentration thereby reducing arterial stiffness. Long-term endurance sport practice has been demonstrated to reduce arterial stiffness, whereas discrepancies exist in the literature on the relationship between chronic strength sport practice and arterial stiffness.

Materials and methods: Healthy recreational master athletes (aged 35-64) who have been active in endurance (bicycle) and strength sports (judo, wrestling, box) for the last 10 years have been included in this study. Athletes under 35 and older than 65, athletes with previous known arterial hypertension, dyslipidemia, diabetes mellitus, cardiovascular disease, women, smokers were excluded from the study. Several national and local recreational sport club associations are involved in this study. The first phase of the study includes collecting data and fulfilling self-assessment questionnaires about physical activity and lifetime training hours on each sport player. After The International Physical Activity Questionnaire has been fulfilled, questionnaire about their lifetime training hours (average endurance and strength training hours per week × 52 × training years). Later, anthropometry parameters (height, weight, body mass index, waist-to-hip ratio, waist-to-height ratio), electrocardiogram (with Sudden Death Screening Module based on Seattle criteria and investigator independent software), arterial stiffness (which is measured noninvasive by Arteriograph), and standard metabolic parameters have been measured. Until now 10 endurance recreational athletes and one strength athlete have completed the study.

Results: This is a cross sectional study, and all subjects will be stratified according to their lifetime training hours (average endurance and strength training hours per week × 52 × training years) in low, medium, and high training group upon completion of the study. Calculation of average training hours is based on athletes estimation and/or exercise diary. Group thresholds will be determined after subject selection to ensure equal group sizes: low training group, medium training group, and high training group. All athletes provide written informed consent, and the protocol has been approved by the hospital ethics committee.

Discussion: It is well known that endurance sport reduces arterial stiffness but there are conflicting results within the literature on effect that has long lasting strength sport on arterial stiffness. After age of 35, cardiovascular diseases is the leading cause of death in recreational athletes. Atrial fibrillation occurs particularly in athletes with more than 1500 lifetime training hours. More than 4500 lifetime training hours is however associated with longer P wave duration and higher left atrial volume. So far, there are no published papers describing correlation between lifetime training hours and arterial stiffness. With results at the end of this study we expect to find out which number of lifetime training hours is associated with lower vascular age and better metabolic parameters in endurance and strength athletes. However, upon the competition of this study we will see and negative impact that sport can have on organism, which is essential for our better understanding on different sport training on human organism.

MeSH/Keywords: arterial stiffness, cardiometabolic risk, vascular risk

Poster code: R-02-09-085

Poster Title: Association of CD133, CD44, PD-L1 and HNE expression with the outcome of neoadjuvant treatment of rectal adenocarcinoma

PhD candidate: Nikolina Lonjak

Part of the thesis: Association of CD133, CD44, PD-L1 and HNE expression with the outcome of neoadjuvant treatment of rectal adenocarcinoma

Mentor(s): Sanda Bubanović, PhD, research associate, Tajana Silovski, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: The discovery od valid biomarkers in treatment of locally advanced rectal adenoacarcinoma (LARC) could be crucial for personalized and precise medicine.

Materials and methods: Number of 100 patients with LARC are going to be divided into two subgroups, one treated with neoadjuvant radiotherapy and the other with neoadjuvant chemoradiotherapy, then both surgically resected. A comparison of biomarkers (CD133, CD44, PD-L1 and HNE) will be made between biopsy and operative samples, between two subgroups of patients and in relation to the pathological response.

Results: A database of 160 patients was collected and important clinical data from hospital information systems were entered. For about half of patients both colonoscopy and surgery samples were found to be in our hospital centres and for the remaining part only one pathological sample was found, mostly because the diagnostics was performed in regional hospital. The process of obtaining the other sample is undertaken. We gathered most of the acquired antibodies for the IHC analysis.

Discussion: This research could define a subgroup of patients who could potentially be spared of neoadjuvant treatment, thus reducing the toxicity and overtreatment.

MeSH/Keywords: Rectal adenocarcinoma, neoadjuvant treatment, CD133, CD44, PD-L1, HNE

Poster code: R-02-09-091

Poster Title: Microvascular lesions in lupus nephritis

PhD candidate: Tamara Knežević

Part of the thesis: Severe infections in patients with systemic lupus erythematosus and lupus nephritis

Mentor(s): Professor Branimir Anić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Microvascular lesions (MVL) can be found in the kidneys of lupus nephritis (LN) patients and might be associated with worse outcomes. There are very few studies which evaluated MVLs in these patients and we aimed to provide a comprehensive evaluation of all MVLs, their frequency, characteristics and association with renal outcomes one year after kidney biopsy.

Materials and methods: We have conducted a retrospective cohort study to evaluate the characteristics and prognostic significance of MVLs in the kidney of subjects with LN. We have collected data on demographics, clinical and laboratory parameters and histopathology (light, immunofluorescent and electron microsopy). MVLs were characterized according to previous classifications. SLE was diagnosed using the American College of Rheumatology criteria.

Results: A total of 56 patients with biopsy-proven LN were followed up for at least one year after kidney biopsy (79% women, mean age at biopsy 38±13 years). Forty patients (71%) had MVLs in the kidney. The most common MVLs were arteriolar endotheliocyte swelling (54%), arteriolar hyalinosis (25%) and endothelialitis (8%) and the frequency distribution of all microvascular lesions is shown in Figure. Median number of lesions in the MVL group was 1 (interquartile range 1 to 2) and subjects had up to 6 MVLs. There was no difference in the median number of MVLs across LN classes ($p>0.05$). Proteinuria was highest in class V (5.5 g/day, $p=0.06$ vs. all other classes). Subjects with MVLs had lower baseline proteinuria compared with those with no lesions (3.6 vs. 5.4 g/day, $p=0.037$), but there was no difference in serum creatinine (92 vs. 119 umol/L, $p=0.25$). There were no differences in the occurrence or number of MVLs across LN classes ($p=0.63$). The number of MVLs was not correlated with proteinuria ($p>0.05$). There was no difference in the frequency of proliferative lupus between MVL and no MVL groups (79% vs. 60%, $p=0.15$). A total of 48% of subjects achieved complete response (CR), 27% achieved partial response (PR) and 25% had no response (NR). MVLs were not associated with response (defined as CR or PR) in a multivariate regression model (OR 3.5 [0.5, 24.6]).

Discussion: MVLs are common in LN. They were associated with lower baseline proteinuria, but not with proliferative LN or renal outcomes. The association with proteinuria warrants further research.

MeSH/Keywords: Microvascular lesions, Lupus nephritis, Outcomes, Proteinuria

Poster code: R-02-09-112

Poster Title: Impact of Sodium-Glucose Cotransporter 2 inhibitors on chromogranin A concentration in patients with chronic heart failure and reduced left ventricular ejection fraction

PhD candidate: Vanja Ivanović Mihajlović

Part of the thesis: Impact of Sodium-Glucose Cotransporter 2 inhibitors on chromogranin A concentration in patients with chronic heart failure and reduced left ventricular ejection fraction

Mentor(s): Associate Professor Šime Manola, MD PhD, Assistant Professor Mario Udovičić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Heart failure is a clinical syndrome that represents a significant public health problem, especially in patients with reduced left ventricular ejection fraction (HFrEF). A novelty in the European Society of Cardiology guidelines is the introduction of Sodium-Glucose Cotransporter 2 (SGLT2) inhibitors in the first line of HFrEF therapy. This study is the first to investigate chromogranin A as an alternative marker of chronic heart failure in light of the new guidelines.

Materials and methods: All subjects who have been included meet the following inclusion criteria: both genders, over 18 years of age, with EFLV 40%, who are treated with standard optimal drug therapy according to current guidelines and who are in stable functional status and in a stable phase of the disease that did not require a change of therapy in the last month, or a visit to the emergency department. All patients with severe valvular or pericardial disease, infiltrative or hypertrophic cardiomyopathy, history of an acute coronary or cerebral incident in the last 3 months, history of type 1 diabetes, unregulated hypertension, manifest primary lung disease, active malignancy, systemic autoimmune or active infectious disease, significant hepatic or renal insufficiency and assessed glomerular filtration rate were excluded from the study. Subjects have been divided into two groups: the tested and the control. Tested group includes patients who started treatment with SGLT2 inhibitors no more than 2 weeks before potential inclusion in the study: dapagliflozin 10 mg once daily, or empagliflozin 10 mg once daily, in approximately equal proportions. SGLT2 inhibitors have not been introduced to the control group due to their financial limitations. Both groups have been exposed to the same study protocol. Initially, all patients have completed an SF-36 questionnaire. A blood sample has been taken from each subject for planned laboratory analyzes, ie determination of chromogranin A (basal chromogranin A; maximum 2 weeks after introduction of SGLT2 inhibitors) and NT -proBNP. After blood sampling, each subject has performed a 6-minute walk test and then have had an echocardiographic examination of the heart to assess parameters of left ventricular systolic and diastolic function, mitral regurgitation, as well as estimated pulmonary circulation pressures. After 6 months, the same procedure as the initial one will be repeated.

Results: The research plan is to gather a total of 80 subjects (60 in the tested group, 20 in the control group). So far, a total of 12 subjects have been collected, 5 in the tested group and 7 in the control group. Each subject signed an informed consent before being included in the study. All subjects underwent planned initial measurements (laboratory and during the 6-minute walk test) and their planned echocardiographic parameters were measured. A questionnaire on quality of life was filled out. A corresponding database was also formed, and all currently known data for each patient are entered.

Discussion: Chromogranin A is a known marker of heart failure, but it has never been investigated exclusively in patients with reduced left ventricular ejection fraction who are treated with new, modern heart failure therapy, including SGLT-2 inhibitors. This study is aiming to determine the impact of SGLT2 inhibitors on the concentration of chromogranin A, correlation between chromogranin A and NT-proBNP, functional status of the patient and several echocardiographic indicators of left and right ventricle function, mitral regurgitation and pulmonary circulation pressures. This research could possibly contribute to better understanding of chromogranin A as an alternative marker of chronic heart failure.

MeSH/Keywords: heart failure, SGLT2 inhibitors, chromogranin A

Poster code: R-02-09-130

Poster Title: Association of gene variations (single nucleotide polymorphisms rs10455872 and rs3798220) and serum concentrations of lipoprotein (a) with individual cardiovascular risk

PhD candidate: Dunja Leskovar

Part of the thesis: Association of gene variations (single nucleotide polymorphisms rs10455872 and rs3798220) and serum concentrations of lipoprotein (a) with individual cardiovascular risk

Mentor(s): Associate Professor Ivan Pećin, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Lp (a) is an LDL-cholesterol-like lipid particle that additionally contains apolipoprotein (a) with highly thrombogenic properties. Elevated serum lp (a) levels are associated with the occurrence of cardiovascular incidents at an earlier age (myocardial infarction, CVI, peripheral art disease) and increase the cardiovascular risk by 2-3 fold. Unlike other particles, the concentration of lp (a) is genetically determined, and studies show that 20% of the population has elevated concentrations. Gene polymorphisms associated with higher lp (a)levels and the development of a cardiovascular incident have been found. Cardiovascular diseases are the leading cause of death both in the world and in our country. According to data for 2018, 43.7% of the total number of deaths in Croatia was result of cardiovascular disease. Meta-analyses show that increased serum concentration of lp (a) is an independent risk factor for cardiovascular diseases (coronary artery disease, myocardial infarction, aortic stenosis, aortic aneurysm, ischemic cerebral incident). Given that the concentration of lp(a) is exclusively genetically determined, gene polymorphisms were discovered that are associated with higher concentrations of lp(a). Single nucleotide polymorphisms rs10455872 and rs3798220 are associated with higher serum lp(a) levels and coronary disease.

Materials and methods: A total of 250 subjects with elevated serum lp (a) will be recruited into the case-control designed study. The group of cases consists of subjects who developed a cardiovascular incident at an early age in their lifetime, and the control group will consist of subjects who did not have a cardiovascular incident.Cases and controls will be matched by age and sex. Exclusion criterias are subject's age (>65 years), chronic kidney disease (eGFR<60 ml/min/1.73 m2), smoking status, acute infection and autoimmune diseases. Genotyping of LPA gene polymorphisms (SNP rs10455872, rs3798220) will be performed using the real-time PCR method. In individuals with extremely high lp(a) values and an early incident cardiovascular event without other risk factors, next-generation sequencing for the LPA gene will be performed to possibly identify new high-risk SNPs

Results: So far, 246 blood samples have been collected from subjects, and testing for high-risk variants is underway. Of these, 15 samples from high-risk subjects were sent for next-generation sequencing. All statistical methods that will be used in the research are defined. Considering the above, preliminary results are being prepared.

Discussion: Elevated lp(a) as a possible main cause of a cardiovascular event was found in a large number of cases in younger patients (<60 years) in whom all other possible causative factors (hyperlipidemia, obesity, thrombophilia, etc.) were ruled out. Observational and genetic evidence convincingly demonstrates that high Lp(a) concentration is causal for ASCVD, AVS, and cardiovascular and all-cause mortality in men and women and across ethnic groups. There is a large discrepancy between the clinical picture and the serum concentration of lp(a). Such a phenomenon can be explained by different gene variants. Studies suggest that carriers of rs10455872-G-allele had 3.86 and 2.54 higher risk of Lp(a) 30 mg/dL or the presence of aortic valve calcification. Other studies have shown a strong association of LPA rs10455872 and rs3798220 with coronary artery disease

MeSH/Keywords: lipoprotein (a), cardiovascular disease, single nucleotide polymorphisms

Poster code: R-02-09-137

Poster Title: Uric acid concentration as a predictor of recurrence of atrial fibrillation after pulmonary vein isolation

PhD candidate: Kristijan Đula

Part of the thesis: Uric acid concentration as a predictor of recurrence of atrial fibrillation after pulmonary vein isolation

Mentor(s): Associate Professor Šime Manola, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Atrial fibrillation (FA) is the most common arrhythmia in humans, and the incidence is expected to arise significantly in the coming decades due to population aging. FA has been associated with increased mortality, stroke risk, heart failure and other cardiovascular diseases. The cornerstone of FA treatment is the pulmonary vein isolation (PVI) with pharmacological treatment, but often, even after the initial successful procedure, recurrence of FA occurs. Nowadays there is no single factor or combination of factors that can reliably identify patients in whom recurrence of FA is likely. Elevated serum uric acid levels are a proven risk factor for the development of various cardiovascular diseases, including FA. Several studies have attempted to evaluate urate levels as a possible marker of an increased risk of (future) recurrence of FA after PVI, but data are generally scarce. The proposed study seeks to assess whether continuous monitoring of serum uric acid may help predict FA recurrence.

Materials and methods: We are conducting prospective, observational, non-randomized trial which will take a part at Department for Arrhythmology, UHC Sestre milosrdnice (Zagreb). Patients with paroxysmal FA at age of 18-75 years old, in whom PVI are planned will be enrolled. Exclusion criteria are as follow: a) contraindication for oral anticoagulation; b) dilated left atrium (LA PLAX >55mm or LAVI 34ml/m2); c) significant valvular pathology; d) myocardial infarction treated either by PCI or CABG in the last 3 months prior the enrollment; e) stroke or TIA in the last 3 months prior the enrollment; f) acute infectious disease; g) active malignancy; h) systemic connective tissue disorder; i) alcohol abuse; j) gout; k) Henle's loop diuretics or thiazids; l) pregnant or breastfeeding women. Enrolled patients will be continuously monitored (24h holter ECG, echocardiogram, uric acid, etc) every 3 months during total period of 12 months follow-up. Primary outcome will be recurrence of FA after PVI during 12 months follow-up. Secondary outcomes will be recurrence of other atrial tachyarrhythmias and left atrium dimension/function during 12 months follow-up. We are planning to enroll 60 patients. This is the product of a calculated power analysis with an accuracy of 82-96%. Mixed generalized logistic regression model will be used.

Results: We started with enrollment by January, 2023. 6 patients after PVI were enrolled and 2 follow-up were done. Data were collected systematically as defined by study protocol. We are planning to enroll vast majority of patients till end of 2023. The follow-up is expected to be completed by end of 2024, after which data analysis will be performed.

Discussion: Uric acid is known to be associated with cardiovascular disease, including FA. There is no definite pathophysiological explanation, but it seems that oxidative stress could be one of the important factors. And uric acid as one of the markers of oxidative stress could potentially be useful in predicting patients who will develop FA over time. Evidence regarding prediction of FA reccurence after PVI using uric acid is scarce. Canpolet et al. showed in a prospective, cohort study that the recurrence rate of FA in patients after cryoenergy PVI is related to elevated uric acid levels, while He et al in a retrospective analysis confirmed such results in the population after radiofrequency PVI. To our knowledge, this is the first study that will try to find correlation between dynamics of serum uric acid concentration and atrial fibrillation reccurence.

MeSH/Keywords: atrial fibrillation, pulmonary vein isolation, uric acid

Poster code: R-02-09-142

Poster Title: Can we predict long-term functional outcomes in non-high-risk pulmonary embolism patients?

PhD candidate: Tea-Terezija Cvetko

Part of the thesis: Predictive value of right atrial deformation on the functional capacity of patients with pulmonary embolism without high risk

Mentor(s): Associate Professor Nikola Bulj, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Acute pulmonary embolism (PE) is a form of venous thromboembolism (VTE) that is common and sometimes fatal. For survivors of an acute PE, long-term outcomes exist along a continuum ranging from full recovery to severe permanent functional limitations often with right heart dysfunction and chronic thromboembolic pulmonary hypertension. Transthoracic echocardiography (TTE) is a practical and widely used tool for risk stratification in pulmonary embolism (PE). We hypothesized that right atrial (RA) reservoir function, represented by peak RA systolic strain, could be used as a predictive measurement for the clinical (functional) outcome, measured by a 6-minute walk test (6MWT) in non-high-risk PE patients. To the best of our knowledge, this study will be the first to evaluate RA systolic strain in patients with non-high-risk PE and use it as a predictive value for functional outcomes. We believe this study could be a cornerstone in the field of potential rehabilitation for PE patients.

Materials and methods: All patients with diagnosed non-high-risk PE will undergo TTE within 24h. The functional outcome will be determined using a 6-minute walk test (6MWT) after 3- and 6 months. We hypothesise that patients with reduced peak RA systolic strain will have reduced 6MWT. We excluded all patients with suboptimal echocardiographic windows for visualization of the right heart chambers, patients who are unable to perform 6MWT, patients with significant comorbidities (expected survival less than 6 months), patients in New York Heart Association class III-IV, patients with known pulmonary arterial hypertension, and patients who had COVID-19 within 6 months.

Results: So far, we gathered 20 patients with non-high-risk PE. 7 of out 20 withdrew their signed consent. 13 patients had transthoracic echocardiography done within 24h of diagnosing non-high-risk PE. No statistical analysis of the preliminary results has been performed yet.

Discussion: The predictive value of right atrial function assessed by echocardiography speckle tracking in patients with acute non-high-risk PE has never been evaluated. Preliminary results seem to be in concordance with our hypothesis, though no statistical analysis has been performed. Since the beginning of the study, we were faced with many challenges in recruiting the study population and are currently behind the proposed schedule. At least 4 reasons are to be emphasized: the global COVID-19 pandemic has significantly influenced the incidence of PE (many patients were not eligible for our study due to COVID-19 status); the acquisition of adequate echocardiographic windows for right chambers evaluation is often limited due to adiposity; patients find it cumbersome to return for follow-up appointments and would often miss the proposed date; a significant number of eligible patients are severely limited in performing 6MWT and fall below the average for their age and sex even prior to the PE event. More patients are needed to adequately test of hypothesis.

MeSH/Keywords: right atrial strain, non-high risk pulmonary embolism, 6-minute walk test, right ventricular strain

Poster code: R-02-09-144

Poster Title: Thermal changes during clavicle fracture healing in children

PhD candidate: Filip Jurić

Part of the thesis: Thermal changes during clavicle fracture healing in children

Mentor(s): Associate Professor Anko Antabak, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Clavicle fractures are one of the most common fractures in children, accounting for about 15% of all upper limb fractures. Treatment is mostly conservative, with classical radiograph methods used to monitor the healing process. Recently, infrared thermography (IRT) has been proposed as an alternative method for fracture detection, but no study has correlated the temperature difference with callus formation.

Materials and methods: A prospective study was conducted, including 22 children aged between 6 and 17 years. Using a thermal infrared camera, temperatures above the fracture and the healthy side were measured on the 1st, 4th, 8th, 15th, and 22nd day after the trauma. Along with IRT, an ultrasound of the fracture site was performed to determine callus formation. The temperature difference between the fractured and healthy sides was noted for each visit. The data obtained were entered into the database and statistically analysed using the MedCalc software program.

Results: The average age of patients was 12.4 years, with the majority being boys (17) and significantly fewer girls (5). The left side was more often affected than the right side (13 over 9). In our study, we found a correlation between callus formation and the temperature change above the fracture. A maximum temperature difference of an average of 0.7 °C was noted during the reparative phase of callus formation (in most patients during the first 8 days). After the formation of fibrocartilaginous callus, the temperature above the fracture declines until it is equal to that of the healthy side. If we compare the average temperature difference between the broken and healthy sides depending on the visit, we notice that there is a statistically significant difference between the 2nd and 3rd visits (4th and 8th day) compared to other visits. Therefore, the p-value of the temperature difference between the 4th day and the 1st, 15th, and 22nd day is $p < 0.0001$, $p = 0.036$, and $p < 0.0001$, respectively, while the p-value of the temperature difference between the 8th day and the 1st, 15th, and 22nd day is $p = 0.0007$, $p = 0.0068$, and $p < 0.0001$. There were no statistically significant differences between the 4th and 8th day after injury.

Discussion: Skin temperature is mostly affected by blood flow. Bone healing is a complex process that takes place in three phases: inflammatory, reparative, and remodelling phase. The inflammatory phase (phase of angiogenesis) lasts about 4 days and is characterized by the organization of the hematoma, angiogenesis, and the formation of inflammatory granulation tissue rich in newly created capillaries. During this phase, an increase in blood flow is expected to lead to an increase in temperature above the fracture site. In our research, we recorded a significant increase in temperature in the first days after the injury with a peak at day 8, which would correspond to the inflammatory phase of bone healing.Furthermore, the temperature curves follow the formation of callus. In most patients, with the help of ultrasound, we recorded the formation of callus on the eighth day after the injury. When the callus is formed, the temperature curves start to fall and reach values close to zero between the 15th and 22nd day. It is significant that the treatment of clavicle fractures in children lasts 21 days. With this research, we see that IRT has significant potential in the diagnosis of callus formation. This can significantly contribute to reducing the use of X-rays when monitoring patients with fractures. This is particularly significant in the children's population, where it is imperative to reduce the dose of ionizing radiation as much as possible.

MeSH/Keywords: Clavicle Fractures; Fractures, Bone; Upper Extremity; Child; Adolescent; Radiography; Infrared Thermography; Callus

Poster code: R-02-10-008

Poster Title: Supraumbilical skin tightening after laser assisted liposuction/lipolysis

PhD candidate: Ivonne Žgaljardić

Part of the thesis: Supraumbilical skin tightening after laser assisted liposuction/lipolysis

Mentor(s): Professor Davor Mijatović, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Supraumbilical skin laxity is one of the biggest challenges for plastic surgeons. A significant degree of laxity is successfully treated with abdominoplasty with navel transposition. There is no quality solution for mild to moderate degrees of laxity. Surgical methods include abdominoplasty with transposition of the umbilicus, which often ends with a vertical scar cut in the median line infraumbilically, or reverse abdominoplasty, which results in an unacceptably visible scar at the junction of the chest and abdominal wall, especially in men. Previous research on laser-assisted liposuction/lipolysis speaks in favor of thermal injury to the reticular dermis and subdermal connective tissue due to heating to a temperature of 48-50°C, which stimulates neocollagenesis, and in a clinical sense, the aforementioned results in skin tightening.

Materials and methods: Patients with mild or moderate supraumbilical skin laxity undergoing elective laser-assisted liposuction/lipolysis have been included in the prospective study. People with significant supraumbilical skin laxity who are candidates for abdominoplasty with navel transposition, women who undergo simultaneous breast reduction and those who became pregnant at the end of the measurement period were excluded from the study. All patients with previous abdominal surgery were also excluded from the study. Prior to the surgery, height, weight, body mass index, age, sex, skin elasticity, as well as the presence or absence of stretch marks in patients has been recorded . Abdominal circumference has been measured at the level of the navel and distance from the upper umbilical edge and jugulum. These measurements have been performed before the procedure, as well as at intervals of seven days, three weeks and three months after the procedure. Supraumbilical skin tightening has been evaluated by lifting of the upper edge of the navel and the results have been evaluated according to height, weight, body mass index, skin elasticity and presence or absence of stretch marks.

Results: The study lasts until February 7, 2024, and so far 70% of the intended sample has been collected, and the statistical analysis of the collected data is underway. All the mentioned measurements were performed before the procedure, as well as at intervals of seven days, three weeks and three months after the procedure. The data collected so far speak in favor of better skin retraction in younger, thinner patients with better preoperative skin elasticity and without stretch marks but for the accuracy of the data, it is necessary to wait for the completion of the study and the processing of the intended sample.

Discussion: Thermal injury to the deep dermis and subcutaneous connective tissue that occurs during laser-assisted liposuction / lipolysis leads to the production of new collagen and elastin, which results in skin tightening. Previously published research confirms the superiority of the laser technique compared to standard tumescent liposuction when it comes to skin tightening. So far, there are no published papers describing the tightening of the skin above the navel and the lifting of the upper edge of the navel. Also, there are no reports that connect the degree of skin tightening with height, weight, body mass index, skin elasticity and the presence or absence of stretch marks. Younger, non obese patients with better skin quality e.g. better elasticity and without stretch marks have faster skin regeneration process therefore it is expected to obtain better results in these patients.

MeSH/Keywords: lipectomy, lipolysis, elasticity

Poster code: R-02-10-093

Poster Title: Tactile breast sensation after nipple-sparing mastectomy and implant-based reconstruction

PhD candidate: Jana Leskovar

Part of the thesis: Correlation between sensation and anthropometric characteristics of the breast after subcutaneous mastectomy and implant-based breast reconstruction

Mentor(s): Associate Professor Krešimir Bulić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Nipple-sparing mastectomy preserves native breast envelope, but negatively affects breast sensation.

Materials and methods: Patients who underwent nipple-sparing mastectomy and immediate implant-based breast reconstruction in the previous 3 years at the University Hospital Centre Zagreb were invited to participate. Examination consisted of tactile sensory testing and structured interview about the subjective observations on sensation in the reconstructed breast. Tactile perception thresholds were assessed across 6 regions of the reconstructed and contralateral healthy breasts utilizing Semmes-Weinstein type monofilaments. Depending on the respective category of sensation, 6 levels of sensibility were distinguished. Total sensory score was introduced as a measure of an average tactile sensibility in each breast. For the purpose of the preliminary analysis, breast implant volume was used as an indicator of breast volume. The data were statistically analysed using nonparametric tests. P-value < .05 was considered statistically significant.

Results: Tactile sensation examination was performed in 20 reconstructed and 13 healthy breasts of 20 female subjects aged between 34 and 70 (54.10 years ±4.36). All subjects had measurable sensation in at least 3 tested regions of the reconstructed breast. The nipple-areola complex had the poorest sensibility (87.96 g ±36.73). The thickest monofilament was not perceivable in 40% of the subjects in the nipple and 30% of the subjects in the areola. The most sensible were the superior medial (21.2 g ±20.22) and inferior medial (23.68 g ±20.23) regions. No statistically significant correlation was found between overall breast sensation and breast implant volume (r = 0.13). However, there was a significant correlation of overall breast sensation with the time interval from reconstruction to examination (r = 0.82). Finally, total sensory scores were significantly lower in the reconstructed breasts in comparison to the contralateral healthy breasts. Through a structured interview, 85% of the subjects reported they were able to feel touch in the reconstructed breast and found there was a progressive improvement in breast sensibility over time.

Discussion: Our results demonstrate that nipple-sparing mastectomy followed by implant-breast reconstruction significantly impairs breast sensation. The mean tactile perception thresholds across all tested regions correspond to the loss of protective sensation. Moreover, the tactile sensation was not measurable in the nipple-areola complex of 25% of the subjects. There are several possible underlying mechanisms: A. the nipple-areola complex is mainly innervated by nerves accessing it through glandular breast tissue which is removed during mastectomy, B. reinnervation by spontaneous sprouting from the healthy sides takes longer to reach the central parts of the breast, C. the nipple-areola complex has a distinct composition of mechanoreceptors compared to the breast skin. Furthermore, breast implant volume was not associated with the level of tactile sensation contrary to the known negative impact of breast volume on sensation in healthy breasts. This finding suggests that breast volume does not determine tactile sensibility when the breast envelope innervation is severly diminished. To conclude, we find that tactile sensation after nipple-sparing mastectomy and breast reconstruction with implant is based upon partial denervation during surgical procedure and spontaneous reinnervation over time.

MeSH/Keywords: Breast Implants; Mastectomy, Subcutaneous; Sensation; Touch

Poster code: R-02-10-126

Poster Title: Influence of endogenous immunoglobulin G glycosylation on the efficacy of rituximab therapy in patients with B-cell non-Hodgking lymphoma insight into the research stage

PhD candidate: Nevenka Cigrovski

Part of the thesis: Influence of endogenous immunoglobulin G glycosylation on the efficacy of rituximab therapy in patients with B-cell non-Hodgking lymphoma

Mentor(s): Professor Igor Aurer, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: The main goal of this research is to determine whether glycosylation of endogenous immunoglobulin G (IgG) affects the therapeutic efficacy of rituximab in the treatment of patients with B-cell non-Hodgkin's lymphoma (B-NHL).

Materials and methods: At least 72 patients with B-NHL who are scheduled to be treated with rituximab and chemotherapy at the Department of Hematology of UHC Zagreb are planned to be included in the study. Blood samples from patients are obtained twice: before and after the treatment with rituximab. Endogenous IgG will be isolated and its glycosylation pattern will be analyzed. The following clinical outcomes of treatment will be observed: response to therapy (complete remission, partial remission, stable disease, progressive disease), number of infections and typical pathogens, survival without lymphoma progression and overall survival.

Results: So far, 20 patients with B-NHL have been included in the study, of which 11 were female and 9 male patients. The average age at the time of diagnosis was 61,7 years (31-87) and median was 64,5 years. One patient died before the end of the treatment. Twenty blood samples were collected before the start of the treatment and six blood samples were collected after the end of the treatment with rituximab. Tubes with plasma are frozen at -20 °C (freezer) and stored until analysis.

Discussion: After collecting all samples and performing glycosylation analysis, this research could show to what extent the glycosylation of endogenous IgG affects the outcome of the usual way of treating B cell non-Hodgkin's lymphomas. In a practical sense, this means that the glycosylation of endogenous immunoglobulins G could be used as a biomarker for the expected therapeutic response when using rituximab. The research could also help in clarifying the influence of endogenous IgG glycosylation on the immune reactivity regulation.

MeSH/Keywords: B-cell non-Hodgking lymphoma, rituximab, immunoglobulin G, glycosylation

Poster code: R-02-14-107

Poster Title: Systematic review of ethical issues of health professionals related to decision-making in patients at the end of life in oncology and hemato-oncology departments.

PhD candidate: Antonia Kustura

Part of the thesis: Ethical issues of health professionals related to end-of-life decision-making in patients with malignancies

Mentor(s): Professor Ana Borovečki, MD PhD, Associate Professor Dražen Pulanić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: End-of-life care of adult patients with advanced or incurable cancers is filled with ethical challenges. Palliative and end-of-life care has now become the responsibility of not just intensivist, but moreover, internal medicine physicians, oncologists and hemato-oncologist. End-of-life decision-making is a challenging process, where many physicians continue to rely on their own intuition, morality, and judgment, partly because of the lack of guidance and partly because of the difficulty of making such decisions. Withholding and withdrawing of treatment are legally equal processes, but many physicians still report that they do not perceive them as equal.

Materials and methods: Search of Medline was done, with the exclusion of paediatric group of patients. Separate search was done for oncology and hemato-oncology patients at the end of the life.

Results: The physician's approach to the care of a palliative patient and the conversation with the family differs depending on the cultural background of physician, the customs of the people and the expectations of the family which could explain the various ethical dilemmas that arise. Also, gaps in approach were present between oncologists and hemato-oncologists. The individual approach of physicians is more pronounced with lack of defined algorithms and procedures.Difference was also found in the attitudes and approaches of older and younger doctors, where older doctors are more inclined to stop active treatment earlier, while younger doctors, on the other hand, show better communication with family and patients. Oncologists reported more personal comfort with symptom control and ethical/legal issues as compared with hematologists. In some studies hemato-oncologists viewed palliative care as end-of-life care, whereas oncologists were more inclined to include palliative care as early as possible.

Discussion: Lack of systematic approach or algorithms represent important problems which can lead to ethical issues in physicians. Therapeutic limitations and miscommunication with family members often causes ethical dilemmas. Differences between oncologists and hemato-oncologists exist, but are not entirely clear. It is suggested that share of decision making burden and early integration of palliative care can foster a shift in care for palliative patients. Some suggested that patients and their family members should be more included in the decision making process at the end of the life. In this way, decision-making process would be facilitated with less ethical issues. This review had methodological limitations. Formulating a search strategy was challenging due to the broad scope within the topic area. Nevertheless, clear problems have emerged as causes of ethical dilemmas.

MeSH/Keywords: palliative care; withholding treatment; end of life; withdrawing treatment; ethical Issue; professional ethics code

Poster code: R-02-15-119

Poster Title: F-18-fluorocholine PET/CT imaging in primary hyperparathyroidism after negative or inconclusive neck ultrasound and MIBI scintigraphy

PhD candidate: Eva Pasini Nemir

Part of the thesis: The value of positron emission tomography/computed tomography (PET/CT) with fluorine-18-choline in the localization of hyperactive parathyroid glands in patients with primary hyperparathyroidism and negative conventional diagnostic tests

Mentor(s): Professor Dražen Huić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Primary hyperparathyreoidism (pHPT) is an endocrine disorder with parathyroid surgery still representing the only curative approach. Preoperative localisation of hyperfunctioning parathyroid glands is challenging, but crucial for minimally invasive parathyroid surgery. The aim of our study is to assess the value of F-18-fluorocholine positron emission tomography (PET/CT) in patients with negative or inconclusive neck ultrasound and Tc-99m MIBI scintigraphy.

Materials and methods: At 20 to 30 minutes after injection, standard image acquisition was performed, 2-3 minutes of recording per bed position. For semi-quantitative analysis, the SUVmax value was measured. We calculated 18F-choline PET/CT positivity, sensitivity, specificity, accuracy, PPV and NPV on per-lesion and per-patient analysis. PET/CT was reported as a positive study after visual and semi-quantitative analysis if focal uptake was identified. True positive PET/CT studies were histologically proved as adenomas, hyperplasias or parathyroid tissue. The scan was considered negative if there was no focal uptake found on PET/CT tomograms. PET/CT positive lesions were considered as false positive if they were histologically confirmed and they were not corresponding to adenoma or hyperplasia of parathyroid gland. In our study we didn't have any false negative studies. For semi-quantitative analysis, we measured SUVmax values. Results and patients characteristics of our study were expressed as mean ± sd. For the correlation of SUVmax values and biochemical markers and lesion dimensions, we used Pearson correlation test.

