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Preliminary research results

Basic medical sciences – preliminary research results

Poster Title: Role of bone morphogenetic protein 1 and its neutralizing antibody in congenital muscular dystrophy in a mouse model with a mutation in the laminin gene

PhD candidate: Ivona Matić Jelić

Part of the thesis: Role of bone morphogenetic protein 1 and its neutralizing antibody in congenital muscular dystrophy in a mouse model with a mutation in the laminin gene

Mentor(s): Academician Slobodan Vukičević

Affiliation: University of Zagreb School of Medicine

Introduction: Bone morphogenetic protein (BMP) 1 is a secreted, glycosylated, zinc-dependent metalloproteinase. Administration of the BMP1.3 (BMP1 isoform) neutralizing antibody reduced renal fibrosis, preserved renal function and increased survival in rat renal fibrosis model. BMP1.3 antibody therapy reduced fibrosis progression in a rat liver cirrhosis model. Congenital muscular dystrophy (CMD) belongs to a group of neuromuscular disorders and is characterized by muscle weakness and muscle wasting. Merosin deficient congenital muscular dystrophy type 1A (MDC1A) is the second most prevalent CMD form. The responsible gene is lama2, important for production of laminin-211 which contributes to the protein called merosin, extracellular matrix protein that is strongly expressed in the basement membrane of skeletal muscle. The most commonly used animal models for MDC1A are mouse strains Dy-W (dystrophia muscularis) which present a muscle pathology due to a complete and partial deficiency in the 2 chain of laminin. Compared to the other available models for this disease, the severity and disease progression of Dy-W mice more closely resembles human MDC1A. The Dy-W mouse exhibits severe muscular dystrophy and survival is reduced to a range of 5-10 weeks. We decided to perform all experiments till week five.

Materials and methods: The research includes experiments on Dy-W mice that carry the mutation in lama2 gene. The experiment includes therapy with 50 μg/kg anti-BMP1.3 polyclonal antibody administered twice a week (intraperitoneally with 25G needle, injection volume depends on the mass of the mouse individual) from third to fifth week of life. During three weeks time experiment, mice were weighed twice a week and monitored daily. At the end of the experiment, the mice were genotyped and organ samples (muscle, liver, kidney) from all genotypes (Dy-W-/-, Dy-W+/-, Dy-W+/+ and Dy-W-/- anti-BMP1.3) were taken for gene expression analysis (BMP1, BMP1.3, BMP2, BMP5, Smad genes, TGF). Expression of targeted genes in muscle, liver and kidney were evaluated at the RNA basis via RT-PCR. Also, muscle from Dy-W-/-, Dy-W+/-, Dy-W+/+ and Dy-W-/- antiBMP1.3 were taken for histological analysis. Sirius red staining was performed to enable assessment of ratio of fibrosis in each muscle section via quantification with Olympus CellSens Dimensions software.

Results: We found an increased BMP1.3 concentration in muscle, liver and kidney of mice with a mutation in laminin 2 gene with a congenital muscular dystrophy. Further, expression of TGF and Smad genes was also upregulated in Dy-W-/- mice, as well as BMP2 and BMP5. It has been previously reported that inflammation and fibrosis are a very early signature of MDC1A pathology and we noticed upregulation in collagen in muscle tissues isolated from Dy-W-/- mice. It is therefore possible that BMP1.3 molecule could also play a critical role in collagen processing in Dy-W pathology. We continue to examine the BMP1.3 expression in Dy-W mice and determine if inhibition of BMP1.3 could lead to amelioration of fibrogenic pathology associated with MDC1A. We tested the common SMAD4 and TGF specific SMAD2 and -3 and TGF, BMP2 and -5 expression in homozygous Dy-W mice as compared to heterozygote animals and found extreme expression of all genes, indicting a very disrupted system of ECM and TGF family GF signaling. In homozygous Dy-W mice that received therapy we observed reduced expression of target genes, better motility, greater mass and better survival.

Discussion: This study could provide a basis for the use of the proposed antibody in order to reduce the symptoms of MDC1A in humans and enable the development of a new targeted therapy for a condition for which there are currently only empirical palliative solutions.

Acknowledgments: /

MeSH/Keywords: Bone Morphogenetic Protein 1; Regeneration; Congenital Muscular Dystrophy; BMP1 Neutralizing Antibody

Poster code: R-01-01-059

Poster Title: In vitro determination of the potential influence of bone morphogenetic protein 6 (BMP6) and serotonin (5HT) on glucose metabolism

PhD candidate: Marina Milešević

Part of the thesis: Uinak kotanog morfogenetskog proteina 6 (BMP6) i serotonina na metabolizam glukoze i kotani metabolizam

Mentor(s): Tatjana Bordukalo Nikšić, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: BMP6 protein, a member of the TGF- superfamily, is well known for its potent osteoinductive potential that enhances osteoblast differentiation and induces new bone formation in vivo. Beyond the skeletal system, BMP6 signaling is important in the development and homeostasis of various organs and tissues and several studies implicate that BMP6 has a certain impact on the pancreas and liver in maintaining the glucose homeostasis, which needs to be further investigated. Serotonin (5-hydroxytryptamine, 5HT) is a monoamine neurotransmitter that modulates the neural activity and a wide range of neuropsychological processes. Outside the central nervous system, 5HT in the circulation is mainly produced by enterochromaffin cells of the gut shows multiple functions in peripheral organs such as a diverse role in the regulation of the endocrine function of the pancreas under hyperglycemic conditions.

Materials and methods: INS-1 cells were cultured in 48 well plates in a complete medium and were grown in a 37 °C incubator under a humified atmosphere containing 5 % CO2. For the determination of the effects of BMP6 and 5HT on cellular processes related to glucose metabolism and insulin secretion, cells were treated with BMP6 (100 ng/well) or 5HT (1 and 10 mM) for 24 h in a complete medium with or without 5HT respectively. Insulin secretion assay in basal (BIS, 2,8 mM glucose) and high glucose concentration (GSIS, 16,7 mM glucose) was performed after 24 h treatment as described before (Bennet, 2016). Insulin concentration in the collected medium was measured by using rat insulin ELISA kits (Mercodia, Uppsala, Sweden). Total RNA from cells was extracted by using TRIzol reagent and gene expression analysis of genes related to glucose metabolism (PEPCK, G6Pase, Glut2, GCK, INS) was performed by standard RT-qPCR procedure (Heid, 1996).

Results: Under the condition of GSIS, treatment of INS1- cells with BMP6 for 24 h resulted in a statistically significant reduction in the secreted concentration of insulin compared to untreated control. Gene expression results indicate that the expression of G6Pase is significantly increased in the presence of BMP6 protein. Under the condition of BIS, treatment of INS-1 cell for 24 h with 1 mM 5HT resulted in a statistically significant enhancement in the secreted concentration of insulin compared to untreated control. Under the condition of GSIS, treatment of INS-1 cell for 24 h with both concentrations (1 and 10 mM) resulted in a statistically significant increase in the secreted concentration of insulin compared to untreated control. Gene expression results indicate that the expression of G6Pase is significantly decreased in the presence of 1 mM 5HT, and a similar trend is observed at a concentration of 10 mM 5HT but without statistical significance.

Discussion: We analyzed the BMP6 and 5HT impact on insulin output in BIS and GSIS conditions in INS-1 cells and showed that BMP6 significantly decrease, while 5HT significantly increase pancreas insulin output in GSIS. Both have a significant influence on the expression of the enzyme glucose-6-phosphatase (G6Pase) and glucose metabolism in GSIS. G6Pase is expressed mainly in the liver and the kidney cortex, the two most important gluconeogenic tissues, but it is also expressed in the -cells of pancreatic islets of humans, mice, and rats, most particularly in the starved and diabetic states. G6Pase catalyzes the dephosphorylation of glucose-6-phosphate to glucose and inorganic phosphate, an opposite process to glucose utilization, and a step required in the regulation of insulin secretion from pancreatic -cells. G6Pase activity is increased in islets isolated from animal models of type II diabetes where it augments a glucose cycle that consumes one molecule of ATP per cycle which is presumed to be an antagonist to GSIS. BMP6 and 5HT have shown diverse impacts on insulin secretion in INS-1 which seems they regulate through direct influence on gene expression of enzyme G6Pase. BMP6 has shown a negative impact while 5HT has shown a positive impact on glucose metabolism in pancreatic -cells in GSIS conditions by increasing and decreasing G6Pase expression, respectively. The exact mechanism of how BMP6 and 5HT influence on the glucose metabolism, insulin secretion, and the development of type II diabetes will be further explored in the BMP6 knockout model of mice compared to wild type.

MeSH/Keywords: BMP6, serotonin, glucose metabolism, insulin secretion

Poster code: R-01-01-062

Poster Title: T2 relaxation time mapping of articular cartilage in primary OA and DDH-induced secondary OA

PhD candidate: Tea Duvančić

Part of the thesis: Specifics of the structure of the osteochondral unit of the acetabulum, neoacetabulum and femoral head in patients with secondary coxarthrosis caused by developmental disorders of the hip

Mentor(s): Professor Domagoj Delimar, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Developmental dysplasia of the hip (DDH) is an abnormality of the hip joint characterized by mild to complete dislocation of the femoral head. It is one of the leading causes of secondary hip osteoarthritis and as such causes degenerative changes similar to those found in primary osteoarthritis (OA). In advanced stages of DDH, the acetabulum and femoral head are not in physical contact and a new acetabulum (neoacetabulum) is formed. Patients with advanced stages of DDH therefore have two acetabular regions: anatomical acetabulum and neoacetabulum. T2 mapping is a compositional MRI technique used for the analysis of the biochemical composition of cartilage. It reflects collagen content, collagen fiber orientation, and water composition of cartilage extracellular matrix. Longer T2 relaxation times indicate cartilage degeneration. In this study, we compared the degree of cartilage degeneration caused by primary and secondary OA using T2 relaxation time mapping.

Materials and methods: Samples were obtained during total hip arthroplasty using a 10 mm cylindrical chisel (Small Joint OATS Set, 10 mm, Arthrex, Germany) from 2 groups of patients: patients with DDH and those with primary OA. Samples obtained from the DDH group were taken from acetabulum, neoacetabulum, non-weight bearing part of the femoral head and weight-bearing part of the femoral head, and those obtained from the OA group from the acetabulum and femoral head. Samples were washed in saline solution and scanned on a 7.0T micro-magnetic resonance imaging (u-MRI) machine immediately following the surgery. T2 relaxation times of cartilage were calculated. Results were compared between different anatomical regions and experimental groups.

Results: Mean T2 relaxation times for acetabulum, neoacetabulum, non-weight bearing and weight-bearing part of the femoral head of DDH group were 43.05±7.76, 65.92±33.12, 38.09±18.45 and 37.91±18.36 ms, respectively. Mean T2 relaxation times for acetabulum and the femoral head of OA group were 45.96±23.95 and 36.77±11.09 ms. In both the DDH and primary OA groups, the acetabular cartilage had longer T2 relaxation times compared to the femoral cartilage. There was no significant difference neither between T2 relaxation times of femoral head cartilage of DDH and primary OA patients ($p=.42$) nor between the acetabular cartilage of DDH and OA patients ($p=.07$). T2 relaxation time of neoacetabular cartilage was significantly longer than that of the acetabular cartilage of both the DDH and primary OA patients.

Discussion: Longer T2 relaxation times of acetabular cartilage in both groups of patients indicate that primary and secondary OA cause more damage to the acetabular than to the femoral head cartilage. No significant differences were found between neither the femoral head cartilage of DDH and that of primary OA group, nor between the acetabular cartilage of DDH patients and acetabular cartilage of primary OA patients, which suggests that primary and secondary OA cause a similar degree of degeneration. However, the neoacetabular cartilage had a significantly longer T2 relaxation time than all the other studied anatomical regions. This indicates that the neoacetabulum is most affected by DDH-induced secondary OA.

MeSH/Keywords: Hip dysplasia, osteoarthritis, cartilage, micro-MRI, T2 mapping

Poster code: R-01-02-099

Poster Title: Can ex vivo magnetic resonance imaging be used to predict sperm presence in testicular tissue of men with azoospermia?