Results: We gathered study data from 167 patients who underwent an 18F-choline PET scans. All included patients had mean serum PTH level of 14.49 ± 7.84 pmol/l and mean calcium levels of 2.74 ± 0.16 mmol/l. PET/CT scan was performed as second-line imaging. In regards to a per-patient analysis, our study had PET positivity of 92.81% (155/167). 69 patients were excluded due to unavailability for follow-up. Overall, per-patient sensitivity, specificity, accuracy, PPV and NPV were 100%, 70.06%, 95.1%, 94,44%, and 100 % . On a per-lesion analysis, out of 167 PET/CT studies, we identified 182 lesions. 72 PET positive lesions were not included in final analysis (unavailable for follow-up). 12 patients were considered true negatives and it was decided by referred clinicians not to operate on those patients. Overall sensitivity, specificity, accuracy, PPV and NPV on a per-lesion analysis were 100%, 75%, 96.26%, 95,79%, and 100 %. Ninety (90/95) lesions were PET/CT true positives. Eighty-six patients had surgery, and this corresponded to 95 histological lesions. Histologically there were 60 adenomas and 25 hyperplasias. The mean SUVmax values of hyperplasias were 6.29±2.77, while that of adenomas 6.64±2.43. Four false positive lesions were benign lymph node tissue (4/95) and one false positive lesion was follicular thyroid adenoma (1/95). Mean SUVmax value of false positive lesions was 5.45±1.18. Hyperfunctional tissue had a higher SUVmax value than false positive lesions. The most common localization of hyperfunctioning glands was below and posterior of lower poles of thyroid lobes 33.68% (32/95). 8.42% of hyperfunctioning lesions (8/95) were ectopic. After surgery, all patients had PTH serum values measured (15 minutes after extirpation or postoperative on the same day). Mean PTH serum values in patients who had successful surgery decreased for an average of 62.54% (preoperative PTH 14.49±7.84 pmol/l to 5.69±4.87 pmol/l). Mean Ca serum levels decreased from a mean of 2.74±0.16 mmol/l to 2.34±0.16 mmol/l. We didn't find any correlation between serum PTH levels and SUVmax values (Pearson p value = 0.028). A slight positive correlation was found between preoperative Ca serum values and SUVmax values of lesions (Pearson p value = 0.21). Meanwhile, a moderate positive correlation was seen between the dimensions of lesions and SUVmax values (Pearson p value = 0.4).

Discussion: 18 F-fluorocholine PET/CT is a fast and sensitive method for localisation of hyperactive parathyroid glands in patients with long history of primary hyperparathyroidism, representing a substrate for minimal invasive surgery after negative or inconclusive neck ultrasound and MIBI scintigraphy and showing excellent diagnostic performance.

MeSH/Keywords: PET/CT, F-18 fluorocholine, pHPT

Poster code: R-02-17-042

Poster Title: Evaluation of vision-related quality of life in patients after vitrectomy following idiopathic epiretinal membrane

PhD candidate: Gentian Bajraktari

Part of the thesis: Evaluation of vision-related quality of life in patients after vitrectomy following idiopathic epiretinal membrane

Mentor(s): Assistant Professor Tomislav Jukić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Idiopathic epiretinal membrane (IEM) is a common disease in the elderly population, with an overall prevalence of 7 %. It affects the inner surface of the retina over the macula. IEM is responsible for reduced visual functions, as well as the impact on quality of life. Pars plana vitrectomy (PPV) with epiretinal peeling, has been accepted as a standard procedure in patients with IEM. The aim of the study is to evaluate the impact of different intraocular tamponades on the visual-related quality of life (VRQOL) after IEM surgery with epiretinal membrane peeling

Materials and methods: In this randomized, cohort study, we included 50 consecutive patients diagnosed with IEM, which were referred for surgery at the University Clinical Center Rebro Zagreb, Department of Ophthalmology. The study has been approved by the Ethics Committee of the University Clinical Center Rebro Zagreb (no. 380-59-10106-22-111/3; 22 March 2018) and it has been carried out in line with the Helsinki Declaration. All patients were informed about the safety of the study before signing the informed consent. All participants have been equally randomized into two groups. The first group the Air group, was with 25 participants to which vitreous was substituted with air during PPV, and the second group the Balanced Salt Solution (BSS) group, was with 25 participants to which vitreous was substituted with BSS during PPV. To guarantee the randomization effect, the placement of participants into the group has been initiated by a coin toss for the first participant. The upcoming participants have been then divided into groups consecutively. The following data were collected before and after surgery and compared between two groups: VRQOL, best corrected visual acuity (BCVA), intraocular pressure (IOP), metamorphopsia, contrast sensitivity (CS), and central macular thickness (CMT)

Results: PPV was performed in 50 eyes. At baseline, there were no significant differences between the two groups. At 6 months postoperatively, VRQOL ($p < 0.001$), BCVA ($p < 0.001$), CMT ($p < 0.001$), CS ($p < 0.001$), and metamorphopsia ($p < 0.001$) improved significantly in comparison to baseline, without significant differences between the air tamponade and BSS groups.

Discussion: Visual function and VR-QoL improve in the short term after IEM removal. In this study, we have evaluated the usage of Air tamponade and BSS tamponade after PPV surgery in patients with IEM. We have discovered that parameters such as BCVA, CMT, CS, Metamorphopsia, and VR-QoL composite score have improved gradually in both groups throughout the 6 months after surgery. Despite improvements in the observed parameters, there has been no significant difference between the two groups. To our knowledge, this is the first study that compares VRQoL between two groups after iERM surgery.

MeSH/Keywords: Idiopathic epiretinal membrane, intraocular tamponade, pars plana vitrectomy, vision-related quality of life

Poster code: R-02-18-019

Poster Title: REFRACTIVE ERROR MANAGEMENT: NEW INSIGHTS INTO INHIBITING MYOPIA PROGRESSION IN CHILDREN AND ADOLESCENTS OF CENTRAL AND SOUTHEAST EUROPE

PhD candidate: Ana Maria Varošanec

Part of the thesis: Patterns of myopia development dynamics in children and adolescents of Central and Southeast Europe

Mentor(s): Professor Zdenko Sonicki, MD PhD, Associate Professor Mirjana Bjeloš, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Myopia, or nearsightedness, is one of the most significant diseases in ophthalmology, and in medicine in general. It is reaching epidemic proportions. It is predicted that almost half of the world's population will be myopic by 2050, and 30% of them will have high myopia. High myopia is associated with degenerative and irreversible changes in the optic nerve and the macula, due to which such patients remain poor-sighted or blind for life. Therefore, myopia represents a major public health as well as a socioeconomic problem. The aim of the study is to determine the patterns of myopia development in children and adolescents of Central and Southeast Europe.

Materials and methods: Retrospective data review of randomly selected patients diagnosed with primary myopia and followed-up in the pediatric ophthalmology clinics of the University Eye Department of University Hospital Sveti Duh within the period 2007.- 2021. Patient's age at the first and follow-up visits, and visual acuity at near and far monocularly and binocularly were recorded. Refraction was determined subjectively and objectively by retinoscopy in cycloplegia. Patients 0-18 years (y) of age from Central and Southeast Europe origin with primary naïve myopia and 2 follow-up visits over a period of 6 months were included and stratified according to age, sex, and classification of myopia. Analysis of myopia progression was modeled using analysis of variance (ANOVA).

Results: 78 female and 48 male patients (3 18 y) were collected. A total of 685 examinations were recorded. Mean follow-up time was 5.3 y for males and 5.6 y for female patients. Mean age and appropriate standard deviation (SD) was 12.5 with SD 4.1 y and 17.5 with SD 3.7 y for males and 11.5 with SD 3.0 y and 16.5 with SD 3.2 y for females at the first and last examination respectively. At the first examination, 10 female and 5 male patients were diagnosed with premyopia, 63 female and 40 male patients with myopia, and 5 females and 3 males with high myopia. Premyopia onset was at 13.0 with SD 5.2 y for males and 12.3 with SD 3.2 y for females, low myopia at 12.8 with SD 4.0 y and 12.0 with SD 3.0 y respectively, and high myopia at 11.6 with SD 4.9 y and 11.0 with SD 2.5 y respectively. At baseline, there was no difference between sex but at the age of 18 years, more females were myopic ($p<0.05$). Between the first and last examination, the average spherical equivalent (SE) of subjective refraction increased -1.0 with SD 0.4 D RE and -1.0 with 0.4 D LE ($p<0.05$) in female patients and -0.95 with 0.5 D RE and -0.96 with SD 0.4 D LE in males ($p=0.01$). Based on retinoscopy, the increase measured -0.1 with SD 0.1 D RE -0.1 with 0.1 D LE in females ($p=0.005$). For male patients, the average SE increase was -0.1 with 0.2 D RE and -0.1 with SD 0.1 D LE ($p=0.0087$).

Discussion: Females developed premyopia, low, and high myopia earlier than males. We hypothesize that observed sexual dimorphism of myopia development conforms to pubertal maturation. Moreover, at the age of 18 y, more females were myopic, advocating for sex as a modulating factor in the pathophysiology of myopia development. Overall, the earliest was the onset of high myopia, while premyopia outbroke the latest. Within the 5y of follow-up, the mean SE remained stable regardless of sex, while the subjective refraction increased for 1 D. In conclusion, undercorrecting the cycloplegic error for 1 D within the first five years of myopia onset proved to be effective in inhibiting myopia progression. Further studies are needed to affirm this relationship.

MeSH/Keywords: Myopia, Child development, Adolescent development, Retinoscopy

Poster code: R-02-18-073

Poster Title: Enlarged Foveal Avascular Zone in a Patient with Alport Spectrum Disorder Caused by a Heterozygous Variant of Uncertain Significance in COL4A4

PhD candidate: Mira Knežić Zagorec

Part of the thesis: Ocular morphological and biometric characteristics in Alport spectrum disorders

Mentor(s): Professor Tamara Nikuševa Martić, MD PhD, Associate Professor Damir Bosnar, MD PhD

Affiliation: University Eye Department, University Hospital Sveti Duh, Zagreb, Croatia; School of Medicine, University of Zagreb, Zagreb, Croatia

Introduction: Alport spectrum disorders (ASD) are genetically heterogeneous disorders characterized by ultrastructural abnormalities in type IV collagen 345 heterotrimers found in glomerular basement membrane, basement membrane of the organ of Corti, and basement membranes of the eye (Bowman's layer, Descemet's membrane, lens capsule, internal limiting membrane, and Bruch's membrane) due to mutations in COL4A3, COL4A4, and COL4A5 genes encoding alpha3, alpha4, and alpha5 chains of type IV collagen, respectively. The aim of this study was to investigate ocular morphological and biometric characteristics in ASD, and to determine if different types of mutations contribute to different phenotypes.

Materials and methods: This is a cross-sectional study. Patient data from Croatian Science Foundation's project Genotype-phenotype correlation in Alport syndrome and thin basement membrane nephropathy database was reviewed. Patients aged >= 18 years with identified mutation in COL4A3, COL4A4 or COL4A5 gene and matched controls underwent a comprehensive eye examination, including best-corrected visual acuity, slit-lamp examination, Goldmann aplanation tonometry, and dilated fundus examination. Furthermore, specular microscopy (CEM-530, Nidek, Gamagori, Japan), optical biometry (IOLMaster 700®, Zeiss, Oberkochen, Germany), and Scheimpflug imaging (Oculus Optikgeräte GmbH, Wetzlar, Germany) were performed. Moreover, multimodal retinal imaging was performed, including ultra-wide field color fundus photography and fundus autofluorescence (California, Optos®, Marlborough, MA, USA) as well as optical coherence tomography (OCT) and OCT-angiography (Spectralis®, Heidelberg Engineering, Heidelberg, Germany).

Results: Preliminary OCT-angiography study showed enlarged foveal avascular zone in both superficial and deep capillary complex in both eyes in a patient with a heterozygous variant of uncertain significance in COL4A4 (c.446G>A, p.Gly149Glu) and no observed OCT changes in macular morphology or thickness. In silico prediction tools supported a deleterious effect on the protein. In addition, best-corrected distance visual acuity was 0.0 logMAR in both eyes. Slit-lamp examination and dilated fundus examination were unremarkable.

Discussion: This finding highlights the importance of complementary role of comprehensive multidisciplinary clinical evaluation and genetic testing in ASD suspects. Defining the phenotypic spectrum of COL4 variants plays a key role in early diagnosis, clinical management, and follow-up. This is particularly important in the light of novel heterozygous variants in COL4A4 or COL4A3 due to wide variance of associated phenotypes. OCT-angiography offers an opportunity for non-invasive imaging of the retinal and choroidal vasculature, thus providing an another perspective for better understanding of microvascular changes in ASD.

MeSH/Keywords: Alport syndrome; type IV collagen; optical coherence tomography angiography

Poster code: R-02-18-124

Poster Title: Association between the expression of immunohistochemical markers BAP1 and histone deacetylases with uveal melanoma patients' survival

PhD candidate: Domagoj Vlašić

Part of the thesis: Association between the expression of immunohistochemical markers BAP1 and histone deacetylases with uveal melanoma patients' survival

Mentor(s): Associate Professor Tomislav Jukić, MD PhD, Professor Marijana Ćorić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Uveal melanoma (UM) is adults' most common primary intraocular malignancy. UMs with the BAP1 mutation have a worse outcome. Histone deacetylases (HDACs) are overexpressed in various malignancies, and BAP1 modulates HDAC-2 expression in mesothelioma cells.

Materials and methods: Clinical data on 60 patients in whom UM enucleation was performed from 01.01.2006-31.12.2016 will be extracted from the Ophthalmology Clinic database, University Hospital Center Zagreb, and the Croatian Cancer Registry. In addition, paraffin blocks of UM from the UHC Zagreb Pathology and Cytology Clinical Institute archives will be used for BAP1, HDAC-2, and -4 immunohistochemical analysis.

Results: HDAC-2 expression was mainly nuclear, with only a few cases (7/59, 12%) displaying also cytoplasmic immunoreactivity (Figure 1.). Nuclear HDAC-2 expression was observed in 39 (66%) of the 59 examined cases. HDAC-4 expression was cytoplasmic in 21 of the examined 59 cases (36%) (Figure 2.). The majority of positive cases displayed a moderate or strong HDAC-4 expression (14/31, 45%).

Discussion: Preliminary results indicate that there can be the possible existence of a predictive BAP1 value for HDAC amplification.

MeSH/Keywords: uveal melanoma, BAP1, HDAC-2, HDAC-4, overall survival, disease-free survival

Poster code: R-02-18-145

Poster Title: The effect of reduced muscle mass index on the treatment outcome of patients with metastatic colorectal cancer

PhD candidate: Domina Kekez

Part of the thesis: The effect of reduced muscle mass index on the treatment outcome of patients with metastatic colorectal cancer

Mentor(s): Professor Stjepko Pleština, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Reduced muscle mass index (MMI) and sarcopenia are present in a large percentage of patients with advanced malignancy during the treatment period, and is one of the major causes of morbidity and mortality.

Materials and methods: This is a prospective cohort study that included patients with metastatic colorectal cancer (mCRC) who started treatment for metastatic disease with chemotherapy at the UHC Zagreb from January 1, 2020 to December 31, 2021. Follow-up will last until December 31, 2023. BIA scale was used to assess reduced MMI in patients with first-line treatment of mCRC. The association of reduced MMI with treatment outcomes was monitored, ie, sex, age, type of first line treatment (chemotherapy and biological therapy), time to disease progression to the first line of treatment including maintenance therapy, association with primary tumor location (left or right), previous adjuvant treatment, number of organs affected by metastases, association of biological tumor characteristics (RAS and BRAF mutational status) with reduced MMI. All relevant data were collected from medical records. BIA estimates body composition using mathematical equations that use the difference in electrical conductivity of individual tissues to calculate body composition. The measurement is based on the passage of a low-voltage current through the body, during which tissue resistance and reactance are registered. Method is simple and easy to perform. Patients are weighed with a BIA scale (Tanita PRO) once a month before chemotherapy as part of a complete physical examination. Since there is no consensus on the threshold value of a reduced MMI in patients with mCRC it will be defined as SMI (skeletal muscle index; calculated values of skeletal muscle mass (kg)/squared height in m2) a value of one SD below average of the examined group (for men and women). According to the analysis of the power of the statistical test (used variables 0.05, HR 0.80 with statistical significance p< 0.005), it is necessary to analyze data for 110 patients. Standard statistical methods will be used in accordance with the data distribution, which include the 2 test, t-test, or Mann-Whitney U test, and Spearman's or Pearson's correlation, depending on the data distribution. For the population included in the research, divided according to the presence or absence of a reduced MMI, the Kaplan-Meier analysis (K-M product limit estimator) will be used to estimate the probability of overall survival and the time to disease progression, and for the comparison of individual group, the log-rank test will be used. The Cox proportional hazard model with a 95% confidence interval will be used to evaluate the predictions of the presence of a reduced MMI on overall survival and time to disease progression along with other potential influencing factors.

Results: The study included 112 patients with mCRC over a period of 2 years. The average age was 62 years. Of the 112 patients, 50% were men (56 patients), 50% women (56 patients). 96 patients had a primary tumor of the left colon (85.7%), and 16 patients had a tumor of the right colon (14.3%). RAS MT tumor was present in 54.4% of patients and RAS WT in 43.7%, one patient had proven BRAF mutation, and in one the mutation could not be determined. The number of metastatic sites ranged from 1 to 4 (73% of patients had liver metastases, and 35% had lung metastases). The patient's follow-up period is until the end of 2023, and then the impact of MMI on the patient's treatment outcomes will be assessed.

Discussion: The contribution of this research is the examination of the association of reduced MMI as a separate prognostic and predictive factor in patients with mCRC, and a potential early indicator of worse outcomes in the treatment of these patients in interdependence with other factors conditioned by tumor biology and patient characteristics, and for the first time in prospectively designed research.

MeSH/Keywords: reduced muscle mass index, sarcopenia, colorectal cancer

Poster code: R-02-19-007

Poster Title: Diagnostic and prognostic role of presepsin in febrile neutropenia in patients with Non-Hodgkins lymphomas

PhD candidate: Karla Mišura Jakobac

Part of the thesis: Diagnostic and prognostic role of presepsin in febrile neutropenia in patients with Non-Hodgkins lymphomas

Mentor(s): Associate Professor Gordana Pavliša, MD PhD, Professor Slobodanka Ostojić Kolonić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Febrile neutropenia (FN) and sepsis are common complications of intense cytotoxic therapy in patients with Non-Hodgkins lymphomas. Early diagnosis of sepsis is crucial in the prevention of serious complications, but it is still a challenge for the clinician because there is a lack of appropriate rapid diagnostic methods. Presepsin (PSP) is an immunologic biomarker, the circulating soluble form of the CD14 subtype.

Materials and methods: Subjects are NHL patients who develop FN during treatment. After laboratory, microbiological, and radiological processing, the subjects will be classified into one of two groups, depending on whether there is evidence of an infectious event. PSP levels will be determined on the first day and 72 hours after antimicrobial therapy.

Results: in the period from 2019 to 2023, a total of 102 serum samples of patients with non-Hodgkin's lymphoma diagnosed with FN were collected. A total of 61 patients participated in the study, of which 20 patients were the control group. The following demographic and clinical data were collected: age, gender, performance status, BMI, comorbidities, basic diagnosis, chemotherapy regimen, day since the start of chemotherapy of the last chemotherapy cycle, highest measured body temperature, blood pressure, prophylactic use of G-CSF, source of infection, presence of central venous catheter. Among the laboratory parameters, the following parameters were determined: leukocyte count, absolute neutrophil count, lymphocyte count, monocyte count, hemoglobin concentration, platelet count), sedimentation, AST, ALT, GGT, potassium, sodium, urea, creatinine, CRP, procalcitonin, LDH, proteins, albumins, immunoglobulins. Immune status was determined by flow cytometry in each patient. Routine biochemical parameters were determined on fresh biological samples of venous blood. Microbiological processing was also performed (blood cultures, urine culture, sputum). Each patient underwent a chest X-ray. Depending on the findings of the processing performed, the respondents were classified into one of the following groups: group 1 (patients with proven infection) or group 2 (patients without proven infection). On the 1st day of illness and 72 hours after the introduction of antibiotic therapy, venous blood was sampled for the determination of presepsin. The serum sample was centrifuged and stored in a freezer at -80°C. The samples were analyzed by the ELISA method. After taking blood for laboratory tests, the patient was adequately treated, including empiric antibiotic therapy according to the guidelines for the treatment of FN, along with all other necessary supportive treatment measures.

Discussion: The data analysis is still in progress.

MeSH/Keywords: febrile neutropenia, sepsis, non-Hodgkin lymphoma, presepsin

Poster code: R-02-19-101

Poster Title: Polo-like kinase 1 expression effect on treatment outcome in patients with metastatic colorectal cancer

PhD candidate: Lana Jajac Bručić

Part of the thesis: Polo-like kinase 1 expression effect on treatment outcome in patients with metastatic colorectal cancer

Mentor(s): Professor Slavko Gašparov, MD PhD, Assistant Professor Vesna Bišof, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Colorectal cancers that occur at an earlier age (younger than 50 years) in most cases have poorer treatment outcome and poorer overall survival. Overexpression of polo-like kinase 1 (PLK1) has been found in CRC and is associated with poorer survival.

Materials and methods: This retrospective study will include patients with metastatic colorectal cancer treated from January 1st, 2015, to December 31st, 2020. at the General Hospital of ibenik-Knin County or at the Clinical Hospital Center Zagreb. Patients will be divided into two groups; the first group will contain patients of younger age (at the time of diagnosis younger than 50 years), and the second group will contain patients of older age (at the time of diagnosis aged 50 or more). The sample size in the study will be a minimum of 140 patients (two groups of 70 patients). Differences in the frequency of individual clinical and pathological features between the two examined groups will be determined using the chi-square test or, if necessary, Fisher's exact test. The Wilcoxon-Mann-Whitney test will be used to compare continuous characteristics. The survival analysis will be performed by the Kaplan-Meir method. The existence of a difference in survival between the examined groups of patients will be tested by a log-rank test. The impact of individual variables on experience will be analyzed using the Cox model with proportional hazard. Values of $p < 0.05$ will be considered statistically significant.

Results: From May 2022., a database of a total of 162 patients was created. All relevant clinical data as well as tumor morphological characteristics and molecular status, were entered into an excel table. The data contains: age, gender, date of diagnosis of malignant disease (age at diagnosis of malignant disease), comorbidities, localization of primary tumor, histological type of tumor, tumor grade, TNM stage, K-RAS, N-RAS, BRAF status (presence or absence of mutation in the mentioned genes), microsatellite status (stability vs instability). The data about applied treatment also collected; whether local treatment of the primary tumor was preformed or not, whether adjuvant treatment was administered or not, if so, what type of therapy was used, which chemotherapy treatment the patient received, how many lines of treatment were applied, whether the patient was treated with biological therapy and which, duration of response to a particular line of therapy. ECOG performance status during treatment was also recorded as well as dynamics of tumor marker values. The data on metastases sites, number of metastases, local treatment of metastases, are also has been entered. At the departments of pathology paraffin tissue tumor blocks for all patients were collected. Then the tissue blocks were sliced up and prepared for the ongoing immunohistochemical examination. The expression of PLK1 protein will be determined by standard immunohistochemical staining on tumor tissue samples using a specific antibody. Samples will be dewaxed, antigens will be unmasked using high temperature, samples will be incubated with a specific antibody, and visualization will be done by an indirect polymer-based method with Dako commercial kit, EnVisionTM FLEX Hgh pH with chromogen 3,3'-diaminobenzidine (D) in the Dako Autostainer automated system (Dako). Immunohistochemical (IHS) staining results will be interpreted based on the IHS score where nuclear and cytoplasmic positivity of tumor cells will be considered.

Discussion: PLK 1 overexpression has been found in many types of cancer, including CRC, and is associated with poorer survival. As mCRC in the younger population are more aggressive and have worse treatment results, we expect that our data will support the hypothesis that PLK-1 expression is higher in the age under the 50. PLK1 overexpression could be a new predictive factor, ie a good target for targeted treatment with PLK1 inhibitors.

MeSH/Keywords: Polo-like kinase 1, young colorectal cancer, metastatic colorectal cancer

Poster code: R-02-19-125

Poster Title: The tourniquet effects on postoperative bleeding and the outcomes of elbow arthroscopy

PhD candidate: Igor Knežević

Part of the thesis: The tourniquet effects on postoperative bleeding and the outcomes of elbow arthroscopy

Mentor(s): Professor Ivan Bojanić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Elbow arthroscopy has become a gold standard in treating numerous elbow disorders. However, elbow arthroscopy is almost exclusively performed with the use of a tourniquet leaving it prone to known tourniquet complications.

Materials and methods: Our randomised prospective research would be conducted over a period of 24 months in a single institution. All the procedures will be performed by a single experienced surgeon in a standardised manner. The patients between the age of 18 and 65 in whom an elbow arthroscopy was indicated, would be included in the research. All under-aged patients and patients older than 65 as well as ones who had previous surgery on the elbow would be excluded. The patients with tumours around the elbow, who have coagulation disorders, systemic neuromuscular disorders, irreversible neural or vascular injuries and signs of local or systemic infections would also be excluded. Statistical analysis yielded a minimum required sample size of 40 patients randomly divided into 2 independent groups. The first (NT) group would consist of patients who underwent elbow arthroscopy without the use of a tourniquet, while the second (T) group would consist of patients who underwent elbow arthroscopy while a tourniquet was applied. Before the procedures, an independent examiner would obtain informed consent, and collect and anonymise the data. Detailed anamnesis and clinical examination precede filling out the Mayo Elbow Performance Score and the 36-Item Short Form Health Survey questionnaires. The same practice would be repeated 6 months after the surgery. The patients would record the highest pain intensity during the first 14 postoperative days on a visual-analogue scale. On the second postoperative day, an independent examiner would inspect the operated elbow and the postoperative bleeding volume would be recorded. A standardised postoperative rehabilitation protocol was identical for all the patients. During the follow-up period of a minimum of 6 months, all the reported complications would be noted and categorised as mild or serious according to Nelson et al. publication.

Results: Our preliminary results pretend to the first 27 participants, 12 in the NT group who were operated on without the use of a tourniquet and 15 in the T group who were operated on with the use of a tourniquet. The average tourniquet time was 107 minutes. The average procedure time in the NT group was 106.3 minutes, while in the T group it was 88.7 minutes. Mean systolic blood pressure was 117.5 mmHg in the NT group compared to 114 mmHg in the T group. Mean diastolic blood pressure was 68.3 mmHg in the NT group compared to 69 mmHg in the T group. Mean intraoperative irrigation fluid use was 12725 ml in the NT and 11000 ml used in the T group. Mean postoperative pain was 3.6 in the NT group compared to 4.3 in the T group. Mean postoperative bleeding volume was 81.5 ml in the NT group and 116.1 ml in T group.

Discussion: The preliminary results show that elbow arthroscopy without the use of a tourniquet lasts longer and a consumption of the irrigation fluid is higher than in the equivalent procedure with the use of a tourniquet. However, postoperative pain and postoperative bleeding volume is lower when the tourniquet was not used. These results are in line with our assumptions, still, the participants recruitment should be finished and completed results should be statistically analysed.

MeSH/Keywords: arthroscopy, elbow, tourniquet, bleeding

Poster code: R-02-20-087

Poster Title: Anatomical study of a quadruple gracilis tendon graft for the anterior cruciate ligament reconstruction

PhD candidate: Ivan Levaj

Part of the thesis: Biomechanical testing of a graft made of quadruple-folded gracilis muscle tendon for the reconstruction of the anterior cruciate ligament

Mentor(s): Professor Ivan Bojanić, MD PhD, Associate Professor Ana Pilipović, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Anterior cruciate ligament (ACL) tear is one of the most common injuries of the knee joint, and one of the most important prerequisite for successful ACL reconstruction is choosing a graft that restores the anatomical and biomechanical properties of the native ACL and has a minimal donor site morbidity. In this part of the research aim was to establish feasibility of using quadruple gracilis tendon graft for ACL reconstruction based on its anatomical specifications.

Materials and methods: Removal of gracilis and semitendinosus tendons from total of thirty cadavers (sixty knees) was performed, the tendons were debrided and stored in the freezer at a temperature of -20°. Tendons were divided into two separate groups and in each group quadruple gracilis tendon graft was compared to one of the two standard quadruple hamstring grafts: double-folded semitendinosus and gracilis tendon graft, and quadruple semitendinosus tendon graft. Before testing, the tendons were taken out of the freezer and left to thaw at room temperature. After thawing, overall length of each tendon was measured with a tape measure, after which the width and thickness of each tendon, at four predetermined points, were measured with a digital calliper. After construction of aforementioned grafts, dimensions of each graft were noted. Length was measured with digital calliper, while the diameter was determined by passing the quadruple grafts through a specially designed metal instrument, the graft sizer, with differences in hole diameter of 0.2 mm.

Results: At this point of our research we completed the anthropometric measurement of ten matched tendon pairs and their respective grafts for both groups. Total of twenty gracilis tendons were measured with average tendon length of 266.8 mm (SD 26.9), while the average length and diameter of their corresponding quadruple grafts were 65.89 mm (SD 6.41) and 6.48 mm (SD 0.61). Average length of semitendinosus tendons was 284.3 mm (SD 32.3), while the average length and diameter of their corresponding quadruple grafts were 70.30 mm (SD 7.84) and 8.16 mm (SD 0.51). Average length and diameter of quadruple hamstring graft composed of double-folded semitendinosus and gracilis tendons were 138.16 mm (SD 14.13) and 7.72 mm (SD 0.83).

Discussion: Preliminary results showed that average length of the quadruple gracilis tendon graft is above the required mark of 60 mm for all-inside ACL reconstruction technique. In only three out of twenty cases (15%) the tendon graft was under recommended length. Therefore, we can conclude that in the majority of cases gracilis tendon is sufficient in length to replace the intra-articular portion of the ACL and also to provide adequate tendon graft insertion into bone sockets at femoral and tibial ACL footprints. Average diameter of a quadruple gracilis tendon graft is just above the diameter of the native ACL recorded in the literature (6.2 mm, SD 0.7), however, it is significantly smaller compared to quadruple semitendinosus and double-folded semitendinosus and gracilis tendon grafts from this study. After confirmation of satisfactory anatomical specifications and in order to determine if quadruple gracilis tendon graft is sufficient for ACL reconstruction, adequate biomechanical study of aforementioned tendon grafts is being performed simultaneously.

MeSH/Keywords: anterior cruciate ligament, gracilis muscle, hamstring muscles, reconstructive surgical procedures

Poster code: R-02-20-118

Poster Title: PRAME expression in invasive breast carcinoma

PhD candidate: Lea Korša

Part of the thesis: Expression and prognostic value of PRAME antigen in invasive breast carcinoma

Mentor(s): Zlatko Marušić, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: PRAME (PReferentially expressed Antigen in MElanoma) is a carcinoma testis antigen expressed in numerous tumor types. The aim of this study was to assess PRAME expression in different surrogate subtypes of breast carcinoma and its correlation with other prognostic factors.

Materials and methods: This retrospective study included total of 89 patients who were diagnosed with invasive breast carcinoma on core needle biopsy at Clinical Department of Pathology and Cytology, University Hospital Centre Zagreb and had available archived tissue samples. Based on the immunophenotype of invasive breast cancer, samples were divided into four groups (25 Luminal A like, 31 Luminal B like, 18 HER2 positive and 15 triple negative breast carcinoma). All data relevant for the research were obtained from informatic system at University Hospital Centre Zagreb (age of the patient, pathohistological report, oncological treatment). Expression of PRAME antigen was assed in all core needle tissue samples by immunohistochemistry (IHC) using the EPR20330 (ab219650; Abcam) monoclonal antibody. Evaluation of the immunohistochemical analysis of PRAME reactivity was performed in consensus by two pathologists who were blinded to other information. Expression of PRAME was quantified as positive (nuclear and/or cytoplasmic staining) or negative, and as a percentage of tumor cells expressing PRAME. Sections of normal human testis tissue was used as positive control for PRAME reaction (nuclear PRAME expression).

Results: A significantly higher expression of PRAME was detected in HER2 positive carcinomas and TN breast carcinomas compared to ER positive (luminal like) subtype of breast carcinomas. PRAME expression was detected in 53% (8/15) TN carcinomas and 72% (13/18) HER2 positive carcinomas, as opposed to luminal A and B like breast carcinomas, where it was expressed in 32% (8/25) and 26% (8/31) of cases, respectively. Percentage of PRAME positive tumor cells showed positive correlation with tumor size, Ki67 proliferation index, HER2 status, nuclear grade and presence of metastasis, and negative correlation with ER status.

Discussion: Previous studies on PRAME in breast carcinoma were mainly based on RT-PCR detection, with immunohistochemical studies limited to polyclonal antibody results. Our study showed that HER2 positive and TN breast carcinomas more commonly express PRAME than ER positive carcinomas and that PRAME expression shows positive correlation with certain prognostic factors. The importance of PRAME expression in breast carcinoma lies in its potential use as an immunotherapeutic target, particularly in patients with limited therapeutic options (e.g. in TN carcinomas).

MeSH/Keywords: breast cancer; PRAME antigen; immunohistochemistry

Poster code: R-02-23-001

Poster Title: Fecal Calprotectin as a Biomarker of Food Allergy and Disease Severity in Children with Atopic Dermatitis without Gastrointestinal Symptoms

PhD candidate: Alen Švigir

Part of the thesis: Significance of fecal calprotectin in children with atopic dermatitis

Mentor(s): Professor Suzana Ljubojević Hadžavdić, MD PhD, Marta Navratil, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Fecal calprotectin (FCP) is a biomarker of intestinal inflammation and has recently been proposed as a diagnostic biomarker of food allergy (FA) in children.

Materials and methods: The aim of this study was to compare FCP level in infants and children under 4 years old with 1) atopic dermatitis (AD) with food allergy (FA) and 2) children with AD and without FA with the results in healthy controls. In total, 46 infants and children (mean age 14 months ± 12) diagnosed with AD were divided into two groups: G1, children with atopic AD with FA (n=28) and G2, children with AD without FA (n=18). The control group (G3) was made up of healthy children of the same age (n=18).

Results: The median FCP was significantly higher in G1 compared with G2 (G1: median 154, IQR 416 µg/g vs G2: median 41.3, IQR 59 µg/g; P=0.0096). The median FCP in children with AD and FA was significantly higher before elimination diet in comparison with FCP after 3 months of elimination diet (median 154, IQR 416 µg/g vs median 35, IQR 23 µg/g; P=0.0039). The level of FCP was significantly positively correlated with the SCORAD score (r=0.5544, P=0.0022).

Discussion: Our study showed a significant difference in level of FCP in patients with AD without FA compared with patients with AD and FA. We also found a positive correlation of FCP with SCORAD score, a biomarker of AD severity. New studies are needed to investigate the role of FCP as a biomarker of FA in children with AD.

MeSH/Keywords: allergy, atopic dermatitis, inflammatory disorders

Poster code: R-02-24-032

Poster Title: Dose of X-ray radiation during pediatric electrophysiological interventions on the heart

PhD candidate: Nikola Krmek

Part of the thesis: Dose of X-ray radiation during pediatric electrophysiological interventions on the heart

Mentor(s): Assistant Professor Vjekoslav Radeljić, MD PhD, Ivana Kralik, PhD, research associate

Affiliation: University of Zagreb School of Medicine; Gottsegen Cardiovascular Center, Budapest, Hungary; University hospital center Sestre milosrdnice, Zagreb, Croatia

Introduction: In electrophysiology (EP), the gold standard for displaying the position of a catheter in the patient's body is using X-rays. Determining the dose of radiation a patient receives, especially the impact of that radiation on individual tissues and organs, is very important. Although 3D mapping systems for displaying catheter position, which can lower the X rays use, have been in use for years, there are no papers that provide typical dose area product (DAP) values, estimated organ doses and effective doses for pediatric EP procedures for cases when X-ray radiation is also used. Data on X rays use are missing when determining guidelines in the treatment of arrhythmias, but also when providing information to patients or their caregivers.

Materials and methods: The research is a multicentric cross sectional study. The subjects are children under the age of 18 years who underwent a pediatric EP study with or without ablation in the period from 1.1.2016. until 31.12.2021. in Gottsegen Cardiovascular Center, Budapest, Hungary and from 1.1.2018. until 31.12.2021. in University hospital center Sestre milosrdnice, Zagreb, Croatia. Children with all types of arrhythmias were included. Children with congenital heart defects were also excluded since they represent a much smaller group that has its own specifics regarding the need to use X-ray radiation. All the procedures were done with the use of 3D mapping systems. Conversion factors for effective dose (ED) were used from the literature for interventional cardiology since the irradiated area, so also tissues and organs, in interventional cardiology and electrophysiology are the same.

Results: The research included a total of 914 children who underwent an electrophysiological study in the previously mentioned period. There were 509 (55.7%) male and 405 (44.3%) female children. The average age of the patients was 13.25 years. There were 80 (8.8%) younger than 7.5 years, 248 (27.1%) between 7.5 and 12.5 years, and 586 (64.1%) older than 12.5 years. The most common indication for the procedure was supraventricular tachycardia in 348 (38.1%) patients, followed by frequency: preexcitation 210 (23.0%), WPW syndrome 149 (16.3%), palpitations 138 (15.1 %), premature ventricular contractions (PVC)/ventricular tachycardia (VT) 69 (7.5%). The most common arrhythmological substrate determined by electrophysiological testing was right-sided accessory pathway in 303 (33.15%) subjects. They are followed by frequency: AVNRT 246 (26.92%), left sided accessory pathways 232 (25.38%), ventricular substrate 64 (7.00%), atrial tachycardia in 32 (3.50%). X rays were used in 346 (37.86%) patients, and in 568 (62.14%) the electrophysiological procedure was performed without radiation. The most common reason for using X rays was transseptal puncture in 218 patients (63.01 % of irradiated). The second most common reason was the positioning of a wire, introducer or catheter in 73 patients (33.49 % of irradiated), followed by mapping with a catheter in 40 (11.56 % of irradiated) and angiography in 15 (4.34 % of irradiated). Median DAP, when X rays were use, was 9 cGycm2 (minimum 0,01 cGycm2, maximum 642 cGycm2). Effective dose median was 32,82 µSv (minimum 0,03 µSv, maximum 4700 µSv).

Discussion: In this paper we showed frequency and doses of X rays use in pediatric electrophysiology while using 3D mapping systems. These doses are the contribution to reference levels in pediatric electrophysiology when using 3D mapping system. Compared to the diagnostic reference levels for pediatric interventional cardiology, doses shown in our paper are quite smaller thanks to 3D mapping systems. Also, values in this paper are lower when compared to pediatric electrophysiology procedures using low fluoroscopy frame rate, but without 3D mapping systems. Values of ED in pediatric electrophysiology are comparable to a single X ray of abdomen. In todays time, pediatric electrophysiology can be done mostly without use of X ray radiation, and when used, dose of radiation is minimal.

MeSH/Keywords: electrophysiology, heart, children, X-rays, radiation dose

Poster code: R-02-24-058

Poster Title: The relationship between the composition of the bacterial intestinal microbiota and the development of active disease in children with celiac disease

PhD candidate: Mario Mašić

Part of the thesis: The composition of the intestinal bacterial microbiota in children with celiac disease and their healthy relatives and analysis of the relationship with the occurrence of the disease

Mentor(s): Assistant Professor Zrinjka Mišak, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: The mechanisms of the development of celiac disease are not fully understood. Recent studies are focused on bacterial intestinal microbiota as an additional factor that favors the development of active disease. We aim to compare the composition and diversity of the bacterial intestinal microbiota in children newly diagnosed with active celiac disease with the intestinal microbiota of their healthy siblings and healthy unrelated children.

Materials and methods: This is a prospective, observational cohort study, which included children diagnosed with active celiac disease, their healthy siblings, and healthy peers. Patients were recruited at Children's Hospital Zagreb. Celiac disease was diagnosed according to current ESPGHAN guidelines in children younger than 18 years of age. We acquired fecal samples from the patients who were diagnosed with CD and from the control groups. The fecal samples were taken before the diagnosis and after the induction of remission with a gluten-free diet. All the fecal samples were frozen at the temperature of -80°C. The analysis of the intestinal microbiota is performed by fecal DNA extraction from a 150 mg stool sample and by PCR amplification of 16S ribosomal RNA. Afterward, we determine the polymorphisms of the length of the terminal restriction fragments. Terminal restriction fragments are joined by OTU (operational taxonomic units). Assignment of OTU to bacterial taxonomy is done according to the RDP (Ribosomal Database Project) 16S rRNA database. The comparison of the diversity of the OTUs obtained between the study groups will be done by the Shannon-Wiener diversity index, cluster analysis will be performed using BioNumerics software, and the dendrogram (showing the similarity of the intestinal microbiota between groups) will be calculated using the Pearson correlation coefficient and the unweighted array method. The difference in the distribution of taxonomic units will be analyzed by PERMANOVA (permutational multivariate analysis of variance) analysis.

Results: We included 20 children diagnosed with CD at Children's Hospital Zagreb (45% male, 55% female), with median age of 7,88 years (1,25y 16,5y). On the presentation, 40% of children were asymptomatic and discovered due to active screening, having a first-degree relative with CD. Others were symptomatic, and the most common symptom was abdominal pain (35%), and anemia (10%). On the presentation, the children were mildly malnourished, with a median bodyweight z-score of -0,69 (-2,13 1,54), and body height z-score 0.05 (-2,52 2,18). Children were diagnosed with CD due to ESPGHAN criteria, with 35% of them being diagnosed with a no-biopsy approach. Others went through diagnostic small bowel biopsy, and two patients had Marsh 2, four had Marsh 3a, three Marsh 3b, and three had Marsh 3c lesions on biopsy samples. After diagnosis, fecal samples were taken from all patients before GFD was introduced and frozen at -80°C temperature. After a minimum of 12 months on GFD, adherence to the diet was checked with a Biagi score, and another fecal sample was taken from the participants and frozen for further analysis. In the first control group, the group of first-degree relatives, a fecal sample was taken from 10 participants. In the second control group, a group of non-related healthy peers, a fecal sample from 20 participants was obtained. All samples are awaiting analysis.

Discussion: This research will determine the composition and diversity of the bacterial microbiota in celiac patients during active disease and during remission in relation to their healthy siblings and healthy unrelated peers. It will be determined whether the introduction of a gluten-free diet leads to changes in the composition and diversity of the intestinal microbiota, which could improve our understanding of the disease development.

MeSH/Keywords: celiac disease; microbiota; gluten-free diet, 16S ribosomal RNA

Poster code: R-02-24-083

Poster Title: Analysis of HMGB-1, RAGE, PCDH1 and Gd-IgA1 in patients with IgA vasculitis

PhD candidate: Martina Held

Part of the thesis: Analiza HMGB-1, RAGE, PCDH1 i Gd-IgA1 u bolesnika s IgA vaskulitisom

Mentor(s): Professor Marija Jelušić, MD PhD, Ana Kozmar, PhD

Affiliation: University of Zagreb School of Medicine

Introduction: IgA vasculitis (IgAV) is the most common childhood vasculitis, clinically characterized by a palpable purpuric rash, which predominantly affects the lower extremities, diffuse abdominal pain, arthritis/arthralgias, nephritis and/or IgA deposition in biopsy specimen (skin, intestinal tract, kidney). Although it is a self-limiting disease with favorable outcomes, various acute and chronic complications can occur. The most important chronic complication and the main cause of morbidity and mortality among children suffering from IgAV is IgAV nephritis (IgAVN), which therefore represents the main prognostic factor. However, there are currently no biomarkers in routine clinical use for IgAV that can indicate active disease and predict acute and chronic complications with an emphasis on the risk of developing kidney disease progression. In this study, we assessed a noninvasive multi-biomarker approach in the diagnostic test for IgAV.

Materials and methods: Demographic and clinical data, as well as routine laboratory tests, were analyzed, and venous blood and urine samples were collected in the acute phase and after 6 months in IgAV patients and once in the control group. Serum and urine concentration of the following biomarkers: galactose-deficient immunoglobulin A1 (Gd-IgA1), high mobility group box 1 (HMGB1), receptor for advanced glycation end products (RAGE) and protocadherin 1 (PCDH1) were examined and measured by enzyme-linked immunosorbent assay (ELISA) and urine values were corrected for urinary creatinine.

Results: Among 63 pediatric IgAV patients 34 were girls and 29 were boys with median (range) age 6.25 (4.5-7.38) at the time of the diagnosis. Purpuric rash was present in all patients, 90,4% had joint involvement, 41.27% had gastrointestinal involvement, while 34.92% of them developed IgAVN. Purpuric rash extended from the lower extremities and gluteal region had 39,68% patients, 10.87% had relapses of purpuric rash while 4.76% developed the most severe skin symptoms which included ulcerations and necroses. NSAIDs (non-steroidal anti-inflammatory drugs) were the most frequently prescribed medicines in 90.47% patients, followed by glucocorticoids administered in 44.44% and ACE (angiotensin converting enzyme) inhibitors in 19.05% patients while 14.28% patients received immunosuppressants. Urine PCDH1 concentrations were statistically higher in IgAV patients compared to the control group [57.79 (4.14-246.29) ng/mmol vs. 2.06 (1.54-5.17) ng/mmol, $p<0.001$]. Urine PCDH1 concentrations were lower among IgAV patients who developed GI manifestations compared to the rest of the patients [4.14 (3.17-6.40) ng/mmol vs. 179.86 (88.14-389.39) ng/mmol, $p<0.001$].

Discussion: This prospective cohort study furthers our understanding of the pathophysiology of IgAV since children with IgAV have increased urinary PCDH1. We suggest that PCDH1 has a role in the immunoinflammatory process of IgAV, especially in the acute phase. The reasons for the increased urine PCDH1 observed in IgAV patients can be multifactorial. PCDH1 is a membrane protein that makes a subgroup of cadherins and has an important role in maintaining connections between keratinocytes and glomerular cells endothelium and epithelium of proximal tubules of the kidney. Statistically significant difference of lower urine PCDH1 concentrations among IgAV patients who developed GI manifestations compared to the rest of the patients was observed. Therefore, PCDH1 may be involved in the pathophysiology of gastrointestinal manifestations of IgAV and could serve as a biomarker in distinguishing those patients who will develop GI symptoms. Further studies are needed to clearly establish the roles of PCDH1 in the pathogenesis of IgAV.

Acknowledgments: Croatian Science Foundation Project IP-2019-04-8822

MeSH/Keywords: IgA vasculitis; biomarker; protocadherin-1

Poster code: R-02-24-102

Poster Title: Monitoring the effect of therapy in patients with lung cancer using ultralow-dose CT examination - a pilot study

PhD candidate: Ivana Kuhtić

Part of the thesis: Sensitivity and specificity of ultra-low-dose chest and proximal abdominal computed tomography in the follow up of the patients with lung cancer during the therapy

Mentor(s): Assistant Professor Maja Hrabak Paar, MD PhD, Associate Professor Marko Jakopović, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: To determine the sensitivity and specificity of ultra-low-dose computed tomography (ULDCT) compared to standard computed tomography (CT) with a full radiation dose in the detection of measurable lesions in patients with lung cancer, and to compare the radiation dose of these two types of examinations.

Materials and methods: The pilot study included 30 subjects with lung cancer who were referred for a follow-up chest and abdomen CT scan with intravenous administration of a contrast agent. All participants filled out informed consent for inclusion in the study. CT scan was done on a Revolution Apex 160 scanner after intravenous iodine contrast agent administration. The delay for the standard CT protocol was 60 seconds with the tube peak voltage of 120 kVp and automatic exposure control. The ULDCT examination was performed immediately after the standard CT examination with a corrected tube peak voltage (100 kVp) and automatic exposure control. The sensitivity and specificity of the ULDCT examination in the detection of measurable lesions (tumors, enlarged lymph nodes, distant metastases) were calculated in comparison with the standard CT protocol. The Wilcoxon signed-rank test was used to compare the dose-length product (DLP) between the two imaging protocols.

Results: The sensitivity of the ULDCT examination in the detection of measurable lesions is 88%, and the specificity is 80% compared to the CT protocol with a full dose of radiation. DLP with the ULDCT protocol (median 52 mGy*cm, IQR 40-55 mGy*cm) was significantly lower as compared to the standard CT protocol (median 190 mGy*cm, IQR 132-210 mGy*cm; $W=465$, $p<0.001$).

Discussion: According to the results of the preliminary study, the sensitivity and specificity of the ULDCT examination in the detection of measurable lesions in patients with lung cancer is acceptable, with a significantly lower radiation dose. It is necessary to conduct further research on a larger number of subjects to determine for which type of lesions ULDCT imaging is sufficient, intending to reduce the cumulative radiation dose during prolonged active follow-up.

MeSH/Keywords: lung cancer, the effect of therapy, ultralow-dose CT protocol

Poster code: R-02-25-003

Poster Title: Orbital measurements for sex estimation in Croatian population

PhD candidate: Elvira Krešić

Part of the thesis: Craniometric analysis of sexual dimorphism of the modern Croatian population using MSCT

Mentor(s): Igor Erjavec, PhD, research associate, Associate Professor Željana Bašić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: As a part of my doctoral thesis, we published a study aimed to investigate sexual dimorphism of the orbital region in the contemporary Croatian population using MSCT images from the virtual collection of the University Department of Forensic Sciences, University of Split.

Materials and methods: This retrospective study included head CT scans of 414 adult patients (214 male and 200 female). Excluding criteria were: condition after skull trauma, condition after craniotomy, and slice thickness >1 mm. A total of 217 patients (115 male and 102 female) were scanned at the University Hospital Centre Split, Department of Diagnostic and Interventional Radiology, Split, Croatia.197 patients (98 male and 99 female) were scanned at the University Hospital Centre Zagreb, Department of Diagnostic and Interventional Radiology, Zagreb, Croatia. Images were reconstructed to the original slice thickness with a soft tissue convolution kernel. DICOM files obtained by scanning were imported into Stratovan Checkpoint Software Version 2020.10.13.0859. Files were loaded and viewed in 2D (axial, sagittal, and coronal plane) and 3D using semi-transparent 3D volume rendering. Twelve landmarks were included, as follows: zygomaxillare anterior (zma), zygoorbitale (zo), dacryon (da), ectoconchion (ec), and landmarks on lower and upper orbital margin that define the orbital height. Landmarks defined by zygomaxillary sutures: zygomaxillare anterior (zma) and zygoorbitale (zo) were defined in axial view and simultaneously controlled on 3D model. Dacryon (da) was marked directly on 3D model and checked on the axial plane. To mark ectoconchion (ec), the axial plane was first moved through the 3D model/coronal view and rotated until the plane was aligned with the superior orbital border. This plane was lowered until it bisected the orbit into two equal halves. The landmark was finally marked in axial view. Unnamed landmarks on inferior and superior orbital borders were located by moving the sagittal plane on 3D model until it bisected the orbit into equal medial and lateral halves. Then the landmarks were placed in sagittal view. Landmarks collected on each specimen were exported as .nts files and loaded into the R (version 3.6.2), Rstudio (version 1.2.5033) using geomorph package. The same package was then used to define eight interlandmark distances as standard orbital measurements. We used principal component analysis (PCA) to explore sexual dimorphism and possible regional differences in orbital measurements. Differences between males and females in Split and Zagreb regions and sex-related differences were tested using an independent samples t-test. To develop sex classification models, we used linear discriminant analysis. The models were developed for single variables, while the multivariate model was built using a stepwise feature selection. Sex estimation accuracy was tested using a leave-one-out cross-validation method and provided separately for males and females. Data was visualized in the R using ggbiplot package. The statistical analysis was conducted using IBM SPSS software (version 22, SPSS Inc., Chicago, IL, USA), with a level of statistical significance set at $P<=0.05$.

Results: The PCA analysis showed separation based on sex and region, and additional analysis demonstrated that the females and males in Split and Zagreb differed in four orbital measurements ($P<=0.001$). Only those measurements that did not show regional differences were analyzed and all of them showed statistically significant sexual dimorphism. The accuracy of univariate functions for sex estimation ranged from 53.43% to 71.88% and for multivariate functions, the accuracy was 73.45%.

Discussion: The orbital measurements of the Croatian population showed restricted forensic relevance in sex estimation. Also, interpopulation variability was found (PCA analysis showed the separation of Split and Zagreb samples). This is could be an interesting finding as previous studies did not take interpopulation differences into consideration, even when larger populations were considered. The interpopulation differences can be explained by several factors such as historical events and natural selection through

climate adaptation. These differences can be observed both in some features of the neurocranium and viscerocranium.

MeSH/Keywords: MSCT, orbital area, sexual dimorphism; Croatia; forensic anthropology

Poster code: R-02-25-018

Poster Title: Expression of PD-L1 in molecular subtypes of Muscle-invasive bladder cancer

PhD candidate: Matej Knežević

Part of the thesis: Expression of TIGIT, LAG-3 and PD-L1 immune ligands in molecular subtypes of Muscle-invasive bladder cancer

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

Affiliation: University of Zagreb School of Medicine

Introduction: Muscle-invasive bladder cancer (MIBC) is an aggressive disease that requires multimodal treatment. MIBC is a also a heterogeneous tumor with different molecular profiles and accordingly different behavior. The two main molecular subtypes are luminal and basal MIBC. Additionally, MIBC is an immunoreactive tumor and recently immunotherapy has been developed as a novel promising therapeutic option. The purpose of immunotherapeutic approach modalities is to restore the anti-tumor immunity, which has led to a change in the paradigm of MIBC treatment.

Materials and methods: The expression of PD-L1 biomarker in the main molecular subtypes of MIBC was investigated. Based on a random selection of 43 patient samples with material obtained after transurethral resection of urinary bladder cancer and a diagnosis of MIBC, luminal and basal MIBC subtypes were distinguished. Phenotyping was performed by immunohistochemical analysis for Cytokeratin 5/6 and GATA3.

Results: In the basal subtype cancer group, a higher expression of PD-L1 in immune cells was obtained. In the positive samples of basal MIBC, expression of 10-90 % was detected. Samples of the luminal subtype cancer group predominantly showed a reaction of less than 5 %, indicating a negative PD-L1 response.

Discussion: With the aim to analyze the immune microenvironment in the molecular subtypes of MIBC, we performed immunohistochemistry focused on PD-L1 intensity in all cancer specimens. Benefit of the immunotherapy application based on immune checkpoint inhibitors is usually expected in patients with strong immune response identified in the tumor microenvironment. Difference in positivity can suggest changes in treatment approach. The result of this study potentially advances the understanding of the MIBC immune context. This could also potentially contribute to easier selection of a subgroup of patients who could benefit from immunotherapy.

Acknowledgments: /

MeSH/Keywords: Biomarkers, Cancer microenvironment, Immunohistochemistry, Muscle-invasive bladder cancer.

Poster code: R-02-28-098

Poster Title: Study of psychological personality indicators in female patients with obesity and anorexia nervosa

PhD candidate: Filip Mustač

Part of the thesis: Psychological indicators of personality in obese patients and patients with anorexia nervosa

Mentor(s): Associate Professor Darko Marčinko, MD PhD, Martina Matovinović, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Despite the relatively well-known problem of eating disorders in today's society, modern pharmacological and non-pharmacological therapy of anorexia nervosa and obesity, the mentioned diseases still represent a significant challenge in treatment since the mentioned disorders significantly impair the quality of life and predispose the individual to numerous somatic risks and diseases. The role of personality structure in these entities is increasingly mentioned as very important in the treatment process and one that can prevail over the development of the disorder itself, i.e. work on personality as crucial in the therapeutic process.

Materials and methods: Female patients aged 18-45, divided into two clinical groups (patients with anorexia nervosa and patients with obesity), participate in this cross-sectional study. Patients are included in the research consecutively. Personality factors (dimensions of temperament, especially novelty seeking and harm avoidance, uncertain reflective functioning, displacement, internal shame, vulnerable narcissism) in two clinical groups are measured with validated questionnaires: TCI-140, DSQ-40, B-PNI, RFQ-8 , EISS, DASS-21, CTQ-SF and DEBQ. Multivariate analysis of covariance (MANCOVA) will be used for comparisons between clinical groups in personality indicators, while controlling for appropriate sociodemographic and clinical variables.

Results: Data were collected using the above-mentioned validated questionnaires for a larger part of the planned sample in both clinical groups. Data were collected live (face to face) at the Department of Psychiatry and Psychological Medicine at the University Hospital Centre Zagreb and at the Centre for Eating Disorders BEA in direct consultation with patients, during which they had the opportunity to ask all the time if a question was not entirely clear to them. A database containing socio-demographic data was created, which will be correlated with the results of validated questionnaires in statistical analysis.

Discussion: We expect that the results of this research will recognize the similarities and differences in psychological personality indicators between female patients with anorexia nervosa and obesity, especially in the dimensions of temperament, vulnerable narcissism and internal shame, highlight the importance of recognizing psychodynamic phenomena in these disorders and, through deeper knowledge, indicate the possible need for psychotherapy in given groups of patients.

MeSH/Keywords: anorexia nervosa, obesity, psychological personality indicators, shame, narcissism

Poster code: R-02-29-086

Poster Title: Cognitive functions and regional cerebral blood flow in patients with early schizophrenia

PhD candidate: Ivona Orlović

Part of the thesis: Cognitive functions and regional cerebral blood flow in patients with early schizophrenia

Mentor(s): Assistant Professor Tomislav Jukić, MD PhD, Associate Professor Dalibor Karlović, MD PhD

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

Introduction: Cognitive impairment in schizophrenia is considered to be the main domain of the disease and a significant predictor of the patient's functional outcome. Patients with the first episode of schizophrenia show cognitive function deficits, which could remain relatively stable for years after the first episode, regardless of clinical symptom fluctuations. One-photon emission computed tomography (SPECT) can be used to examine the relationship between cognitive deficits and abnormal patterns of regional cerebral blood flow (rCBF) in regions responsible for cognitive functions.

Materials and methods: This cross-sectional study included 100 subjects of both sexes aged 19 to 30 who were treated at the Psychiatric Clinic of University Hospital Centre Sestre Milosrdnice. Subjects were divided into two groups, 50 subjects with the first episode of schizophrenia and 50 subjects with an early course of schizophrenia, i.e. those who had more than one episode and disease duration less than five years. The term early schizophrenia will be used for the total sample of subjects. Subjects with the first episode of schizophrenia were drug naive. Subjects with an early course of schizophrenia were drug free for at least three months prior to actual treatment. Socio-demographic and anamnestic data were examined by a structured questionnaire. The severity of the clinical picture was assessed using the Schizophrenia Positive and Negative Symptoms Scale (PANSS), cognitive functions were measured using a 5-KOG cognition screening test, and rCBF was scanned using SPECT with Tc-99m-HMPAO. Changes in brain activity were quantified with commercial software and the deviation was calculated for each region of interest activities from normal values for age (MIM neuro, MIM Software Inc., Cleveland, OH, USA; https://www.mimsoftware.com). For each subject were determined the same regions of interest in both cerebral hemispheres (superior and inferior medial frontal gyrus, middle frontal gyrus, medial orbital gyrus, gyrus rectus, anterior cingulate gyrus, thalamus, hippocampus, caudate, lateral and medial temporal lobe, orbitofrontal region), and the deviation of activity of standard SPECT rates for certain age was calculated for each region. Statistical analysis was performed using SPSS software. Sociodemographic, clinical characteristics, cognitive functions and rCBF were presented with descriptive statistics. The Kolmogorov-Smirnov test was used to estimate the normality of the distribution of continuous values. Differences in quantitative values were assessed by the t-test or the Mann-Whitney U test. Differences in categorical variables were analyzed by X2 test or Fisher's exact test. The correlation coefficients between changes in regional cerebral blood flow and cognitive functions were calculated, and regression models assessed the multivariate effect of individual variables on cognition.

Results: The preliminary results indicate that subjects with early schizophrenia show moderate to severe function deficits in the examined cognitive domains. These domains included short and long term memory, attention, processing speed, working memory, verbal fluency and executive functions. Preliminary results also confirm that subjects with early schizophrenia show deviation of rCBF activity, mainly expressed in fronto-temporal region. Based on the current results, it is possible to conclude that the hypothesis of this research has been confirmed, two groups of subjects differ significantly in the certain deficits of cognitive functions and reduction of fronto-temporal cerebral blood flow.

Discussion: Abnormal patterns of the brain perfusion especially in fronto-temporal region could be associated to cognitive function deficits presented already in the first episode of schizophrenia. It is possible that reduction in fronto-temporal cerebral blood flow affects impairment of cognitive functions considering their anatomical background. Studies show contradictory results regarding rCBF abnormality, which is noticeable when comparing cognitive deficits of the first episode schizophrenia and longer duration of the disorder.

MeSH/Keywords: Schizophrenia, Cognitive dysfunction, Psychopathology, Single-Photon Emission-Computed Tomography, Regional Cerebral Blood Flow

Poster code: R-02-29-121

Poster Title: Art therapy as adjuvant treatment for depression in adults - a randomized controlled clinical trial

PhD candidate: Ivan Barun

Part of the thesis: Art therapy as adjuvant treatment for depression in adults - a randomized controlled clinical trial

Mentor(s): Associate Professor Igor Filipčić, MD PhD, Assistant Professor Sonja Vuk, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Depressive disorders are mental disorders characterized by pervasive low mood, low self-esteem, and loss of interest or pleasure in activities a person used to enjoy in. The combination of pharmacotherapy and psychotherapy is regarded as optimal strategy for treating depression. One of the possible treatments for depressive disorders is art therapy a form of psychotherapy which uses art as a medium for expression and communication.

Materials and methods: Randomized controlled trial of 65 adults with depressive disorders: experimental group 12 weekly art therapy sessions + ambulatory care; control group ambulatory care alone (psychopharmacotherapy and supportive conversation). Battery of standardized tests at three points (T1=beginning of the study; T2= end of the period of twelve weeks; T3=after 12 weeks follow-up) consisting of (BDI-II) Beck Depression Inventory, (HRSD) Hamilton Rating Scale for Depression, (BAI) Beck Anxiety Inventory (BAI) and (HRSA) Hamilton Rating Scale for Anxiety will be used.

Results: The trial is currently in the phase of participant recruitment where 35 patients with depressive disorders (22 with moderate depressive disorder and 13 with major depressive disorder) in the ambulatory care have been recruited and assesed with battery of tests. Intervention art therapy session have been designed to target the symptoms of depression and anxiety specifically.

Discussion: Validation of specific effect of psychodinamically oriented art therapy on symptoms of anxiety and depression in patients suffering from varying severity of clinical manifestation of depressive disorders with the possibility of designing specific art therapy programmes with the aim of improving mental health of this population.

MeSH/Keywords: art therapy, depressive disorder, anxiety, randomized controlled trial, creativity

Poster code: R-02-29-146

Poster Title: Sonoelastography in the assessment of skeletal muscle rigidity in patients with Parkinson's disease

PhD candidate: Vanja Vojnović

Part of the thesis: Sonoelastography in the assessment of skeletal muscle rigidity in patients with Parkinson's disease

Mentor(s): Associate Professor Gordana Ivanac, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: The diagnosis of Parkinson's disease (PD) is clinical, based on the main clinical characteristics (UK PD Society Brain Bank diagnostic criteria). There is no specific diagnostic method or laboratory finding that can unequivocally confirm the diagnosis of PD; therefore, today much attention is paid to PD biomarkers. Recently, sonoelastography has also been mentioned as a possible biomarker of PD. The most prominent symptom of PD is muscle rigidity. Currently, the main methods of clinical assessment of muscle rigidity are physical examination and palpation, most often expressed through the UPDRS-III score. We will try to prove that sonoelastography can be used to objectively assess muscle rigidity in patients with PD and therefore, be a possible biomarker for PD.

Materials and methods: For the purpose of presenting preliminary results of our research, we included the results of 20 patients with PD and 20 healthy controls, matched in sex and age. In the PD group inclusion criteria were age 18 years and a diagnosis of PD according to British Brain Bank Criteria. Exclusion criteria were other neurological diseases or conditions that may clinically manifest with muscle rigor or spasticity. In the control group, we included adults (age 18 years), and here the exclusion criteria were history of illness or medication that could affect their muscle tone. Before the ultrasound examination, each subject was given a UPDRS-III score. The ultrasound examination was performed on ultrasound machine, Aixplorer Mach 30 (SuperSonic Imagine, France), in shear-wave elastographic (SWE) mode, with a high-frequency L18-5 probe. We compared the elastographic values of the biceps brachii muscle of patients with PD and the healthy population. Within the group with PD, we also compared the symptomatically dominant arm (the arm particularly affected by rigor) and the symptomatically non-dominant arm (the arm less affected by rigor). The correlation of the UPDRS score and the obtained elastographic values was tested with the Spearman rank correlation. The significance of the difference in the elastographic values of the biceps brachii muscle in the healthy population and the PD patients (and in the symptomatically dominant and non-dominant arm) was tested using t test. The level of significance was set at $P<0.05$

Results: We are presenting our preliminary results in this research. We found that the elastographic value of biceps brachii muscle in symptomatically dominant arm in patients with PD was 176.8±61.5 kPa, in symptomatically non-dominant arm in patients with PD was 104.6±29.8 kPa and in healthy controls was 54.6±10.5 kPa. There was a significant difference between elastographic values of biceps brachii muscle in patients with PD and healthy controls ($p<0.05$), and no significant difference between elastographic values in symptomatically dominant and non-dominant arm in patients with PD. A positive linear correlation was found between elastographic values of biceps brachii muscle in patients with PD and their UPDRS-III scores ($rs=0.84$).

Discussion: Our results show that the elastographic value of biceps brachii muscle in patients with PD was higher than in healthy controls. However, there was no significant difference between remarkably and mildly symptomatic arms. This goes to show that PD is a systemic disease, with unilateral presentation only at the early beginning of the disease. The large standard deviation of elastographic values in PD group may result from gender, different disease duration and duration of medication treatment. There was a positive correlation between the elastographic value and UPDRS-III score in patients with PD. In conclusion, our results show that sonoelastography can quantify, i.e. objectify, the rigidity of skeletal muscles. With that in mind, we are of the opinion that sonoelastography could be used to monitor the treatment and progression of PD, and therefore, be a potential biomarker of PD.

MeSH/Keywords: Parkinson's disease, biomarker, muscle rigidity, sonoelastography

Poster code: R-02-30-024

Poster Title: Results after two-year follow up of autonomic nervous system impairment in people with early multiple sclerosis

PhD candidate: Berislav Ruška

Part of the thesis: Long-term follow up of autonomic nervous system impairment in people with multiple sclerosis from the stage of clinically isolated syndrome

Mentor(s): Associate Professor Mario Habek, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Research has shown that autonomic dysfunction (AD) occurs commonly in people with multiple sclerosis (pwMS). It is believed that demyelinating lesions, which are main feature of MS, can affect any part of central nervous system including parts that are important for normal autonomic nervous system function. Neuroimmune interactions that occur at the same time also contribute to AD, but exact pathophysiological mechanisms need to be elucidated. AD in pwMS is very important because research shows that AD affects quality of life of pwMS, Additionally research shows that AD can also be predictor of the future course of the MS in a single patient. Although studies have shown that subjective and objective AD is present in pwMS, it is not well known how AD changes in pwMS over the disease course because studies investigating long-term development of AD during the disease course are scarce. Therefore this PhD research aims to track changes of AD in pwMS form the earliest stage of the disease for a longer time period which is certainly a novelty in understanding of AD in pwMS.

Materials and methods: This prospective cohort study enrolled 121 participants who have been diagnosed with clinically isolated syndrome at the baseline. Enrollment of participants occurred between August 2014 and February 2016. In this timeframe initial test of AD has been performed in each participant. Participants have then been followed for six years during which tests have been repeated in a two-year intervals. Follow-up of all of the participants ended in July 2022. All of the tests were performed in Laboratory for ANS testing at the Clinic of Neurology, University Hospital Centre Zagreb, Croatia. All of the 121 participants were initially screened for drugs and diseases that could affect ANS function. At the baseline, each participant had a thorough neurological exam with EDSS evaluation, completed COMPASS-31 questionnaire, completed brain MRI, and underwent a battery of neurophysiological tests for objective evaluation of ANS function. Battery of ANS tests included Quantitative Sudomotor Axon Reflex Test (QSART), heart rate and blood pressure responses to the Valsalva maneuver, heart rate response to deep breathing test and blood pressure response to passive tilt test. Results of these tests are then expressed with ten-point Composite Autonomic Scoring Scale (CASS) divided into three domains- sudomotor (0-3 points), cardiovagal (0-3 points) and adrenergic (0-4 points), with each point higher meaning more severe impairment.

Results: Main preliminary analysis has been performed after two years of follow up. At the time, data was available for 84 participants. There was a high drop-out rate because lot of patients were not from Zagreb. However complete CASS data was available for 62 patients. In 24 (38.7%) patients there was worsening, in 16 (25.8%) there was improvement and in 22 (35.5%) there was no change in CASS score. If we look at data separately, in 90% of participants, for which appropriate data was available, there was no change in parasympathetic nervous system tests. In 47.3% there was either worsening or improvement in sympathetic adrenergic function. In the end 28.6% had similarly either worsening or improvement in sudomotor function.

Discussion: Results show that significant number of pwMS developed worsening of ANS function during a two-year follow-up, whereas only a quarter of patients improved in the same time frame. Results also show that in those first two years changes were mainly seen in adrenergic and sudomotor indices of CASS, therefore indicating that in early stages of MS both adrenergic and cholinergic parts of the sympathetic nervous system experience significant fluctuations. This results are in line with current opinion that parasympathetic nervous system is relatively spared in early disease stages. It will be very interesting to see how these results change over six-year disease course when the final analysis is completed.

MeSH/Keywords: multiple sclerosis, autonomic dysfunction, COMPASS-31, CASS, clinically isolated syndrome

Poster code: R-02-30-088

Poster Title: Long-term follow up of autonomic nervous system impairment in people with multiple sclerosis from the stage of clinically isolated syndrome

PhD candidate: Berislav Ruška

Part of the thesis: Dugorono praenje poremeaja autonomnog ivanog sustava u osoba s multiplom sklerozom od stadija kliniki izoliranog sindroma

Mentor(s): Associate Professor Mario Habek, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Research has shown that autonomic dysfunction (AD) occurs commonly in people with multiple sclerosis (pwMS). It is believed that demyelinating lesions, which are main feature of MS, can affect any part of central nervous system including parts that are important for normal autonomic nervous system function. Neuroimmune interactions that occur at the same time also contribute to AD, but exact pathophysiological mechanisms need to be elucidated. AD in pwMS is very important because research shows that AD affects quality of life of pwMS, Additionally research shows that AD can also be predictor of the future course of the MS in a single patient. Although studies have shown that subjective and objective AD is present in pwMS, it is not well known how AD changes in pwMS over the disease course because studies investigating long-term development of AD during the disease course are scarce. Therefore this PhD research aims to track changes of AD in pwMS form the earliest stage of the disease for a longer time period which is certainly a novelty in understanding of AD in pwMS.

Materials and methods: This prospective cohort study enrolled 121 participants who have been diagnosed with clinically isolated syndrome at the baseline. Enrollment of participants occurred between August 2014 and February 2016. In this timeframe initial test of AD has been performed in each participant. Participants have then been followed for six years during which tests have been repeated in a two-year intervals. Follow-up of all of the participants ended in July 2022. All of the tests were performed in Laboratory for ANS testing at the Clinic of Neurology, University Hospital Centre Zagreb, Croatia. All of the 121 participants were initially screened for drugs and diseases that could affect ANS function. At the baseline, each participant had a thorough neurological exam with EDSS evaluation, completed COMPASS-31 questionnaire, completed brain MRI, and underwent a battery of neurophysiological tests for objective evaluation of ANS function. Battery of ANS tests included Quantitative Sudomotor Axon Reflex Test (QSART), heart rate and blood pressure responses to the Valsalva maneuver, heart rate response to deep breathing test and blood pressure response to passive tilt test. Results of these tests are then expressed with ten-point Composite Autonomic Scoring Scale (CASS) divided into three domains- sudomotor (0-3 points), cardiovagal (0-3 points) and adrenergic (0-4 points), with each point higher meaning more severe impairment.

Results: Main preliminary analysis has been performed after two years of follow up. At the time, data was available for 84 participants. There was a high drop-out rate because lot of patients were not from Zagreb. However complete CASS data was available for 62 patients. In 24 (38.7%) patients there was worsening, in 16 (25.8%) there was improvement and in 22 (35.5%) there was no change in CASS score. If we look at data separately, in 90% of participants, for which appropriate data was available, there was no change in parasympathetic nervous system tests. In 47.3% there was either worsening or improvement in sympathetic adrenergic function. In the end 28.6% had similarly either worsening or improvement in sudomotor function.