PhD candidate: Ana Planinić

Part of the thesis: Predictive model of sperm presence in the testis of men with azoospermia based on magnetic resonance imaging

Mentor(s): Professor Davor Ježek, MD PhD, Siniša Škokić, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Azoospermia is a condition where there are no spermatozoa in the ejaculate and men who wish to have children undergo testicular sperm extraction (TESE). The success rate of TESE in nonobstructive azoospermia (NOA) is only around 50%. Histological analysis is the only method used for predicting sperm retrieval. The shortcomings are that histology in NOA is heterogeneous and the method is invasive. Magnetic resonance imaging (MRI), on the other hand, can provide whole testis imaging and parameter mapping and is also a non-invasive method. Could ex-vivo magnetic resonance imaging of testicular tissue be useful in designing a predictive model for sperm presence?

Materials and methods: 35 samples of testicular tissue were obtained via TESE and all of them underwent 7T MRI, specifically diffusion tensor imaging (DTI) and magnetization transfer imaging (MTI). Samples were then histologically processed, analyzed, and divided into groups based on their mean Johnsen score: JS<2, JS2, JS4, 4<JS<8, and JS8.

Results: The apparent diffusion coefficient (ADC) was higher in samples with a lower mean Johnsen score which reflects the higher level of water diffusion in tissue with lower cell density. The difference was statistically significant ($p<0,05$) between each group. Fractional anisotropy (FA) was significantly lower ($p=0,018$) in the group with a mean Johnsen score below 2 compared to the other samples which could reflect less restricted water diffusion in tissues with the lowest cell density. Magnetization transfer ratio (MTR) which reflects the density of macromolecules in tissue did not differ significantly between histological groups.

Discussion: Higher ADC values are associated with a lower mean Johnsen score and could be useful in predicting histological groups of testicular tissue while FA values could be useful in identifying samples with the lowest mean Johnsen scores. Ex vivo magnetic resonance imaging could be useful in designing a predictive model for sperm presence in testicular tissue.

MeSH/Keywords: testis, spermatozoa, azoospermia, sperm retrieval, diffusion tensor imaging, anisotropy

Poster code: R-01-02-125

Poster Title: Status of F2 isoprostane, malondialdehyde, vitamin E and superoxide dismutase in the follicular fluid of patients with idiopathic infertility

PhD candidate: Ivana Zec

Part of the thesis: Status of F2 isoprostane, malondialdehyde, vitamin E and superoxide dismutase in the follicular fluid of patients with idiopathic infertility

Mentor(s): Professor Marina Šprem Goldštajn, MD PhD, Associate Professor Krunoslav Kuna, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Follicular fluid (FF) represents the microenvironment surrounding the oocyte. Oxidative imbalance causes macromolecule damage and thus can affect oocyte quality. Gonadotropin stimulation may have a direct impact on oxidation stress markers. This study presents preliminary findings of redox homeostasis in FF with idiopathic infertility in the natural cycle and after ovarian stimulation. Oxidative stress is obtained through the measurement of F2-isoprostane, malondialdehyde, vitamin E and superoxide dismutase.

Materials and methods: The study will include sixty women under the age of 42 with unexplained infertility. So far, the surplus specimens of FF are collected during the oocyte retrieval from the 24 women undergoing ovarian stimulation and 10 women in the natural modified cycle. The majority of samples were analysed for F2-isoprostane (ELISA), total proteins and vitamin E. Malondyaldehyde (HPLC) and superoxide dismutase (ELISA) will be determined additionally after sample collection. Oocyte and embryo quality are also assessed during the sample collection. Results are expressed as the median and interquartile range (IQR).

Results: The median age of all subjects is 36 (min/max: 27-42) years. Total proteins were assessed in 28 samples with a median concentration of 45,5 (±9,5) g/L. After extraction in SPE columns, F2-isoprostanes were assessed only within-group undergoing stimulation (n=26) Median concentration was 35,48 (±48,46) pg/g of protein. Vitamin E was measured only in 7 samples from the stimulated cycle and 7 samples from natural modified cycles. Median values were 5,33 (±0,56) mol/L and 5,48 (IQR±0,4) mol/L, respectively. Within the stimulated group the median number of oocytes retrieved was 7 (±10). Seventeen oocytes were ranked with MII stage where 15 achieved normal fertilization (2PN). Thirteen embryo transfers were obtained in the group with ovarian stimulation.

Discussion: Significant variability is observed in concentrations of F2-isoprostanes within-group undergoing ovarian stimulation. Vitamin E presents a similar concentrations in both groups. Although, the study is in progress and the number of subjects is too small for comparison between groups. After sample collection, data results will provide better insight in alterations of redox homeostasis during ovarian stimulation.

MeSH/Keywords: Oxidative Stress, Follicular Fluid, Ovulation Induction, F2-Isoprostanes, Vitamin E

Poster code: R-01-02-157

Poster Title: SUPERIOR SAGITTAL SINUS OCCLUSION AND PENTADECAPEPTIDE BPC 157 THERAPY IN RAT

PhD candidate: Slaven Gojković

Part of the thesis: SUPERIOR SAGITTAL SINUS OCCLUSION AND PENTADECAPEPTIDE BPC 157 THERAPY IN RAT

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

Affiliation: University of Zagreb School of Medicine

Introduction: The aim of this study is to resolve the consequences of the permanently occluded superior sagittal sinus (SSS) and central venous occlusion in rats (i.e., multiorgan failure syndrome). The resolving focus was on the stable gastric pentadecapeptide BPC 157, its therapy effect shown to overwhelm peripheral venous occlusion syndromes. The activated bypassing key was the rapid vessels recruitment; collateral pathways (i.e., left ovarian vein, inferior mesenteric vein, azygos vein), reliant on the injurious occlusion, reestablished blood flow that compensated the vessel occlusion.

Materials and methods: This study was conducted with 12-week-old, 200 g body weight, male albino Wistar rats, randomly assigned at 6 rats/group/interval. We administered the stable gastric pentadecapeptide BPC 157, L-NAME, L-Arginine and combination of the latter two agents was used to investigate the stated effects of the sinus ligation. For the rats euthanized at 15 min, 24 h and 48 h ligation-time, medication was at 1 min ligation-time, 10 μg/kg BPC 157, 10 ng/kg BPC 157, or 5 mL/kg saline. Mean blood pressure recordings were made in anesthetized rats with a cannula connected to pressure transducer inserted into the SSS, portal vein, inferior vena cava and abdominal aorta at 15 min, 24 h or 48 h post-ligation. ECGs were recorded continuously in anesthetized rats at 15 min, 24 h or 48 h ligation time. Tissue specimens from the brain, liver, spleen, stomach, duodenum, lungs and heart were obtained from rats with superior sagittal sinus ligation at 15 min, 24 h and 48 h post-ligation. On being euthanized, the SSS, and peripherally, in major veins and abdominal aorta were removed from the rats, and clots were weighed. The presentation of the brain, and peripheral veins (azygos, superior mesenteric and inferior caval) was recorded in deeply anaesthetized rats, with a camera attached to a USB microscope, before procedure in normal, and then, in rats with ligated SSS 15 min after procedure, before and after therapy as well as at the 15 min, 24 h and 48 h ligation-time before sacrifice. The presentation of the gross lesions in gastrointestinal tract and vessels was recorded in deeply anaesthetized rats, with a camera attached to a USB microscope. At 15 min, 24 h and 48 h post-ligation, we assessed hemorrhagic congestive areas in the stomach and duodenum. To determine the serum levels of aspartate transaminase, alanine transaminase and total bilirubin, blood samples were collected immediately after euthanasia. Statistical analysis was performed by parametric one-way analysis of variance (ANOVA), with post-hoc NewmanKeuls test and non-parametric KruskalWallis test and subsequently the MannWhitney U test to compare groups. To compare the frequency difference between groups, the chi-square test or Fischers exact test was used. $p < 0.05$ was considered statistically significant.

Results: BPC 157 completely counteracts phenomena related to the sinus ligation.

Discussion: The occluded SSS rapidly develops brain swelling in rat and peripheral vessels failure. Addition of L-NAME, L-arginine and the combination of these agents provided further insight, with L-NAME reducing the effects, L-arginine aggravating the effects and the combination having a similar tied effect. Ligation of the SSS immediately overwhelms normal (negative) pressure, and induces the increased (positive) pressure, along with the severe portal and caval hypertension (portal hypertension exceeding caval hypertension), and aortal hypotension, persisting throughout the entire experimental period. Thrombosis rapidly appears (i.e., 15 min), progressing both centrally, in the SSS, and peripherally, in portal vein, inferior caval vein, superior mesenteric vein, lienal vein and abdominal aorta. BPC 157 fully counteracts these disturbances consistent with the evidenced counteraction of the increased pressure in the SSS, portal and caval hypertension and the counteraction of the additional venous hypertension.

MeSH/Keywords: BPC 157, superior sagittal sinus, occlusion, therapy, vascular recruitment, rat

Poster code: R-01-03-121

Poster Title: The Effect of Acute Oral Galactose Administration on the Redox System of the Rat Small Intestine

PhD candidate: Jan Homolak

Part of the thesis: Pathophysiological alterations of gastrointestinal system in animal models of Alzheimers and Parkinsons disease

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

Affiliation: University of Zagreb School of Medicine

Introduction: Galactose is a ubiquitous monosaccharide with important yet incompletely understood nutritive and physiological roles. Chronic parenteral d-galactose administration is used for modeling aging-related pathophysiological processes in rodents due to its ability to induce oxidative stress (OS). Conversely, chronic oral d-galactose administration prevents and alleviates cognitive decline in a rat model of sporadic Alzheimer's disease, indicating that galactose may exert beneficial health effects by acting in the gut. The present aim was to explore the acute time-response of intestinal redox homeostasis following oral administration of d-galactose.

Materials and methods: Male Wistar rats were euthanized at baseline (n = 6), 30 (n = 6), 60 (n = 6), and 120 (n = 6) minutes following orogastric administration of d-galactose (200 mg/kg). The overall reductive capacity, lipid peroxidation, the concentration of low-molecular-weight thiols (LMWT) and protein sulfhydryls (SH), the activity of Mn and Cu/Zn superoxide dismutases (SOD), reduced and oxidized fractions of nicotinamide adenine dinucleotide phosphates (NADPH/NADP), and the hydrogen peroxide dissociation rate were analyzed in duodenum and ileum.

Results: Acute oral administration of d-galactose increased the activity of SODs and decreased intestinal lipid peroxidation and nucleophilic substrates (LMWT, SH, NADPH), indicating activation of peroxidative damage defense pathways.

Discussion: The redox system of the small intestine can acutely tolerate even high luminal concentrations of galactose (0.55 M), and oral galactose treatment is associated with a reduction rather than the increment of the intestinal OS. The ability of oral d-galactose to modulate intestinal OS should be further explored in the context of intestinal barrier maintenance, and beneficial cognitive effects associated with long-term administration of low doses of d-galactose.

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MeSH/Keywords: oxidative stress; galactose; reduction-oxidation, gastrointestinal system

Poster code: R-01-03-168

Poster Title: Long-term Motor Central Effects of Botulinum Toxin Type A in Rats

PhD candidate: Petra Šoštarić

Part of the thesis: Peripheral and central effects of botulinum toxin in the 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 type A (BoNT-A) is a potent presynaptic neurotoxin and a standard therapy in hyperkinetic movement disorders, presumably due to its local muscular anticholinergic effect. However, recent experimental data point to possible central effects in the CNS. The present aim was to examine the contribution of the transcytosis-dependent central toxin action on the long-term muscular function recovery in rats, as well as TeNT-evoked spastic paralysis.

Materials and methods: Rats were bilaterally injected with BoNT-A into the gastrocnemius muscle (2 U/kg) or sciatic nerve (5 U/kg). The following day, the animals were injected intrathecally (i.t.) with BoNT-A-neutralizing antitoxin (5 I.U.). Different motor tests were assessed (gait ability score, digit abduction score (DAS), rota-rod, beam walking (BW) and swimming performance) for 77 days. On day 62, animals were injected i.m. with tetanus neurotoxin (TeNT). Electrophysiology was measured on day 62, 69 and 77 to record the M-wave (CMAP) and monosynaptic stretching reflex (H-reflex). At the end of the experiment, rats were sacrificed by anaesthetic overdose and intracardially perfused by tissue-fixation perfusion. Immunohistochemistry of cl-SNAP-25 was analysed by staining m. gastrocnemius and spinal cords of perfused animals.

Results: The recovery of rotarod performance and gait ability was accelerated in animals i.n. injected with BoNT-A and antitoxin when compared to other treatments as the DAS score assessment recovery of toe spreading reflex flaccid paralysis. Moreover, a trend of impaired swimming velocity was present with i.n. injected BoNT-A and HS animals which is also pointing to central effects of BoNT-A. Beamwalk performance was also faster recovered in antitoxin injected animals. Developed dorsiflexion resistance after evoked spastic paralysis with i.m. TeNT was less evoked in animals injected with horse serum. In comparison to HS animals, BoNT-A i.n. injected animals with administered antitoxin developed much more evident spastic paralysis, very similar to paralysis of control animals.

Discussion: In different motor tests (gait ability score, digit abduction score, rota-rod, beam walking and swimming performance), i.t. antitoxin significantly accelerated the flaccid paralysis and motor performance recovery. BoNT-A reduced the lower hind-limb diameter and muscle size without significant recovery during the entire experiment, which resulted in reduction of CMAP and H-reflex. The TeNT-evoked increase in muscle tone was reduced by BoNT-A dependently on its central effect. However, the H-reflex, when corrected for reduced muscle size or reduced CMAP, was not affected by the toxin treatment, suggestive of the lack of the toxin's direct effect on monosynaptic reflex. The enzymatic activity of the toxin, examined by cleaved synaptosomal-associated protein 25 (cSNAP-25) immunohistochemistry, was still present in neuromuscular junctions and spinal cord. The central occurrence of the cSNAP-25, present in second order spinal cord cholinergic neurons, depended on the toxin's central transcytosis. Long term motor effects of BoNT-A both on normal motor performance (day 1-62), as well as the spastic paralysis (days 62-78), are influenced by the toxin's ongoing central action mediated by retrograde transport and transcytosis. These data suggest that clinically relevant beneficial effect of BoNT-A result from toxin's combined peripheral and central effects.

Acknowledgments: Funding: Croatian Science Foundation (project ID: UIP-2019-04-8277)

MeSH/Keywords: Botulinum toxin type A, central motoric regions, cl-SNAP-25, electromyography

Poster code: R-01-03-171

Poster Title: The role of checkpoint kinase 1 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

Introduction: Cytarabine is known to induce differentiation of AML (acute myeloid leukemia) cells at low doses, but the mechanism is not completely elucidated. We have previously reported that pyrimidine synthesis inhibitors induced differentiation of AML cells by activating ataxia telangiectasia and RAD3 related (ATR) checkpoint kinase 1 (Chk1) mediated signaling pathway. The murine bone marrow stromal cell line MS5 is known to attenuate cytarabine mediated cytotoxicity, but the role of stromal cells on AML differentiation is unknown. The aim of this study is to test the role of Chk1 in cytarabine mediated differentiation and to determine the effects of stromal cells in a coculture system.

Materials and methods: Leukemia cell lines U937 (85011440, ECACC) and THP1 (ACC 16, DSMZ) and stromal cell line MS5 (ACC 441, DSMZ) were incubated in the presence of cytarabine (10, 100, 1000 nM) and pyrimidine synthesis inhibitors AICAr (0.2 mM) and brequinar (0.5 μM) for 72 hours. Viable cells were counted using hemocytometer and trypan blue exclusion. The expression of differentiation markers and cell cycle analysis were determined by flow cytometry. Chk1 activation was determined by western blot, and its role in differentiation by use of pharmacological inhibitors. Morphology of May Grunwald Giemsa stained cells was analyzed by AxioVert 200 microscope and Axiocam MRc 5 camera.

Results: Cytarabine reduced number of viable cells, stimulated expression of differentiation markers, induced cell cycle arrest and morphological changes in AML cell lines. The addition of nucleosides completely prevented cell cycle arrest and differentiation of AML cells induced by pyrimidine synthesis inhibitors, but had no effects on cytarabine induced changes. Western blot analysis revealed that cytarabine induced Chk1 activation. Torin2 and VE821, pharmacological inhibitors of ATR Chk1 signaling pathway reduced differentiation and cell cycle arrest in response to cytarabine, AICAr and brequinar. The presence of MS5 cells reduced cytarabine mediated toxicity and inhibited the expression of differentiation markers in U937 cells treated with low dose cytarabine.

Discussion: Our results suggest that low dose cytarabine induces differentiation of AML cells by activating Chk1 and thus shares mechanism with pyrimidine synthesis inhibitors. The presence of stromal cells decreased cytotoxic and differentiating effects on cytarabine on AML cell. Understanding the mechanism of differentiation effects of low dose cytarabine 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, cell differentiation, AICA ribonucleoside, cytarabine, pyrimidine synthesis, stromal cells

Poster code: R-01-04-129

Poster Title: DNA methylation status and expression of pluripotency related genes in testicular development of rat

PhD candidate: Dajana Krsnik

Part of the thesis: Epigenetic status and expression of SALL4 gene in normal and impaired testicular development

Mentor(s): Associate Professor Ana Katušić Bojanac, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Epigenetic changes, like DNA methylation are responsible for gene expression modifications and can affect testicular cell development. SALL4 and LIN28A/B are pluripotency related markers, being highly expressed in testis in undifferentiated spermatogonia respectively, where they play an essential role in maintaining their self-renewal properties. However, data about their DNA methylation dynamics during testicular development are limited. The aim of this study was to analyze and compare expression dynamics with DNA methylation status of SALL4 and LIN28A/B in different stages of early fetal and neonatal testicular development of rat. It is known that methylation status of SALL4 as well as LIN28A/B CpG islands located in Exon1 region affect their expression.

Materials and methods: DNA and RNA were isolated from the fresh fetal and neonatal samples of rat testis taken successively from GD20.5 to PND5.5. QPCR method was applied to determine expression status of SALL4 as well as LIN28A/B while the pyrosequencing method was used to determine the level of CpG methylation in promotor regions of mentioned genes

Results: The results had demonstrated that SALL4 and LIN28A/B were expressed in all examined testicular developmental stages with significantly higher mRNA expression in the fetal compared to the early neonatal stages of testicular development. Furthermore, promoter regions of both genes were highly hypomethylated, however, without a difference in the level of methylation between the individual developmental stages.

Discussion: Decreased mRNA expression of Sall4 and Lin28 in postnatal compared to fetal testis, but no differences in methylation level between the individual developmental stages, indicate that analyzed region of Sall4 and Lin28 is not involved in controlling the expression of these genes or perhaps DNA methylation is not mechanism for controlling expression of Sall4 and Lin28. We assume that the expression and related function of spermatogonial related genes could be controlled at the posttranscriptional level, which is a much quicker way to activate/deactivate genes than the DNA methylation mechanism. But further studies are needed to elucidate this claim. For example, it would be useful to analyze the methylation status of another region of these genes that could play a role in controlling the expression and/or analyze the expression of known transcriptional regulators of Lin28 and Sall4 mRNA.

Acknowledgments: This study was supported by the Scientific Center of Excellence for Reproductive and Regenerative Medicine, Croatia, and through the European Regional Development Fund, under grant agreement KK.01.1.1.01.0008.

MeSH/Keywords: Sall4, Lin28, DNA methylation, testis development

Poster code: R-01-05-001

Poster Title: Signaling through Notch 1 decreases osteoclast progenitor activity in the mouse model of rheumatoid arthritis

PhD candidate: Maša Filipović

Part of the thesis: Notch osteoclast progenitor signaling pathway in a mouse model of rheumatoid arthritis

Mentor(s): Professor Danka Grčević, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Periarticular and systemic bone loss in rheumatoid arthritis is mediated by increased osteoclast activity. Osteoclast progenitor cells (OCPs) derived from the myeloid lineage are susceptible to regulation through Notch signaling. Murine bone marrow and splenic OCPs, identified as CD45+Ly6G-CD3-B220-NK1.1-CD11blo/+CD115+ cells, are specifically increased in arthritis. We aimed to determine the effects of OCP Notch signaling inhibition and Notch 1 signal activation on OCP activity and arthritis-induced bone resorption in murine collagen-induced arthritis (CIA).

Materials and methods: Male C57Bl/6, CX3CR1CreERT2xRBP-J (Notch inhibition) and CX3CR1CreERT2xNICD1 (Notch 1 constitutive activity) mice were immunized with chicken type II collagen, treated with i.p. injections of tamoxifen (75 mg/kg) to induce Cre-mediated recombination and sacrificed at day 35 following immunization. Cre negative littermates were used as controls. Expression of Notch receptors 1 through 4 on OCPs was analyzed by flow cytometry in periarticular bone marrow (PBM) and spleen (SPL). FACS sorted OCPs were stimulated by osteoclastogenic factors (M-CSF and RANKL) and stained for TRAP expression. Murine hindpaws were scanned with Bruker SkyScan 1076 µCT and talar bones were analyzed. Research was approved by the Ethics Committee.

Results: We confirmed the expression of Notch receptors on OCPs by flow cytometry with Notch 1 and 2 being most abundantly expressed (around 45% and 60% positive OCPs in PBM and 35% and 20% in SPL respectively), with a significant increase of Notch 2 expression in arthritis. Notch 1 signal activation in OCPs differentiating in vitro leads to reduced numbers of TRAP+ osteoclasts while Notch deletion stimulated osteoclastogenesis. Arthritic CX3CR1CreERT2+xNICD1 mice had lower OCP numbers and an increase in expression of all four Notch receptors on OCPs, while arthritic CX3CR1CreERT2+xRBP-J mice had decreased OCP expression of Notch 1 through 3. Talar bone volume was reduced in arthritic CX3CR1CreERT2+xRBP-J mice.

Discussion: Our results confirm that Notch signaling may represent an important therapeutic target for the regulation of osteoclast activity in arthritis. Both in vitro and in vivo Notch 1 constitutive signal activation suppressed while Notch deletion enhanced osteoclastogenesis in CIA model. Taken together with our previous results of enhanced osteoclast formation by using neutralizing Notch 1 antibodies we confirmed an inhibitory role of Notch 1 signaling in osteoclast differentiation during arthrtis.

Acknowledgments: We thank prof. Kalajzić, prof. Wensveen and prof. Honjo for generously providing transgenic mouise strains. The work has been supported by Croatian Science Foundation projects IP-2018-01-2414, UIP-2017-05-1965 and DOK-2018-09-4276.

MeSH/Keywords: Notch; osteoclast progenitor; rheumatoid arthritis; collagen-induced arthritis; bone loss

Poster code: R-01-06-006

Poster Title: Regional differences of the primate-characteristic subplate formation period in human corticogenesis

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

Introduction: The human fetal neocortex is characterized by a transient fetal lamination as a result of diverse neurogenetic processes, i.e. neuronal proliferation, migration, cellular molecular specification, and synaptogenesis. The most prominent and voluminous transient zone of the human fetal cortex is the synapse-rich subplate (SP) compartment. SP is essential for the early fetal neuronal circuitry establishment, serving as a "waiting compartment" for the thalamocortical afferents invading the cortical plate (CP) at 24 postconceptional weeks (PCW). Between 13 and 15 PCW, the SP zone begins to form from the deep portions of the delaminating CP, where SP cells are secondarily dispersed downwards toward fetal brain ventricles (CP cell "spread down"). The loose part of the CP was initially described as the "second" CP by Poliakov (1949). Secondary dispersion of CP cells is mediated by the tangential and oblique penetration of thalamocortical and basal forebrain afferents into the CP.

Materials and methods: We utilized immunohistochemistry and immunofluorescence of diverse molecular markers (neuronal, glial, synaptic, fibrillar, extracellular matrix, neuronal laminar and projection neuron markers) on postmortem prenatal human brain tissue between 13 and 15 PCW to follow dynamic processes of the SP formation.

Results: Our results revealed expression pattern dynamics of diverse molecular markers during the process of SP formation, i.e. transcription factors were used as suitable neuronal laminar and projection neuron markers, showing the cell "spread down" from the deeper parts of the CP and consequently, the SP expansion period. In addition, our results suggest that the transcription factor Tbr1, one of the projection neuron markers, is a reliable marker of CP cells` "displacement" and final laminar destination during a human-specific key event of corticogenesis - SP formation period. Furthermore, we analyzed regional differences in the subplate formation period between the frontal and the occipital cortex. Previously known cytoarchitectonic differences were further correlated using projection neurons and additional molecular markers to reveal the dynamics of SP formation in different brain regions.

Discussion: The expanded subplate zone formation is a major event that distinguishes cortical development in humans and non-human primates compared to rodents, the most commonly used animal models in neuroscience. Importantly, analyzed prenatal period (around 13 PCW) is one of the vulnerable developmental windows for growing thalamo-SP circuitry. Given that the SP is an important component of early prenatal neuronal circuitry, abnormal subplate formation is associated with the potential pathogenesis of neurodevelopmental disorders where early neuronal circuitry may be disturbed.