Discussion: Results show that significant number of pwMS developed worsening of ANS function during a two-year follow-up, whereas only a quarter of patients improved in the same time frame. Results also show that in those first two years changes were mainly seen in adrenergic and sudomotor indices of CASS, therefore indicating that in early stages of MS both adrenergic and cholinergic parts of the sympathetic nervous system experience significant fluctuations. This results are in line with current opinion that parasympathetic nervous system is relatively spared in early disease stages. It will be very interesting to see how these results change over six-year disease course when the final analysis is completed.

MeSH/Keywords: multiple sclerosis, autonomic dysfunction, COMPASS-31, CASS

Poster code: R-02-30-089

Poster Title: Genetic and epigenetic features of NLRP3 gene in people with myasthenia gravis

PhD candidate: Hrvoje Bilić

Part of the thesis: Genetic and epigenetic features of NLRP3 gene in people with myasthenia gravis

Mentor(s): Professor Ervina Bilić, MD PhD, Professor Fran Borovečki, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Inflammasomes and genes encoding inflammasomes are being increasingly studied in various autoimmune diseases. Myasthenia gravis (MG) is a neurological autoimmune disorder while the NLRP3 inflammasome is the most researched inflammasomes until now.

Materials and methods: In this study a sample of 65 patients diagnosed with MG and a control group of 65 healthy subjects (age and sex matched) will be analysed. A database of MG patients has been created with various clinical and paraclinical features of the patients. Blood samples (5 ml in a tube with EDTA for DNA isolation) will be taken from subjects, and the most common SNPs (rs3806265, rs35829419) and the methylation status of the promoter region of the NLRP3 gene will be analysed.

Results: Demographic, clinical and paraclinical characteristics of 65 MG patients is presented. Out of 65 patients, 44 were female (67,7%) and 21 male (31,3%). The average age of all myasthenic (MG) patients was 50 years (64 years for males, and 43 years for females). Antibody sera finding showed a large prevalence of N-AChR positive patients (84,6%). MuSK positive antibodies were found in 4,6% patients while seronegative patients made up 10,8% of the patient sample studied. Regarding disease onset (early versus late onset), 40 patients (61,5%) had on early disease onset, while 25 (38,5%) started having myasthenic symptoms later in life (50 years of age being the cut off value). When analysed by gender, young onset disease type was much frequent in females (82%, 36 out of 44 female patients) then in male patients (19%, 4 out of 21 males). When looking at the clinical characteristics of the patients, majority had a predominantly generalized MG (70.8%, 46 patients), followed by patients with bulbar difficulties (21,5%, 14 patients) and purely ocular patients consisting of 7,7% (5 patients). When thymic pathology was analysed, we found that 27 patients (41.5%) underwent thymectomy, while the remaining 38 patients (58.5%) did not have a thymic pathology and were not candidates for thymic surgery, while the average of the patients that underwent thymectomy was 43,4 years.

Discussion: Based on our preliminary data myasthenia gravis (MG) affects both males and females, with a higher prevalence in females. The majority of patients presented with early onset disease, which was more frequent in females. The most common type of MG was predominantly generalized, followed by bulbar and ocular types. In terms of antibody sera findings, N-AChR positive patients made up the majority, while MuSK positive patients were relatively rare and a significant proportion of patients' sample were seronegative. It is worth noting that thymic pathology was observed in almost half of the patients studied, and over 40% of patients underwent thymectomy. The average age of patients who underwent thymectomy was 43.4 years, which suggests that thymectomy may be more commonly performed in younger patients with MG. Overall, these findings are mostly in line with previous studies that looked into demographic, clinical, and paraclinical characteristics of MG patients. Once we incorporate and analyse these preliminary findings combined with genetic and epigenetic analysis of MG patients and healthy controls, we will be able to determine if there is a greater frequency of occurrence of SNPs (rs3806265 and rs35829419) in MG patients compared to healthy controls and what are the specificities of certain MG subtype groups.

MeSH/Keywords: Myasthenia gravis, NLRP3 gene, inflammasomes, SNP, methylation

Poster code: R-02-30-092

Public health and healthcare – preliminary research results

Poster Title: Breast magnetic resonance imaging (MRI) versus mammography for National Breast Cancer Screening Program in women with family history of breast cancer: a prospective observational study

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: University of Zagreb School of Medicine, Croatian Institute of Public Health and Zagreb Clinical Hospital Center

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. Our hypothesis is that an abbreviated MRI protocol, in addition to mammography, will increase the number of detected breast cancer cases in women with family history of breast cancer who are included in the National Breast Cancer Screening Program.

Materials and methods: The study included 46,505 women who underwent mammography on call within the 6th cycle of the National Breast Cancer Screening Program implemented in the City of Zagreb. Data on the increased risk of breast cancer 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; and one first-degree relative with breast cancer, one second-degree relative with breast cancer, regardless of age and three or more second-degree relatives with breast cancer, regardless of age. Recognized women with a family history of breast cancer were invited for mammography and MRI at the Clinical Institute for Diagnostic and Interventional Radiology, Zagreb Clinical Hospital Center. Subjects who had had mammography or MRI done earlier the same year were excluded from the study. The women who accepted the invitation were examined in the Selenia® Dimensions® digital mammography unit (Hologic, Bedford, MA) in two standard projections as part of the 7th cycle of the screening program, while those who agreed to participate in the study underwent MRI on a 1.5 T scanner (Avanto, Siemens, Erlangen, Germany), according to an abbreviated protocol. The standardized BI-RADS (Breast Imaging Reporting and Data System) classification, developed by the American College of Radiology, was used for breast imaging reporting. The analysis was carried out using descriptive epidemiology methods.

Results: According to the inclusion criteria, 318 women with family history aged 50-69 were recognized as high risk and were invited firstly for mammography exam. Out of 318 women invited for a mammographic examination, 192 women responded. 178 of them also underwent a MRI, according to an abbreviated protocol, and the remaining 13 women did not agree to a MRI, or were unable to appear for the examination for medical reasons, while one had had a mammography done earlier the same year. Eleven breast cancer cases were detected in 178 women who were screened by mammography and MRI, where of 6 cancer cases were positive only on MRI, 1 only on mammography and 4 on both methods.

Discussion: The application of MRI has increased the number of detected breast cancer cases in women with family history who were included in the National Breast Cancer Screening Program, compared to mammography alone. Mammographic screening, despite the emergence of other diagnostic methods, remains the most acceptable method of early detection of breast cancer for women at average risk. However, for women with family history of breast cancer who were included in the population-based screening program, as the results of this study would indicate, the application of MRI as a supplementary diagnostic method should be considered.

Acknowledgments: The authors would like to thank all the women who agreed to participate in the research.

MeSH/Keywords: High risk for breast cancer, family risk for breast cancer, early detection of breast cancer

Poster code: R-03-01-009

Poster Title: Knowledge about and prevalence of chlamydia infection among Emerging Croatian Adults: a bio-behavioral cross_sectional study

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, Croatian Institute of Public Health, Zagreb, Teaching Institute for Public Health "Dr. Andrija Stampar"

Introduction: Chlamydia infection is one of the most frequent sexually transmitted infections (STI) in young people and may cause complications in both women and men. The aim of the study was to determine the prevalence of genital chlamydia infection, assess the knowledge and correlates of chlamydia-related knowledge and willingness to provide urine sample for chlamydia testing in emerging adults aged 18-25 in Croatia.

Materials and methods: A national cross-sectional probability-based bio-behavioural survey in young people aged 18-25 years in Croatia from November 2021 to February 2022 was carried out. Participants (n=1197), members of a national online panel, completed an online questionnaire on socio-demographics, sexual behaviours and knowledge about STI. The sampling was from the panel database of research company. Multi-stage double stratification by region and settlement size and quota-based by gender, age and education was used to randomly draw participants. For representativeness, adjusting according to the data of Croatian Bureau of Statistics and post-hoc weighting for gender and age was applied. Participants were asked for informed consent before behavioural and biological (chlamydia testing) part of the survey. All the procedures were carried out electronically respecting data protection standards. The anonymity of the participants was ensured by randomly generated codes to link biological and behavioral data. After completion of the questionnaire, for which they received a small token of appreciation (5 EUR voucher), participants were offered to participate in a biological part of the study, which included provision of a urine sample for chlamydia testing (awarded with 20 EUR voucher). Sampling kit with return prepaid postage package and illustrated instructions for self-collecting urine and application for the results was sent by post (carried out for the first time in Croatia). Samples were mailed to the laboratory at the Teaching Institute for Public Health "Dr. Andrija tampar" and tested for chlamydia using Cobas 4800 CT/NG test. Descriptive analysis, multivariable linear regression to determine correlates of knowledge about chlamydia infection and logistic regression to assess correlates of the willingness to test for chlamydia was carried out.

Results: From the 921 participants (failed on attention trap questions and missing responses were excluded) who took the behavioural part, 448 participants sent their urine specimens (40.4% female and 34,3% male). Chlamydia prevalence was 2.5% (95% CI 1.2-5.1) in women and 1.0% (0.3-3.2%) in men. Of the total, 21.6% respondents didn't have a single correct answer of the six questions about chlamydia-related knowledge, while only 9.6% had five or six correct answers. In regression analysis, significantly higher odds of willingness to test for chlamydia were found in females (OR = 1.34, p = 0.024), those with better knowledge about the infection (OR = 1.11, p = 0.005), and those with lower religiosity (OR = 0.91, p = 0.017). In the case of knowledge about chlamydia, participants who self-reported being tested for chlamydia before this survey had significantly better knowledge about chlamydia (B=0.87, s.e.= 0.21; p<0.001).

Discussion: The results show a substantially lower chlamydia prevalence compared to the national study among emerging adults in 2010 in Croatia, when 5.3% of female and 7.3% of male participants tested positive, and similar to the prevalence (3.0%) among students in the city of Zagreb tested for chlamydia in screening program from 2017 to 2019. Chlamydia prevalence in this study was relatively similar than that found in young adults in other EU/EEA countries in women (3.6%), but lower in men (3.5%). Those with better knowledge about chlamydia were more likely to provide urine samples, which shows that interventions for increasing knowledge about chlamydia could lead to scalling up testing. Efforts for chlamydia control should focus on primary prevention and targeted testing with effective case management.

Acknowledgments: I would like to express gratitude to Goran Koletić, Ivan Landripet and Kristina Stepusin Seferovic for their help with data collection, and Jasmina Vraneš, microbiological Investigators to their contributions in laboratory testing of samples and Iskra Ale

MeSH/Keywords: Chlamydia infection, prevalence, Croatia, knowledge, testing, young adults

Poster code: R-03-01-120

Poster Title: Association of Anti-Diabetic Drugs and COVID-19 Outcomes in Patients with Diabetes Mellitus Type 2 and Cardiomyopathy: Real World Evidence

PhD candidate: Jelena Dimnjaković

Part of the thesis: Analysis of the relationship between different preparations for the treatment of diabetes and infection with the SARS-CoV-2 virus in people with type 2 diabetes

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

Affiliation: University of Zagreb School of Medicine

Introduction: There is scarcity of information on population with diabetes and cardiomyopathy in context of COVID-19, and especially on association of antidiabetic medications and COVID-19 outcomes.

Materials and methods: Study is designed as retrospective cohort analysis covering years 2020 and 2021. Data from National Diabetes Registry (CroDiab) data were linked to hospital data, primary healthcare data, SARS-CoV-2 vaccination database and SARS-CoV-2 test results database. Study outcomes were cumulative incidences of SARS-CoV-2 positivity, COVID-19 hospitalizations and COVID-19 deaths. For outcomes predictors, logistic regression models were developed.

Results: Of 231 796 patients with diabetes mellitus type 2 in database, 14 485 patients had cardiomyopathy. Two-year cumulative incidences of all 3 study COVID-19 outcomes were higher in these patients than in general diabetes population (positivity 15,3% vs 14,6%, p= 0,01; hospitalization 7,8% vs 4,4%, p<0,001; death 2,6% vs 1,2%, p<0,001). SGLT2 therapy was found to be protective of SARS-CoV-2 infections [OR 0,722 (95% CI 0,610-0,856)] and COVID-19 hospitalizations [OR 0,555 (95% CI 0,418-0,737)], sulphonylureas to be risk factors for hospitalization [OR 1,184 (95% CI 1,029-1,362)] and insulin to be risk factor for hospitalization [OR 1,261 (95% CI 1,046-1,520)] and death [OR 1,431 (95% CI 1,080-1,897)].

Discussion: Patients suffering DM type 2 and cardiomyopathy are at greater risk of acquiring SARS-CoV-2 infection and having worse outcomes than general diabetic population. SGLT-2 therapy was a protective factor against SARS-CoV-2 infection and against COVID-19 hospitalization, sulphonylurea was COVID-19 hospitalization risk factor while insulin was a risk factor for all outcomes. Further research is needed in this diabetes sub-population.

MeSH/Keywords: Diabetes Mellitus, Type 2, COVID-19, Hypoglycemic Agents, Sodium-Glucose Transporter 2 Inhibitors, insulin

Poster code: R-03-02-060

Poster Title: A Qualitative Study on the Experiences of Pediatricians in the Processes of Care for Preschool Children with Complex Needs

PhD candidate: Iva Lukačević Lovrenčić

Part of the thesis: Determinants of coordination, integration and continuity of the care process for preschool children with complex needs

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

Affiliation: Andrija Stampar School of Public Health, University of Zagreb School of Medicine

Introduction: In order to define determinants of coordination, integration, and continuity of the care process for children with complex needs, it is necessary to explore the dynamic context in which the inputs (people, equipment, space) coexist and the manner in which they are utilized in the processes of care. All of this impacts the providers' capacity to ensure care tailored to the child's and caregivers' needs. Pediatricians play a significant role in the coordination of care for children with complex needs, and thus in maintaining the continuity of care.

Materials and methods: The research was conducted in two phases. As part of the first phase of the research, a search of MEDLINE, Scopus, and Web of Science Core Collection, with a document analysis on selected strategic and legal documents, standards, and reports related to the topic was completed. Two focus groups with pediatricians were conducted in the second part of the research. The participants were selected via purposive sampling through the "Health Observatory" project, professional associations and collaborators, and using the snowball method. The selected criteria for the sampling of participants were: specialist pediatric experience longer than 5 years; active work at the primary or hospital level; personal experience of working with preschool children with complex needs. 6 primary and 6 hospital pediatricians participated, territorially covering all 4 Croatian regions. The focus groups were held on the Zoom platform, and each participant previously sent an informed consent via email. The research domains upon which the specific semi-structured questions were formed were rooted in the results of the first phase of the research. The sessions were audio-visually recorded, and anonymized transcripts were made immediately after. The thematic analysis method, as described by Braun and Clarke, was used to analyze the transcripts.

Results: Based on the developed model of analysis and data processing of focus groups with pediatricians of the primary, secondary, and tertiary levels of health care, the results that emerged were shaped by the descriptive codes and the subthemes and themes derived from them. Based on the statements of participants, the profile and needs of children with complex needs, the role of pediatricians in the process of caring for a child with complex needs, the role of parents in the care process from the perspective of pediatricians, problems of a family with a child with complex needs, determinants of the cooperation inside and outside the health system, the mitigating circumstances in the planning and implementation of care for children with complex needs, and the aggravating circumstances in the planning and implementation of care for children with complex needs from the pediatricians' point of view were identified.

Discussion: This research aimed to identify the experiences of pediatricians in the processes of care for preschool children with complex needs. International examples of good practice show that it is necessary to integrate health and social aspects of care, with strong support from the early and preschool education system. In Croatia, a concrete legal basis for the establishment of counseling centers for complex patients at community health centers has recently been introduced, in which all relevant stakeholders can participate in care planning and implementation, thus creating an opportunity for improving care for children with complex needs.

Acknowledgments: The research was conducted within the project "Health Observatory" (UP.04.2.1.06.0045).

MeSH/Keywords: child, preschool; delivery of health care, integrated; continuity of care; health services needs; social care; informal care, patient care management; quality of care; process assessment, health care

Poster code: R-03-02-062

Poster Title: Pesticide residues in adolescents' diet in the Zagreb region effect of food processing

PhD candidate: Marija Macan

Part of the thesis: Relationship between exposure to pyrethroid and organophosphate insecticides in food with pubertal development and hormonal status in boys

Mentor(s): Katja Dumić Kubat, PhD, senior research associate, Veda Marija Varnai, PhD, research advisor

Affiliation: University of Zagreb School of Medicine, Institute for Medical Research and Occupational Health, Backweston Laboratory - Department of Agriculture, Food and the Marine (DAFM), Ireland, School of Public Health "Andrija Štampar"

Introduction: The main objective of the PhD thesis is to assess the risk of adverse effects of exposure to pyrethroid (PYR) and organophosphate (OP) insecticides on sexual development in (pre)pubertal boys. Within this research, Total Diet Study (TDS) was conducted in a region of Zagreb, Croatia, to assess exposure to pesticide residues in the adolescent population. Food samples for a TDS study are analyzed as table-ready, but certain food could be consumed with and without previous processing. It is known that food processing can alter the number of pesticide residues, but so-called processing factors for specific pesticide residue-food combinations are still under development by the European Food Safety Agency (EFSA) and other bodies. The effect of food processing was also studied within the TDS framework.

Materials and methods: Food was either bought as already processed (e.g. fruit compotes, pickled and canned vegetables), or raw and then prepared as consumed. Certain raw commodities were analyzed both before and after processing (peeling and thermal processing): apples, carrots, courgettes, cucumbers, pears, plums, tomatoes, and Swiss chard, which enabled us to study the effects of pealing and thermal processing on pesticide residues. After the samples preparation, up to 5 subsamples were combined into 1 composite sample, homogenized, stored frozen at -20 °C, and shipped on dry ice to Backweston Laboratory Complex, DAFM, Celbridge, Ireland, where the samples were analyzed for residues of 288 pesticides and their metabolites. The samples were extracted using the mini-Luke method, and a solvent exchange into ethyl acetate was done after extraction with acetone/dichloromethane/petroleum ether at 4060 °C. A portion of the ethyl acetate fraction was diluted 1/20 with methanol and subjected to LC-QQQ analysis, while the remainder underwent GC-High Resolution Accurate Mass Spectrometry and GC-QQQ analysis without further processing. The analyses underwent Quality Control in accordance with SANTE/11312/2021.

Results: When comparing a total number of unprocessed and processed food samples, a higher percentage of positive results was found in unprocessed compared to processed food (43% vs. 21%). Compared to processed foods, a higher percentage of positive results was found both in unprocessed vegetables (38% vs. 16%) and fruits (68% vs. 52%). Values above MRL were found in 11% of unprocessed fruit and in 3% of processed fruits, and in 3% of unprocessed and 1.4% of processed vegetable samples. Pealing decreased residue level in 71% of samples. For example, pealing decreased pirimicarb by 74% in raw apples, while azoxystrobin decreased by 96% and boscalid by 76% in tomatoes that were peeled before cooking, compared to cooked unpeeled tomatoes. On the other hand, pealing did not affect level of carbendazim and thiacloprid in pears, and oxamyl in cucumbers. Thermal processing decreased residue level in 64% of samples. For example, thermal processing decreased acetamiprid in cooked chard by 91%, prosulfocarb in cooked carrots by 72%. On the contrary, thermal processing increased residue level in 14% of samples. For example, oven baking increased residues of boscalid and pirimicarb above the limit of quantification in plums and in apples. Then again, thermal processing did not affect level of boscalid and pirimicarb in apples and mandipropamid in spinach.

Discussion: Lower percentage of positive results in processed food samples, compared to unprocessed foods, is related to the peeling of food as well as dilution (e.g. boiling in water), and possibly also to thermal degradation and/or hydrolysis of pesticide residues. In our food samples that were analyzed both before and after processing, pealing and thermal processing decreased residue concentrations in a vast majority of cases. Nevertheless, there were exceptions, described above. Increased residue levels in baked food samples could be due to water loss and consequent pesticide residue concentration in the sample. In the European

database of processing factors for pesticides, we could not identify processing factors for pesticide residue-food combinations observed in our study. Further research is warranted on developing processing factors for a wide variety of foods and pesticide residues.

Acknowledgments: This research is funded by the Croatian Science Foundation (HrZZ-IP-2019-04-7193) and the Department of Agriculture, Food and the Marine, Ireland.

MeSH/Keywords: pesticide residues; food processing; total diet study

Poster code: R-03-02-108

Poster Title: Determining benzodiazepines prescribing pattern as a first step to guide rational pharmacotherapy - 3-year national observational study

PhD candidate: Katarina Gvozdanović

Part of the thesis: Applicability of the Central Health Information System of the Republic of Croatia for making regulatory decisions about drugs on the example of benzodiazepine therapy management

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

Affiliation: University of Zagreb School of Medicine, Croatian Agency for Medicinal Products and Medical Devices

Introduction: If used properly benzodiazepines are considered safe, however, there are concerning reports of their extensive overuse worldwide. In Croatia, diazepam and alprazolam are continuously among first ten most used medicines, with continuously increasing DDD/TID (defined daily dose/1000 inhabitants). The aim of our study was to obtain a detailed benzodiazepine-prescribing pattern in Croatia in order to support effective decision-making that will ensure rational prescribing, better health outcomes and reduced cost for healthcare system. For this purpose we used Croatian Central Health Information System (CEZIH) as a novel source of real-world data in pharmacoepidemiology in Croatia.

Materials and methods: This is a retrospective longitudinal observational study analyzing electronic prescriptions for medicines with ATC class N05BA prescribed and issued in Croatia in 3-years period (1.1.2015-31.12.2017). Prescriptions were retrieved from CEZIH who is operated by the Croatian Health Insurance Fund (CHIF). We analysed data on number of prescriptions, type and quantity of prescribed medications over time, patient and prescribers' characteristics as well as compliance with applicable guidelines and product information with regards to treatment duration and indication for use. Data were stored in SQL Server and SQL queries were used in order to filter and group data as well as to calculate values.

Results: In a 3-year period 4.619 prescribers issued 13.848.802 prescriptions for benzodiazepines for 1.237.635 individual patients. Median number of prescriptions per patient was 11.2 ranging from 1 to up to 575. Diazepam was prescribed in 44% of all prescriptions. 787.152 (63.6%) patients were female and the sex difference was even more pronounced in older age groups. The oldest patient was born in 1909. and the youngest in 2017. The most common indication was Generalized anxiety disorder (2.531.201, 18.3%) followed by Major depressive disorder (1.136.397, 8.21%). For over 66 % of patients prescriptions were made off-label or for a longer time period or age group than defined by the product information (summary of product characteristics).

Discussion: The preliminary results characterize the extent of the problem with benzodiazepine overuse in Croatia. Over 1/4 of Croatian population received some type of benzodiazepine during the study period, many of them outside the approved conditions. CEZIH proved as valuable source of information with enough granularity to allow us to check different aspects of drug prescribing practices. Reliable data on drug prescribing pattern as well as details on patients and prescribers characteristic will help us pinpoint weak spots and focus educational or regulatory actions which will consequently improve patient safety

MeSH/Keywords: benzodiazepines, over prescribing, electronic prescriptions

Poster code: R-03-02-109

Poster Title: Professionalism and Loneliness as Predictive Factors of Burnout in International Medical Students

PhD candidate: Ivan Pavao Gradiški

Part of the thesis: Changes in elements of medical professionalism in medical students according to Jefferson's model

Mentor(s): Professor Ana Borovečki, MD PhD, Marko Ćurković, PhD, research associate

Affiliation: University of Zagreb School of Medicine, University Psychiatric Hospital Vrapce

Introduction: Burnout among students is typically described as a general state of exhaustion, followed by a cynical devaluation of their studies and uncertainty of their ability to perform them correctly. Its a common mental health concern among medical students with international students being more susceptible to suffer from it due to additional demands of adjusting to their new living environment on top of the usual academic stress.

Materials and methods: A cross-sectional study was performed in the School of Medicine of the University of Zagreb on students enrolled in the English-language medical program, which comprises largely of international students. The general version of the Maslach Burnout Inventory (MBI-GS) was used as a dependent variable, while Jefferson Scales of empathy (JSE-S), teamwork (JSAPNC), and physician lifelong learning (JeffSPLL-MS) as well as the Social and Emotional Loneliness Scale for Adults (SELSA-S) were used as predictive variables. In addition, socio-demographic information was collected which included sex, country of birth, native language, age, academic achievement, and living situation. Linear regression models were applied to identify predictors of burnout.

Results: In a sample of 188 medical students (38 Croatians and 144 foreigners from 28 countries), 18% of the global score in the MBI-GS was explained by lifelong learning and family loneliness. A separate analysis for each of the three domains of the MBI-GS allowed the creation of the following models. The first model explained 19% of the variance of the exhaustion domain by country of birth, living with parents, academic year, and cynicism. The second model explained 24% of the variance of the cynicism domain by academic year, empathy, lifelong learning, and exhaustion. Finally, the third model explained 24% of the variance of the professional efficacy domain by lifelong learning, family loneliness, and cynicism. All obtained models presented an effect size between medium and large, and matched the required conditions for statistical inference.

Discussion: These findings provided valuable insight into the international students stress due to ongoing medical studies as well as separation from their family support. They confirmed the important protective role of family support for medical students as well as outlining two specific elements of medical professionalism, empathy and lifelong learning, as protective factors in preventing burnout in international students. In order to minimize and prevent students burnout as well as ensure adequate professional development during their academic period, interventions could be implemented during studies focusing on fostering these attributes and skills.

MeSH/Keywords: international medical students; burnout

Poster code: R-03-02-132

Research proposals

Basic medical sciences – research proposals

Poster Title: The effect of a new microbiota-based emollient plus formulation on the skin microbiota composition and safety, efficacy and tolerability assessment in the treatment of mild atopic dermatitis

PhD candidate: Dora Hrestak

Part of the thesis: The effect of a new emollient plus formulation based on ectoin, Lactobacillus spp. and Bifidobacterium spp. bacteria on skin microbiota composition and safety, efficacy and tolerability assessment in mild atopic dermatitis treatment

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, relapsing, inflammatory skin disease characterized by a typical distribution of changes - dry skin, intense itching, redness, excoriations and lichenification. The use of emollient plus preparations (EPP) on diseased skin alleviates the symptoms and, potentially, the need for topical corticosteroids.

Hypothesis: The new EPP based on ectoine, Lactobacillus spp. and Bifidobacterium spp. will change the skin microbiota composition and thus reduce skin inflammation, alleviate symptoms and signs of the disease and contribute to improving the quality of life of patients with mild AD.

Aims: The main objective of the study is to assess safety and efficacy of active components in in vitro preclinical models and determine the safety, efficacy and tolerability of the final EPP formulation based on ectoine, Lactobacillus spp. and Bifidobacterium spp. in altering the skin microbiota composition, reducing signs of skin inflammation, as well as alleviating disease symptoms and signs in adult patients with mild AD during 4 weeks of administration.

Materials and methods: The study is designed as a comparison of two emollient therapies, a new emollient plus and control emollient (formulation without active ingredients), on adult patients with diagnosed mild AD who do not use anti-inflammatory therapy and meet all inclusion and exclusion criteria. Both treatments will be applied twice daily, each on one side of the body (left/right on the same patient). Study visits will be scheduled on day 0, week 2 and 4 during which microbiota, safety, efficacy and patient-reported outcomes will be assessed. Effects on skin microbiota will be evaluated by determining the bacterial taxonomic content from skin swabs after DNA isolation, amplicon library preparation and sequencing of 16S rRNA using the next-generation sequencing (NGS) Illumina MiSeq technology. Efficacy will be assessed by determining changes between groups and all three time-points. Parameters measured will be: modified objective SCORAD index, transepidermal water loss, hydration, erythema (mexameter), redness (3D camera), lichenification, elasticity, pH, scaliness and skin microtexture. To assess the patient-reported outcomes two questionnaires, Dermatological Life Quality Index (DLQI) and Numeric Rating Scale (NRS) for average and worst pruritus (itching), will be completed by enrolled subjects. Additionally, the patients will report the regularity, frequency and type of emollient use.

Expected scientific contribution: Expected results of the research will contribute to the development of a new local preparation that will successfully change the skin microbiota composition and thus reduce the signs of skin inflammation, alleviate symptoms and signs of the disease and improve the quality of life of patients with mild AD. Clinical and preclinical testing will contribute to the safety and efficacy assessment of the new EPP in the treatment of mild AD, the advancements of the knowledge about the disease, available tools in its treatment and the patients' quality of life.

MeSH/Keywords: atopic dermatitis, emollient plus, microbiota

Poster code: T-01-03-029

Poster Title: Effect of BPC 157 on hemodynamic changes caused by severe ulcerative colitis in rats

PhD candidate: Dinko Bekić

Part of the thesis: Effect of BPC 157 on hemodynamic changes caused by severe ulcerative colitis 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.

Hypothesis: Severe ulcerative colitis leads to vascular dysregulation syndrome and multiorgan damage. Pentadecapeptide BPC 157 leads to reduction of intracranial, portal and caval hypertension, aortic hypotension, opening of collateral blood vessels and reduction of lesions of secondary organs.

Aims: To demonstrate ulcerative colitis in rats as a syndrome of vascular dysregulation and multiorgan damage as in large vessel occlusion and other similar nociceptive procedures, and the effect of BPC 157 on antagonizing the entire syndrome (reduction/elimination of intracranial (superior sagitalis sinus), portal and caval hypertension, and aortic hypotension). Also, we want show the interdependence between the use of BPC 157 and the NO-agent, the NO synthase inhibitor (NOS) L-NAME and/or the NOS-substrate L-arginine, inhibition of the NO-system (L-NAME), stimulation (L-arginine), and immobilization (L-NAME+L-arginine).

Materials and methods: During the experiments, the animals will be under deep general anesthesia induced by intraperitoneal administration of thiopental 40 mg/kg and diazepam 10 mg/kg. After washing the distal bowel with 10 ml of 0.9 NaCl, 1 ml of 9% acetic acid will be administered intrarectally. The animals will be divided into 8 groups and in all tested groups the only difference in the post-intervention procedure will be in the applied agent. A total of 144 animals will be included in the research. 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. Also, in order to specifically monitor the effect on hemodynamics and heart function, an electrocardiographic record will be recorded. At the end of the hemodynamic evaluation, the animals are sacrificed using an excessive dose of the anesthetic thiopental. 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.

Expected scientific contribution: To determine severe ulcerative colitis as a syndrome of vascular dysregulation and multiorgan damage and the therapeutic effect of BPC 157 on antagonizing the entire syndrome

MeSH/Keywords: BPC 157; Hemodynamics; Rat; Ulcerative colitis.

Poster code: T-01-03-038

Poster Title: Protective Effects of Rhus Coriaria Fruit Ethanolic Extract in Sodium Iodate-induced macular degeneration in rats

PhD candidate: Genista Mustafa

Part of the thesis: Protective Effects of Rhus Coriaria Fruit Ethanolic Extract in Sodium Iodate-induced retinal degeneration in rats

Mentor(s): Professor Ante Tvrdeić, MD PhD, Professor Armond Daci, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Age-related macular degeneration is a progressive disease that causes damage to the macula and loss of central vision. There are two forms the AMD, "wet" or neovascular form and 'dry' or geographic atrophy, which is presented in more than 80% of patients with AMD, but without any adequate treatment.

Hypothesis: Rhus coriaria ethanolic extract has protective, antioxidant, and anti-inflammatory effects in sodium iodate-induced macular degeneration in rats.

Aims: Our aim is to evaluate the protective effect as well as anti-inflammatory and antioxidant activity of Rhus coriaria extract on the structure of the retina in sodium iodate-induced macular degeneration in rats.

Materials and methods: We will use male Wistar rats provided by our Faculty of Medicine in Prishtina,1214 weeks of age and weighning 250-300g. 32 animals will be randomly divided into 4 groups, with 8 rats in each group. The rats will be divided into the Control Group , the Experimental Group (treated with sodium iodate, single bolus dose IV) and 2 Experimental Groups treated with different doses of RCEE (250 and 500 mg/kg, PO) in 28 days. In our study, we will use immunopathology, immunohistochemistry, and gene expression analysis of retinal structure, inflammation mediators, oxidative stress markers and statistical analysis. For normally/parametric distributed data we will use ANOVA and post-hoc Tukey-Kramer test and for nonparametric data, we will use Kruskal-Wallis and post-hoc Dunn test.

Expected scientific contribution: Our objective is to explain the further medical uses of Rhus coriaria, including also traditional application in eye diseases, by adding new pharmacological investigation and mechanistic effects for the first time in Sodium Iodate induced macular degeneration in rats, which might contribute to the treatment perspective in Age-related Macular Degenerative (AMD) and in this way to globally contribute in prevention of blindness around the world.

MeSH/Keywords: Age-related macular degeneration, Rhus coriaria ethanolic extract, animal model, protective effect, antioxidant effect, anti-inflammatory effect

Poster code: T-01-03-128

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 of stable pentadecapeptide BPC 157 on tissue healing has been described in many scientific papers. The studies describe its effect on wound healing such as burns and mucosal fistulas, but also in case of muscle tissue, ligament and bone injury. It has a cytoprotective effect, heals mucosal ulcers and has a role in inflammatory bowel disease treatment. It also stimulates tendocyte growth, improves healing and functional recovery after Achilles tendon transection, MCL transection and quadriceps muscle injury. Recent studies in rats describe the onset od Occlusion and Occlusion-like syndrome, which are a result of endothelium damage and vessel thrombosis. Both are characterized by hypertension in superior sagittal synus, portal vein and inferior caval vein, alongside with aortal hypotension. The syndromes differ in mechanical occlusion ie. the occlusion is present in Occlusion syndrome, but absent in Occlusion-like syndrome. Lower leg fracture in rats is a model for long bone fractures, and results in damage to the surrounding soft tissues. The onset of Occlusion and Occlusion-like syndrome was described in gastrointestinal, circulatory and neurologic system, but not in osteomuscular system. In this study we will examine the Occlusion-likesyndrome after lower leg fracture in rats, its effect on vital organ systems and the potential benefit of pentadecapeptide BPC 157.

Hypothesis: Pentadecapeptide BPC 157 enhances local healing in rat lower leg fracture, from hematoma formation to utter bone healing and functional recovery. It also eliminates the onset of Occlusion-like syndrome and its systemic consequences after the fracture.

Aims: Demonstrate the Occlusion-like syndrome and the therapeutic effect of pentadecapeptide BPC 157 after lower leg fracture in rats, from hematoma formation to complete bone healing.

Materials and methods: In male Wistar albino rats with lower leg fracture, with or without BPC 157 therapy, we will demonstrate the onset of Occlusion-like syndrome, ECG disturbances, multiorgan failure; brain, heart, lungs, liver, kidney and GI tract lesions; intracranial (superior sagittal synus), portal and caval hypertension and aortal hypotension, from hematoma formation to bone healing and functional recovery. Results will be presented macroscopically, pathohistological and with gene expression.

Expected scientific contribution: Occlusion-like syndrome is a result of lower leg fracture in rats, and is counteracted by pentadecapeptide BPC 157 which also leads to faster fracture healing and functional outcome.

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

Poster code: T-01-03-140

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

PhD candidate: Sarah Meglaj

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 slowly 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 -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 inflammatory processes in the patient's brain are associated with increased permeability of the blood-brain barrier, which enables the entry of immune cells and the gradual acceleration of the degeneration of dopaminergic neurons. In addition to the role of immune cells, the role of inflammasomes in neurological diseases has recently been emphasized. Given that inflammasomes are still not fully characterized in PD, we believe that their characterization, as well as the characterization of immune cells, would contribute to the timely diagnosis and development of targeted therapies.

Hypothesis: Gene variants associated with immune response and inflammasome activation and expression profiles of dendritic cells and CD4+ T-lymphocytes differ between PD patients and control subjects.

Aims: The goal of this study is to identify and characterize the genetic background of the immune response in PD using advanced genomic methods such as next-generation sequencing (NGS) and single-cell sequencing.

Materials and methods: This case-control study will include 75 subjects suffering from PD and 25 control subjects aged 50 to 70, selected at the University Hospital Centre Zagreb. In the first part of the research, a comparison of the gene variants of PD patients and control subjects will be carried out through clinical exome sequencing. The DNA of all participants will be isolated from whole blood samples and used for library preparation. The main steps of library preparation include DNA fragmentation, ligation and cluster formation. The formed clusters will be sequenced by next-generation sequencing (NGS). A comprehensive statistical analysis will be performed on the obtained sequences. The next stage of the research will be the analysis of transcriptomes of dendritic cells and CD4+ T-lymphocytes using a single-cell sequencing approach. Cells of interest will be selected by magnetic separation from blood samples and will be used in the preparation of samples for gene expression analysis. The cell suspension, together with 10X gel beads and reagents (10X Genomics, USA) is applied to a microfluidic chip that is placed in a device that enables the creation of gel beads-in-emulsion. The beads formed this way contain barcodes and capture cells with the same mRNA. Marked mRNA molecules undergo the process of reverse transcription, resulting in cDNA fragments that will be used for the preparation of libraries. The prepared libraries will be sequenced by NGS. Obtained results will be compared with the results of expression analysis and validated on the SH-SY5Y cell model by silencing selected genes via transduction of lentiviral particles, whereby our goal is to examine the cause-and-effect relationship between the accumulation of -synuclein and the immune response.

Expected scientific contribution: We expect to show possible genetic changes in underlying immune mechanisms involved in the development of PD and indicate possible target sites for the action of potential immunomodulatory therapy.

Acknowledgments: This study will be conducted as a part of the Immune PD research project (HrZZ IP-2020-02-8475), funded by the Croatian Science Foundation.

MeSH/Keywords: Parkinson's disease, alpha-Synuclein, Inflammasomes, Next-generation sequencing, Dendritic cells, CD4-positive T-lymphocytes, Single-cell sequencing

Poster code: T-01-05-012

Poster Title: The role of pharmacogenetics in an individualised approach to apixaban treatment

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: Cardiovascular (CV) diseases are the leading cause of death in the world. CV patients are usually exposed to polypharmacy which comes with risks of undesirable drug effects such as side effects or inefficiency. Therefore, a personalised approach to treatment is needed and pharmacogenetics is an ideal approach. Apixaban is a direct oral anticoagulant primarily used for the treatment of deep vein thrombosis (DVT), pulmonary embolism (PE), and prevention of thromboembolic events in patients diagnosed with atrial fibrillation (AF) whose frequent adverse drug reactions (ADRs) are minor and major bleedings. Polymorphisms in genes which encode CYP3A4/5, CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2J2 metabolic enzymes and transmembrane proteins ABCG2 (BCRP, breast cancer resistance protein) and P-glycoprotein (ABCB1/MDR1, multidrug resistance protein) are potential factors in the variability of the activity and safety profile of apixaban. There is a special emphasis on the need for studies which should clarify the association of these polymorphisms with concrete clinical outcomes.

Hypothesis: Gene polymorphisms for transporters ABCB1 and ABCG2 and metabolizing enzymes CYP2C9, CYP2C19, CYP2J2 and CYP3A4/5 are associated with a risk of bleeding in patients treated with apixaban.

Aims: To determine which pharmacogene variants ABCB1, ABCG2, CYP2C9, CYP2C19, CYP2J2 and CYP3A4/5 are associated with risk for development of side effects, primarily bleeding, in patients treated with apixaban. Further, to examine the role of drug interactions that share the same metabolic pathways with apixaban in the development of apixaban side effects (interactions drug-drug) and to identify overall clinical and genetic parameters that can serve as predictors of the development of apixaban side effects (interactions drug-drug-gene).

Materials and methods: The research is prospective with a duration of 3 years. It will include a minimum of 470 subjects, aged over 18, with new indications for apixaban treatment for at least a 3-month duration. Excluding criteria is a contraindication for apixaban treatment. Subjects are grouped into cases (with ADRs) and controls (without ADRs). Pharmacogenetic analysis of chosen pharmacogenes will be conducted on 7500 Real-Time PCR System Applied Biosystems (Applied Biosystems, Foster City, CA, USA) with the TaqMan® PCR method. Drug-drug interactions will be tested with the LexiComp® system.

Expected scientific contribution: This research should bring new scientific knowledge about the association of pharmacogenes with the safety of apixaban administration, especially in patients with comorbidities and polytherapy, and should contribute to the assessment of the patient population for which it is of clinical importance to conduct pharmacogene analysis before the introduction of therapy.

MeSH/Keywords: pharmacogenetics, adverse drug reactions, cardiovascular drugs, anticoagulants, drug-drug-gene interactions, genetic polymorphisms, precision medicine

Poster code: T-01-05-070

Poster Title: Comparison of hemodynamic changes in the frontal cortex during anesthesia and perioperative behavioral and cognitive changes in patients receiving ketamine analgesia

PhD candidate: Nikola Prpić

Part of the thesis: Comparison of hemodynamic changes in the frontal cortex during anesthesia and perioperative behavioral and cognitive changes in patients receiving ketamine analgesia

Mentor(s): Professor Neven Henigsberg, MD PhD

Affiliation: University of Zagreb School of Medicine, Clinical hospital Merkur

Introduction: Near-infrared spectroscopy (NIRS) offers a unique chance for observations of cerebral hemodynamic changes due to neural metabolic activity. Previous research has shown a connection between cortical metabolism and changes in anesthesia which have in turn had a beneficial effect on post anesthesia cognitive and behavioral recovery.

Hypothesis: Administering ketamine will have a positive effect on hemodynamic changes in the frontal cortex measured by NIRS which will result in a more beneficial cognitive and behavioral side effect profile after anesthesia.

Aims: To examine the relationship between hemodynamic changes in the frontal cortex during anesthesia and perioperative cognitive and behavioral changes.

Materials and methods: In 50 subjects we will gather data routinely gathered during anesthesia and NIRS signals, as well as behavioral and cognitive test results. Data will be analyzed via time series analysis.

Expected scientific contribution: Resolving the relationship between hemodynamic changes in the frontal cortex during anesthesia and perioperative behavioral, cognitive and functional changes.

MeSH/Keywords: NIRS, anesthesia, intraoperative awareness, cerebrovascular circulation

Poster code: T-01-08-015

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

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.

Hypothesis: The cranial dura has a richer capillary network and a greater amount of aquaporins than the spinal dura and the outer layer of the dura is richer in capillaries and aquaporins than the inner layer.

Aims: The general aim of this research is to show the morphological differences of the capillaries of the cranial and spinal dura. Specific aims are: (1) to prove the existence of aquaporin in the capillary walls of the cranial and spinal dura, (2) to determine the morphometric characteristics of the capillaries in the cranial and spinal dura, (3) to determine the amount of aquaporin in the capillaries of the inner and outer layers of the cranial and spinal dura and (4) to show whether there is a morphological similarity between the pig and human dura both in the cranial and spinal parts.

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.

Expected scientific contribution: This research would show that the dura is not only a collection of connective tissue with a mechanical role of protecting the brain and spinal cord but also participates in neurofluid physiology. This would open new avenues in the research of pathological processes within the craniospinal space, such as trauma, edema, bleeding i.e. ways of treating them.

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

Poster code: T-01-08-035

Poster Title: Relationship between glycemia and the volume of peritumoral brain edema in patients with metastases of central nervous system

PhD candidate: Martina Štenger

Part of the thesis: Relationship between glycemia and the volume of peritumoral brain edema in patients with metastases of central nervous system

Mentor(s): Professor Nives Pećina-Šlaus, MD PhD, Associate Professor Goran Mrak, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: One of the most common symptoms in patients with brain metastases, which causes significant morbidity, is peritumoral brain edema. So far, routine therapy has been dexamethasone, however, with long-term use, refractoriness of edema develops. The exact causes of both edema and the development of refractoriness to therapy have not yet been discovered.

Hypothesis: Patients with secondary neoplasms of the central nervous system who have prolonged hyperglycemia have a larger volume of peritumoral brain edema.

Aims: The general objective of this research is to compare the values of preoperatively measured HbA1c with the volume of peritumoral brain edema on preoperative MRI of the brain in patients with metastases of the central nervous system

Materials and methods: About 80 patients will be included in this research and will be treated surgically in the KBC Zagreb Neurosurgery Clinic for the removal of a brain metastases. Pathohistological diagnosis of all removed tissue is routinely performed at the Clinical Institute for Pathology and Cytology of KBC Zagreb. Only subjects with first central nervous system surgery will be included. Patients who received a blood transfusion three months ago or underwent major surgery will not be included in the study. Patients will be recorded whether they have previously been diagnosed with diabetes or not. HbA1c measurement will be done in the way that is currently routinely used in the Clinic for Neurosurgery. The device analyzes blood samples using standard high-performance liquid chromatography. HbA1c is calculated as a ratio to total hemoglobin with the help of a chromatogram. MRI of the brain will be done in a manner that is routinely used for neuronavigation purposes. All MR scans will be done at the Clinical Institute for Diagnostic and Interventional Neuroradiology of KBC Zagreb. Thin-slice high-resolution T2 scans with a layer thickness of 0.9 mm will be used. These images will be correlated with T1 MR images. For each tumor process, its location in relation to the tentorium will be determined, and accordingly, the processes will be divided into two categories. A volumetric analysis of the tumor process and peritumoral edema will be performed semi-automatically in such a way that the tumor and edema are manually delineated on the axial sections and finally the volume of the aforementioned is calculated. The obtained values of tumor volume and peritumoral edema will correlate with each other and with glycated hemoglobin values. Both obtained values will be correlated with the pathohistological diagnosis of the tumor. It will be determined whether there is a significant correlation between the obtained volumes, considering whether the process is supratentorial or infratentorial, and whether there is a correlation if the patient has been diagnosed with diabetes. The collected data will be described using the usual procedures of descriptive statistics, and the results will be presented tabularly and graphically.

Expected scientific contribution: The expected contribution would be a better explanation of the cause of edema. So far, the cause of peritumoral brain edema has not been explained, and we cannot influence it with targeted therapy, but it is treated in all patients with dexamethasone. It is known that dexamethason leads to hyperglycemia in the blood. It is also known that the effectiveness of dexamethasone therapy decreases with time and higher doses are needed, which is also in line with our hypothesis. The clinical importance of euglycemia in patients with brain metastases would be defined.

MeSH/Keywords: brain metastases, peritumoral brain edema, HbA1c

Poster code: T-01-08-043

Poster Title: Molecular profile of electrophysiologically defined neuron subpopulations in the subthalamic nucleus of the rat

PhD candidate: Tin Luka Petanjek

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

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 biconvex diencephalic nucleus whose role is to control the motor system as part of the extrapyramidal motor system. Although research on the STN has grown significantly, there are still many unknowns about its development, structure and function. A special problem of STN research are electrophysiological studies. Electrophysiological changes in the STN have been discovered in Parkinson's disease, however it is unclear how these changes occur at the cellular level in humans. Unfortunately, electrophysiological studies of the human STN are limited to experiments during deep brain stimulation surgeries. Therefore, data on the electrophysiological characteristics of neurons in the human STN are lacking. Based on electrophysiological characteristics in animal studies, STN neurons can be divided into two main subgroups - burst and tonic firing neurons. In order to successfully translate electrophysiological data from animal models to humans, it is necessary to determine the electrophysiological profile and molecular markers of rat STN neurons and to determine in which subgroups of human neurons these molecular markers are found.

Hypothesis: Different electrophysiologically defined subgroups of cells in the rat STN also have unique molecular markers on the basis of which they can be identified.

Aims: The aim of the study is to determine the unique markers of different electrophysiologically defined subgroups of neurons in the rat STN by determining the electrophysiological profile, morphological appearance, molecular profile and unique molecular markers of neurons in the rat STN.

Materials and methods: The research will consist of two parts. In the first part, electrophysiological experiments of STN neurons will be conducted in order to determine their characteristics and classify them into subgroups accordingly. In the second part neurons filled with biocytin will be visualized with streptavidin to analyze the morphology, and then molecular markers will be determined using the immunofluorescence method. MATERIALS: The research will be carried out on acute brain slices of the STN in adult male rats. Rats will be anesthetized and confirmed dead. Brain tissue will carefully be removed and cut on vibratome into 300 micrometer slices. Up to 80 rats will be used for this study. PATCH CLAMP METHOD: After preparation of acute brain slices of the STN, each slice will be transferred to the recording chamber and placed on a microscope for electrophysiological measurements under constant perfusion with oxygenated artificial cerebrospinal fluid. A micropipette will be brought to the neurons of the brain slice under a constant current pulse until the giga Ohm connection is established. After breaking through the neuron membrane, the whole cell configuration will be established. The firing pattern of an individual neuron will be recorded and biocytin from the pipette will freely pass from the pipette and fill the neuron. After recording, the brain section will then be fixed in paraformaldehyde and incubated in streptavidin to fluorescently visualize the neuron. MORPHOLOGICAL ANALYSIS Streptavidin incubated neurons will be recorded using confocal microscope then reconstructed and anlysed using Neurolucida computer program. IMMUNOFLUORESCENCE To determine the expression of molecular markers in STN neurons we will use markers such as neuronal markers, functional proteins (parvalbumin, calretinin), synaptic markers (synaptophysin), transcription factors and neurotransmitter receptors. RNA SCOPE METHOD In cases where high-quality antibodies are not available for protein expression analysis, the RNAscope method will be used, which enables the analysis of mRNA expression in the tissue even in cases where the mRNA quality is reduced.

Expected scientific contribution: The expected scientific contribution of this research is the determination of molecular markers of electrophysiologically defined cell subpopulations of the subthalamic nucleus. The established markers could enable the translation of electrophysiological results obtained in experimental animals to subgroups of neurons of the human subthalamic nucleus.

MeSH/Keywords: subthalamic nucleus, electrophysiology, molecular markers

Poster code: T-01-08-056

Poster Title: Reorganisation of dendritic spines of hippocampal granule cells in the mouse brain following entorhinal cortex lesion in vivo

PhD candidate: Fran Božić

Part of the thesis: Reorganisation of dendritic spines of hippocampal granule cells in the mouse brain following 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 aims to use 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 that is tightly linked to the dendritic spines. It has been observed that synaptopodin interacts with calcium signalling molecules in the spines, and it promotes actin molecule polymerisation. These functions are the basis of spine structural plasticity and they promote dendritic spine growth and structural stability.

Hypothesis: Synaptopodin is positively associated with dendritic spine head size and dendritic spine survival of dentate granule cells following entorhinal cortex lesion in vivo in mice.

Aims: To analyse dendritic spines (density, head size, and SP content) in the denervated and non-denervated portion of the dentate gyrus molecular layer, to compare spine morphology of distinct dendritic segments of the same granule cell and to determine whether SP+ spines suppress the formation of new spines in their neighbourhood by analysing the distances between SP+ and SP- spines and their neighbouring spines.

Materials and methods: This research uses C57BL/6-J male mice aged 10-24 weeks which are perfused at several time points following the steretaxic entorhinal lesion (3, 7, 14, and 28 days post-lesion (dpl)) (n = 5 per group). Additionally, sham-operated mice are used as 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 realigned, 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, surgically stitched, and the antibiotic prophylaxis is administered intraperitoneally. The same procedure is used for the sham-operated mice except for lowering the wire knife. Upon reaching the planned time point, the mouse is transcardially perfused and the brain is then sectioned on a vibratome in 100 m 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 micropipette is pulled using a vertical puller, loaded with fluorescent dye, and then guided into the hippocampal granule cell layer where the cells are impaled and filled 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 only if it is placed inside the spine boundary.

Expected scientific contribution: Extant data suggest that the reorganisation of the brain contributes to the recovery of function, which is significant for rehabilitation. This research will contribute to this body of knowledge by examining how granule cells reorganise after entorhinal denervation and by examining the role of the plasticity protein synaptopodin in this context.

MeSH/Keywords: dendritic spines, denervation, reorganisation, synaptopodin, morphology, hippocampus

Poster code: T-01-08-065

Poster Title: Molecular and morphological characteristics underlying neuronal specialization in the human anterior cingulate cortex

PhD candidate: Matija Vid Prkačin

Part of the thesis: Molecular and morphological characteristics underlying neuronal specialization in the human anterior cingulate cortex

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

Affiliation: University of Zagreb School of Medicine

Introduction: Current knowledge about the specific cells of the anterior cingulate cortex is insufficient to make clear conclusions about its functional specialization in humans. Therefore, the goal of this research is to determine the morphological and molecular characteristics of projection neurons and interneurons in the anterior cingulate cortex and to provide insight into the functional organization of the cortical network in this region.

Hypothesis: Molecular and morphological characteristics of neurons differ between the anterior cingulate cortex and other areas of the prefrontal cortex.

Aims: GENERAL AIM: To determine the morphological and molecular characteristics of neurons in the anterior cingulate cortex (Brodmann area 24). SPECIFIC AIMS: (1) to determine the morphological and molecular specialization of cells by layer in Brodmann area 24; (2) to develop an algorithm for the identification of specialized cells in the Brodmann area 24; (3) to compare the morphological and molecular characteristics of the cells of the anterior cingulate cortex (Brodmann area 24) with the cells of the dorsolateral (Brodmann area 9) and orbital (Brodmann area 14) prefrontal cortex.

Materials and methods: Sections of the frontal and the anterior cingulate cortex (Brodmann areas 9, 14 and 24) of 5 adult brains from the Zagreb neuroembryological collection will be used in this research. Anti-NeuN antibody and fluorescent Nissl staining will be used to show cytoarchitectonics. Anti-SMI-32 and MAP2 antibodies will be used to detect projection neurons, and double labeling using anti-NeuN, SMI-32, MAP2 and anti-AnkG antibodies will be used to visualize their initial axon segments. Anti-calretinin, calbindin, parvalbumin, somatostatin will be used for the detection of GABAergic interneurons.Anti-versican and neurocan antibodies and Wisteria floribunda agglutinin will be used to show the condensed extracellular matrix. RNAscope in situ hybridization will be used for the detection of target RNA. For the molecular characterization of neurons, probes for the following genes will be used: SLC32A1 (VGAT) and SLC17A7 (VGLUT1). Histological preparations processed by immunohistochemistry and RNA in situ hybridization will be imaged on a confocal microscope. Quantitative analysis will be performed in the Neurolucida 2020 software. The proportion of projection neurons and the distribution of perineural networks in all layers of Brodmann area 24 will be determined. For morphometric analysis, neuron body contour will be outlined on confocal images of histological sections stained with anti-NeuN antibody. Based on molecular (expression of SMI-32 and MAP2 proteins), morphological (position of initial axon segment) and morphometric (area, aspect ratio, roundness) parameters, an algorithm will be created that will be able to clearly distinguish the specialized cells of the anterior cingulate region from other neurons in the cerebral cortex. The acquired quantitative data will be statistically analyzed (Kruskal-Wallis test) to determine the differences in morphometric parameters between neurons of different regions of the cerebral cortex.

Expected scientific contribution: Analysing the morphological and molecular properties of neurons of the anterior cingulate cortex is the basis for understanding the functional organization of this area. The development of an algorithm for the identification of neuron specialization would enable objectively determine the structural specialization of an individual cortical area. The aforementioned would also have translational significance, as it would enable to objectively identify changes in the organization of the neural network in pathological conditions.

Acknowledgments: This work was supported by the Croatian Science Foundation Grants No. IP-2019-04-3182 (Brain Extracellular Matrix in Development and in Perinatal Hypoxia, PI: Nataša Jovanov Milošević).

MeSH/Keywords: anterior cingulate cortex, von Economo neurons, interneurons, neural networks

Poster code: T-01-08-071

Poster Title: The effect of cellular aging on protein quality control 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: University of Zagreb School of Medicine

Introduction: Neurodegenerative diseases such as Alzheimers, Parkinsons, Huntingtons and many other are characterized by the accumulation of protein aggregates within the affected neurons. Although aging is a major risk factor for the disease, the molecular basis of the effect that cellular aging has on the accumulation of protein aggregates is unclear. To better understand why protein aggregates accumulate in neurodegenerative diseases, it is important to investigate the fundamental molecular pathways by which cells maintain protein homeostasis under normal conditions, and how these processes are affected during aging.

Hypothesis: Cellular aging has a negative effect on the selective degradation of misfolded proteins in yeast Saccharomyces cerevisiae.

Aims: Aim: To examine the effect of cellular aging on the selective degradation of misfolded proteins in yeast Saccharomyces cerevisiae. Specific aims: (1) to examine the degradation of misfolded proteins during chronological aging in yeast cells; (2) to investigate the effect of a disrupted ubiquitin-proteasome system on the chronological aging in yeast cells; (3) to determine whether there is a correlation between the localization of proteasomes and the degradation of misfolded proteins during chronological aging in yeast cells; (4) to examine the effect of replicative aging in yeast cells on the degradation of misfolded proteins.

Materials and methods: The experimental model used in this study is the yeast Saccharomyces cerevisiae. Strains available from the strain bank (Euroscarf, Bad Homburg, Germany) and constructed in this study are isogenic to wild-type S288C strain. Chronological aging of yeast cells will be achieved using the standard method of cell growth in a complete or selective liquid medium, for the period 2 to 7 days without media change, during which cell culture enters the stationary phase. Growth and purification of replicative aged yeast cells will be achieved by using a protocol for a mother enrichment program. Briefly, cell culture will be grown to the exponential phase and cells will be labeled with EZ-Link Sulfo-NHS-LC-biotin. The biotinylated mother cells will then be purified using magnet beads conjugated with streptavidin, labeled with modified biotin-binding protein avidin and examined using fluorescence microscopy. Protein degradation will be investigated by cycloheximide chase followed by Western blot. After the addition of cycloheximide to the cell culture, which inhibits protein synthesis de novo, cells will be collected at specific time points and total cellular lysates will be prepared. Western blot method will be used to analyze the proteins by antibodies specific to the antigen epitope. The proteins will be visualized using chemiluminescence. The signal intensity of the model proteins will be normalized to the total amount of protein in the cell lysates using the Stain-free imaging method (BioRad). Intracellular localization of proteasomes and model proteins will be examined using gene fusions of proteins with fluorescent proteins and widefield or confocal microscopy. Viability of yeast cells will be assessed by a semi-quantitative method of analyzing colony formation from decimal dilutions of the culture inoculated onto a solid medium. Cell fractionation will be performed to separate quiescent and non-quiescent cells from the stationary phase culture by the Percoll density gradient centrifugation.

Expected scientific contribution: We expect that this study will show whether degradation of misfolded proteins is impaired in old cells, which will contribute to our understanding how protein quality control is affected during cellular aging.

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

Poster code: T-01-08-072

Poster Title: Washing brains or how to see things more clearly Fluorescence microscopy can be used for visualization of blood vessels and neurons in the cleared mouse brain

PhD candidate: Dominik Hamer

Part of the thesis: Light sheet fluorescence microscopy in the assessment of morphological characteristics of the mouse brain after ischemic lesion

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

Affiliation: University of Zagreb School of Medicine, Department of Histology and Embryology

Introduction: Stroke is one of the leading causes of death and disability in Croatia and Europe, and is caused by disorder in the blood supply of certain brain regions which reduces or completely cuts off the supply of nutrients and oxygen. The complexity of stroke research comes from the dependence of morphological, functional and molecular mechanisms that occur immediately after ischemic damage. In the evaluation of new medical interventions in the treatment and rehabilitation of patients who survived stroke, it is necessary to conduct experiments on laboratory animals. This provides relevant data related to morphological, functional and molecular events, which results in the expansion and improvement of preclinical studies and increases the possibility of their potential translation into clinical trials.

Hypothesis: The characteristics of ischemic brain damage visualized using magnetic resonance imaging correspond to the morphological characteristics of ischemic damage visualized with light sheet fluorescence microscopy in cleared mouse brain.

Aims: MAIN AIM: Describe the microscopic characteristics of ischemic damage in the mouse brain using light sheet fluorescence microscopy and compare them with the characteristics obtained by magnetic resonance imaging. SPECIFIC AIMS: 1. introduction and evaluation of histological methods for the preparation of cleared mouse brain samples, which achieves a satisfactory transparency of organs and the preservation of fluorescently marked structures of interest 2. analysis of fluorescently labeled neurons within the cleared mouse brain and reconstruction of a three-dimensional image obtained using light sheet fluorescence microscopy after inducing ischemic damage 3. data quantification obtained from the three-dimensional reconstruction of fluorescently labeled neurons within the mouse brain using a computer program for image processing after inducing ischemic damage 4. comparison of morphological characteristics of ischemic damage using magnetic resonance imaging and light sheet fluorescence microscopy

Materials and methods: Mouse brains were isolated from two months old mouse (Thy1 YFP 16 strain), which naturally produce yellow fluorescent protein (YFP) in neurons. Blood vessels staining was achieved by using Lycopersicon Esculentum Lectin Texas Red (Invitrogen) dye which was injected in the left heart ventricle of living mouse prior perfusion. Subsequently, mice were perfused with 1xPBS and 4 % formalin solution and cleared. Different clearing methods for making transparent brain samples can be applied. For whole brain tissue clearing, three methods were used: ECi (optical clearing using ethyl cinnamate), PEGASOS (polyethylene glycol associated solvent system) and FluoClearBABB (fluorescent protein preserving using benzyl alcohol and benzyl benzoate). Cleared mouse brain samples were cut on approximately 1 mm thick slices using cutting mold, mounted subsequently on the glass slides in the drop of the final clearing solution, covered by coverslips, and imaged using inverted fluorescence microscope (The EVOS FL Auto Imaging System, ThermoFisher Scientific) and confocal microscope (Olypus FV3000). Additionally, antibody labeling method was used for neuronvisualization. Neurons in mouse hemisphere were labeled with primary conjugated antibody (NeuN Antibody Vio R667 , Miltenyi Biotec) and cleared using MACS Clearing Kit (Miltenyi Biotec). For visualization of antibody labeled neurons in cleared mouse brain hemisphere light sheet fluorescence microscope (LSFM) was used (Ultramicroscope II, LaVision Biotec).

Expected scientific contribution: A comparison of these methods will provide insight into the morphological consequences of brain ischemia which is a prerequisite for the development of new therapeutic procedures after a stroke.

Acknowledgments: This study was supported by the European Union through the European Regional Development Fund, under Grant Agreement No KK.01.1.1.07.0071 project Sinergy of molecular markers and multimodal in vivo imaging during preclinical assessment of the consequence

MeSH/Keywords: ischemic stroke, magnetic resonance imaging, light sheet fluorescence microscopy, tissue clearing, ischemic lesion

Poster code: T-01-08-078

Poster Title: Features of the functional damage of the mouse brain collected by monitoring animals in their home cage and kinematic analysis of gait after ischemic lesion

PhD candidate: Daniela Petrinec

Part of the thesis: Features of the functional damage of the mouse brain collected by monitoring animals in their home cage and kinematic analysis of gait after ischemic lesion

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

Affiliation: University of Zagreb School of Medicine

Introduction: Stroke is one of the biggest health problems. It is the first cause of disability and the second leading cause of death in developed countries, including Croatia. Acute therapy of ischemic stroke includes thrombolysis or thrombectomy and there are no other targeted therapies for patients, only symptomatic therapy and rehabilitation which may or may not be successful. Therefore additional research and understanding of the functional recovery process is necessary. Neuroimaging methods such as magnetic resonance imaging and optical imaging of live animals have allowed insight into the events in the brain after an ischemic lesion, however, such data need to be supplemented with other methods of assessment of living animals in order to be able to conclude something more about functional damage.

Hypothesis: Functional features of mouse brain damage assessed by monitoring animals in their own cages and kinematic analysis of gait correspond to ischemic lesions recorded by magnetic resonance imaging.

Aims: The general aim of this research is to clarify the relationship between ischemic brain lesions and functional damage in mice. The specific aims of this research are: to establish and optimize the procedure of recording mice in a device for kinematic gait analysis, to establish a longitudinal procedure for monitoring mice in their own cages, to determine the functional damage of mice during the acute and chronic phases after MCAO, and to compare the size and volume of the ischemic lesion shown by magnetic resonance imaging with the functional deficit of the mouse after MCAO.

Materials and methods: Functional damage of the mouse brain (wild type) after ischemic lesion will be determined by kinematic analysis of gait and the activity of the mice will be monitored day and night in their home cages. Methods such as magnetic resonance imaging and optical imaging of live animals have enabled an understanding of the development of ischemic lesions in the mouse brain. In contrast to neuroimaging, the technological possibilities of assessing functional impairment after an ischemic lesion were limited to neurological tests. In addition to the well examined acute phase, recent research also focuses on the chronic phase through longitudinal research, as will be the case with this research.

Expected scientific contribution: With the aim of collecting as relevant data as possible, and standardizing and improving the quality of research, the features of automated methods in the functional assessment of ischemic brain damage will be evaluated.

Acknowledgments: This study was supported by the European Union through the European Regional Development Fund, under Grant Agreement No. KK.01.1.1.07.0071, project Sinergy of molecular markers and multimodal in vivo imaging during preclinical assessment of the consequenc

MeSH/Keywords: ischemic lesion, size of ischemic lesion, functional damage, kinematic analysis, home cage monitoring

Poster code: T-01-08-099

Poster Title: Histological and in vivo MRI markers of extracellular matrix and cortical connectivity reorganization in the brain of a rat model of moderate perinatal hypoxia

PhD candidate: Matea Drlje

Part of the thesis: Histological and in vivo MRI markers of extracellular matrix and cortical connectivity reorganization in the brain 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: This study aims to identify specific changes in the organization of the extracellular matrix (ECM) and cortical connectivity in a rat model of moderate perinatal hypoxia. The multimodal approach in this research (comparison of in vivo magnetic resonance images with histological and immunohistochemical findings) should allow a better understanding of the development of cerebral connectivity, synaptogenesis, and plasticity in the immature brain. This approach can potentially identify novel specific patterns for in vivo magnetic resonance imaging (MRI) of perinatal brain injuries to improve MRI methods in diagnosing brain injuries and the early outcome prognosis of such injuries.

Hypothesis: Moderate perinatal hypoxia causes reorganization of the rat brain extracellular matrix and neocortical connectivity that can be detected at postnatal day 15 (P15) by molecular, histological, and in vivo MRI methods.

Aims: The overall aim is to identify and characterize the consequences of early (P1) moderate perinatal hypoxic injury on the rat brain ECM and cortical connectivity. Specific aims are to evaluate early moderate perinatal hypoxic injury in rats at P15 to identify and characterize changes in: 1. Regional brain volumes, by in vivo structural MRI 2. Regional brain diffusivity and connectivity by in vivo diffusion tensor MRI 3. Expression of markers of neurons, glia, and ECM, by histology and immunohistochemistry 4. Cross-correlate the observed changes to improve understanding of the nature of the grey and white matter injury.

Materials and methods: For this study, 48 P15 Wistar rats (RccHan: WIST), both sexes (24 females, 24 males) will be used). Newborn rats will be separated from dams at the P1 and exposed to controlled normobaric hypoxia in a hypoxic chamber (8% O, 92% N, n=24) for 2 hours or to control conditions (21% O, 78% N, 2 hours, n=24). At P15, a subset of animals (n=16, 8 per group) male animals will be used for the in vivo MRI protocol and later will be added to 32 animals of both sex (24 females and 8 males) involved only in the ex vivo histological protocol. Based on the MRI findings, we will target the most affected cortical areas, and histological and immunohistological staining of those cortical areas will be performed. Light and confocal microscopy will be used for image acquisition, and ImageJ or Neurolucida software will be used for the analysis. The in vivo MRI, histochemical and immunohistochemical results will be compared to achieve the aim of the study.

Expected scientific contribution: This study is expected to provide new insights into the brain reorganizational capacity and in a clinically relevant rat model of moderate perinatal hypoxic brain injury. Moreover, it might optimize and validate a novel animal model for further research into perinatal hypoxia for pharmacological studies and new MRI diagnostic modalities design.

Acknowledgments: This research is co-financed by the Scientific Centre of Excellence for Basic, Clinical, and Translational Neuroscience (project "Experimental and clinical research of hypoxic- ischemic damage in perinatal and adult brain"; GA KK01.1.1.01.0007 funded by t

MeSH/Keywords: lumican, subplate, MRI volumetry, neocortex, immunohistochemistry

Poster code: T-01-08-105

Poster Title: Histological and in vivo MRI markers of extracellular matrix and cortical connectivity reorganization in the brain of a rat model of moderate perinatal hypoxia

PhD candidate: Matea Drlje

Part of the thesis: Histoloki i in vivo-MRI biljezi reorganizacije izvanstanine tvari i kortikalnih veza u mozgu takorskog modela umjerene perinatalne hipoksije

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

Affiliation: University of Zagreb School of Medicine

Introduction: This study aims to identify specific changes in the organization of the extracellular matrix (ECM) and cortical connectivity in a rat model of moderate perinatal hypoxia. The multimodal approach in this research (comparison of in vivo MR images with histological and immunohistochemical findings) should allow a better understanding of the development of cerebral connectivity and synaptogenesis, and plasticity in the immature brain. This approach can potentially identify novel specific patterns for in vivo MRI of perinatal brain injuries to improve MR imaging methods in diagnosing brain injuries and the early outcome prognosis of such injuries.

Hypothesis: Moderate perinatal hypoxia causes reorganization of the rat brain extracellular matrix and neocortical connectivity that can be detected at postnatal day 15 (P15) by molecular, histological, and in vivo MRI methods.

Aims: The overall aim is to identify and characterize the consequences of early (P1) moderate perinatal hypoxic injury on the rat brain extracellular matrix (ECM) and cortical connectivity. Specific aims are to evaluate early moderate perinatal hypoxic injury in rats at P15 to identify and characterize changes in: 1. regional brain volumes, by in vivo structural MRI 2. regional brain diffusivity and connectivity by in vivo diffusion tensor MRI 3. expression of markers of neurons, glia, and ECM, by histology and immunohistochemistry 4. Cross-correlate the observed changes to improve understanding of the nature of the grey and white matter injury.

Materials and methods: For this study, 48 15-day-old (P15) Wistar Han rats (RccHan: WIST) will be used (24 per group, mixed sex). Newborn rats will be separated from dams at the age of the first postnatal day (P1) and exposed to controlled normobaric hypoxia in a hypoxic chamber (8% O, 92% N) for 2 hours or to control conditions (21% O, 78% N, 2 hours). At 15 days of age, A subset of animals (n=16, 8 per group) male animals will be used for the in vivo MRI protocol and later will be added to 32 animals involved only in the ex vivo histological protocol. Based on the MRI findings, we will target the most affected cortical areas, and histological and immunohistological staining of those cortical areas will be performed. Light and confocal microscopy will be used for image acquisition, and ImageJ or Neurolucida software will be used for the analysis. The in vivo MRI, histochemical and immunohistochemical results will be compared to achieve the aim of the study.

Expected scientific contribution: This study is expected to provide new insights into the brain reorganizational capacity and in a clinically relevant rat model of moderate perinatal hypoxic brain injury. Moreover, it might optimize and validate a novel animal model for further research into perinatal hypoxia for pharmacological studies and new MRI diagnostic modalities design.

MeSH/Keywords: lumican, subplate, MRI volumetry, neocortex, immunohistochemistry

Poster code: T-01-08-106

Poster Title: Specific transduction of mouse brain neurons and astrocytes with lentiviral vectors for amplification of the Spry2 gene

PhD candidate: Monika Berecki

Part of the thesis: Specific transduction of mouse brain neurons and astrocytes with lentiviral vectors for amplification of the Spry2 gene

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

Affiliation: University of Zagreb School of Medicine

Introduction: Lentiviral vectors (LVs) are used to modify gene expression in the central nervous system (CNS) to understand its physiology. Genetic modifications are achieved through viral vectors, which allow for the study of development, function, and pathogenesis of cells. Lentiviruses are the only retroviruses that can infect postmitotic cells. LVs can be modified using strong promoters and interference RNA to avoid unwanted transduction. These vectors have minimal inflammatory response and maintain the normal morphology and cellular composition of the area of injection. LVs can be identified in tissue through immunofluorescent histochemical staining using fluorescence confocal microscopy and fluorescence light field microscopy. The Spry2 protein, which regulates cell differentiation and signal transduction, is used in studies on CNS pathology. Sex chromosome genes, hormones, and steroids can affect glial cell migration, release of neurotrophic factors, and inflammation. Although sex differences in brain function and response to pharmacological intervention exist, they are not well-studied, leading to bias in treatment and different therapeutic effectiveness in men and women.