Acknowledgments: This work was supported by Croatian Science Fundation project DOK-2020-01-5029 (AJ).

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

Poster code: R-01-08-013

Poster Title: Molecular diversity among adult hippocampal and entorhinal cells

PhD candidate: Daniel Franjić

Part of the thesis: Molecular diversity among adult hippocampal and entorhinal cells

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

Affiliation: University of Zagreb School of Medicine

Introduction: The hippocampal formation (HIP) and entorhinal cortex (EC) are critical components of a widespread neural network for memory and integration of space and time. Based on cytoarchitectonic, cellular, and circuit-related features, the hippocampal-entorhinal system can be divided into subregions that include the simpler three- layered allocortex of the dentate gyrus (DG), hippocampus proper (cornu ammonis [CA]), and subiculum (Sub) and the more complex laminated periallocortex (mesocortex) of the pre- and parasubicular areas and the EC. The molecular basis of cell type diversity in these subregions and their homology with bordering neocortical cell types remains poorly understood. Neurogenesis of granule cells in the adult DG has been documented across mammalian species and studied extensively in rodents, propelling a number of hypotheses about its functional role in cognitive processes and its potential for regenerative approaches. However, there is no consensus regarding the existence of significant neurogenesis in the adult human DG. Within the human hippocampal-entorhinal system, some cell types and circuits are selectively vulnerable to certain pathological processes, including ischemia or AD pathology and age-related neuronal loss. A more detailed molecular profiling of this system will aid our understanding of human brain development and neuropsychiatric diseases.

Materials and methods: We performed high-coverage single-nucleus RNA sequencing (snRNA-seq) on five anatomically defined sub- regions of the hippocampal-entorhinal system from adult human donors (DG, CA2CA4, CA1, Sub, and EC). We also profiled DG cell populations from adult rhesus macaques (Macaca mulatta) and all hippocampal fields from young adult pigs (Sus scrofa). Additionally, we investigated underlying species-level distinctions within this region by cross-species integrative comparisons with parallel previously published sample dataset from young adult mice (Mus musculus). Single nucleus capture was performed on microfluidic system (10x Genomics), followed by cDNA synthesis, RNA sequencing on HiSeq 4000 platform (Illumina), and data analysis.

Results: We identified highly diverse cell populations with clear regional distinctions within the hippocampal-entorhinal system. Integrated cross-species analysis revealed robust transcriptomic and histologic signatures of neurogenesis in the adult mouse, pig, and macaque but not humans. Doublecortin (DCX), a widely accepted marker of newly generated granule cells, was detected in diverse human neurons, but it did not define immature neuron populations. We characterized subregion-specific, transcriptomically defined cell types and transitional changes from the three-layered archicortex to the six-layered neocortex. Notably, METTL7B defined subregion-specific excitatory neurons and astrocytes in primates, associated with endoplasmic reticulum and lipid droplet proteins, including Alzheimers disease-related proteins.

Discussion: We report an extensive single-cell transcriptomics analysis of several anatomically defined cell populations in the adult human, macaque, and pig hippocampal-entorhinal system. Our findings reveal fundamental species differences in adult hippocampal neurogenesis and delineate the molecular diversity of the cytoarchitectural transition from the allo- to the neocortex. These results also outline genes that are selectively enriched in certain species and cell types that may have a role in the specific biology and/or pathology of the hippocampal-entorhinal system.

MeSH/Keywords: Single-cell; RNA-seq; hippocampus; entorhinal cortex; evolution; adult neurogenesis; neocortex; aging

Poster code: R-01-08-016

Poster Title: Inhibition of Tlr2 reduces inflammatory response after ischemic lesion of adult mouse brain

PhD candidate: Sanja Srakočić

Part of the thesis: Features of Tlr2 receptor mediated inflammation after ischemic lesion of mouse brain

Mentor(s): Professor Srećko Gajović, MD PhD

Affiliation: Laboratory for Regenerative Neuroscience, Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia

Introduction: Toll like receptor 2 (Tlr2) is surface receptor of microglia and astrocytes. After ischemic lesion of the mouse brain Tlr2 activates microglia-mediated inflammatory response. Activation of Tlr2 causes production of inflammatory cytokines and apoptosis, which aggravates neuroinflammation after brain ischemia. The aim of the study was to determine features and dynamics of Tlr2-mediated inflammatory response after ischemic lesion of adult mouse brain.

Materials and methods: Wt and Tlr2-/- transgenic mice (3-month-old male, n=25 for each group) with bioluminescent marker expressed under Tlr2 gene promoter underwent 30 min transient Middle Cerebral Artery Occlusion (tMCAO) followed by reperfusion. Size of ischemic lesion and Tlr2 gene activity was longitudinally monitored by magnetic resonance imaging (MRI; Bruker 7T BioSpec) and bioluminescent imaging (BLI; Perkin Elmer IVIS Spectrum) respectively. Mice were sacrificed and their brains were isolated before the surgery and on days 4 and 8. Neuroinflammation was assessed by flow cytometry and immunohistochemistry using inflammatory microglial markers (CD68, Tlr2, CD11b and CD45). Blood was drawn and concentration of inflammatory cytokines in blood plasma was determined using multiplex ELISA (LegendPlex).

Results: Tlr2 deficient mice had larger ischemic lesion and brain edema, yet there were no differences in neurological deficit score and survival. BLI showed higher Tlr2 promoter activity after brain ischemia in Wt mice, and Tlr2-mediated inflammation reduced more quickly in Tlr2-/- mice. Majority of BLI signal was located on ipsilateral brain hemisphere, however activation on contralateral hemisphere was also observed. Correlation between size of ischemic lesion and Tlr2 promoter activity was observed on days 2 and 4 in both mouse strains. When BLI signal was corrected according to lesion size Wt mice had higher Tlr2 promoter activity per size of ischemic lesion. Flow cytometry showed accumulation of macrophage and lymphocytes was more persistent in Wt mice compared to Tlr2-/- animals. Moreover, percentage of resting microglia after ischemic lesion was higher in Tlr2-/- animals. Presence of inflammatory markers CD68 and Tlr2 on contralateral and ipsilateral brain hemisphere after ischemia was confirmed by flow cytometry and immunohistochemistry. CD68 had higher expression in Wt mice compared to Tlr2-/- animals. ELISA showed higher concentration of cytokines in blood plasma of Tlr2 deficient animals.

Discussion: Although Tlr2 deficient mice had larger ischemic lesion functional outcome was not affected and Tlr2-/- mice recovered more quickly compared to Wt animals. Tlr2 deficiency caused reduced inflammatory response after brain ischemia as shown by BLI, flow cytometry and immunohistochemistry. In addition, inflammatory response reduced more quickly in Tlr2 deficient animals. Inflammation was also observed in peripheral blood due to blood-brain barrier destruction after ischemia.

Acknowledgments: The study was supported by the Croatian Science Foundation project RepairStroke (IP 06 2016 1892). The work of doctoral student Sanja Srakočić has been fully supported by the ˝Young researchers career development project training of doctoral students˝.

MeSH/Keywords: ischemic stroke, mouse, inflammation, microglia, Toll like receptor 2

Poster code: R-01-08-017

Poster Title: The effect of ganglioside composition on enzyme activity, protein expression and submembrane localization of Na+ /K+ -ATPase in mouse brain.

PhD candidate: Borna Puljko

Part of the thesis: Influence of ganglioside composition on expression, submembrane localization and activity of Na +, K+ -ATPase and Ca2 + -ATPase cell membrane in mouse brain

Mentor(s): Assistant Professor Kristina Mlinac Jerković, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: The Na+/K+-ATPase (NKA) is an enzyme that asymmetrically distributes Na+ and K+ ions across the plasma membrane to generate and maintain the membrane potential, also having a role in signaling processes. NKA dysfunction has been implicated in several neurodegenerative disorders. Its positioning as well as different functions of active and inactive pools depend on interactions with neighboring membrane lipids. Ganglioside enriched lipid rafts (LR) are housing active NKA pool, while the bulk membrane contains the inactive pool. Gangliosides are known to modulate the structure, function and localization of membrane proteins thus having an impact on ion homeostasis. The aim of this study was to investigate the effect of altered ganglioside composition on activity, protein expression and submembrane localization of NKA, using St8sia1 null mice with impaired synthesis of gangliosides.

Materials and methods: Adult wild type (WT) and null mice littermates were sacrificed, brains neuroanatomically dissected and cortical and cerebellar homogenates prepared. NKA activity was measured spectrophotometrically. Protein expression of NKA in homogenates was analyzed by Western blotting. LR and non-raft (nLR) fractions from cortices and cerebella were isolated by ultracentrifugation in discontinuous sucrose gradients, and submembrane localization of NKA analyzed by Western blotting.

Results: Data revealed statistically lower NKA activity in cortices of null mice compared to the WT mice, whilst there was no disparity in the cerebella. Total protein amount of NKA was statistically lower in null mice cortices compared to their WTs, whilst it was unvaried in the cerebella. Analysis of submembrane localization has shown higher amount of NKA to be positioned within LR of cortices than those derived from the cerebella.

Discussion: These results demonstrate that altered ganglioside composition may contribute to lower NKA expression influencing NKA activity and cellular ion homeostasis.

MeSH/Keywords: gangliosides, Na+/K+-ATPase, lipid rafts, neurodegeneration

Poster code: R-01-08-032

Poster Title: Left Hemisphere vs. Right Hemisphere and Language

PhD candidate: Lara Pilepić

Part of the thesis: Correlation between Language Skills and Ishemic Lesion Localisations of Both Hemispheres in Acute Phase of Stroke

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

Affiliation: University of Zagreb School of Medicine

Introduction: For years it has been known that left hemisphere is dominant for language. Newer neurolinguistic research point out the importance of both hemispheres working together to bring out undisturbed language and latest research even emphasise a more dominant role of right hemisphere in some language activities.

Materials and methods: In this research 120 patients with ishemic stroke in the anterior brain circulation will be enrolled. Patients will be divided in two groups: with stroke in the right branch of anterion brain circulation and with stroke in left branch of anterior brain circulation. An MRI scan and a CAT-HR examination will be done within a standard neurological and speech-language scope. Statistical analysis for the preliminary results was done on 80 patients.

Results: We found no significant difference in different language tasks in patients with stroke in right and left branch of anterior brain circulation except in naming. Patients with stroke in right branch of anterior brain circulation showed more disturbances in complicated language tasks.

Discussion: These preliminary results agree with latest neurolinguistic research. Future analysis will be focused on finding out specific brain localisations responsible for language tasks.

MeSH/Keywords: language functioning, stroke, lesion localisation

Poster code: R-01-08-034

Poster Title: Re-initiation of antithrombotic treatment following chronic subdural hematoma evacuation - a systematic review and network meta-analysis of controlled studies

PhD candidate: Andrija Bitunjac

Part of the thesis: Re-initiation of antithrombotic treatment following chronic subdural hematoma evacuation - a systematic review and network meta-analysis of controlled studies

Mentor(s): Professor Vladimir Trkulja, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Chronic subdural hematoma (cSDH) is predominantly a disease of the elderly, a population burdened with comorbidities and consequent antithrombotic therapy. Commonly, cSDH requires surgical treatment and the procedure carries a risk of acute bleeding. It is unclear whether antithrombotic treatment should be re-installed in these patients and under which circumstances. We aim to evaluate outcomes in patients who underwent surgical treatment for cSDH in relation to re-installment of their antithrombotic treatment.

Materials and methods: This systematic review and network meta-analysis will be made according to the PRISMA guidelines (PRISMA-NMA extension). Repeated literature searches (in 6-month intervals) will be conducted over a period of 18 months by two independent researchers using an agreed search strategy. Electronic databases ((PubMed Medline, Ovid Medline (including Cochrane Database), Scopus, Elsevier Science Direct, Web of Science)) and reference lists (manual search) will be included. Inclusion criteria : all randomized control trials, non-randomized controlled trials, stratified cohort studies and case-control studies: PICO MODEL: - P (population) cSDH patients treated surgically treated with antithrombotic drugs - I (intervention) in whom, due to indication, antithrombotic treatment is re-installed after cSDH surgery - C (comparison) in comparison to patients with an indication for antithrombotic treatment which, whoever, is not re-installed, or is re-installed under a different protocol (e.g., early vs. late) - O (outcome) reporting on post-surgical bleeding events and/or occlusive incidents Primary outcomes are (i) incidence of post-surgical occlusive events; (ii) incidence of post-surgical intracranial bleeding events (acute or recurrence of cSDH) and (iii) death. Secondary outcomes are extracranial major or clinical relevant bleedings. Risk of bias will be evaluated by two independent researchers using the Cochrane risk-of-bias tool for RCTs (RoB2) an for non-randomized studies of interventions (ROBINS-1) and the Newcastle-Ottawa scale (NOS). Frequentist and Bayesian generalized hierarchical (mixed) models will be fitted to event probabilities that allow for direct, indirect and mixed (combined direct and indirect) treatment comparisons with covariate adjustments (meta-regression). Treatment effects will be summarized as odds ratios (ORs) with respective confidence/credible intervals.