Hypothesis: Upregulation of Sprouty 2 gene expression will affect the morphology of neurons more than astroglia, and will result in a lower extent of neuroinflammation in females than in males.

Aims: The study aims to investigate differences in the response to gene transduction by lentiviral vectors in astrocytes and neurons of female and male mice. The specific goals of the study are to establish a production platform for lentiviral vectors, confirm the specificity of transduction in brain cells of male and female mice, examine morphological changes induced by lentiviral manipulation of the Sprouty 2 gene, and investigate gender differences in the molecular response to gene manipulation. These aims will be achieved by using specific markers for neurons and astrocytes, confocal and light-field microscopy, and by measuring the levels of Spry2, pERK/ERK, pAKT/AKT, and pPLC/PLC.

Materials and methods: Heat-shock method will be used to multiply plasmids in DH5- E. coli for LV production. Plasmids were provided by collaborators and are third generation, i.e. HEK293T cell line will be transfected with four plasmids. LV concentration and resuspension will be done with sterile PBS. ELISA and One-Wash, HIV-1 p24 ELISA kit will be used for LV production titration. Experiments will be conducted on 32 wild-type and Thy1-YFP mice. The LV suspension will be injected into the brain at two depths using the stereotactic frame, and the animals will be monitored for 3 days with intraperitoneal analgesia and glycemic regulation. Euthanasia will occur on the 7th, 14th, or 21st day from the injection. Fixed and cryoprotected tissue will be cut and processed by the free-floating IF-IHC method to analyze the colocalization with fluorescent marker proteins from LV. Statistical analysis will be performed with the IBM SPSS Statistics version 26.0.0 program.

Expected scientific contribution: The establishment of a platform for the production of lentiviral vectors should enable progress in the research of the central nervous system of mice. Successful specific transduction should open the possibility of future therapeutic intervention using diverse constructs. Furthermore, research that equally includes both sexes should indicate potential differences in the response to this type of genetic manipulation in the early stages of research, which should enable timely optimization of procedures, and the introduction of preclinical interventions that consider both sexes.

MeSH/Keywords: lentivirus therapy, neurons, astrocytes, sex dimorphism

Poster code: T-01-08-138

Clinical medical sciences – research proposals

Poster Title: Effect of a transversus abdominis plane block on operative wound healing after a cesarean delivery

PhD candidate: Mirta Ciglar

Part of the thesis: Effects of the anterior abdominal wall regional anesthesia on operative wound healing after a cesarean delivery

Mentor(s): Associate Professor Dinko Tonković, MD PhD, Assistant Professor Marinko Vučić, MD PhD

Affiliation: University of Zagreb School of Medicine, Sestre milosrdnice University Hospital Center

Introduction: Cesarean section is one of the most frequently performed surgical procedures in the world. It is often accompanied by severe postoperative pain that leads to a complex immune and stress response in the body. Neutrophil to lymphocyte ratio (NLR) is a reliable and easily available marker of immune response to various infectious and non-infectious stimuli. Elevated NLR values are predictive in the early detection of wound infection after cesarean section. Wound healing involves three stages: inflammation, proliferation, and remodeling. REEDA scale is a valid tool for assessing obstetric wound healing. Many factors can affect the wound-healing process. Consistent overlap between pain-related dysregulation of neuroendocrine and immune function and the aspects of the neuroendocrine and immune function shown to be critical in wound healing suggests that pain may play a role in delayed wound healing. According to previous research, the transversus abdominis plane (TAP) block significantly reduces pain and cortisol, epinephrine, and norepinephrine levels after cesarean section. Adding dexmedetomidine to the local anesthetic in the TAP block has a more pronounced analgesic effect and prolongs the duration of analgesia. The author found only one study demonstrating that adding a TAP block to standard analgesia positively affects wound healing after cesarean delivery. The influence of the addition of dexmedetomidine in TAP block on wound healing after cesarean delivery has not been investigated so far.

Hypothesis: The use of bilateral transversus abdominis plane block just after cesarean delivery will lead to lower values of the REEDA scale compared to standard analgesia, where the addition of dexmedetomidine to levobupivacaine will have a more pronounced effect compared to TAP block with levobupivacaine.

Aims: The main aim is to examine whether the application of the bilateral TAP block with levobupivacaine, with and without the addition of dexmedetomidine, affects wound healing after cesarean delivery. Specific aims are to determine the influence of bilateral TAP block with levobupivacaine and levobupivacaine with the addition of dexmedetomidine on the postoperative stress response by measuring serum cortisol levels, the postoperative immune response by determining neutrophil to lymphocyte ratio and the intensity of postoperative pain by using a pain numerical rating scale. Additional aims are to compare postoperative stress and inflammatory responses between groups and establish whether there is a correlation between these responses and wound healing.

Materials and methods: This study will be conducted as a prospective, randomized study at Sestre milosrdnice University Hospital Center. A total of 115 healthy parturients undergoing elective cesarean section will be included. Participants will be randomly allocated to either a group SA receiving the standard postoperative analgesia or groups receiving the TAP block with levobupivacaine (group L) or levobupivacaine with the addition of dexmedetomidine (group D). An independent observer will assess wound healing using the standardized REEDA scale 72 hours after the cesarean delivery. Serum cortisol levels and neutrophil to lymphocyte ratio will be measured for all participants before the cesarean delivery and on the third postoperative day. Each participant will be assessed at 4, 8, 12, 18, 24, and 72 hours after surgery for pain by using the pain numerical rating scale.

Expected scientific contribution: The results of this study could be used in the future for the standardization of protocols for the treatment of postoperative pain after cesarean delivery with the most favorable postoperative inflammatory and stress response and influence on wound healing.

MeSH/Keywords: Cesarean section, wound healing, nerve block

Poster code: T-02-01-004

Poster Title: Serum concentrations of lipocalin-2, interleukin-8 and calprotectin in patients with hidradenitis suppurativa

PhD candidate: Joško Miše

Part of the thesis: Serum concentrations of lipocalin-2, interleukin-8 and calprotectin in patients with hidradenitis suppurativa

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

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

Introduction: Hidradenitis suppurativa (HS) is an inflammatory disorder characterized by recurrent painful nodules, abscesses and sinus tracts on axillary, groin, gluteal and anogenital regions. It is a skin disease that severely impacts patients quality of life with pain, malodour, sleep and sexual dysfunctions and poor mental health as consequences of uncontrolled disease. HS has an estimated global prevalence between 0.1-1%. However, the exact prevalence could be even higher since the patients still experience a significant diagnostic delay without a pathognomonic test. The fundamental mechanism of HS consists of three main aspects: follicular hyperkeratosis and dilatation, follicular rupture and chronic inflammation. Disease severity is determined by using the Hurley clinical grading system which categorizes HS into three stages. Hurley stage I is characterized by single or multiple abscesses without sinus tracts and permanent lesions or cicatrization (scarring). Hurley stage II includes single/multiple, widely separated and recurrent abscesses with sinus tracts and cicatrization. Stage III features are multiple interconnected abscesses, sinus tracts and cicatrization that involves an entire anatomical area. The lack of objective criteria for disease diagnosis and severity classifications has prompted researchers to identify biomarkers that could be used in clinical practice to guide treatment decisions. Lipocalin-2, interleukin-8 and calprotectin have been chosen due to their role in the pathogenesis of inflammatory skin diseases, including hidradenitis suppurativa.

Hypothesis: Serum concentrations of lipocalin-2 (LCN-2), interleukin-8 (IL-8) and calprotectin are elevated in patients with hidradenitis suppurativa compared to healthy controls.

Aims: Primary aim of the thesis is to examine the association between the serum concentrations of LCN-2, IL-8 and calprotectin and the presence of mild and moderate-to-severe and severe forms of hidradenitis suppurativa. Additional thesis objectives include comparing the serum concentrations of LCN-2, IL-8 and calprotectin between patients with mild and moderate-to-severe and severe forms of hidradenitis suppurativa, as well as healthy controls; and assessment of association between serum concentrations of LCN-2, IL-8 and calprotectin and sociodemographic factors of HS patients.

Materials and methods: Type of study is cross-sectional study. The thesis will include 60 HS patients: 30 patients with mild (Hurley stage I) and 30 patients with moderate-to-severe HS (Hurley stages II and III), as well as 30 healthy individuals. Excluding criteria are individuals younger than age 18, previous history of chronic infections, malignancies and autoimmune diseases, and patients who underwent systemic therapies within 4 weeks of sampling. Disease severity will be determined using Hurley classification, IHS 4 classification (International Hidradenitis Suppurativa Severity Score System). Quality of life will be determined by using DLQI (Dermatology Life Quality Index). The values of serum concentrations of LCN-2, IL-8, and calprotectin will be determined using commercially available ELISA tests.

Expected scientific contribution: 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: T-02-02-104

Poster Title: Sarcopenia and psoriatic arthritis

PhD candidate: Sanda Špoljarić Carević

Part of the thesis: Sarcopenia in patients with psoriatic arthritis

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. Sarcopenia (SP) is a progressive generalized muscle disorder characterized by loss of strength and mass of skeletal muscle. SP causes a decrease in physical ability and increases the risk of various poor outcomes .According to our findings, there are no published papers that investigated the prevalence of SP and its association with the disease activity (DA) and functional status in PsA, that used the new EWGSOP2 criteria.

Hypothesis: SP in patients with PsA is associated with greater DA and poorer quality of life (QoL).

Aims: General aim is to determine the prevalence of SP in patients with PsA.Specific aims are :1.Determine the association between SP and physical activity levels in PsA 2.Establish an association between SP and DA in PsA 3.Determine the association between SP and functional status in PsA 4.Determine the association between SP and the level of vitamin D in PsA 5.Determine the association between SP and severity of psoriasis in PsA 6.Determine the association between SP and disease treatment with modifying antirheumatic drugs in patients with PsA.7.Establish an association between SP and QoL in PsA

Materials and methods: Patients diagnosed with PsA will be included in the cross-sectional study. The research will be conducted at the Special Hospital Naftalan. CASPAR criteria will be used to diagnose PsA. The diagnosis of SP will be made according to the of EWGSOP2 criteria.The inclusion criteria: PsA and 18 years and older. The non-inclusion criteria: age less then 18 years, other inflammatory rheumatic diseases, malabsorption , eating disorders, inflammatory bowel disease, malignant disease in the previous 5 years, long-term immobility, neuromuscular diseases, cerebrovascular disease and pregnancy. Before the start of the research, a permit will be obtained from the Ethics Committee of the Special Hospital Naftalan and the Ethics Committee of the School of Medicine in Zagreb. At least 90 participants will be included in the study. After signing informed consent, all subjects will be taken a medical history ,sociodemographic data, anthropometric measurements and clinical examination will then be performed. Each subject will be assigned the value of C-reactive protein (CRP) and vitamin D. The level of pain and the subjective impression of the severity of the disease will be determined using a visual analog scale All subjects will be assessed for muscle strength with a dynamometer, mass with Bioelectric impedance and functional ability with Timed up and go test. Assessment of their level of physical activity and health-related QoL will be by using standardized questionnaires: The international physical activity questionnaire and the SF-36 self-assessment questionnaire. DA will be assessed with ASDAS or DAPSA index. Functional status will be evaluated by BASFI or HAQ-di index. The severity of psoriasis with the Psoriasis area severity index, and fatigue with FACIT-F index. The statistical significance level is set at 0.05. The values of variables measured by the nominal or ordinal scale will be represented by contingent tables. The values of variables measured by the interval or ratio scale will be represented by appropriate measures of central tendency and dispersion. The normality of the distribution of these variables will be examined by the Shapiro-Wilk test, after which the analysis will be carried out using appropriate parametric or nonparametric methods.

Expected scientific contribution: Determining the prevalence of SP in PsA and introducing SP diagnostics in PsA care will contribute to early recognition of SP, improving the QoL and preventing poor outcomes.

Acknowledgments: None

MeSH/Keywords: sarcopenia, psoriatic arthritis, quality of life, vitamin D

Poster code: T-02-03-122

Poster Title: vNOTES AUTOLOGOUS TISSUE RECONSTRUCTION VRS TRADITIONAL VAGINAL SURGERY

PhD candidate: Luka Matak

Part of the thesis: COMPARISSON BETWEEN AUTOLOGOUS TISSUE RECONSTRUCTION USING vNOTES AND STANDARD SURGERY IN TREATMENT OF PELVIC ORGAN PROLAPSE

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

Affiliation: University of Zagreb School of Medicine Croatia, Imelda Hospital Bonheiden Belgium

Introduction: The National Institute of Health and Care Excellence(NICE) guidelines recommend that women be offered lifestyle interventions, physical and behavioral therapies, and medication before surgical options are considered. Still 11-19% of women are estimated to undergo surgery for pelvic organ prolapse (POP) and 30% will require a reintervention.

Hypothesis: Patient with autologous graft transplantation will have less relapse than standard vaginal surgery group

Aims: Compare the efficacy of vNOTES (vaginal natural orifice transluminal surgery) and traditional vaginal surgery between the two groups by determining the risk of reoperation for anterior, posterior or apical prolapse within the study period.

Materials and methods: Patients with a POP-Q of 3 or 4 and an indication for hysterectomy and prolapse repair will be selected and randomly divided in two groups. One group will be operated with standard technique for pelvic organ prolapse and in the other autologous graft will be used using vNOTES. Informed consent will be obtained for all patients. The following criteria will be also taken into the account: patients older than 18 years old with no desire to preserve fertility, POP-Q 3 or 4 stage of prolapse with indication for hysterectomy (abnormal uterine bleeding, enlarged fibrous uterus, premalignant cervical findings), no contraindication for general anaesthesia, pneumoperitoneum or Trendelenburg position, no rectovaginal endometriosis or history of rectal surgery, no history of severe pelvic inflammatory disease, no suspicion of malignancy, no pelvic radiotherapy. Virginity, pregnancy, acute urinary infection were considered contraindications; absence of vaginal delivery and obesity were not. All patients will be operated by the same surgeon. Antibiotic prophylaxis will be given preoperatively according hospital protocols 30 minute before surgery. In standard surgery group of patients vaginal hysterectomy will be done with anterior and/or posterior repair depending on the judgment of the surgeon at the time of the operation. Uterosacral and cardinal ligaments will be repositioned and attached to the vaginal vault with re-absorbable sutures (Vicryl 1; Ethicon, Somerville, NJ, USA) after hysterectomy at the time of vault closure. A modified McCall culdoplasty (obliteration of pouch of Douglas by plication and approximization of the uterosacral and cardinal ligaments using re-absorbable sutures (PDS II 0; Ethicon), and elevation of the vaginal apex) will be done in this group to prevent enterocele and further apical prolapse. Technique description for patients in vNOTES PREFAP (posterior rectus fascia prolapse repair) group. The patient is placed in lithotomy position under general anaesthesia. The procedure consists of 4 parts: vNOTES hysterectomy, harvesting of PREFAP native tissue graft, anterior repair with PREFAP native tissue graft, posterior repair with native tissue graft. Data for the whole study population subject to descriptive statistics. For the general aim of a dichotomous variable of either having reoperation for anterior or apical prolapse, a Chi-squared test will be performed. This will be used for all other dichotomous variables. Measurement data will be expressed as mean±standard deviation (x±SD), and compared using independent-sample t test. KaplanMeier survival analyses will be made using the general aim as a failure variable. We used R software to calculate Phi of 0.26 on the basis of publication. With this Phi middle size effect is positioned on 0.3. Using Chi Square for study power on effect size 0.3,p 0.05 and power of 0.8, sample size is 54.

Expected scientific contribution: This research is important to the international scientific community because it explores the relatively new field of autologous tissue transplantation in prolapse surgery. It has original contribution because a new minimally invasive technique in the preparation and application of autologous tissue in patients with POP is applied.

Acknowledgments: I am deeply grateful to my advisors, Professor Slavko Orešković and Professor Jan Baekelandt, for their guidance and support throughout my research

MeSH/Keywords: vNOTES vaginal vault prolapse autologous transplantation

Poster code: T-02-05-026

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. 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.

Hypothesis: Anxiety and depressive symptoms and reduced quality of life are equally present in all PCOS phenotypes, are independent of clinical and biochemical determinants and represent an intrinsic characteristic of PCOS. Hormonal contraceptives reduce anxiety and depressive symptoms and improve the quality of life in women with PCOS.

Aims: The aim of this prospective interventional study is to examine the frequency of anxiety and depressive symptoms, to assess QoL, investigate the influence of determinants of PCOS and the values of serotonin and allopregnanolone in women with PCOS compared to the healthy population, before, 3 and 6 months after HC usage.

Materials and methods: It is planned to include 120 women aged 18-45 with a 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.

Expected scientific contribution: Further clarification of the relationship and etiology of anxiety and depressive symptoms and the reduction of QoL in women with PCOS, which can contribute to further research in this area and the implementation of early screening and treatment in the daily care of women with this common and health burdening condition.

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

Poster code: T-02-05-068

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, Associate 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 are presently identified. Much researched factors of satisfaction with childbirth are pain relief and the way the pregnancy is completed. Some authors found that psychosocial aspects and the approach of health personnel had the greatest impact on the level of satisfaction, while others mention the importance of strengthening interventions in the field of women's participation in decision-making and supporting the natural course of childbirth. The question remains, can some traits of character and temperament and even shame have an impact on the satisfaction of childbirth? And if so, do they have an influence on satisfaction regardless of the perception of social support and satisfaction with one's own life or is it related? If sp, to what extent are they related? Based on the observed connections, can the healthcare staff be suggested in ways to increase mother's satisfaction with childbirth and reduce their traumatic experience?

Hypothesis: The degree of satisfaction with childbirth is related to mother´s temperament, character and shame, and greater satisfaction will contribute to greater satisfaction with life and social support of the mother.

Aims: The general objective is to show the connection between the degree of satisfaction with childbirth and the temperament and character of the mother. Specific goals are to determine the connection between certain characteristics of the character, temperament and shame of the mother in labor with satisfaction with childbirth, to determine the risk factors related to temperament and character traits and satisfaction with life and social support of the mother to the satisfaction with childbirth and to propose preventive measures to reduce the dissatisfaction of the mother with childbirth.

Materials and methods: The subject research will be interdisciplinary, cohort, prospective, and multicenter, carried out with questionnaires on a convenient sample. Within a year, the research is planned to be carried out in three maternity hospitals of different sizes in the Republic of Croatia (in the cities of Zagreb, akovec and Nova Gradika). It is necessary to collect about 200 respondents, but a larger sample is planned. The City-BiTS scale will be used to examine satisfaction with childbirth, and the BSS-R 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 with the maternity scale SWLS. The PSS scale will be used for assessment of the perception of social support of the mother. Surveys will be handed out to respondents the first day after giving birth, with a request to fill it out within three days from the day of giving birth. Exclusionary criteria include minors, business incapacity, unsigned informed consent, established severe somatic or any mental illness of the mother, existence of serious fetal diseases, emergency caesarean section, premature birth, surveys for which may doubt the veracity of the filled-in data, or there is information about any interpersonal conflict. In addition, all relevant data related to the health status of the mother and fetus/newborn related to the course of pregnancy, week and method of termination of pregnancy, perinatal outcome, anthropometric data and mother and newborn and data related to possible therapy during pregnancy will be collected.

Expected scientific contribution: The research could contribute to the elucidation of the connection between character and temperament traits and the shame of childbirth satisfaction with childbirth and that it will contribute to the possibility of proposing preventive measures to reduce dissatisfaction with childbirth. .

MeSH/Keywords: Childbirth, parturient, satisfaction, character, temperament, social support

Poster code: T-02-05-139

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. One of the methods for rhythm control is pulmonary vein isolation. Despite the development of ablation techniques, the FA 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.

Hypothesis: Plasma concentration of miRNA-21 in patients with AF recurrence will be higher then in those without AF recurrence after catheter ablation of paroxysmal AF. Plasma concentration of miRNA-150 in patients with AF recurrence will be lower then in those without AF recurrence after catheter ablation of paroxysmal AF.

Aims: To determine the association of circulating miRNA-21 and miRNA-150 with AF recurrence in paroxysmal AF patients treated with catheter ablation.

Materials and methods: The study will include 90 patients with paroxysmal AF. After admission to hospital we will determine them circulatory (plasma) levels of miRNA-21 and miRNA-150 by quantitative qRT-PCR. All patients will then undergo catheter crioablation as method of pulmonary vein isolation. The incidence of AF recurrence will be monitored by ambulatory follow-up 12 months following the procedure. We will analyze connection between AF recurrence and miRNA concetration in blood.

Expected scientific contribution: Biomarker application of miRNA-21 and miRNA-150 will detect patients with a higher risk of FA recurrence.

MeSH/Keywords: Paroxysmal atrial fibrillation, pulmonary vein isolation, miRNA

Poster code: T-02-09-006

Poster Title: Influence of continuous positive airway pressure in patients with severe obstructive sleep apnea on pentraxin 3 value

PhD candidate: Marko Perčić

Part of the thesis: Influence of continuous positive airway pressure in patients with severe obstructive sleep apnea on pentraxin 3 value

Mentor(s): Academician Miroslav Samaržija, Associate Professor Edvard Galić, MD PhD

Affiliation: University of Zagreb School of Medicine, Clinical Hospital Sveti Duh, University Psychiatric Hospital Vrapče

Introduction: Obstructive sleep apnea (OSA) is a disorder caused by reduced muscle tone during sleep and independently increases the risk for non-fatal cardiovascular events and cardiovascular mortality. Continuous positive airway pressure (CPAP) treatment reduces the risk for cardiovascular events and mortality in the long term. OSA leads to increased secretion of inflammatory mediators. Pentraxin 3 (PTX3) is a mediator of inflammation, which affects the progression of atherosclerosis and is also elevated in heart failure.

Hypothesis: The use of CPAP therapy in patients with severe OSA leads to a reduction in PTX3 values.

Aims: General objective of this research is to investigate the effect of CPAP on PTX3 values in patients with severe obstructive sleep apnea. Our specific goals are (1) to investigate the correlation of PTX3 and inflammatory mediators before and after CPAP therapy in patients with severe obstructive sleep apnea, (2) to investigate the correlation of PTX3 and hematological markers before and after therapy with CPAP in patients with severe OSA, and (3) to measure the impact of sleepiness before and after using the CPAP device with a questionnaire (Epworth Sleepiness Scale, Epworth).

Materials and methods: In this prospective cohort study, subjects will be selected after polysomnography, which will be done at the University Psychiatric Hospital Vrape, with a confirmed diagnosis of severe OSA (AHI30). Data will be collected at two time points before the introduction of CPAP therapy and after 6 months of using the CPAP device for at least 4 hours a night. The patients will have the samples necessary for the analysis of laboratory parameters, which will include a complete blood count, C-reactive protein, urea, creatinine, potassium, sodium, glucose in blood, HbA1c, complete lipidogram and coagulogram, NT-proBNP, fibrinogen, albumin, pentraxin 3 (ELISA). After 6 months, the subjects will be analyzed again for the same laboratory parameters, and Holter EKG, Holter RR and echocardiography. Exclusion criteria will be AHI<30, younger than 18 years, pregnant women, those suffering from severe chronic obstructive pulmonary disease, severe chronic renal insufficiency, atrial fibrillation, acute heart failure, who have overcome cerebrovascular insult or transient ischemic attack in the last 6 months, acute coronary syndrome in the last six months, those taking anticoagulant therapy. The required number of subjects determined by the t-test for dependent samples with a test power of at least 90% with an effect power of d=0.66 and a significance level of 0.05 is 28, with an expected decrease in PTX3 values by 25% after 6 months of CPAP therapy considering to its initial values. In the research, the data will be presented tabularly and graphically. An analysis of the normality of the distribution of numerical data will be made and corresponding parametric and/or non-parametric statistical analyzes and methods of data presentation will be applied to the obtained results. Quantitative data will be presented through ranges, arithmetic means and standard deviations, or medians and interquartile ranges in cases of non-parametric distribution. Categorical data will be presented through absolute frequencies and corresponding shares. Differences in quantitative values between individual measurements will be evaluated with the t-test for dependent samples, that is, with the Wilcoxon test, and differences in categorical variables. Appropriate correlation coefficients between observed clinical variables will be calculated. All P values less than 0.05 will be considered significant.

Expected scientific contribution: To determine the effect of therapy with a CPAP device for a longer duration (6 months) in patients with exclusively severe OSA on the values of PTX3, which have not been examined in previous studies, and in addition investigate the influence of the therapy on other

pathophysiological mechanisms that lead to increased cardiovascular risk and inflammatory condition in the same subjects.

MeSH/Keywords: OSA, CPAP, PTX3

Poster code: T-02-09-044

Poster Title: Association between electrocardiographic characteristics and clinical outcomes in hospitalized COVID-19 patients treated with remdesivir

PhD candidate: Petra Bistrović

Part of the thesis: Association between electrocardiographic characteristics and clinical outcomes in hospitalized COVID-19 patients treated with remdesivir

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

Affiliation: University of Zagreb School of Medicine

Introduction: COVID-19 is a viral disease that typically presents with respiratory symptoms, however practically any organ system can be affected. Patients with prior cardiac comorbidities are at a particularly higher risk of complications. As the pandemic began, there were many uncertainties regarding treatment, however, soon remdesivir, the first approved antiviral drug for treatment of COVID-19, emerged on the market. Following a wide distribution of the drug, first cardiac manifestations were observed in post-marketing, most notably sinus bradycardia (attributed to structural similarity to adenosine). However, possible effects on T wave axis in the electrocardiogram were also noted. This begs a question whether it is safe to apply remdesivir in patients with prior cardiac comorbidity, as well as in those with acute cardiovascular complications of COVID. The electrocardiogram is a routinely used test that gives an insight into chronic and acute cardiac disease. The aim of our study is to see if there is an association between use of remdesivir, mortality and specific changes in the electrocardiogram recorded at admission.

Hypothesis: Remdesivir use is associated with increased mortality in patients with ST-T segment changes present in the ECG at admission.

Aims: The general aim is to determine the association of ST-T segment changes in ECGs recorded at admission with outcomes of hospitally treated COVID-19 patients. Specific aims include defining ECG characteristics of patients with severe COVID-19 at admission, analyze the association between ST-T segment changes at admission with demographic and clinical data and symptoms, analyze the association between ST-T changes at admission with outcomes of hospitalized patients with severe COVID-19 and finally analyze the interaction between the presence of ST-T segment changes at admission, use of remdesivir and death.

Materials and methods: We plan to conduct a retrospective cohort study of patients hospitalized at Clinical Hospital Dubrava from October 2020 to June 2021. All participants were SARS-CoV-2 positive and over the age of 18 years old. A control group of participants was defined from a group of patients who did not receive remdesivir due to shortage of drug or due to physician's choice, matched based on age, sex, Charlson comorbidity index, COVID severity and then further selected by maximum oxygen requirement during hospitalization (oxygen supplementation, high flow oxygen therapy and mechanical ventilation). Patients' sociodemographic data and clinical characteristics will be obtained from electronic medical records. ECGs recorded at admission will be obtained from medical archives and analyzed per ACC/AHA's recommendations and other relevant literature. Primary outcome of interest is death during hospitalization. Data analysis will be conducted in two phases, first clinical and prognostic values of ECG changes will be assessed for all of the patients included in the study, then followed by the analysis of interaction between ST-T segment changes, remdesivir treatment and death.

Expected scientific contribution: This research could potentially improve understanding of COVID-19 and expand current knowledge of effectiveness and safety of remdesivir use in patients with ST-T segment changes. This can potentially help in identifying patients at risk of adverse effects, allowing for a change in therapeutic approach or an increase in monitoring. Results from this research could be applicable to real-life patients who are advanced in age and have a higher comorbidity burden.

MeSH/Keywords: COVID-19; electrocardiogram; remdesivir; ST-T segment; SARS-CoV-2

Poster code: T-02-09-046

Poster Title: Toxicity and effectiveness of bendamustine treatment in patients with indolent nonHodgkin lymphomas, chronic lymphocytic leukemia and mantle cell lymphoma

PhD candidate: Barbara Dreta

Part of the thesis: Toxicity and effectiveness of bendamustine treatment in patients with indolent nonHodgkin lymphomas, chronic lymphocytic leukemia and mantle cell lymphoma

Mentor(s): Professor Igor Aurer, MD PhD, Assistant Professor Josip Batinić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Bendamustine is alkylating agent that is used in treatment of indolent non-Hodgkins lymphoma. In phase III clinical trials combination of bendamustine and monoclonal antibody showed high efficacy with low toxicity. Rummel et al in StiL clinical trail compared treatment with R-CHOP and R-bendamustine in indolent non-Hodgkin lymphoma and showed increase in median progression free survival (69.5 mo vs 31.2 mo) and lower levels of hematological toxicities (30% vs 68%) and infections (37% vs 50%). Results of phase III clinical trials made treatment with bendamustine in combination with monoclonal antibody preferred regimen for treatment of indolent lymphomas. Longer follow up in clinical trials and clinical observations documented higher levels of infections then previously reported. Gallium phase III clinical trial showed higher number of infections grade 3 - 5 in patients treated with bendamustine compared to CHOP or CVP.

Hypothesis: Bendamustine toxicity is dependent on patients characteristics (age, ECOG, CIRS), disease type and line of treatment.

Aims: Our goal is to determine bendamustine toxicities and risk factors for their development. Specifically, we aim to assess correlation between toxicities and patient characteristics: age, sex, ECOG and CIRS scores, correlations between toxicities and type of disease and line of treatment. Aims of this study are also to determine relationship between treatment toxicities and serum immunoglobuline values, grade of neutropenia and prophylactic use of antimicrobials and/or granulocytes growth factors. Evaluation of effectiveness of treatment that will be correlated to those reported in the available literature.

Materials and methods: Patients with indolent non Hodgkin lymphomas, chronic lymphocytic leukemia and mantle cell lymphoma, who were treated with bendamustine based therapy and received at least one dose of bendamustine in our center from January 1st 2013 to June 30th 2022 will be included. We will collect demographic data (age, sex, ECOG, CIRS), data on disease type, lines and type of prior treatment, laboratory data (CBC, immunoglobuline levels, LDH value), before and during bendamustine treatment, as well as data from imaging methods (CT scans). Data about side effects will be collected: hematological toxicities (anemia, neutropenia, thrombocytopenia, hypogammaglobulinemia), infections (number, grade, site) and secondary malignancies. Effectiveness of treatment will be evaluated by RECIL criteria.

Expected scientific contribution: Determination of risk factors for development of bendamustine treatment toxicities

MeSH/Keywords: Bendamustine, Lymphoma, Non-Hodgkin, Safety, Infections

Poster code: T-02-09-111

Poster Title: Graft rejection detection in heart transplantation recipients by synchrotron X-ray phase contrast imaging

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: Graft rejection remains the most important complication after heart transplantation (HTx). Endomyocardial biopsy (EMB) is the gold standard in HTx follow-up, however, conventional histopathological (HP) analysis is limited by tissue damage during preparation, 2-dimensional (2D) analysis, and low inter-observer agreement in rejection grading. X-ray phase contrast imaging (X-PCI) has shown potential for non-destructive imaging of the myocardium, enabling high-resolution 3D analysis with fibrosis and fibre orientation quantification.

Hypothesis: The X-ray phase contrast imaging (X-PCI) produced by synchrotron represents a valid and effective method in the assessment of cardiac graft cellular rejection and provides a more detailed 3D visualization of cellular and tissue morphology.

Aims: Main objective is to compare X-PCI and classical histopathology with light microscopy in the detection of cellular rejection of heart grafts. Some other specific aims are to compare EMB tissue analysis after heart transplantation by classical histology compared to X-PCI in the assessment of cardiac graft rejection grading, to compare clinical outcomes of patients assessed for cellular rejection by classical histology versus X-PCI assessment, as well as to further investigate the characteristics of myocardial tissue morphology in EMB samples using X-PCI together with light and electron microscopy.

Materials and methods: This is designed as a multi-centric, prospective observational study that aims to collect approximately 400 EMB samples from patients in standard post-HTx follow-up at 3 clinical centres: University Hospital Centre Zagreb (UHCZG), University Hospital Dubrava and Hospital Clinic Barcelona. Collected data, besides the EMB samples, will also include patient history, current clinical status, laboratory, ECG, coronarography and echocardiographic data. EMB specimens will be initially imaged by X-PCI at the Paul Scherrer Institute (Villigen, Switzerland), producing digital 3D imaging datasets (at 0.65 um pixel resolution size) for computational analysis, and then prepared for light microscopy and classical histopathology analysis at the University of Zagreb School of Medicine. Three datasets will be generated for analysis - 2D X-PCI dataset, 3D X-PCI dataset, and 2D HP images. Acquired X-PCI images and HP slides will be diagnostically graded (ISHLT 2004. grading system), comparatively assessed by at least two observers (including an experienced cardiovascular pathologist) in a blinded fashion, and further analysed in conjunction with gathered clinical data. Another additional output will be the potential development of computational methods for the automatic and semi-automatic analysis of digital imaging datasets.

Expected scientific contribution: We expect to prove the value of 3D analysis of EMB samples and improve the understanding of heart transplant rejection that will encourage the integration of new research tools, use of X-PCI analysis in other heart diseases in future research, and research progress of transplantation medicine in Croatia. Investigations in this study aim to prove the clinical relevance of the X-PCI method, and with the ongoing development of imaging devices miniaturization, their clinical application in the form of human heart scanners in toto.

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

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

Poster code: T-02-09-123

Poster Title: Predictive factors of histological changes on blood vessels of the kidney in glomerular diseases

PhD candidate: Patricia Kačinari

Part of the thesis: Predictive factors of histological changes on blood vessels of the kidney in glomerular diseases

Mentor(s): Associate Professor Mario Laganović, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Along with arterial hypertension and diabetes, glomerular diseases are the most common cause of chronic kidney disease (CKD). Histological changes in blood vessels are a very common finding in renal biopsies. Such changes are often seen as an additional finding in addition to glomerular disease and they also occur independently and are then traditionally associated with the existence of arterial hypertension. However, numerous studies have shown that they are also present in normotensive patients. In recent years, several studies have been conducted that individually analyzed the association with some of the parameters that are planned to examined in this study in glomerular diseases, but the studies were are individual.

Hypothesis: Histological changes in kidney blood vessels in glomerular diseases confirmed by kidney biopsy can occur regardless of arterial hypertension.

Aims: GENERAL AIM: to determine the predictive factors of the occurrence of histological changes on the blood vessels of the kidney in glomerular diseases confirmed by kidney biopsy. SPECIFIC AIM : 1. To investigate the frequency and degree of histological changes in the blood vessels of the kidneys in confirmed glomerular diseases kidney biopsy 2. Correlate the occurrence of histological changes in the blood vessels of the kidney in glomerular diseases confirmed by kidney biopsy with the duration, control and therapy of hypertension 3. Correlate the occurrence of histological changes on the blood vessels of the kidney in glomerular diseases confirmed by kidney biopsy kidneys with basic glomerular disease 4. Correlate the occurrence of histological changes in the blood vessels of the kidney in glomerular diseases confirmed by kidney biopsy with stiffness parameters of large blood vessels 5. Correlate the occurrence of histological changes on the blood vessels of the kidney in glomerular diseases confirmed by kidney biopsy with other cardiovascular risk factors.

Materials and methods: The subjects in the research are patients who were previously indicated by a nephrologist for a kidney biopsy in the Department of Nephrology and Dialysis of the Dubrava Clinical Hospital. Subject are are persons of both sexes, with the exception of the pediatric population (age < 18 years). In all patients, blood and urine would be sampled for laboratory analysis . Furthermore, a detailed history and clinical status with all anthropometric measurements would be taken from each patient for evaluation and cardiovascular risk according to the SCOR cardiovascular risk questionnaire. The key criterion for entering the research is a kidney biopsy, which is indicated for the patient according to absolute clinical indication. Kidney biopsy is performed with a kidney biopsy device. A kidney biopsy is then taken preparation for histopahtological analysis by light microscopy (LM), immunofluorescence (IF) and electron microscopy (EM). The study would include patients diagnosed with Ig nephropathy (IgAN), membranous nephropathy (MN) and focal segmental glomerulosclerosis (FSGS). Arteriosclerotic changes will be described according to degrees as follows: fibrointimal thickening artery and the degree of hyalinosis of arterioles. Non-invasive methods as part of the research that would be performed are the following: color doppler analysis of renal arteries, sOscillometric measurement of the velocity of pulse wave velocity and echocardiogram.

Expected scientific contribution: The results of this research would contribute to a better understanding of histological changes in arteries in glomerular diseases as well as potential predictive factors in their occurrence, which until now were assumed to be only a consequence of hypertension. Positive correlation of changes in the arteries with the type and severity of the underlying kidney disease, as well as the connection

with to other predictive factors of cardiovascular risk would represent the possibility of creating new prognostic tools in these diseases as well as a potential new target site in treatment with new smart drugs.

MeSH/Keywords: angionephrosclerosis, hyalinosis of arterioles, fibrointimal thickening, glomerulonephritis, arterial hypertension

Poster code: T-02-09-133

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

PhD candidate: Maja Vizjak

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.

Hypothesis: Open surgical repair of aortic abdominal aneurysm with vascular prosthesis will increase pulse wave velocity and cardio-ankle vascular index.

Aims: Our primary goal is to determine the change in arterial stiffness in patients with abdominal aortic aneurysm before and after open surgical replacement of aneurysm with vascular prosthesis. Specific goals are to measure preoperative and postoperative values of carotid-femoral pulse wave velocity and cardio-ankle vascular index, to analyse the relationship between aneurysm diameter and arterial stiffness and to determine preoperative and postoperative central hemodynamic parameters that are influenced by arterial stiffness.

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. 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.

Expected scientific contribution: This research will help us examine the correlation between the replacement of the diseased aortic wall with vascular prosthesis and the cardiovascular risk using arterial stiffness. On this basis we could conclude that the cardiovascular risk after open surgical procedure stays the same, decreases or even increases and this will affect our decision on more aggressive treatment of other cardiovascular risks.

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

Poster code: T-02-10-010

Poster Title: The role of pharmacogenomics in an individualized approach to rivaroxaban treatment

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 has large interindividual trough concentration variability affecting its efficacy and safety, especially bleeding events. This variability could be associated with age, liver and kidney function, concomitant illness and therapy, and pharmacogenetic predisposition. Rivaroxaban is a substrate of ABCB1 and ABCG2 transporters, and CYP2J2, CYP3A4/5 enzymes. The polymorphisms of these genes may affect the pharmacokinetics of rivaroxaban and its safety profile.

Hypothesis: The incidence of adverse events is more frequent in patients who are carriers of variant alleles CYP2J2, CYP3A4, CYP3A5, ABCB1 and ABCG2 compared to patients with the wild-type genotype for each of the mentioned genes.

Aims: To determine which CYP2J2, CYP3A4, CYP3A5, ABCB1, and ABCG2 gene variants can serve as predictors of rivaroxaban adverse events.

Materials and methods: 3-year prospective study will include a minimum of 375 subjects, aged 18 years, with an indication for rivaroxaban for at least 3 months. The subjects will be divided into 2 groups: a group with adverse events, and a control group without adverse events. They will be monitored during regular control visits and if necessary; clinical and laboratory data will be collected and genotyping will be done.

Expected scientific contribution: This research will contribute to new knowledge about the association of gene polymorphisms with the effectiveness and safety of long-term use of rivaroxaban.

MeSH/Keywords: polymorphisms, rivaroxaban, side effects

Poster code: T-02-13-049

Poster Title: 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

Affiliation: University of Zagreb School of Medicine; University Hospital Centre Zagreb, Clinical Department for Transfusion Medicine and Transplantation Biology Croatian Tissue and Cell Bank, Human Milk Bank; University of Zagreb Faculty of Food Biotechnology

Introduction: Bacteria from the genera Bifidobacterium and Lactobacillus and other probiotic bacteria are effective in the prevention of necrotizing enterocolitis (NEC), to which premature children are susceptible. Feeding with infant formula almost doubles the risk of developing NEC compared to feeding with pasteurized donated human milk (DHM) in premature children. There is not enough data on the presence and representation of certain genera of bacteria in DHM.

Hypothesis: The bacterial microbiota of DHM from donors who gave birth vaginally and were not exposed to antibiotics will have a higher proportion of bacteria within the genera Lactobacillus and Bifidobacterium.

Aims: The general aim is to identify the composition of the bacterial microbiota of DHM using the next generation sequencing (NGS) method. Specific aims are to investigate whether there are characteristics of the donors, their children or DHM that can potentially influence the representation of certain bacterial genera.

Materials and methods: Archival samples from pools of raw donated milk expressed in the first 6 months after birth from at least 70 different donors will be included in the research. The samples will meet the quality requirements for clinical use. According to the characteristics of the donors, the tested milk samples will be divided into two groups: (1) samples from donors who gave birth vaginally and were not exposed to antibiotics in the previous 6 months and (2) samples from donors who gave birth by caesarean section or vaginally, but were exposed to antibiotics in the previous 6 months. The second group of donors will be divided into subgroups of those who received only peripartum prophylaxis and those who received antibiotic therapy. Data will be collected on: (1) donors - age, body mass index, use of antibiotics within 6 months of donation, geographical area of residence, mode of delivery and weeks of gestation; (2) children - sex, birth order, age at the time of breastfeeding and the method of feeding with breast milk and (3) milk - method of expression, type of milk, length and temperature of storage, nutritional value and microbiological culture findings before pasteurization. Bacterial deoxyribonucleic acid (DNA) will be isolated from the DHM samples using the commercial kit, according to the manufacturer instructions. Hypervariable region V1-V3 of the 16S ribosomal RNA (r RNA) gene of the isolated DNA will be amplified with appropriate primers and polymerase chain reaction (PCR) will be performed. NGS will be performed on an Illumina platform, which will yield raw data containing information about nucleotide sequences within the V1-V3 region. The analysis will be carried out using the Quantitative Insights Into Microbial Ecology (QIIME) 2 program in order to obtain a taxonomic classification of bacteria within the samples at the level of phylum, class, order, family, genus and eventually species with the number and percentage of individual sequences.

Expected scientific contribution: The research would expand knowledge about the representation and diversity of bacterial microbiota in DHM depending on the characteristics of the donor, her child and milk. A better knowledge of the composition of the microbiota of DHM and the characteristics that influence the composition is significant because of the possibility of restoring the microbiota of pasteurized milk.

MeSH/Keywords: bacteria, microbiota, human milk, next generation sequencing

Poster code: T-02-16-002

Poster Title: Association of table salt intake with iodine intake and the thyroid gland function

PhD candidate: Tea Delić

Part of the thesis: Association of table salt intake with iodine intake and functional status of the thyroid gland in the adult population

Mentor(s): Assistant Professor Sandra Karanović Štambuk, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Excessive table salt intake is associated with arterial hypertension, cardiovascular, cerebrovascular and renal disease and mortality. WHO recommends a daily salt intake of 5 grams and calls on all countries to determine the extent of additional salt iodisation in parallel with salt reduction in order to keep the iodine concentration in the population satisfactory.

Hypothesis: Reducing daily table salt intake to 8 grams does not lead to insufficient iodine intake in general population and consequently does not affect functional status of thyroid gland.

Aims: To determine iodine intake in Croatian general adult population using 24-hour urine and to determine the association of table salt intake, iodine intake and the functional status of the thyroid gland. Secondary goals are to determine table salt and iodine intake from 24-hour urine, compare the iodine intake among the group with an average salt intake of 8 g/day and the group with an average salt intake of 11 g/day and to analyze the association of thyroid hormones and iodine intake in these two groups. Based on the results, we aim to assess whether and by how much the table salt should be additionally iodized in order to achieve recommended amounts of iodine intake if salt intake was decreased to 8 g and 5 g per day. The data will be analyzed for the whole population, men and women, different regions in Croatia and urban and rural area.

Materials and methods: 1850 subjects enrolled in the survey EHUH-2 will be divided into 2 groups: salt intake 8g/day and salt intake 11 g. In addition to medical history, height, weight and blood pressure measurements, blood and 24h urine samples will be taken for serum creatinine, fasting blood glucose, lipids, uric acid, serum electrolytes, TSH, FT3, FT4, antiTPO, antiTg and urine iodine, sodium, potassium and creatinine concentrations.

Expected scientific contribution: We will obtain data on iodine intake in adult Croatian population, its association with salt intake and functional status of the thyroid gland and possible need for additional iodization of table salt if target values of salt intake of 8g or recommended 5g per day were reached.

MeSH/Keywords: sodium chloride, iodine, thyroid gland, thyroid hormones

Poster code: T-02-17-052

Poster Title: Identification and characterization of potential protein biomarkers of cancer cachexia in saliva

PhD candidate: Ivan Vičić

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

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

Affiliation: University of Zagreb School of Medicine, UHC Zagreb

Introduction: Cancer cachexia is a multifactorial syndrome characterized by loss of muscle mass, with or without the loss of adipose tissue, which is incorrigible by nutritional support and leads to functional impairment. It is of theoretical and practical importance to recognize the early signs of the development of cachexia based on molecular features before the appearance of a clinically overt condition and regarding the uneven and unpractical methods of evaluation there is an ongoing search for a cachexia biomarker. Most studies research plasma proteome, but due to practical, affordable, non-traumatic sampling and repeatability, saliva, as a plasma ultrafiltrate, has shown promising results. Recent advancements in liquid chromatography and mass spectrometry techniques enable the identification of salivary (and plasma) proteins, which could serve as potential biomarkers of cancer cachexia.

Hypothesis: The salivary proteome of cachectic cancer patients differs from the proteome of the saliva and plasma of non-cachectic cancer patients and healthy individuals.

Aims: This study aims to analyze the proteomic profile of saliva and plasma of cachectic cancer patients. To compare the results between the cachectic patients and non-cachectic cancer patients and healthy individuals and to quantify selected biomarker candidates specific to the cancer cachexia group by immunodetection tests.

Materials and methods: Sixty subjects will be recruited in this proof of concept study. Twenty subjects will be cachectic patients with metastatic tumor diseases (cachexia diagnostic criteria by Evans et al), twenty patients with the same metastatic tumor diseases without cachexia, and twenty subjects will be healthy controls. Due to known variations in the salivary proteome, samples will be collected at the same time of the day (after one hour of abstaining from food and drinks), and subjects will be of a specific age group (between the age of 50 and 70 years old). Given that it is impossible to identify the number of proteins that will be detected in advance, the number of participants cannot be determined by power analysis a priori. Instead, it will be done as a post hoc analysis. The number of samples is comparable to the ones used in previous similar research. Exclusion criteria are the presence of an active infection, systemic autoimmune diseases, liver failure, salivary gland conditions, head and neck cancer diagnosis, intestinal obstruction, and chemotherapy administration in the last seven days. Blood and saliva samples will be collected from all subjects and frozen at 80 °C. The saliva collection procedure will be carried out by collecting successive sputum specimens directly into a Petri dish, which will be transferred with a pipettor into Eppendorf tubes. In case of organoleptic changes in the color of saliva, blood clots, or food residues, the subject will be excluded from the study. After sample purification and digestion, high-performance liquid chromatography separation will be performed (Thermo Scientific UltiMate 3000 nano HPLC system integrated with LTQ-Orbitrap Discovery mass spectrometer). Mass spectrometry scans (full scan MS) will be analyzed using MaxQuant 1.6.0.16. software package. Proteins will be quantified using the intensity-base absolute quantification (iBAQ) algorithm and identified in silico using the UniProt human proteome database. After identification of a potential biomarker in cachectic patients by mass spectrometry, immunodetection tests will be used to confirm and quantify selected candidates/potential biomarkers. The study will last for two years.

Expected scientific contribution: A potential protein biomarker of cancer cachexia (additionally validated by further research) could be used in the early diagnosis of the condition, distinguishing the etiology of weight loss in cancer patients and guiding the nutritional therapeutic interventions.

MeSH/Keywords: biomarker candidates, cancer cachexia, saliva proteomics

Poster code: T-02-19-040

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): Assistant Professor Marija Milković Periša, MD PhD

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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. Neoadjuvant treatment is an offshoot of systemic treatment before surgery and is the standard in the treatment of locally advanced breast cancer.

Hypothesis: 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. Elevated immunohistochemical expression of SphK1 and COX2 proteins in biopsy samples of triple-negative and HER2-positive breast cancer before neoadjuvant chemotherapy is associated with a worse response to neoadjuvant treatment

Aims: The main aim of the study is to analyze the immunohistochemical expression of SphK1 and COX2 proteins in the biopsy diagnostic samples of triple-negative and HER2-positive breast cancer treated with neoadjuvant chemotherapy and biological therapy, to determine their mutual relationship and the connection of their expression with the pathohistological response to chemotherapy treatment determined on the operative sample.

Materials and methods: A retrospective study will be conducted on archived biopsy tissue samples of breast cancer patients diagnosed and treated with neoadjuvant chemotherapy at KBC Zagreb in the period from January 1, 2018 to December 31, 2022. Clinical data and data on pathohistological features of the tumor will be downloaded from the hospital information system, and immunohistochemical analysis of SphK1 and COX2 protein expression will be performed on tumor samples. The clinical data that will be collected will include the patient's age at diagnosis, tumor size and axillary lymph node status before chemotherapy treatment determined by radiological methods, histological tumor subtype, estrogen and progesterone receptor status, HER2 status, nuclear grade, Ki67, type of chemotherapy protocol and data on the return and/or progression of the disease. Patients will be divided into 3 subgroups depending on the immunophenotype of the tumor: 1) HER2 positive, 2) luminal B HER2 positive and 3) triple negative. Immunohistochemical expression of SphK1 and COX2 proteins will be determined on a biopsy sample of tissue obtained by a broad needle biopsy before the treatment. The marker of treatment effectiveness will be the pathohistological response determined on a sample of breast tissue obtained by surgery after chemotherapy treatment and expressed using the RCB index (residual cancer burden): RCB 0 - complete pathological response, RCB I and RCB II - partial response, RCB III - poor response to 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.

Expected scientific contribution: To obtain data on the immunohistochemical expression of SphK1 and COX2 proteins and their interrelationship in breast cancer, as well as their possible predictive feature in neoadjuvant treatment.

MeSH/Keywords: breast cancer, SphK1, COX2

Poster code: T-02-19-094

Poster Title: Correlation of changes in serum S100 concentration and metabolic parameters in positron emission tomography computed tomography with outcomes of treatment with immune checkpoint inhibitors in patients with metastatic melanoma

PhD candidate: Luka Simetić

Part of the thesis: Correlation of changes in serum S100 concentration and metabolic parameters in positron emission tomography computed tomography with outcomes of treatment with immune checkpoint inhibitors in patients with metastatic melanoma

Mentor(s): Associate Professor Natalija Dedić Plavetić, MD PhD, Assistant Professor Davorin Herceg, MD PhD

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

Introduction: Melanoma is a malignant neoplasm of melanocytes. Advanced disease has a very poor prognosis. With the development of immunotherapy drugs such as nivolumab or pembrolizumab, significant improvement has been observed in the treatment outcomes (progression-free survival, PFS and overall survival, OS). The use of nuclear medicine methods such as positron emission tomography computed tomography (PET/CT) enables better monitoring of response.

Hypothesis: Patients with metastatic melanoma treated with immune checkpoint inhibitors who, after 12 weeks of treatment, achieve an increase in the serum concentration of the S100 marker, along with an increase in the ratio of metabolic parameters of bone marrow to liver (BLR), or spleen to liver (SLR) in positron emission tomography computed tomography, have a shorter progression free survival.

Aims: Aim: to analyse the serum concentration of the S100 marker and the metabolic parameters of PET/CT, BLR and SLR before and after 12 weeks of immunotherapy treatment, to determine their mutual relationship and connection with treatment outcomes (PFS) Specific aims are to analyse the metabolic parameters of PET/CT, BLR and SLR before and after 12 weeks of immunotherapy in patients: 1) who have a mutation in the BRAF gene and those who do not 2) with autoimmune side effects 3) in relation to the number and type of organs affected by metastasis

Materials and methods: The analysis will include patients with metastatic melanoma treated with immune checkpoint inhibitors in the period from January 1, 2017. to December 31, 2024. Inclusion criteria: patients older than 18 years, pathological confirmation of melanoma, confirmed metastatic disease by PET/CT, institutional approval for immune checkpoint inhibitors treatment, imaging with PET/CT after 12 weeks of treatment and laboratory sampling of serum S100 concentration before and after 12 weeks of immunotherapy treatment. It is planned to include at least 120 patients. Exclusion criteria: the existence of a previous malignant disease, the existence of an autoimmune disease before the start of treatment, any liver, spleen or bone marrow condition that could interfere with metabolic parameters analysis, the use of corticosteroids or other immunosuppressive drugs. The immunotherapy protocols are pembrolizumab 200 mg every 3 weeks i.v. (or 2mg/kg), nivolumab 240 mg every 2 weeks i.v. (or 3mg/kg). The analysis of the serum concentration of S100 marker will be performed by the electrochemiluminescence method at two points, before the start and after 12 weeks of treatment with immune checkpoint inhibitors. Parameters obtained on PET/CT include the metabolic activity of tissues by evaluating the SUV (standardized uptake value) of 18-fluorodeoxyglucose. SUV will be measured in pixels as the ratio of radioactivity to the ratio of injected dose to body weight. The ratio of SUV in hematopoietic and non-hematopoietic tissue (BLR and SLR) will be analyzed as the ratio of the maximum SUV in the mentioned tissues. The specified maximum SUV will be calculated by analyzing the spherical volume of interest (VOI), namely a 3-cm sphere in the liver and spleen, and a 1.5-cm sphere in the centers of the first to fourth lumbar vertebrae, and taking the average of the SUV values in the said vertebrae. Demographic and clinical data on patients will be collected from the hospital information system. PFS is the time from the patient's inclusion in the study to disease progression or death from any cause.

Expected scientific contribution: Serum S100 marker concentration and metabolic parameters of PET/CT can be individually useful in evaluating the effect of immunotherapy treatment in patients with metastatic melanoma, but there is no data on their combination.

MeSH/Keywords: melanoma, immunotherapy, PET/CT, S100, BLR, SLR

Poster code: T-02-19-117

Poster Title: Can Ultrasound Be Used to Improve the Accuracy of Clinical Methods for Measuring Leg-Length Discrepancy?

PhD candidate: Sven Šimunić

Part of the thesis: Influence of ultrasound on the reliability and precision of measuring the difference in the length of the lower extremities by the direct clinical method in children and adolescents

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

Affiliation: University of Zagreb School of Medicine

Introduction: Methods used to measure leg length discrepancy (LLD) provide great insight into health of the musculoskeletal system. LLD is one of the most common findings affecting more than 2/3 of the population. Literature search will reveal lack of consensus regarding different aspects of LLD, such as defining degree of LLD necessary to cause musculoskeletal problems, associations between LLD and certain pathological conditions and validity and reliability of different measuring methods for clinical assessment of LLD. Clinical methods such as use of a tape measure and standing blocks are useful screening tools, but not reliable and accurate enough for clinical decision making. Radiographic measurement is considered current reference standard. Studies showed excellent reliability of ultrasound, which could prove to be an alternative to the radiographic measurement of LLD, because of its low cost and high practicability while avoiding negative effect of ionizing radiation.

Hypothesis: Using ultrasound in measuring leg length discrepancy will increase inter and intraexaminer accuracy and reproducibility for the assessment of LLD when compared to commonly used direct and indirect clinical methods.

Aims: The aim of this study is to compare the reliability and accuracy of ultrasound guided method to common clinical and radiological methods for the measurement of leg length discrepancy in children and adolescents, depending on their body mass index.

Materials and methods: Suggested study is a comparative study; study comparing three different diagnostic methods against a common gold standard. Subjects for the study are 50 children between the ages of 5 and 18 who are diagnosed with LLD and who will receive LLD measurement using radiographic method (scanogram) as a part of diagnostic workup due to their primary diagnosis. Patients are divided into groups based on their body mass index according to World Health Organization Child Growth Standards. Each patient will undergo LLD measurements using 3 different clinical methods. Methods used for evaluating LLD are direct and indirect clinical method, as well as ultrasound guided method. They will be compared for accuracy and reliability against one standardized radiological method to measure LLD. Direct clinical method is measuring distance between the anterior superior iliac spine (ASIS) and the medial malleolus, while in indirect method LLD is obtained by placing blocks of known height under the shorter limb. Ultrasound guided method uses ultrasound to determine anatomic landmarks in order to measure the distance between them while patient is in supine position. Ultrasound used is SonoScape S22 with high frequency (4-16 MHz) linear transducer. Each measurement is performed 3 times by two independent examiners. Radiographs are obtained using X-ray tube as a source of radiation at the distance of 200 cm from the detector, voltage of 70-150 kVp. Radiographs are archived and evaluated using PACS system. Accuracy of a given method is defined as measurement variance from the golden standard, which is radiographic measurement. Reliability is defined as extent to which examiners using the same diagnostic method on the same group of patients, agree on the results. Data collected also includes patients' age, sex, height, weight and primary diagnosis. Patient stratification is based on body mass index (BMI) categories according to the World Health Organization for children aging 5 to 19 years.

Expected scientific contribution: We believe using ultrasound guided method increases accuracy and reproducibility for the assessment of LLD in clinical routine, especially in patient population with higher body mass index, where such measurement is challenging. Although ultrasound guided method has lower accuracy when compared to radiographic methods, our aim is to evaluate whether described method is

reliable and accurate enough to be used not only as a screening tool but also in serial monitoring of LLD thus minimizing unnecessary radiation exposures.

MeSH/Keywords: leg length discrepancy, ultrasound, reliability, sonographic leg length measurement, lower extremity

Poster code: T-02-20-082

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

PhD candidate: Iva Botica

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

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

Affiliation: University of Zagreb School of Medicine

Introduction: At the end of 2019, in the Chinese county Wuhan, a new disease occurred called COVID-19. It was caused by a new strain of the coronavirus called SARS-CoV-2. Symptoms were mostly respiratory but a great number of patients had olfactory dysfunction. The smell disappeared within 3-4 days after the onset of the disease, within 28 days it recovered in 98% of cases. The mechanism of olfactory dysfunction after viral infections usually is the swelling of the nasal mucosa, which prevents the connection of odor molecules and receptors. The smell usually recovers spontaneously or with olfactory training and use of decongestant. The mechanism for developing olfactory dysfunction 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 syndrome. Long-term olfactory dysfunction is present in 11% of patients with this syndrome. Previous research has not examined the impact of olfactory training on the recovery of the sense of smell in patients with post-acute COVID-19 syndrome. The research plan is to examine the sense of smell in patients suffering from post-acute COVID-19 syndrome, to compare it after treatment, and to compare the results of the recovery with the control group.

Hypothesis: Olfactory training recovers olfactory dysfunction in patients with post-acute COVID-19 syndrome.

Aims: The general aim is to examine the impairment of the sense of smell in patients with post-acute COVID-19 syndrome and analyze the effect of olfactory training on the recovery of the sense of smell. Specific aims are to describe the specifics of disorders of the sense of smell in patients with post-acute COVID-19 syndrome, to quantify the olfactory dysfunction and to compare the subjective impression of the sense of smell with the objective finding

Materials and methods: The research started in October 2021. The respondents are divided into two groups. The first group consists 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. The control group consists of patients who have a disorder of the sense of smell, but it is not related to the COVID-19 infection. A detailed medical history and examination will be taken. For olfactory dysfunction, a standardized Sniffin Sticks test (Burghardt®, Wedel, Germany) will be used. 3 subtests will be tested - the threshold, discrimination and identification. Each subtest is evaluated and the final value of the olfactory function is determined. A visual analog scale (VAS) will be provided to the patient for self-assessment of olfactory function. Olfactory training is exposing patients frequently to familiar smells. After 6 months of olfactory training and mucous membrane irrigation, another olfactory testing will be arranged. The results of the control test will be compared with the initial test. Results of olfactory recovery in group of patients with post-acute COVID-19 syndrome will be compared with olfactory recovery in the control group. According to previous studies, that investigated use of olfactory training in patients with non-COVID-19 olfactory dysfunction, the recovery of the olfactory function after 6 months of olfactory training is up to 20%.

Expected scientific contribution: Previous studies have investigated the recovery of olfactory dysfunction in the acute phase of COVID-19. In patients with post-acute COVID-19 syndrome were investigated, only the level of olfactory impairment was investigated. The scientific contribution of this research could be knowledge of the use of olfactory training and the possibility of recovery of an impaired sense of smell in patients with post-acute COVID-19 syndrome.

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

Poster code: T-02-21-096

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.

Hypothesis: Surgical treatment of OSA affects salivary cortisol concentration in patients with moderate and severe obstructive sleep apnea

Aims: The main objective of this study in to determine the range of cortisol values in patients with moderate and severe obstructive sleep apnea before and after treatment. Also to compare salivary cortisol values in patients with severe obstructive sleep apnea treated conservatively with CPAP versus surgically

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.

Expected scientific contribution: 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: T-02-21-131

Poster Title: Association of PD-L1 expression with clinicopathological characteristics of thyroid carcinoma

PhD candidate: Borna Miličić

Part of the thesis: Association of PD-L1 expression with clinicopathological characteristics of thyroid carcinoma

Mentor(s): Professor Drago Prgomet, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Immunohistochemistry markers have a rising significance in the diagnostics and treatment of thyroid cancer. One such marker is PD-1 ligand (PD-L1), which acts as a negative immunoregulatory molecule that enables immunological hiding of tumour cells. The aim of this study is to assess the association of PD-L1 expression with clinicopathological characteristics of four types of thyroid carcinoma.

Hypothesis: The level of PD-L1 expression is associated with clinicopathological characteristics of poorly differentiated thyroid carcinomas

Aims: Main goal: to assess the association of PD-L1 expression and clinicopathological characteristics of 4 main types of thyroid cancer. To find a model which could determine the characteristics of the tumours suitable for treatment with immunotherapy. Specific goals: 1. To immunohistochemically assess the occurrence of PD-L1 in thyroid carcinoma patients ; 2. To immunohistochemically determine the occurrence of PD-L1 in surrounding dysplastic and/or healthy tissue; 3. To immunohistochemically determine the occurrence of PD-L1 in the stroma, blood vessels, and inflammatory cells surrounding the tumour.

Materials and methods: This is a retrospective study and will be conducted on archive bioptic material of the Clinical Department of Pathology and Cytology, University Hospital Centre Zagreb. Paraffin blocks from 2010-2022 will be used. The group that will be analysed consists of 100 papillary carcinomas, 45 follicular, 30 medullar, and 15 anaplastic carcinomas. The control group consists of the same number of healthy thyroid tissue samples. Immunohistochemical staining will be performed on paraffin blocks to asses the PD-L1 expression.

Expected scientific contribution: We expect to demonstrate whether there is a difference in the PD-L1 expression in 4 main thyroid carcinoma types depending on clinicopathological characteristics. If the hypothesis that the increased PD-L1 expression is associated with certain tumour features is confirmed, it could be defined which of those tumours are suitable for the application of immunotherapy.

MeSH/Keywords: Thyroid carcinoma, PD-L1, immunohistochemistry, clinincopathological characteristics

Poster code: T-02-21-143

Poster Title: The effect of high glucose concentration on the expression of UPRmt genes and proteins of LONP1, OMA1 and HSP60 in myocardial samples of patients with failing heart

PhD candidate: Vid Mirošević

Part of the thesis: The effect of high glucose concentration on the expression of UPRmt genes and proteins of LONP1, OMA1 and HSP60 in myocardial samples of patients with failing heart

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

Affiliation: University of Zagreb School of Medicine

Introduction: Heart failure is a progressive condition and the mortality rate is 31% in patients with diabetes. Despite this, there is not enough data to explain the mechanism of cardiac injury caused by hyperglycemia, which is also reflected in lack of targeted therapeutic measures. Cellular stress can change mitochondrial function, damage the mitochondrial network and lead to increased production of reactive oxygen species (ROS) and cell death. The mitochondrial unfolded protein response (UPRmt), part of the mitochondrial quality control system (MQC), serves to maintain normal mitochondrial function. Disturbance of mitochondrial protein homeostasis activates UPRmt, whose main goal is to repair unfolded or misfolded proteins with repair proteins (chaperones) or to remove them with proteases. UPRmt includes a complex of signaling molecules, transcription factors, proteases, antioxidant enzymes and repair proteins. It is not known how high glucose concentration affects the UPRmt.

Hypothesis: High glucose concentration reduces the expression of HSP60 and LONP1 genes and proteins, and increases the expression of OMA1 gene and protein in myocardial samples of patients with heart failure.

Aims: Primary aim is to investigate the effect of high glucose concentration on the expression of LONP1, OMA1 and HSP60 in myocardial samples from patients with heart failure. Secondary aims are to compare the expression of LONP1, OMA1 and HSP60 genes and proteins in myocardial samples of patients with failing heart with normal and elevated values of glycosylated hemoglobin HbA1c and to investigate the effect of high glucose concentration on cell survival in cell culture with the addition of UPRmt activators or inhibitors.

Materials and methods: From heart transplant patients, samples will be taken from the diseased heart that has been removed. Samples of the left ventricle will also be acquired from patients who were implanted with the left ventricular assist device (LVAD). The effect of high glucose level on the expression of individual UPRmt elements in myocardial samples of patients with failing heart will be examined. The expression of LONP1, OMA1 and HSP60 genes and proteins in myocardium of patients with normal and elevated levels (>6.5%) of glycosylated hemoglobin HbA1c will be compared. Quantitative real-time PCR will be used to determine gene expression and ELISA will be used for protein quantification. The mechanism of action of high glucose concentration on the viability of human cells and the impact of changes in the expression of UPRmt elements will be investigated by experiments that will be carried out in cell culture of human aortic endothelial cells (HAEC). The influence of pharmacological UPRmt activators (SRT1720 and bortezomib) and UPRmt inhibitors (CDDO, O-phenanthroline and nonactin) on the survival of cells in cell culture exposed to high glucose will be investigated.

Expected scientific contribution: Discovering the mechanism of mitochondrial dysfunction caused by the harmful effects of high glucose concentration on the heart during heart failure is crucial for the discovery of potential pharmacological agents that could reduce these harmful effects. Such drugs could significantly improve health and quality of life of people suffering from diabetes and heart failure. This dissertation could contribute to these efforts by elucidating the role of individual elements of the UPRmt in the process of the harmful effects of high glucose concentration on the heart.

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

Poster code: T-02-22-115

Poster Title: Thymus gland structure in children with congenital heart disease

PhD candidate: Dražen Belina

Part of the thesis: Thymus gland structure in children with congenital heart disease

Mentor(s): Professor Zdenko Kovač, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Thymus is a central organ for the development of T lymphocte cellular immunity. For the proper selection and maturation of T cells in thymus important role have stromal and epithelial cells.

Hypothesis: Thymus structure is altered in children with congenital heart diseases.

Aims: Selection of T cells in thymus can be compromised with cellular stress imposed on the whole gland because of tissue hypoenergosis. Cellular energy limitation may be caused by hypotension, hypoxemia, volume and/or pressure overload and prostaglandin infusion in these patients

Materials and methods: Thymus tissue bank collected from the year 2013. in the Immunology Laboratory of prof Mariastefania Antica at the Department of Molecular Biology Institute Ruer Bokovi Zagreb. Classical serial hystology sections of thymic cortex and medulla and immunohystochemistry analysis of thymocytes, epithelial cells and stroma will be performed.

Expected scientific contribution: Knowledge of the human thymus structure in children with different congenital problems can provide better selection of the tissue for human thymus transplantation in immunocompromised hosts.

Acknowledgments: Prof Mariastefania Antica, RBI

MeSH/Keywords: thymus, energy limitation, T cells selection, hypoxemia

Poster code: T-02-22-134

Poster Title: Immunohistochemical expression of PAGE4, HIF-1 alfa and TNF alfa in prostate cancer and hyperplasia

PhD candidate: Sanja Cesarec Augustinović

Part of the thesis: Immunohistochemical expression of PAGE4, HIF-1 alfa and TNF alfa in prostate cancer and hyperplasia

Mentor(s): Professor Božo Krušlin, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Prostate cancer (PC) represents the most common site of cancer in Croatia and is the second most common cause of cancer related death among males. Hypoxia and inflammation play an important role in creating a suitable microenvironment influencing prostate cancer development and progression. This study investigates the influence of the expression of PAGE4, HIF-1 (both influencing oxidative stress mechanisms) and TNF- (redirecting inflammation) in prostate cancer compared to hyperplasia.

Hypothesis: In prostate cancer the immunohistochemical expression of PAGE4 and HIF-1 is increased and TNF is decreased compared to hyperplasia.

Aims: The aim of this study is to investigate the immunohistochemical expression of PAGE4, HIF-1 and TNF proteins in the epithelial and the microenvironment component (stroma) of prostate cancer and hyperplasia, and to assess their correlation with the other prognostic factors.

Materials and methods: A cross-sectional prospective study will be conducted with retrospectively collected material and data at the Clinical Department of Pathology and Cytology "Ljudevit Jurak" of the University Hospital Center "Sestre milosrdnice" in Zagreb. Paraffin-embedded archive tissue blocks obtained during routine diagnostic and therapeutic procedures (radical prostatectomy and transurethral resection) as well as all relevant clinical data of at least 70 patients in both groups will be included in the study. For each individual case, hematoxylin eosin (HE) slides will be made, and a representative paraffin block with a minimum of 30% tumor tissue will be selected for immunohistochemical analysis. Immunohistochemical analysis will be performed using antibodies against PAGE4, HIF-1, TNF- on 3-5 µm thick recuts and an adequate positive and negative control will be applied. After immunohistochemical staining, the material will be analyzed in its entirety, and the expression of antibodies will be determined under high magnification (400x), in the area of strongest reaction (the so-called "hot spot"). Signal expression in the epithelial and stromal components of tumors and prostate hyperplasia, as well as signal localization (nucleus/membrane/cytoplasm), will be analyzed. The results of the immunohistochemical analysis for PAGE4, HIF-1 and TNF will be evaluated semiquantitatively by light microscopy, determining the percentage of immunoreactive cells in tumor tissue, tumor stroma and prostatic hyperplasia. The intensity of the staining reaction (IR) will be determined as follows: "0" no reaction, "1" weak reaction, "2" moderate reaction, "3" strong reaction. The percentage of immunoreactive cells was determined as follows: "0" no reactive cells; "1" <10% positive cells; "2" 10 50 % positive cells; "3" >50% positive cells. We will compare these results with other clinico-pathological features.

Expected scientific contribution: A review of the published literature shows no data on the immunohistochemical expression and potential interrelationship of PAGE4, HIF-1, TNF- in prostate cancer cells and hyperplasia. The results of this research may contribute to a better understanding of the influence of the mentioned markers on the biological behavior of prostate cancer. In perspective these molecules could additionally be used as an incentive for further research into potential targeted therapy.

MeSH/Keywords: prostate carcinoma, prostatic hyperplasia, PAGE4, HIF-1 alfa, TNF alfa

Poster code: T-02-23-011

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

Introduction: Renal cell carcinoma 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. These checkpoints include LAG-3, TIM-3 and TIGIT which could be potential targets for the next line of ICI therapy.

Hypothesis: Expression of inhibitory receptors LAG-3, TIM-3 and TIGIT is higher in clear cell renal cell carcinoma cells and their microenvironment than in non-clear cell renal cell carcinoma.

Aims: Primary aim is to determine the expression of inhibitory receptors LAG-3, TIM-3 and TIGIT in clear cell and non-clear cell renal cell carcinoma cells and their respective microenvironments. Specifically we aim to determine the expression of inhibitory receptors LAG-3, TIM-3 and TIGIT in clear cell renal cell carcinoma and non-clear cell renal cell carcinoma cells and its respective immune microenvironments, to determine the difference in expression between the two groups and to investigate a possible connection between inhibitory receptor expression and clinical features of the patient (age, sex, disease stage, survival).