Results: The PubMed Medline, Ovid Medline (including Cochrane Database), Scopus, Elsevier Science Direct, Web of Science databases were searched using the following terms: ("chronic subdural hematoma" OR "chronic subdural haemorrhage" OR "chronic subdural haematoma") AND ("antithrombotics" OR "anticoagulants" OR "anticoagulation" OR "antiplatelet" OR "vitamin K antagonist" OR "aspirin" OR "dabigatran" OR "apixaban" OR "edoxaban" OR "rivaroxaban" OR "fondaparinux" OR "heparin" OR "LMWH" OR "low molecular weight heparin" OR "low-molecular-weight-heparin" OR "P2Y12 antagonist" OR "clopidogrel" OR "prasugrel" OR "ticagrelor" OR "dipyridamole"). The initial search date was 17.11.2021 ((PubMed Medline 259, Ovid Medline (including Cochrane Database) 639, Scopus 394, Elsevier Science Direct 93, Web of Science 198; Overall 1583)). Deduplication was performed using Mendeley Reference Manager, after deduplication 757 studies were identified. The titles and abstracts were screened for relevant papers and 34 studies were selected and read. The bibliographies of these papers are also being scrutinized for other relevant articles following which inclusion and exclusion criteria will be applied and data synthesis and a network meta-analysis continued.

Discussion: The results highlight the paucity of evidence for restarting anticoagulation in patients following CSDH evacuation.

MeSH/Keywords: chronic subdural hematoma, chronic subdural haemorrhage, antithrombotic treatment

Poster code: R-01-08-037

Poster Title: Laminar dynamics of CELF1 expression in early and mid fetal human cerebral wall

PhD candidate: Janja Kopić

Part of the thesis: Laminar expression pattern and regional distribution of CELF1 RNA-binding protein in human fetal cerebral cortex

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

Affiliation: University of Zagreb School of Medicine

Introduction: Precise regulation of gene expression is a prerequisite for normal brain development. Unlike regulation of transcription, which has been studied for decades, the importance of regulating mRNA translation during prenatal brain development has been studied only recently. It was previously shown that Celf1 is required for the development of early neuronal progenitors and glutamatergic neurons during mouse corticogenesis. However, the dynamics of CELF1 expression in the human brain is less known. RNA binding proteins (RBP) are involved in the regulation of neurogenetic processes, such as proliferation, cell specification, migration, and neuronal maturation. Therefore, we analyzed CELF1 expression pattern utilizing immunofluorescence throughout prenatal postmortem human brain tissue in order to reveal laminar shifts and molecular specification of major classes of neurons. In addition, we performed CELF1 double labeling with the upper cortical layer-enriched markers such as SATB2, CUX1, CUX2, and the lower cortical layer enriched markers, such as TLE4, CTIP2, SOX5, TBR1.

Materials and methods: FFPE sections of postmortem human fetal brain tissue, that are part of Zagreb Neuroembryological Collection were stained using immunofluorescence (IF) protocol from our lab. Immunofluorescence staining was used to show the distribution of CELF1 in transient fetal zones of neocortical dorsolateral portions in the developing human frontal lobe during the early fetal (9 to 13 PCW) and mid fetal (15 to 20 PCW) period. Double immunofluorescence labeling was used to determine whether CELF1 colocalizes with cortical layer-enriched markers. All images were acquired using inverted confocal microscope Olympus FV3000. Here, we used secondary antibodies suitable for AF488 and AF555 filters. Utilizing FIJI (ImageJ) software, images were processed and then analyzed.

Results: Our results showed the laminar expression dynamics of RNA binding protein CELF1 during the early and mid fetal period. Moreover, the distribution of cortical layer enriched markers slightly differs in human brain than in mouse brain where they are more layer specific. Double immunofluorescence showed that CELF1 colocalizes with various cortical layers-enriched markers. Accordingly, our results suggest CELF1 involvement in various neurogenetic processes during human corticogenesis such as neuronal identity, their molecular specification, and laminar destination.

Discussion: RNA-binding proteins (RBPs) play a key role in mRNA translation. One of these proteins, CELF1 is required for the development of early progenitors and glutamatergic neurons during the development of the mouse cortex. The dynamics of brain development, time course, and spatial organization are controlled by the regulation of gene expression at several levels. A number of factors are involved in posttranscriptional and translational regulation, including RNA-binding proteins (RBPs). The role of RNA-binding proteins is to control gene expression by creating ribonucleoprotein complexes. RNA-binding proteins have modulatory mechanisms by which may affect the outcome of alternative mRNA transcription, which contributes to the formation of protein isoforms during translation or protein synthesis. The human cerebral cortex is a complex structure of a high degree of the organization whose prenatal development is accompanied by numerous and strictly regulated processes. Despite recent advances in knowledge about the role of mRNA translation in the regulation of cortical development, a number of questions in human corticogenesis remain unanswered.

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MeSH/Keywords: human fetal brain, corticogenesis, cortical layers, projection neurons

Poster code: R-01-08-053

Poster Title: NLRP1 and ASC expression in Alzheimer's disease: correlation with neuropathological changes in the hippocampal formation

PhD candidate: Ena Španić

Part of the thesis: Association of biomarkers of Alzheimer's disease with inflammatory mediators and activation of microglia and inflammasome

Mentor(s): Professor Goran Šimić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Aberrant immune response has been implicated in the pathogenesis of Alzheimer's disease (AD), but it is not clear how it leads to neuronal dysfunction and cognitive decline. One of the possible mechanisms could be the overactivation of the inflammasomes. The main goal of this study was to compare the expression of NLRP1 and ASC molecules in the different areas of the hippocampal formation.

Materials and methods: NLRP1 and ASC were visualized with the immunohistochemistry method in 12 AD and 12 control samples, and the quantification was performed blind to the experimental group and the identity of the cases. Tissue section analysis and images of the slides were obtained with an Olympus BX53 microscope (Olympus, Tokyo, Japan). The total number of all positive cells was divided by the area (mm2) of the hippocampal field analyzed determined using Image J software (U. S. National Institutes of Health, Bethesda, Maryland, USA) and the results were presented as the density of the positive cells per surface of the hippocampal tissue. NLRP1 inflammasome activation was compared between postmortem brain tissue from subjects with AD and control samples. Immunopositivity was correlated with the duration of AD, age of the subjects, and numbers of amyloid plaques and neurofibrillary tangles (NFT) in the same hippocampal regions.

Results: Immunoreactivity (-ir) of both NLRP1 and ASC protein was significantly higher in the hippocampal formation of the AD samples. The number of NFTs significantly correlated with the NLRP1-ir but the correlation with the ASC-ir wasnt significant. The expression of both ASC and NLRP1 did not correlate with the number of the amyloid plaques, age of the subjects, and duration of the disease.

Discussion: Our results show that the expression of NLRP1 and ASC is higher in AD brains compared to controls, suggesting that NLRP1 inflammasome is more active in the AD brains. The significant statistically positive correlation between the number of NFTs and NLRP1 expression suggests that increased NLRP1 expression might be associated with tau protein pathology. The changes in NLRP1 expression are irrespective of age and disease duration. In conclusion, our preliminary findings confirm the previous reports of increased NLRP1 expression in AD (Kaushal et al., Cell Death Differ., 2015, Saresella et. al., Mol. Neurodegener., 2016) and additionally reveal that NLRP1 expression patterns in the hippocampal formation of the AD subjects positively correlate with the extent of neurofibrillary degeneration, but not the number of amyloid plaques. These findings warrant further investigations on the role of the inflammasome in the development of AD.

MeSH/Keywords: Alzheimer's disease, inflammation, NLRP1 inflammasome, hippocampal formation, neurofibrillary tangles

Poster code: R-01-08-056

Poster Title: MRI perfusion quantification of mouse brain using dynamic susceptibility contrast imaging

PhD candidate: Rok Ister

Part of the thesis: Neuroradiological properties of penumbra relative to onset of reperfusion in the mouse ischemic stroke model

Mentor(s): Professor Srećko Gajović, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Localized perfusion measurement of brain tissue plays a crucial role in research and diagnosis of various brain pathologies. Important of note, blood perfusion is a property of a living organ or tissue in situ, and as such, its measurement can only be obtained in vivo. Certain MRI scanning techniques offer minimally invasive methods from which perfusion can be calculated on pixel-by-pixel basis. One such approach is dynamic susceptibility contrast echoplanar imaging (DSC-EPI) which, by utilizing an IV contrast bolus, established itself as a golden standard for perfusion-weighted imaging due to its superior signal-to-noise ratio. In this work we present a newly established workflow and perfusion maps calculated from pilot DSC-EPI scans.

Materials and methods: A C57BL/6 mouse was anesthetized using isoflurane in O2/N2 mixture (1:2 relative ratio). The animal was positioned in a heated animal bed and IV access was established through tail vein. As MRI contrast agent we used Gadovist diluted to 0.1mmol/mL. T2 weighted images for anatomical reference and DSC-EPI images were acquired on Bruker Biospin 70/20 MR scanner for small animals. For T2 weighted image acquisition we used TurboRARE with following parameters: TE 33ms, TR 3000ms, NA 8, matrix size [160x100], FOV [16x10] mm, BW 217Hz/px. For DSC-EPI image acquisition we used single shot spin-echo EPI with following parameters: TE 20ms, TR 1500ms, NA 0, NR 200, matrix size [133x107], FOV [20x16] mm, BW 3524Hz/px, partial FFT [1x1.5], resulting in temporal resolution of 1.5s. During DSC-EPI image acquisition, the contrast bolus was manually administered through improvised IV system. For post-processing, DSC-EPI images were converted to Nifti format and imported into MATLAB. Samples for AIF extraction were automatically extracted and time-series data was deconvoluted using oSVD method. Lastly, CBV, CBF, MTT and TTP perfusion maps were calculated and enhanced for publishing-ready presentation.

Results: Calculated perfusion values acquired from brain tissue are well within physiological parameters according to available literature. CBF maps proved to be particularly useful in visually highlighting cortical and subcortical grey matter structures. In this work, we established a robust workflow capable of producing valuable perfusion data from a mouse brain, ready for future correlation or intervention studies.

Discussion: In recent years, MRI perfusion weighted imaging (PWI) gained significant traction in brain research. Increasing number of brain pathologies are starting to be described in terms of blood perfusion irregularities. That brings a valuable diagnostic tool to clinicians as well as an indispensable outcome measure for researchers. Our future goals are to describe brain perfusion deficits in animal stroke models which could be used in combination with MRI diffusion weighted imaging to measure PWI-DWI mismatch, commonly referred to as penumbra.

Acknowledgments: The study was supported by the Croatian Science Foundation projects RepairStroke (IP-06-2016-1892) and BRADISCHEMIA (UIP-2017-05-8082).

MeSH/Keywords: Magnetic Resonance Imaging, Cerebrovascular Circulation, Mice

Poster code: R-01-08-079

Poster Title: Developmental changes of the vascular network of human fetal brain - a rapid Golgi study

PhD candidate: Goran Ivkić

Part of the thesis: Developmental changes of the vascular network of human fetal brain - a rapid Golgi study

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

Affiliation: University of Zagreb School of Medicine

Introduction: Previous studies have shown a clear correlation between development of vascular network and neurogenesis. They also have displayed stages, which roughly correspond to embryonic, foetal and perinatal period. However, the developmental changes of the brain angioarchitecture during the prenatal period are still poorly investigated. Mature vascular trees typically develop from immature, capillary-like vasculogenic networks. Within a mature organ or tissue, capillary networks are necessarily continuous with their arterial and venous trees. First stage of this doctoral thesis was the descriptive analysis the blood vessels on serial rapid Golgi-impregnated sections of major zonal compartments and layers of the developing telencephalic wall. For each developmental stage, characteristic patterns of vascularisation were extensively documented by Camera lucida drawings. The second part of this work, which is still in progress, is a quantitative analysis with free available program for image analysis Image J, with 'Vessel Analysis' sub-programme for vascular system, with a main goal to define: a. Density of blood vessels (surface area that encompasses the blood vessels / surface area of developing zone x100) and b. Density of length of blood vessels Analysis need to encompass the whole space from pia do fetal ventricle, to show all the developing zones of telencephalic wall and vascular network inside.

Materials and methods: For this purpose, we decide to use a more complex computer programme, with more quantitative options; The computer software VESsel GENeration Analysis (VESGEN 2D), which maps and quanties major parameters of angiogenesis in vascular trees and networks. This software (version 1.0) analyzes a number of two-dimensional (2D) research and clinical applications. VESGEN 2D is released as an easily installed software plug-in for the widely utilized image processing program ImageJ. Currently, we are waiting for permission of the manufacturer for use of this quantitative software

Results: In early fetal period, represented by brains from 10th and 13th postovulatory weeks, there are two vascular patterns within the telencephalic wall. The deeper half consists of intermediate, ventricular and subventricular zone, and this part is characterised with irregular and profusely branching vascular network, while the upper half of telencephalic wall is composed of the wide Subplate zone and the cortical plate, and consists of a number of radially oriented vessels. Starting with 18 week of gestation and throughout the whole preterm period, radial orientation of vessels dominates within the whole telencephalic wall and one can clearly delineate three vascular compartments: outer and inner dense vascular network, separated by the intermediate zone of significantly lower vascular density, and displaying radial alignment and orientation of blood vessels. In the period from 20 to 25 weeks of gestation, the radial orientation of vessels dominates within the entire telencephalic wall; however, the upper third appears denser and more profusely branched. The same pattern of the vascularization remains up to 30 weeks of gestation.

Discussion: The vascular networks in the telencephalic wall are specific transient structures which undergo developmental reorganization in late fetal and perinatal period. Development and reorganization of the transient Subplate vascular network, was ignored by most of authors until recently. It is our hypothesis that the Subplate-zone and its vascular network play a key role in the differential plasticity and recovery after hypoxic-haemorrhagic lesions in premature and newborn infants. The quantification of density and extent of ramification of vascular network will provide a more precise insight of developmental changes and dynamics of all these processes.

MeSH/Keywords: development, vascularization, telencephalon, Subplate zone

Poster code: R-01-08-081

Poster Title: Specifically designed lentiviral vectors for targeted expression of neurons and astrocytes in the mouse brain

PhD candidate: Laura Skukan

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

Mentor(s): Professor Srećko Gajović, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: The introduction of gene modifications mediated by a viral vector is a method which allows the transfer of genetic material or molecular system to change the activity of genes into cells of interest. Lentiviral vectors (LVs) are useful for in vivo applications, because of their efficiency in gene delivery. They have a large cloning capacity, sustained transgene expression and can be engineered to restrict gene expression to particular cellular subtypes through pseudotyping and specific promoters. Therefore, we generated LV encoding Spry2 specific shRNA with specific tropism restricted to transduce astrocytes and neurons. 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 main aim of this study is to confirm the transduction of neurons or astrocytes by specifically designed LVs.

Materials and methods: A four-plasmid system is used to generate LVs in transiently transfected HEK293T cells. Titration of lentiviral particles was performed by measurement of viral p24 antigen using ELISA method. LV encoding shSpry2 was implemented by intracranial injection into striatum and cortex in a 1µL volume into the each brain region. The animals were sacrificed 1 week after injection. To identify the LV infected cell types and colocalization with GFP signal, the imunohistochemical staining of mouse brain sections with antibodies against cellular markers of neurons (NeuN) and astrocytes (GFAP) were performed.

Results: The images of mouse brain sections obtained by confocal microscope 7 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.

Discussion: According to our results, successful transduction of targeted cells in the mouse brain with a specifically designed LVs has been confirmed. Also, Mokola pseudotyped LVs with GS promoter mostly transduce astrocytes while VSV-G pseudotyped LVs with CMV promoter mostly transduce neurons which confirmed our expectation.

Acknowledgments: This study was supported by the European Union through the European Regional Development Fund, under grant agreement No. KK.01.1.1.04.0085, project "CasMouse"

MeSH/Keywords: Lentiviral vector, Sprouty2, shRNA

Poster code: R-01-08-095

Poster Title: Rostro-caudal differences of GABAergic interneurons in the rat cerebral cortex

PhD candidate: Andrea Blažević

Part of the thesis: Obiljeja GABA-ergikih neurona u kori velikog mozga takora

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

Affiliation: University of Zagreb School of Medicine

Introduction: GABAergic interneurons are a highly diverse group of cortical neurons that are more numerous in parietal and occipital regions than in frontal regions of the rat cerebral cortex. Although numerous studies on rodent interneurons were performed, a systematic assessment of their number and laminar position in different cortical regions is still lacking. In rodents parvalbumin neurons are regarded as the most numerous interneuron population, followed by somatostatin and calretinin neurons, while calbindin neurons are regarded as a subpopulation of somatostatin neurons. The level of overlap between the aforementioned interneuron populations is still controversial since some studies have shown major overlap between certain groups.

Materials and methods: In this study, a comprehensive qualitative analysis of double labeled immunofluorescent histological sections of the rat brain was performed. We analyzed four GABAergic interneuron populations (calretinin, calbindin, parvalbumin and somatostatin) in frontal, fronto-parietal and occipital cortical region and assessed the level of overlap between calretinin neurons and the remaining three interneuron subpopulations.

Results: There were no major rostro-caudal differences in the number and laminar distribution of calbindin, parvalbumin and somatostatin neurons, while calretinin neurons were more abundant in occipital region. No substantial overlap between calretinin neurons and other major interneuron populations was observed. Parvalbumin, somatostatin and calretinin neurons were evenly distributed within a cortical column, while calbindin neurons were more numerous in upper cortical layers.

Discussion: The finding that calretinin neurons are generally more abundant in caudal than in rostral regions of the rat cerebral cortex contradicts findings in primates. Possible explanation lies in the fact that increase in calretinin neurons number will lead to increase in parallel processing between cortical fields which is especially important and pronounced in associative areas. Associative areas in humans and primates are in frontal regions, while in rodents are in occipital region. Even though there are a lot of studies focused on similar topics, each of them was performed using different methods so unambigous conclusions about regional and laminar differences can not be determined using existing literature. Well understanding of those differences, number and distribution is important because knowing well the structure of rodents neural circuits will enable better understanding of human neural circuits and, therefore, better understanding of the biological basis of the highest cognitive functions and etiopathogenesis of some neural diseases associated with GABA interneurons.

Acknowledgments: This research is supported 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 KK 01 1 1 01 0007 PI Miloš Judaš)

MeSH/Keywords: rodents, GABA neurons, interneurons, calretinin

Poster code: R-01-08-100

Poster Title: Neural stem cells-derived exosomes decrease damage in a chemical model of hypoxia

PhD candidate: Denis Jagečić

Part of the thesis: Role of neural stem cells on mitophagy regulation after ishemic damage of cells of the neural tissue

Mentor(s): Associate Professor Dinko Mitrečić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Stroke is the third highest cause of death worldwide which affects millions of people and present not only devastating emotional challenge for individuals and their families but also huge financial burden for the health system. In the recent years a lot of effort was made on developing new approaches, therapies and treatments to reduce detrimental effects of stroke. One of the most promising are based on Neural stem cells (NSC) with proven beneficial effect on many different pathological conditions. One of the interesting features of NSC is that they secrete small extracellular vesicle called exosomes, which can influence target cells.

Materials and methods: Neural stem cells obtained from the telenchephalic wall of 14.5 days old mouse embryos were used to obtain exosomes, which were isolated from proliferation medium in which cells were growing by using Norgen Plasma/Serum exosome purification kit. Exosomes were quantified and analyzed using qNano device and Izon software. In addition, they were labeled with lipophilic membrane PKH26 dye. Chemical model of hypoxia was induced by 50µM deferoxamine in 3 specific time-points of NSCs differentiation; day 1, day 5 and day 7. Mitochondrial function and oxidative stress levels were assessed by using the voltage- sensitive dye TMRM and the mitochondrial superoxide indicator MitoSox, respectively.

Results: After presence of NSC-derived exosomes within treated cells was confirmed, we detected a high decrease in a level of mitochondrial superoxide anions and increase in mitochondrial membrane potential. This suggests that developing neural cells affected by hypoxic damage can be successfully treated by exosomes, which improve metabolic parameters of mitochondria.

Discussion: The main objective of this work was to analyze effects of NSC - derived exosomes on mitochondrial parameters in differentiating neural stem cells affected by a chemical model of hypoxia. Exosomes carry many different molecules including genetic materials, proteins and lipids which can enter surrounding cells and affect its metabolism. Indeed, in our work we clearly visualize exosomal accumulation in the perinuclear region of the cells. Healthy mitochondrial network and activity are absolutely crucial for normal cellular function. Hypoxia induce metabolic stress and reduce bioenergetic activity of the cells mostly by influencing mitochondrial population where there is increase of superoxide anion and decrease of membrane potential that results with releasing of cytochrome c and other proapoptotic molecules and subsequently cell death. Mitochondrial manipulation that can reduce its damage and stress can present potential novel therapeutic approach.

Acknowledgments: This work is funded by Croatian Science Foundation (IP-2016-06-9451 and PhD grant for DJ) and co-financed by the Scientific Centre of Excellence for Basic, Clinical and Translational Neuroscience, European Union through the European Regional Development

MeSH/Keywords: neural stem cells, exosomes, mitochondria, hypoxia

Poster code: R-01-08-131

Poster Title: The role of Mbd1 and epigenetic changes in hypoxic and degenerative damage of cells of the nervous system

PhD candidate: Dražen Juraj Petrović

Part of the thesis: The role of Mbd1 and epigenetic changes in hypoxic and degenerative damage of cells of the nervous system

Mentor(s): Associate Professor Dinko Mitrečić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: The most common brain diseases are divided into those caused by hypoxia (lack of oxygen) and those in which neurodegeneration occurs. Nevertheless, there is growing evidence that these pathophysiological events share common mechanisms that lead to cell damage. Our goal is to investigate the hypothesized role of the Mbd1 protein in process of neuronal damage mediated by hypoxia and neurodegeneration. Mbd1 protein participates in the control of gene expression through binding to methylated DNA and is expressed during development of the nervous system. Its deficiency causes an autism like phenotype. In the model of nerve stem cells exposed to hypoxia and in the hyperhomocysteinemic model of neurodegeneration, we will investigate whether the expression of Mbd1 and epigenetic changes of the most important markers of nerve tissue cells (Gfap, Tubb3, Sox2) are common to damage caused by hypoxia and neurodegeneration.

Materials and methods: Neural stem cells (NSC) were isolated from telencephalon of 14 day old mouse embryo. NSC were differentiated in vitro using a protocol by which most of the cells will differentiate to neurons and astrocytes. For the neurodegeneration model cells were exposed to L-Homocystein during differentiation at three different concentrations: 20 M, 100 M and 300 M. We analysed cells on Day 1, Day 3 and Day 7. During differentiation we analysed cell number (DAPI), epigenetic marker (Mbd1) and a marker of DNA damage (γH2A.X) on protein level (immunocytochemistry and Western Blot). Fixed and stained cells were imaged by confocal microscopy, analyzed in Imaris software and statistics was calculated using R studio.

Results: Cell number determined by counting cell nuclei was used as a measure of L-Homocystein cytotoxicity. Cell number decreased by Day 3 of differentiation, however this was less pronounced with added L-Homocystein of any concentration, as the treatment itself caused a reduction in cell number from Day 1. Mbd1 expression increased with differentiation days peaking at day 7 of differentiation. L-Homocystein treatment generally reduced the cell number. γH2A.X expression was not significantly affected by L-homocystein treatment, however it had a small tendency to increase in 300 M treated cells on Day 1 and Day 3.

Discussion: L-homocystien might have cytotoxic effects on NSCs possibly by altering metabolic pathways, affecting cellular methylation processes or even chemically binding to proteins therefore changing their function. A small percentage of NSCs that have highest expression of Mbd1 in their nuclei tend to have smaller cytoplasm and express less differentiation markers such as Tubb3 and Gfap. This observation suggests increased Mbd1 expression is some NSCs puts breaks on their differentiation therefore preserving NSCs pool into adulthood. γH2A.X is a posttranslational phosohrylation of serine 132 of Histone 2A and it is associated with double stranded DNA break. There was only a small tendency for increased γH2A.X expression in in 300 M L-Homocystein treated cells and only on day 1, which could be explained that NSCs adapt to L-Homocystein over time.