Materials and methods: Planned study is a cross-sectional, prospective one. Proposed study will investigate inhibitory receptor (LAG-3, TIM-3 and TIGIT) expression in clear cell and non-clear cell groups of renal cell carcinoma, in tumour cells themselves, and their microenvironment, mainly in immune cells surrounding the tumour. Using sample size calculating methods we determined that at least 28 cases per group (clear cell and non-clear cell) will need to be analysed. Main inclusion criteron was renal cell carcinoma while exclusion criteria include: other kidney tumours (benign lesions, metastatic lesions, urothelial carcinoma, etc.) and small tumour volume (tumours with less then 5 paraffin blocks). Samples of renal cell carcinoma were obtained by nephrectomy or excision of renal tumours. Histomorphological analysis will be performed. After initial analysis, one paraffin block from each patient will be chosen for immunohistochemical analysis using antibodies against inhibitory receptors being investigated (LAG-3, TIM-2 and TIGIT). Semi-quantitative immunohistochemistry will be 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. Kolmogorov-Smirnov test will be used to determine data distribution. Student t test and Mann-Whitney U test will be used in numeric variable analysis, and Chi-square and Fisher exact test will be used in categoric variable analysis. Pearson or Spearman correlation coefficient will be used between individual clinical variables. Kaplan-Maier survival analysis will be used to estimate the impact of inhibitory receptor expression on survival.

Expected scientific contribution: Study results might be beneficial in better understanding of renal cell carcinoma's immune microenvironment and the expression of inhibitory receptors LAG-3, TIM-3 and TIGIT in its different subtypes. As these inhibitory receptors are potential immunotherapy targets their expression distribution could be of importance in future research and, hopefully, one day in clinical practice by way of immunotherapy optimization.

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

Poster code: T-02-23-027

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, University Hospital Centre Zagreb

Introduction: Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy. The survival rate of pediatric ALL has improved significantly in last 30 years. ALL is treated according to international protocols in which asparaginase is one of the most important drugs. Currently, in most countries, polyethylene glycol-conjugated E. coli asparaginase (PEGasparaginase) is used as a first-line formulation and Erwinia asparaginase as a second line. 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.

Hypothesis: In the treatment of children with acute lymphoblastic leukemia, asparaginase activity in the initial period of treatment will be lower in patients who develop an infection.

Aims: The aim of this research is to compare the activity of asparaginase in the induction protocol depending on the presence of infection.

Materials and methods: The study will include patients with newly diagnosed acute lymphoblastic leuekemia 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 o-pthaldialdehyde. 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. Infection is considered if patient become febrile (axillary temperature > 38,0 C) between 12th and 40th day of treatment. The end of infection is considered if both of this conditions are met: discontinuation of intravenous antimicrobial therapy and axillary temperature 38.0C at least 48 hours without applying antipyretic measures. Exclusion criteria are: allergic reaction, silent inactivation, serious adverse event (sepsis, pancreatitis).

Expected scientific contribution: The results will show whether there is a difference in asparaginase activity depending on the presence of infection in the induction protocol. Furthermore, this study could contribute in creating new protocols in which one of the goals is to individualize the dose of asparaginase.

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

Poster code: T-02-24-053

Poster Title: Interrelation between ciliary apparatus and outcomes in patients with biliary atresia

PhD candidate: Matea Kovačić Perica

Part of the thesis: Interrelation between ciliary apparatus and outcomes in patients with biliary atresia

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

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

Introduction: Biliary atresia is a rare infantile disease of unknown etiology characterized by the obliteration of extrahepatic bile ducts leading to liver cirrhosis if not treated promptly. Patients develop cholestasis and acholic stools in the first two months of life. Early Kasai portoenterostomy (KPE) is the only possible 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 native liver survival. The etiopathogenesis is still not elucidated. Recently, the role of primary cilia in the etiopathogenesis of biliary atresia ("ciliary hypothesis") has been increasingly investigated. In the liver, primary cilia are present on cholangiocytes, where they act as mechano-, osmo- and chemoreceptors. Several studies found that primary cilia are abnormal in intrahepatic and extrahepatic bile ducts in syndromic and nonsyndromic forms of biliary atresia.

Hypothesis: Intrahepatic bile duct primary cilia (IHBC) are abnormal in patients with biliary atresia, and there is a correlation between the degree of IHBC disruption and the disease severity and outcomes.

Aims: First, to detect and quantify IHBC changes in patients with biliary atresia at the time of diagnosis and investigate the interrelation between the degree of IHBC disruption and short-term and long-term outcomes. Moreover, to investigate whether IHBCs are more significantly disturbed in patients with the fetal form of the disease.

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) will be included. The control group will consist of infants with cholestasis in whom the liver biopsy has been performed. Medical records will be a source for various patient data (demographic, initial presentation, age at KPE, medications, laboratory results, outcomes, etc.). Liver tissues obtained by surgical biopsy during KPE will be stained by the indirect immunofluorescence (IF) method. Two primary antibodies will be used: mouse monoclonal antibody to cytokeratin-7 to visualize cholangiocytes and rabbit monoclonal antibody to acetyl tubulin to visualize primary cilia. Two fluorescently labeled antibodies will be used as secondary antibodies. The presence and characteristics of IHBC will be analyzed visually and additionally assessed by computer-generated quantification method. The interrelation between changes in IHBC and short-term (the resolution of jaundice and stool color 3 and 12 months after KPE), and long-term clinical outcomes (native liver survival), as well as the form of the disease (perinatal or fetal), will be analyzed.

Expected scientific contribution: Answer to the question whether IHBC changes play a role in the development of biliary atresia, and its association with the disease severity and outcomes in the largest number of patients so far.

MeSH/Keywords: Biliary atresia; Cholestasis; Primary cilia

Poster code: T-02-24-076

Poster Title: The role of psyllium fiber in the treatment of functional constipation in children

PhD candidate: Mia Šalamon Janečić

Part of the thesis: The role of psyllium fiber in the treatment of functional constipation in children

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

Affiliation: University of Zagreb School of Medicine

Introduction: Constipation is a common problem in pediatric population with a worlwide prevalence of 9.5%, but more than 90% of these children have functional constipation as defined by the Rome IV criteria. According to the current guidelines, along with education and toilet training, osmotic laxative polyethylene glycol is the primary preferred medication for the treatment of functional constipation in children. Unfortunately prolonged treatment is often neccessary and it would be usefull to determine if some nonpharmacological measures could shorten the treatment duration or lower the dosage of laxative medication. Although higher fiber intake is recommended in adults with constipation, in pediatric population the existing studies are nonconclusive and further well designed studies are needed to define the potential role of fiber in the treatment of functional constipation.

Hypothesis: The addition of pyllium fiber to the osmotic laxative, polyethylene glycol, in the treatment of functional constipation in children leads faster to treatment success.

Aims: The aim of this study is to compare the time needed to achieve treatment success defined by Rome IV criteria between the two study groups.

Materials and methods: The study is designed based on the previously published Recommendations for pharmacological clinical trials in children with functional constipation: The Rome foundation pediatric subcommittee on clinical trials by Koppen et all. The participants will be children aged 4 to 7 years with functional constipation defined by the Rome IV Criteria diagnosed after a visit in a pediatric gastroenterology outpatient clinic in a pediatric tertiary care hospital. Prior to starting the trial, all participants will receive education, information and reassurance as standard care for functional constipation, and a treatment-free run-in period of 2 weeks is planned. The study is designed as a double blind clinical trial. After the run-in period, the participants will be randomised into 2 groups: 1st group will receive polyethylene glycol and placebo and the 2nd group will receive polyethylene glycol and psyllium fiber. After desimpaction participants will start taking polyethylene glycol in the dose of 0,5/kg/day and 10g of psyllium fiber or 10g of placebo (maltodextrin) as determined by randomisation. The participants parents will fullfill a daily diary which will document the administration of the laxative and psyllium fiber/placebo, the number and consistency of stools based on Bristol stool scale, fecal incontinence and gastrointestinal symptoms/side effects (abdominal pain, bloating, nausea). The study will be conducted throughout 14 weeks. 4, 8 and 12 weeks after the start of the intervention a clinical control, diary check and evaluation will be performed. We will also measure the rectal diameter by ultrasound at the beginning of the study and at the controls. If the patient at one of the controls achieves treatment success, based on the Rome IV criteria, a 50% reduction of polyethylene glycol dose is planned. If the child doesn't defecate for 3 or more days rescue therapy (an increase of polyethylene glycole dose) will be recommended. The specific goals of this study are to determine the difference in days needed to achieve treatment success between the two study groups, to determine the difference in number of children who were able to stop the treatment with laxatives, to determine the difference in frequency of bowel movements and fecal consistency, to determine the difference in rectal diameter and to document side-effects of pysllium fiber intake in children. Data will be analyzed using intention-to-treat principle.

Expected scientific contribution: This is the first randomized, double-blind, placebo-controlled study that would compare the effectivness of osmotic laxative with or without psyllium fiber in children with functional constipation

MeSH/Keywords: functional constipation, children, psyllium fiber

Poster code: T-02-24-090

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

PhD candidate: Paola Blagec

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: Irritable bowel syndrome (IBS) is included in the group of functional gastrointestinal disorders (FGID). IBS is characterized by abdominal pain and changes in the number and consistency of the stool, which significantly affects the quality of life of patients. Inflammatory Bowel Disease (IBD) is a chronic inflammatory disorder of the gastrointestinal tract characterized by a relapsing-remitting course. It has been observed that many patients in remission still have symptoms from the digestive tract, symptoms that are considered functional. Until now, the pathogenesis of functional disorders has not been elucidated. Moreover, currently, there is no universal treatment modality for these disorders. However, disorders of the autonomic nervous system and the composition of the intestinal microbiota are considered important factors in the development of functional gastrointestinal disorders. In addition, separate studies have shown that exercise has a positive effect on autonomic dysfunction and dysbiosis.

Hypothesis: Structured 6-month physical activity leads to the correction of dysbiosis, improvement of autonomic nervous system function, and reduction of symptoms in children with irritable bowel syndrome.

Aims: General aim is to determine the impact of 6-month structured physical activity on the function of the autonomic nervous system, the composition of the intestinal microbiota and symptoms in children with irritable bowel syndrome. Specific aims are to compare the impact of 6-month physical activity on the parameters mentioned above in children with irritable bowel syndrome versus children with chronic inflammatory bowel disease in remission. In addition, we aim to assess whether 6-month physical activity in children with irritable bowel syndrome leads to function of the autonomic nervous system and composition of intestinal microbiota comparable to that in healthy controls.

Materials and methods: Children between the ages of 10 and 18 years will be included in this study. Based on the following criteria, they will be divided into three groups. The first group will consist of children who had been diagnosed with irritable bowel syndrome according to the Rome IV criteria. The second group will consist of children with inflammatory bowel diseases in remission. Remission is defined as Pediatric Crohn's Disease Activity Index <12.5 for Crohn's disease and Pediatric Ulcerative Colitis Activity Index <10 for ulcerative colitis. The third group consists of healthy controls. The exclusive criteria for participation are regular structured physical activity at least 2 times a week and coexistence of another chronic disease. The function of the autonomic nervous system, composition of the intestinal microbiota, and quality of life will be determined for all three groups. Interpretation of autonomic nervous system testing will be done by using Composite Autonomic Scoring Scale (objective method) and a Composite autonomic symptom score-31 questionnaire (subjective method). Intestinal microbiota will be determined via 16S ribosomal RNA. Quality of life will be assessed using the PedsQL 4.0 questionnaire for all groups of subjects and IMPACT-III for patients with chronic inflammatory bowel diseases. Then, two groups (IBS and IBD) will be included in a 6-month exercise program, after which the measurement of the mentioned parameters will be repeated.

Expected scientific contribution: Research could examine the role of moderate physical activity in ANS disorders, intestinal microbiota, and disease symptoms in children with irritable bowel syndrome and chronic inflammatory bowel disease (in remission). The aforementioned could validate the therapeutic role of this intervention.

Acknowledgments: This study will be done as a part of the project " Chronic bowel diseases in children: looking beyond the gut " (AUTORUNGUT, IP-2019-04-3028) funded by Croatian Science Foundation.

MeSH/Keywords: functional disorders, physical activity, autonomic dysfunction, dysbiosis, pediatrics, gastroenterology

Poster code: T-02-24-113

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, Department for diagnostic and interventional radiology

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 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 which is considered the imaging golden standard.

Hypothesis: The measured values of the elastic modulus using shear wave elastography will be decreasing in patients undergoing neoadjuvant chemotherapy.

Aims: The aim of this study is to analyse the effect of neoadjuvant chemotherapy on breast cancer by using shear wave elastography.

Materials and methods: The golden standard for the assessment of the effect of neoadjuvant chemotherapy in patients diagnosed with breast cancer is magnetic resonance imaging. By using grayscale ultrasound in combination with shear wave elastography it is possible to assess the elastic properties of breast cancer and previous research has shown that they are different in comparison with normal breast tissues. The response to neoadjuvant chemotherapy can be assessed by noticing changes in the values of the elastic modulus of breast cancer lesions. Female patients diagnosed with breast cancer who are candidates for treatment with neoadjuvant chemotherapy will be included in the research. The response will be monitored with both methods before the start and upon completion of the treatment. In addition to imaging, all patients will undergo surgical resection of the tumour bed after completion of the neoadjuvant chemotherapy. The Residual Cancer Burden index will be used for the classification of residual disease.

Expected scientific contribution: Breast ultrasound with shear wave elastography may enable us to monitor the effects of neoadjuvant chemotherapy on breast cancer.

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

Poster code: T-02-25-005

Poster Title: 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

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): Associate Professor Igor Filipčić, MD PhD

Affiliation: University of Zagreb School of Medicine, University Psychiatric Hospital Sveti Ivan

Introduction: Cognitive symptoms are one of key features of schizophrenia, persisting throughout the entire span of the illness and hindering everyday functioning in work and social environment. Transcranial magnetic stimulation, a noninvasive and safe procedure targeting neural networks and neurotransmitters, has shown promising results in relieving negative and cognitive symptoms.

Hypothesis: We hypothesized that patients with negative symptoms of schizophrenia who received twenty days treatment of high-frequency repetitive transcranial magnetic stimulation (rTMS) with H7 coil will on average achieve statistically significant higher results on measures of cognitive functioning than patients who received inactive treatment with sham coil.

Aims: The purpose of this study is to examine the impact of high frequency rTMS with H7 coil on cognitive functioning in patients with negative symptoms of schizophrenia. Specifically, we aim to investigate whether there is a statistically significant difference in average achievement on cognitive tests in patients with negative symptoms of schizophrenia before and after twenty days of high frequency rTMS, independent of change in negative symptoms. Furthermore, we want to examine whether there is a statistically significant difference in average achievement on cognitive tests between patients who received active TMS treatment with H7 coil and patients who received inactive treatment with sham coil.

Materials and methods: A total of 68 patients aged 18 to 55 diagnosed with schizophrenia with predominant negative symptoms will participate in a randomized, controlled, double-blind study conducted in University Psychiatric Hospital Sveti Ivan in Zagreb. Patients who meet the inclusion criteria will be interviewed by psychiatrists using The Positive and Negative Syndrome Scale (PANSS) and The Scale for the Assessment of Negative Symptoms (SANS) to evaluate their symptoms at enrollment and after the end of treatment. At same time points, psychologists will assess patients' cognitive functioning via Auditory Verbal Learning Test (verbal memory and learning), verbal-logical memory subtest (verbal memory) and digit span forward (attention) and backward (working memory) task from Wechsler Memory Scale, Benton Visual Retention Test (visual memory), block design subtest (executive and visuospatial functions) and digit symbol substitution subtest (speed of processing) from Wechsler Adult Intelligence Scale, Trail Making Test version B (executive and visuospatial functions), FAS Verbal Fluency Test, and Penn Emotion Recognition Test (social cognition). After initial evaluation patients will be randomized into two groups using adaptive randomization by minimization method with 1:1 ratio. Over the course of twenty workdays, 34 patients will undergo high frequency rTMS treatment with H7 coil, while other 34 patients will undergo inactive treatment with sham coil. To test our hypothesis, we will conduct a 2x2 mixed model analysis of covariance for each cognitive test, with results on SANS scale as a covariate to control potential influence of change in negative symptoms on cognitive functioning results. The first factor will be the within-subject factor of measurement point, and the second factor will be the between-subject factor of treatment type. Level of statistical significance will be set at $p<0.05$.

Expected scientific contribution: Results could help clarify prior inconsistent findings in this field and contribute to development of effective treatment options targeting cognitive symptoms in schizophrenia.

MeSH/Keywords: schizophrenia, cognitive functions, transcranial magnetic stimulation

Poster code: T-02-29-081

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. So far, 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.

Hypothesis: Using evoked potentials in patients with spinal muscular atrophy, we can recognize and quantify changes in sensory pathways in the spinal cord, brainstem and cerebrum.

Aims: 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, to examine whether there is a difference in results within individual types of SMA as well as to examine the difference in the therapeutic approach and length of treatment.

Materials and methods: We will include 50 adult subjects of both sexes with SMA diagnosis and a control group of 50 healthy subjects. We will observe subjects with SMA type 1, 2 and 3 who are treated with nusinersen or risdiplam. Upon study the following data will be obtained: VEP, BAEP, SSEPm and SSEPt. Subjects will be followed-up in 6-month intervals for three years.

Expected scientific contribution: We believe we will prove that in SMA sensory pathways are also affected as well as that the results of evoked potentials will depend on the type of SMA, type and length of treatment.

MeSH/Keywords: Spinal muscular atrophy, evoked potentials, nusinersen, risdiplam

Poster code: T-02-30-037

Poster Title: Genetic variants in patients with focal epilepsies

PhD candidate: Sven Županić

Part of the thesis: Genetic variants in patients with focal epilepsies

Mentor(s): Professor Fran Borovečki, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Although focal epilepsies are more often caused by a clear etiological mechanism, more and more evidence supports the paradigm that genetic factors significantly contribute to the pathogenesis of focal epilepsies.

Hypothesis: Specific genetic variants are associated with the occurrence of focal epilepsy.

Aims: The aim of the study is to determine which genetic variants are present in adult patients with focal epilepsy of unknown etiology. We plan to compare the genetic variants present in patients with focal epilepsy of unknown etiology with the genetic variants present in healthy subjects, determine whether there is a statistically significant difference in the occurrence of any of the genetic variants between the two groups, link variants that are found to have a higher incidence in the group of patients with focal epilepsy with the clinical features of the patients available from the medical records.

Materials and methods: In this cross-sectional study 70 subjects will be in the study group while the other 30 subjects will be in the control group. We determined that a sample of 58 patients would be enough to provide the trial with a power of 80% (two-sided alpha 0.05; event rate of 23%). Subjects in the study group will be patients diagnosed with epilepsy of undetermined etiology over 18 years of age. Subjects included in the control group will be age and gender-matched controls who (1) are over 18 years old (2) have not been diagnosed with epilepsy (3) have never experienced an epileptic seizure or any unexplained crisis of consciousness (4) do not suffer from a progressive neurological disease. All subjects in the study group will be randomly selected from the hospital register of patients at the University Hospital Dubrava. The subjects in the control group will be randomly selected from the general population. All subjects will be tested for genetic variants of the genes which are standard component genes in the epilepsy panel. Patients' blood will be sampled for the purposes of testing. DNA will be isolated from peripheral blood using a commercial DNA isolation reagent kit (Zymo Quick DNA). Sequencing libraries will be prepared using the Nextera Flex for Enrichment (Illumina) reagent kit. Sequencing will be performed on the NextSeq550 sequencing device (Illumina). Sequence alignment will be done using the BWA software package, and data analysis and visualization will be done using the Variant Interpreter software package (Illumina) and searching the following databases: OMIM, ClinVar, HGMD, dbSNP. The presence of different genetic variants in patients with focal epilepsy of unknown etiology and genetic variants present in healthy subjects will be shown by descriptive statistics, and a statistically significant difference in the occurrence of some of the genetic variants between the two groups will be determined by the chi-square test. Furthermore, the variants that we show to have a higher incidence in the group of patients with focal epilepsy will be linked with the clinical features of the patients available from the medical records. This association will be tested by the Mann Whitney U test and the chi-square method. A statistically significant result will be considered the one in which $p<0.05$.

Expected scientific contribution: The present research hopes to describe genetic variants associated with focal epilepsy in subjects from the Croatian geographic area. We hope that the results of this research should contribute to better diagnosis and therapy in patients suffering from epilepsy. We hope this study will be one of the few studies in the world that will analyze genetic variants exclusively in adult patients with focal epilepsies. Furthermore, we hope this will be the first study to compare genetic variants in patients with temporal lobe epilepsy with patients with extratemporal epilepsy.

MeSH/Keywords: Focal epilepsy, Next-Generation Sequencing, Genetic variants

Poster code: T-02-30-041

Public health and healthcare – research proposals

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

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 was the most common cancer in the male population in Croatia in 2020. In 2021. it was the third most common cause of death by neoplasms. A systematic literature review showed a possible connection between socioeconomic factors and prostate cancer. People of higher socioeconomic status have a higher risk of developing prostate cancer, and those of lower socioeconomic status have lower survival rates and higher specific mortality rates. However, there is no research on the connection between socioeconomic and epidemiological factors in Croatia.

Hypothesis: Developed areas of Croatia have a higher incidence and prevalence, lower mortality, and a longer survival of prostate cancer patients.

Aims: General objective is to analyse the association between the regional development indexes and the risk of poverty with epidemiological indicators of prostate cancer in the Republic of Croatia. Specific goals are 1. To calculate the age-standardized rates of incidence, prevalence, and mortality of prostate cancer in Croatia by counties and groups of municipalities and compare the trends according to the area's development. 2. To calculate and compare prostate cancer survival by Croatian counties and groups of municipalities. 3. To compare the distribution of stages at the time of detection and to calculate survival by stage at the time of diagnosis by counties and groups of municipalities. 4. To examine the association of incidence, prevalence, mortality, and survival from prostate cancer with available indices of development and risk of poverty by counties and groups of municipalities.

Materials and methods: This is a cross-sectional study involving the prostate cancer population from 2006-2016. Epidemiological data from the Croatian National Cancer Registry and data on mortality will be collected. For each patient with prostate cancer, data on the patient's age at diagnosis, disease stage at diagnosis, histological diagnosis, county of residence, date and cause of death will be extracted. The risk of poverty is publicly available from the State Bureau of Statistics for 2011. Data on the development index from the Ministry of Regional Development and European Union Funds are published for 2006-2008, 2010-2012, and 2014-2016. Age-standardized incidence, prevalence, and mortality rates of prostate cancer will be calculated by counties and groups of municipalities grouped by development index, as well as the age-standardized net survival of patients with prostate cancer. For calculations of epidemiological indicators, data for five-year age groups (including 85+) and data on the number of inhabitants for each year by age according to the mid-year National Bureau of Statistics estimates for the level of Croatia and counties will be used. For the level of municipalities and groups, since there are no mid-year estimates by age structure, the 2011 census data (the middle of 2006-2016) will be applied to the entire researched period. Counties and municipalities will be further divided into those that remained in the same group according to the first and last development index, those that have improved their status, and those that have worsened, and comparisons will be made between these three groups. Changes compared to the initial state will be shown, differences between groups will be calculated, and correlation coefficients and other appropriate measures of association (ratios of incidence, prevalence, and mortality rates) of the obtained indicators will be presented.

Expected scientific contribution: This research will determine the possible association between the index of development and the risk of poverty with the epidemiological indicators of prostate cancer. In relation to previous research, an additional contribution is comparing changes in these parameters in geographical units that have become poorer, richer, or remained at the same level of development. The research would

provide the information needed by the academic and professional community to direct further preventive, diagnostic, and therapeutic action and reduce existing inequalities.

MeSH/Keywords: prostate cancer, epidemiological factors, socioeconomic factors

Poster code: T-03-01-127

Poster Title: Assessment of the need for deep brain stimulation in patients with Parkinson's disease in Croatia

PhD candidate: Nikolina Sesar

Part of the thesis: Assessment of the need for deep brain stimulation in patients with Parkinson's disease in Croatia

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

Affiliation: University of Zagreb School of Medicine

Introduction: Deep brain stimulation is the gold standard in treating Parkinson's disease. Although it is not widely available globally, it has been used in neurosurgical practice for the past few decades. Global trends tend to lean toward earlier introduction of invasive treatment as well as lowering the indication threshold.

Hypothesis: There is a possibility for greater utilization of deep brain stimulation for Parkinsons disease treatment in Croatia compared to global trends and in the context of Parkinson disease prevalence in our region.

Aims: The aim of this research is to determine the prevalence of deep brain stimulation in Croatia and the general awareness among physicians and patients about this type of treatment.

Materials and methods: To determine the stance of the Croatian medical community on this topic, we conducted structured surveys to assess the awareness of potential patients and physicians about this type of treatment. In order to determine the actual prevalence and availability of deep brain stimulation in Croatia, we analyzed the relationship between the prevalence of Parkinson's disease and the number of procedures performed so far in the country. We compared the obtained data with current global trends related to this type of treatment.

Expected scientific contribution: The results of this research can be used to affirm and expand the application of deep brain stimulation. Determining the potential for growth and development of scientific and professional activities in this highly specialized field will contribute to the development and progress of functional neurosurgery in general. This study will document the scope of work and comprehensiveness of this individual procedure in Croatia. By documenting the current professional activities and prevailing attitudes towards this treatment in the public, it is possible to demonstrate the need to increase the capacity for deep brain stimulation treatment and define a direction in which it will develop in the future in Croatia.

MeSH/Keywords: Parkinson's disease, prevalence, deep brain stimulation, Republic of Croatia

Poster code: T-03-01-141

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

PhD candidate: Ana Ištvanović

Part of the thesis: The association of socioeconomic and demographic indicators 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 is one of the most widespread psychoactive substances. When compared to the world, alcohol consumption in Croatia is above average and is associated with a number of negative outcomes. Harmful use of alcohol ranks among the top five risk factors for disease, disability and death. There are many factors that can be associated with alcohol consumption such as socioeconomic status. COVID 19 pandemic has significantly affected the lives of people and intensified mental health difficulties. There is a possibility that increased anxiety and fear associated with COVID 19 influenced the increase and initiation of psychoactive substance use including alcohol use.

Hypothesis: Demographic indicators: gender and age and socioeconomic indicators: type of household, employment status, level of education, marital status, income and standard of living are directly related to the consumption of alcohol in Croatia. There are changes in alcohol consumption in 2023 after the onset of the COVID 19 pandemic compared to 2019 before the COVID 19 pandemic.

Aims: GENERAL AIM: to investigate the association of socioeconomic and demographic factors with prevalence of alcohol consumption and risky alcohol use in the general population of Croatia, and changes in alcohol consumption between 2019 and 2023 during the COVID 19 pandemic. SPECIFIC AIMS: (1) to investigate the association between demographic factors and alcohol consumption in the general population in Croatia: gender and alcohol consumption, age and alcohol consumption, and (2) to investigate the association between socioeconomic factors and alcohol consumption in the general population in Croatia: type of household and alcohol consumption, employment status and alcohol consumption, level of education and alcohol consumption, marital status and alcohol consumption, income and alcohol consumption, standard of living and alcohol consumption.

Materials and methods: To achieve the aims of proposed research data collected in two survey cycles (third cycle conducted in 2019 and fourth cycle which will be conducted in 2023) of empirical research Substance Abuse among the General Population in the Republic of Croatia will be analysed. This survey collects data on extent and patterns of substance use amongst the general population. General Population Survey is one of key epidemiological indicators developed by the European Monitoring Centre for Drugs and Drug Addiction. It is conducted every four years by the Croatian Institute of Public Health in collaboration with Institute of Social Sciences Ivo Pilar. The last survey cycle was conducted in 2019 using a representative sample of 4,994 citizens of Croatia between the ages of 15 and 64. The basic sample included 4,000 people between the ages of 15 and 64, and oversampling included an additional 1,000 participants between the ages of 15 and 34. Oversampling was carried out in order to include more participants in the group of younger adults because the use of addictive substances is significantly more common in that age group. The survey was conducted on a probabilistic multi-stage stratified sample. The data was gathered by face-to-face interviews. Due to the sensitivity of the topic, it was made possible for the respondents to fill out the questionnaire on their own. The fourth survey cycle will be conducted in 2023 using the same methodology as the previous ones. For the analysis, statistical methods of cross-sectional research, descriptive statistics and regression methods for determination of relationship between selected determinants will be used.

Expected scientific contribution: This research will determine the association between socioeconomic and demographic factors and alcohol consumption in the general population of Croatia and consider whether there are changes in alcohol consumption in 2023 compared to 2019 before COVID 19 pandemic. Based on

this research, it will be possible to plan specific public health interventions to reduce the harmful consumption of alcohol in different population groups.

MeSH/Keywords: alcohol consumption, socio-economic determinants, demographic determinants, Republic of Croatia, Sars-CoV-2

Poster code: T-03-02-059

Poster Title: Risk factors for transitioning from cannabinoids to other drugs as primary drugs among treated psychoactive drug abusers

PhD candidate: Maja Valentić

Part of the thesis: Risk factors for transitioning from cannabinoids to other drugs as primary drugs among treated psychoactive drug abusers

Mentor(s): Professor Mirjana Kujundžić Tiljak, MD PhD, Ivana Pavić Šimetin, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Cannabis is often mentioned in research as the gateway substance for other psychoactive drugs, but the factors of transition from the use of cannabis to other psychoactive drugs are still mostly unknown.

Hypothesis: Male gender, financial condition estimated as below-average, divorced parents, polysubstance abuse, psychiatric comorbidities and psychiatric comorbidities within the family, referral to treatment from a social welfare center, and legal problems are positively associated with the transition from cannabinoids to other psychoactive drugs, whereas the exposure to the greater number of mentioned factors increases the chances of transitioning to another psychoactive drug.

Aims: To determine the characteristics of treated cannabinoid users and to analyze the relationship between their sociodemographic characteristics, drug use patterns, psychiatric comorbidity and legal problems in relation to whether they transitioned to other psychoactive drugs or remained on cannabinoid use.

Materials and methods: The research will include persons recorded in Registry of Psychoactive Drug Abusers since the establishment of the Registry in 1978. Their data will be tracked in the Registry until the last year of data collection, which will be the calendar year before the year in which the analysis will be carried out. People will be divided into two categories:). people whose main substance are cannabinoids and 2) people whose main substance are other psychoactive drugs, and the first psychoactive drug was cannabinoids. The data will be monitored individually and anonymized for each person, and the monitoring of the data for an individual person in treatment ends at the end of the research, the date of death or the date of the last recorded treatment data. Data will be included retrospectively from the Registry, and for each person in the study, the last recorded treatment data will be included in the analysis. The Pompidou questionnaire, a standardized questionnaire proposed by the Pompidou Group of the Council of Europe, is used to record a person in the Registry. The questionnaire includes questions related to socio-demographic characteristics (gender, age, current living conditions children, coexistence with other users of addictive substances, highest level of education, work status, marital status, assessment of financial status, number of children in the family), general details about treatment (treatment with regard to the type of institution, type of contact with the institution of the last treatment, source of referral to treatment, who first found out and exposed the problem with the substance use, when did the parents find out about the problem, the reason for starting the experimentation, assessment of the dominant etiological factor for the development of substance use), drug use patterns, psychiatric comorbidities, substance-related disorders in the family, other clinical data and legal problems which will be analyzed. The characteristics of the sample will be shown by the frequency distribution. Intragroup and intergroup differences will be analyzed, depending on the nature of the probability distribution of the results, using appropriate statistical procedures: statistical tests of significance (ie non-parametric and parametric possibilities) and multivariate association (regression). Joint actions of individual factors will be investigated, with the possibility of excluding "confounding" effects. The results will be presented tabularly and graphically. Data processing and analysis will be done in appropriate programs for data processing and analysis.

Expected scientific contribution: The research could contribute to the timely recognition of cannabinoid users who are at increased risk of transitioning to other psychoactive drugs.

MeSH/Keywords: Cannabinoids, Substance-Related Disorders, Risk Factors

Poster code: T-03-02-063

Poster Title: Exploring Sex-Related Differences in Human B-Lymphocytes Response Induced by Combined Effects of Polystyrene Nanoparticles and Selected Anti-Inflammatory Drugs

PhD candidate: Matija Dvorski

Part of the thesis: Exploring Sex-Related Differences in Human B-Lymphocytes Response Induced by Combined Effects of Nanoplastic Particles and Selected Anti-Inflammatory Drugs

Mentor(s): Professor Robert Likić, MD PhD, Associate Professor Ivana Vinković Vrček, MD PhD

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Introduction: Environmental pollution with nanoplastic particles and endocrine-disrupting chemicals (EDCs) presents a global environmental and public health problem. Nanoplastic particles can adsorb various EDCs, including drugs, present in the environment, easily enter the human body and penetrate cells, causing various cytotoxic effects. Since research shows that women are more sensitive to many drugs than men are, it is important to examine whether women are more susceptible to health risks due to environmental pollutants than men.

Hypothesis: The combined effects of polystyrene nanoparticles and selected non-steroidal anti-inflammatory drugs will cause a different response in B-lymphocytes of male origin compared to the response of B-lymphocytes of female origin, and the biological effects of the mixtures will be different from the effects of single components of the mixture.

Aims: The general aim of this doctoral thesis is to determine sex-related differences in the biological effects of mixtures of each individual anti-inflammatory drug (ibuprofen, I, ketoprofen, K, and diclofenac, D) and polystyrene nanoparticles (PSNPs) suspension in B-lymphocytes of male and female origin. Specific aims are: 1) determine the LC10 of each drug and PSNP suspension (d=20 nm) in B-lymphocytes of male and female origin, 2) analyse the biological effects of each individual drug, PSNPs suspensions, and their mixtures (I + PSNPs, K + PSNPs and D + PSNPs) on cell viability, apoptosis induction, oxidative stress parameters, and changes in gene expression for selected cytokines, and estrogen-dependent immune genes, as well as changes in gene expression of estrogen receptors in B-lymphocytes of male and female origin.

Materials and methods: The study will be conducted in in vitro using naïve B-lymphocytes. Buffy coats from healthy donors (men and women 18 to 45 years of age, who will sign informed consent) will be obtained from the Croatian Institute for Transfusion Medicine. PBMC will be isolated from previously cryopreserved buffy coats using Lymphoprep reagens. Naïve B-lymphocytes will be separated from PBMC by immunomagnetic negative selection and resuspended in fresh culture medium. The minimum toxic concentration (LC10) of each individual drug and the suspension of PSNPs will be determined using the MTS cell viability assay. B-lymphocytes of male and female origin will be treated separately with five different concentrations (lower than LC10) of each drug, PSNPs suspension, and mixtures of each individual drug and PSNPs suspension and analysed after 24 hours of incubation. All treatments and analysis, for each of three individual drugs (with and without PSNPs), will be repeated independently using isolated naïve B-lymphocytes collected from six different male and six different female donors in triplicates. To evaluate the levels of oxidative stress, three different assays (DCFH-DA, mCBM, Rh123) will be used and the fluorescence intensity will be detected by the multilabel plate reader. Cell viability and apoptosis induction will be analysed using the flow cytometry method and the Annexin V-FITC Assay Kit. Changes in gene expression of pro- (IL-1beta, IL-6, TNF-alpha) and anti-inflammatory (IL-10, IL-13, TGF-beta) genes, estrogen-dependent immune genes (TNFRSF17, CAV1), and estrogen receptor genes (ESR1, ESR2) in treated B-lymphocytes of male and female origin will be determined by RT-qPCR analysis using specific primers, SYBR Green dye and two reference genes (GAPDH and beta-actin). Total cellular RNA will be isolated using the Aurum Total RNA Mini kit and converted to cDNA using the High Capacity cDNA Reverse Transcription kit. Sex-related differences will be analysed using two-way ANOVA.

Expected scientific contribution: This research would 1) show whether anti-inflammatory drugs in combination with polystyrene nanoparticles cause different expression of estrogen receptor genes and

estrogen-dependent immune genes in B-lymphocytes of male and female origin, 2) contribute to the development of in vitro models for the early assessment of health risks in men and women exposed to environmental pollutants, including endocrine disruptors.

Acknowledgments: -

MeSH/Keywords: sex, B-lymphocytes, nanoplastics, anti-inflammatory agents, in vitro

Poster code: T-03-06-097