Acknowledgments: The work has been supported by projects Orastem (IP-2016-06-9451) and GlycoDown (PZS-2019-02-4277) awarded by Croatian Science Foundation.

MeSH/Keywords: Hypoxia, neurodegeneration, nerve cells, DNA methylation, epigenetics

Poster code: R-01-08-133

Poster Title: The role of E3 ubiquitin ligases San1 and Ubr1 in the degradation of misfolded proteins in quiescent yeast cells

PhD candidate: Dina Franić

Part of the thesis: Selective degradation of misfolded proteins in quiescent yeast Saccharomyces cerevisiae

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

Affiliation: University of Zagreb School of Medicine

Introduction: Aggregation of misfolded proteins is associated with aging and diseases, such as Alzheimers and Parkinsons. To prevent accumulation of aberrant proteins, cells have developed protein quality control pathways, including selective protein degradation by the ubiquitin-proteasome system (UPS). Previous research on UPS has been predominantly done using proliferating cells, however, many cells, such as neurons, exist in a non-dividing, or a quiescent state, that may be characterized by distinct mechanisms of protein quality control. In my doctoral research I study the specificities of degradation-mediated protein quality control in quiescent cells, using yeast Saccharomyces cerevisiae.

Materials and methods: Yeast Saccharomyces cerevisiae deletion mutants were constructed by homologous recombination of PCR-generated cassettes. Integrative plasmids encoding HA-epitope tagged tGnd1-HA and stGnd1-HA under the control of TEF1- or PIR3-promoter were constructed by molecular cloning and introduced into yeast by lithium acetate-transformation. Genomic integration was confirmed by PCR. To obtain proliferating cells, cells were diluted in a complete liquid medium to optical density (OD600) of 0.2 and grown to OD600 of 0.8. To obtain quiescent yeast, cells were diluted to OD600 of 0.2 as described above and grown for 48 hours without medium change. Protein stability was examined by cycloheximide chase, in which cells are harvested at indicated time points after the addition of the protein synthesis inhibitor cycloheximide. Total protein lysates were prepared by alkaline lysis (Kushnirov, 2000) and analyzed by Western blot using anti-HA antibody and chemiluminescent detection. Anti-HA signal was normalized to the total protein (stain-free method, ImageLab). Results are presented as mean values ± standard deviation.

Results: In quiescent yeast a large pool of the proteasomes is sequestered within cytoplasmic granules in an inactive form (Laporte et al 2008), therefore it has been unclear how misfolded proteins, which are normally proteolytic substrates of the ubiquitin-proteasome system, are managed by the quiescent cells. To study the process of aberrant protein degradation, we expressed two model misfolded proteins, tGnd1 and stGnd1, and a respective wild-type protein Gnd1 in yeast and examined their protein stability. In contrast to Gnd1, which was stable, misfolded tGnd1 and stGnd1 were degraded fast in both proliferating and quiescent cells, demonstrating that quiescent cells are able to target misfolded proteins for selective degradation. Next, we set to determine which molecular pathway mediates selective degradation of tGnd1 and stGnd1. It has been previously shown that in proliferating cells (s)tGnd1 are targeted to proteasomal degradation via a poly-ubiquitin tag generated by E3 ubiquitin ligases San1 and Ubr1. To test whether these E3s are also required for (s)tGnd1 degradation in quiescent cells, we expressed tGnd1 and stGnd1 in deletion mutant strains lacking San1, Ubr1, or both, and examined (s)tGnd1 protein stability. Efficient degradation of stGnd1 in quiescent cells required both San1 and Ubr1, similarly as in proliferating cells. In contrast, degradation of tGnd1 in quiescent cells was almost solely dependent on Ubr1.

Discussion: Together our results indicate that quiescent yeast cells are able to recognize misfolded proteins and selectively target them for degradation. Our finding that selective degradation of misfolded tGnd1 depends on a different set of E3 ubiquitin ligases in proliferating and quiescent cells suggests that distinct pathways operate in protein quality control of certain types of misfolded proteins in proliferating and quiescent cells.

MeSH/Keywords: ubiquitin, proteasome, protein misfolding, quiescence, yeast

Poster code: R-01-08-145

Poster Title: A comparative analysis reveals inter-species phenotypic differences in the adult mammalian subthalamic nucleus

PhD candidate: Tila Medenica

Part of the thesis: A comparative analysis of adult mouse, rat, and human subthalamic nucleus phenotypes

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

Affiliation: University of Zagreb School of Medicine

Introduction: Subthalamic nucleus (STN) is a basal ganglia structure, clinically relevant as a target for deep brain stimulation treatments. Despite of that, little is known about its basic cytochemistry and phenotypical profile. When looking at the existing data, results from different species are often not overlapping and data from one species cannot be extrapolated to another. The aim of this research is to examine the species most commonly involved in STN research (human, mouse and rat) and compare the expression profiles of different transcription factors (TFs) between the species STN.

Materials and methods: To investigate the expression of TFs in the STN, coronal slices containing the STN, from adult male mouse (strain C57BL/6), rat (strain Wistar Han) and human formalin-fixed brains, were used. Double-labelling immunofluorescence was performed with HuC/HuD antibody labelling neuronal cytoplasm and various TFs (FOXA1, FOXP1, FOXP2, NKX2-1, PAX6, DBX1, ISLET1) labelling nuclei on paraffin-embedded tissue (rodent cut into 10 µm and human cut into 20 µm thick sections). All nuclei were coloured with DAPI. Due to higher post-mortem time and poorer quality of human tissue, DAB immunohistochemistry was performed on human fixed-frozen tissue cut into 50 µm thick sections for the TFs to confirm the results.

Results: Our results show differential expression of seven TFs across three species in the STN. TFs belonging to the same forkhead box family share similar expression patterns (FOXA1, FOXP1 and FOXP2) and are present in the mature STN of all three species. NKX2-1 is not expressed in mouse STN, but is expressed in rat and human. Human STN neurons expressed PAX6, DBX1 and ISLET1 transcription factors which are not present in rodent STN neurons.

Discussion: FOX family of TFs are important developmental factors for STN differentiation which explains their overall presence in the STN of all examined species. NKX2-1 is a developmental marker of medial ganglionic eminence and the basal plate. It is expressed in adult human and rat STN, but not in the mouse. According to literature, it is expressed in the mouse STN during development but is silenced at E12.5. This developmental silencing happens only in mouse STN but not in its close relative, the rat. PAX6 is an important regulator of diencephalic neuronal identity and is expressed only in human mature STN. DBX1 and ISLET1 share expression patterns with PAX6 and they seem to be regulated by PAX6 which may explain the similarity in their expression patterns. All examined TFs seem to be expressed in the adult human STN as opposed to rodent STN. This may show that human STN may be more complex in structure and have additional neuronal populations which may imply more complex functions of the human STN. Species differences in the TF expression profiles show that the STN is not strictly phenotypically conserved among mammals. TFs control the expression of many genes and there is likely a whole other spectrum of differences in the expression of their downstream genes. This raises a question of functional significance of STN phenotypic differences, and why has it diverged in mammals. Another concern is the fact that there are many experiments conducted with animal models of STN-related research and caution is needed in translating these results to humans since human and rodent STN are not entirely the same.

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MeSH/Keywords: Subthalamic nucleus, Transcription factors, Basal Ganglia, Immunohistochemistry

Poster code: R-01-08-170

Clinical medical sciences – preliminary research results

Poster Title: The cognitive outcome in carotid thrombendarterectomy with multimodal monitoring cerebral perfusion optimization

PhD candidate: Tina Tomić Mahečić

Part of the thesis: Perioperative complications and cognitive outcome in carotid thrombendarterectomy with multimodal monitoring cerebral perfusion optimization

Mentor(s): Associate Professor Branko Malojčić, MD PhD, Associate Professor Dinko Tonković, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Perioperative multimodal cerebral monitoring (TCD- transcranial Doppler, NIRS- near-infrared spectroscopy, and EEG- electroencephalogram) is discussed to improve outcomes in patients for carotid thrombendarterectomy. Carotid revascularization may improve cognitive dysfunctions resulting from chronic hypoperfusion, but a cognitive decline in time can occur from microembolisation, hyper-, or hypoperfusion during the surgical procedure. We investigated the effect of this monitoring on cognitive functions, and hypothesise that by using these methods we could avoid perioperative complications.

Materials and methods: In this prospective study, we planned to analyze 80 consecutive patients with asymptomatic and symptomatic (TIA-transitory ischemia in anamnesis) stenosis of the internal carotid artery in which carotid thrombendarterectomy is indicated, in general anesthesia. We randomized patients into two groups. The experimental group of patients had expanded intraoperative monitoring involving NIRS and EEG, and the control group had only standard monitoring. We analyzed cognitive tests for all patients preoperatively, first and eighth day postoperatively, and after 8 weeks. BHI (breath-holding index) with TCD was measured preoperatively and postoperatively and after 8 weeks also. Statistical analysis included descriptive statistics, Chi-Square statistics, and repeated measurement ANOVA.

Results: During the research period (starting from January 2019. until now) 224 carotid surgery procedures were performed in our institution. Due to very strict inclusion/exclusion criteria, we had to exclude 94 of the patients with CVI (cerebrovascular insult) in anamnesis, 41 patients had the preprocedural results of the cognitive test less than inclusion criteria (22 points), 10 patients were ASA 2 status, and 14 were excluded because of technical reasons (inoperable, without adequate bone window for measurements, or TCD was in time point not available) from research. The data set from 20 patients were not completed due to Covid 19 pandemic and they were also excluded from our research. Up to the present time we have included 45 patients in our study of whom 38 patients have a complete data set and thus could be analyzed. Data of the remaining 7 patients will be complete after the observation period in 8 weeks. 20 of these 38 patients were included in the experimental group and 18 in the control group. There were no significant differences in those two groups in any of the parameters obtained at the baseline. 30 of included patients are males and 8 are females. All patients measured and tested were right-handed. The patients were 68 +/- 7 years old. 18 of 38 patients were symptomatic (TIA) before surgery. 27 patients had a higher than 70 % stenosis by NASCET criteria on the right side and 15 patients on the left side. We did not find any significant statistical difference neither for any subtest of the MoCA (Montreal Cognitive Assessment) test nor for the sum of these results. Furthermore, there was no difference in the two entities of MBT (month backward test), namely time and mistakes for any time point. We had very similar results with Trail making tests A and B, showing no significant difference in-between experimental and control groups. We also obtained BHI, which also did not differ between time points and groups.

Discussion: The main result of the preanalysis of our data is that we are not able to detect any subtle differences in the cognitive capabilities of our patients. Since due to Covid 19 we were not able to reach our inclusion goal, it cannot be deduced from our results that there is no difference between the two groups. However, we have to mention that all of our patients are right-handed and had the relevant stenosis on the right side. Therefore, it is less likely that differences in perioperative management will result in detectable cognitive impairment. The inclusion of more patients and data analysis will strengthen the quality of our results.

MeSH/Keywords: TEA ACI, cognitive tests, BHI, NIRS,TCD, EEG

Poster code: R-02-01-140

Poster Title: Relationship between parental Ego strength, anxiety, depression, and quality of life of atopic dermatitis in children

PhD candidate: Ivana Martinac Ciglar

Part of the thesis: Relationship between parental Ego strength, anxiety, depression, and quality of life of atopic dermatitis in children

Mentor(s): Professor Mihael Skerlev, MD PhD, Assistant Professor Zrnka Kovačić Petrović, MD PhD

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

Introduction: Atopic dermatitis is a common, chronic, recurrent, inflammatory skin disease that most commonly occurs in childhood. Dermatological chronic diseases significantly affect the quality of life of patients and their family members. Previous research has shown various psychological, social and financial issues of caregivers of children with dermatological diseases, as well as psychological issues of patients. The research would evaluate the Ego strength, anxiety, depression and quality of life of parents of children with atopic dermatitis and correlate the results with the severity of the disease in children. With the results we would try to more precisely define the causes of psychological problems in order to provide parents with lower ego strength and poorer quality of life with adequate help and thus contribute to overall treatment for the child.

Materials and methods: The study will be conducted in the outpatient clinic and the Department of Pediatric Dermatology for 2 years on 90 subjects. The study will include 54 parents of children with atopic dermatitis aged 6 months to 7 years. The control group would be 36 parents of healthy children who do not suffer from chronic skin diseases or other chronic diseases. Here is to present preliminary results on 10 subjects, 5 in each analysed group. The sample excludes subjects whose test results were not valid, parents of children who have atopic dermatitis and other associated chronic disease which does not belong to the group of atopic diseases. The diagnosis of atopic dermatitis is made by a specialist dermatovenerologist based on the clinical picture according to the clinical diagnostic criteria of Hanifin and Rajka. The severity of the disease is assessed with the Scoring Atopic Dermatitis index (SCORAD index). After signing the informed consent, the participants fill in several questionnaires in the presence of the examiner: a structured clinical interview, conducted for the purposes of this research, The Family Dermatology Life Quality Index (FDLQI), Hospital Anxiety and Depression Scale (HADS) and the Ego Identity Scale (EIS), according to Erickson. Data were analyzed using descriptive and analytical methods (t-test).

Results: The pilot phase of the survey had shown that the surveys were well understood, and only minimal wording changes were made based on the discussion after the survey completion. The available data indicated that the survey did not overly encumber parents in terms of duration and that there were no questions that could cause a substantially biased response. The analysis of the pilot data had shown four significant differences between the analysed groups. The most strongly expressed one was the family dermatology quality of life index, which had shown much worse result in the cases group (FDLQI 7.4±4.0 for cases, 1.8±2.4 for controls, $P<0.001$). Additionally, cases had a higher depression scale (HADSD 5.4±2.5 vs 3.2±5.0; $P<0.001$), anxiety scale (HADSA 8.6±3.0 vs 7.2±3.7, $P=0.013$). Lastly, cases had somewhat lower overall Ego identity scale (EISTOT 112.8±14.5 vs 122.4±7.5, $P=0.011$).

Discussion: The completion of the pilot stage had shown that the selected surveys are well-suited for the planned purpose. In addition, the presence of significant differences between the groups even at this sample size suggests that the completed sample size will have sufficient power to detect the expected results and provide a reliable answer to the planned hypothesis.

MeSH/Keywords: atopic dermatitis, pediatric dermatology, caregivers, Ego strength, anxiety, depression, quality of life

Poster code: R-02-02-058

Poster Title: Correlation of Epstein-Barr virus expression on disease prognosis and the incidence of second primary malignant disease in patients with T-cell cutaneous lymphomas

PhD candidate: Mikela Petković

Part of the thesis: Correlation of Epstein-Barr virus expression on disease prognosis and the incidence of second primary malignant disease in patients with T-cell cutaneous lymphomas

Mentor(s): Professor Romana Čeović, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Primary cutaneous lymphomas (PCL) are part of a heterogenous group of non-Hodgkin lymphomas (NHL). PCL, due to the cell of origin, are divided into T-cell cutaneous lymphomas (CTCL) and B-cell cutaneous lymphomas (CBCL). Mycosis fungoides (MF) is an indolent T-cell cutaneous lymphoma which makes approximately 44% of all PCL and 54% of all CTCL. MF usually occurs in adults with a median age of onset between 55 and 60 years, with male sex predominance, with incidence between 6.4-7.7 / 1 000 000. The etiology of MF is still unknown, but possible causative factors are smoking, obesity, chromosome instability and some infections, like Epstein-Barr virus (EBV). EBV is the ubiquitous virus and is assumed that 90% of the population is infected with EBV. EBV is linked to the development of some malignant diseases, like Burkitt's lymphoma. According to previous research, the prevalence of EBV in tumorous skin lesions in MF is between 10-36%. In patients with CTCL, a higher risk of developing second primary malignancies was noticed, like NHL and Hodgkin's lymphoma. Due to now conducted research, approximately 10% of patients with MF will develop another primary malignant disease, representing 2.4 the relative risk for developing a malignant disease compared to the standard population. To this date, there are no epidemiological data in our country about the incidence of second primary malignant disease and the prevalence of EBV infection in the skin lesions in patients with CTCL.

Materials and methods: This retrospective study will be performed at the Department of Dermatology and Venereology, University Hospital Centre Zagreb and Department of Pathology and Cytology, University Hospital Centre Zagreb. This study group will include 80 patients diagnosed with CTCL. The diagnosis of CTCL is based on histological and immunohistochemical criteria. To detect EBV-transcribed RNAs, called EBER, in situ hybridization will be used in the skin tumor samples performed at the Department of pathology and cytology. Stage of the disease will be determined according to TNMB classification for MF/SS according to ISCL and EORTC revised in 2007. From medical documentation and regular follow-ups, the eventual development of second primary malignant tumor will be recorded.

Results: This study group included 80 patients diagnosed with CTCL, more precisely MF. A male: female ratio was 51:29, which means that 63% of the patients were male and 36,25% were female. After in situ hybridization for EBER, none of the 80 patients (0/80) were positive for EBV. Secondary primary malignancy was found in 12/80 patients (15%). Of those 12 patients, 6 were female sex and 6 male sex. Other cancer included prostate cancer (2 patients), smoldering multiple myeloma, tonsillar NHL, lung cancer, breast cancer (2 patients), endometrial cancer, NHL, thyroid cancer and B-cell chronic lymphatic leukaemia. Two patients had 2 second primary malignancy, both female with mammary carcinoma as one of the malignancies, and the other two included planocellular cancer of the floor of the mouth and the monoclonal gammopathy of unknown significance (MGUS).

Discussion: The role of EBV infection in the pathogenesis of MF is still unknown. According to previous research, the prevalence of EBV in tumorous skin lesions in MF is between 10-36%. Some studies included a control group of healthy skin and a prevalence of EBV virus in healthy skin was 0-7%. In our study none of the patients were positive for EBV infection and our hypotheses that EBV infection cause faster progression of the CTCL or development of secondary primary malignant cancer was rejected. To conclude, the prevalence of EBV in patients with CTCL in the Republic of Croatia is 0 so other causative factors, viral and non-viral, are needed further research. Our study group had slightly higher incidence of a second primary malignancy, in total of 15%, compared to data conducted so far which was 10% and two patients form our study group had 2 second primary malignancy. Further studies are required, as well as close follow-up of CTCL patients to recognise a second primary malignancy in time.

MeSH/Keywords: Epstein-Barr virus, primary cutaneous T-cell lymphoma, Mycosis fungoides, in situ hybridization, second primary malignancy

Poster code: R-02-02-077

Poster Title: ADAMTS-4 a possible marker of disease activity and progression of hemophilic arthropathy

PhD candidate: Nataša Kalebota

Part of the thesis: The value of cartilage biomarkers serum chondroitin sulfate epitope 846 and urinary C-terminal telopeptide of type II collagen in assessing the severitiy of hemophilic arthropathy

Mentor(s): Associate Professor Porin Perić, MD PhD, Associate Professor Silva Zupančić-Šalek, MD PhD

Affiliation: University of Zagreb School of Medicine, University Hospital Center Zagreb, Center for Translational and Clinical Research

Introduction: Hemophilic arthropathy (HA) and osteoarthritis (OA) are characterized by articular cartilage degradation. A disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) 4 is an aggrecanase with known local involvement in OA pathogenesis. One of its main targets is the aggrecan, a major structural and functional proteoglycan of the articular cartilage. There has been no study to analyze the role of ADAMTS-4 in HA. Aim is to investigate the role of ADAMTS-4 in the pathogenesis of HA and its discriminatory potential between OA and HA.

Materials and methods: A pilot cross-sectional observational study included N=40 participants between May 2020 and September 2021 in the University Hospital Center Zagreb equally divided across 4 subgroups: 1) hemophilia patients with severe HA, median age 55 (49.5 61.3); 2) hemophilia patients with mild HA, median age 42 (37.5 - 44.5); 3) control subjects with severe OA, median age 59 (45.5 54); and 4) control subjects with no/mild OA, median age 42 (34.3 44.8). We determine ADAMTS-4 plasma concentrations by ELISA in all participants in Center for Translational and Clinical Research. The severity of OA and HA was assessed by a physical medicine and rehabilitation specialist. Ultrasound scanning procedure and scoring method HEAD-US was used to evaluate joints in HA. For all subjects, inclusion criteria were male gender, aged 25 years or older and exclusion criteria were any episode of joint bleeding in the previous month, a bone fracture in the previous 3 months, inflammatory rheumatic diseases, Pagets disease, hyperparathyroidism, hyper- and hypothyroidism, actual glucocorticoid therapy.

Results: The group with severe HA had the highest concentration of ADAMTS-4 in their plasma, with a median of 34.3 (3.41-166) pg/mL. Medians of all other groups amounted to 0 pg/mL, however, a visible trend of declining levels of ADAMTS-4 is visible across the groups: the next being patients with mild HA, followed by patients without hemophilia: firstly, by the group with severe OA and then by patients with no/mild OA. A statistically significant difference among groups was observed (2 13.1; p=0.004). ADAMTS-4 plasma values were shown to be discriminatory of i) hemophiliac vs. non-hemophiliac patients with 95% sensitivity and 50% specificity; ii) between patients with severe vs mild/no disease (regardless of disease pathophysiology) with 80% sensitivity, 25% specificity; iii) between severe HA vs. severe OA patients with 90% sensitivity and 40% specificity.

Discussion: In the present study, we determined for the first time the plasma levels of ADAMTS-4 in patients with mild and severe HA and OA in order to propose its distinguishing diagnostic and prognostic nature. Our study showed a striking elevation in plasma ADAMTS-4 expression levels in HA patients as compared to OA, as well as an increase in patients with severe as compared to mild HA. Our research implicates that HA is a new indication where circulating ADAMTS-4 could to be tested in future research as a marker of disease activity and progression.

MeSH/Keywords: hemophilia, hemophilic arthropathy, osteoarthritis, ADAMTS-4, a disintegrin and metalloproteinase with thrombospondin motifs 4

Poster code: R-02-03-020

Poster Title: The effectiveness of therapeutic ultrasound in the treatment of calcific tendinitis of the shoulder

PhD candidate: Stjepan Čota

Part of the thesis: Efficacy of 4500 Joule therapeutic ultrasound in the treatment of calcifying shoulder tendinitis

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

Affiliation: University of Zagreb School of Medicine

Introduction: Calcific tendinitis of the shoulder (CT-S) is characterized by hydroxyapatite crystals deposits in the tendons of the rotator cuff with pain and acute or chronic shoulder mobility limitations. Therapeutic ultrasound (T-US), due to the tissue warming effect, and therapeutic exercises are commonly used in the treatment. This double-blind, placebo-controlled study aimed to determine whether T-US in combination with therapeutic exercises leads to calcification size and pain reduction, increased shoulder mobility, shoulder functional status improvement, and overall rehabilitation outcome satisfaction in relation to the same therapeutic exercises alone in the treatment of symptomatic CT-S. The primary outcome of the study is the reduction in the calcification size after the treatment.

Materials and methods: A total of 52 consecutive patients (58 shoulders) were enrolled in the study. Six patients (six shoulders) did not complete the treatment. A total of 46 patients (52 shoulders: 26 in the T-US group and 26 in the sham T-US treatment group) completed the treatment. T-US in a continuous mode was administered for 10 minutes per session at a frequency of 1 MHz and an intensity of 1.5 W/cm2 to the area over the calcification. All participants performed the following: shoulder girdle stretching exercises (trapezius, pectoralis, subscapularis, infraspinatus, triceps, serratus, rhomboids) for the first and the last 5 minutes, shoulder girdle strengthening exercises, rotator cuff, and scapular stabilizer strengthening (same muscles plus supraspinatus, deltoid, levator of the scapula) for 20 minutes. Treatment lasted four weeks (5 times a week, a total of 20 times). Before and after the treatment, the following was done: standardized ultrasound examination of the shoulder, assessment of the pain intensity, shoulder mobility measurement, determining the muscle strength of the rotator cuff and the grip force, as well as functional status using the Shoulder Pain and Disability Index (SPADI).

Results: The study is currently in the data processing phase. On a sample of 10 treated participants (5 in the T-US group and 5 in the sham T-US group) the mean VAS pain in the T-US group before the treatment was 4.5 and after 1.6. The mean VAS pain value in the sham T-US group before treatment was 4.9 and after treatment 2.4. The relative change of VAS pain was 65% in the T-US group, and 50% in the sham T-US group.

Discussion: For the first time, the total applied energy of 4500 J per treatment, which is assumed to have a positive effect, will be evaluated. Possible guidelines for a standardized protocol on the use of T-US in the treatment and rehabilitation of symptomatic CT-S could be developed.

Acknowledgments: None

MeSH/Keywords: rehabilitation, ultrasonic therapy, shoulder pain

Poster code: R-02-03-023

Poster Title: Efficacy comparison between Kegel exercises and extracorporeal magnetic innervation in treatment of female patients with stress urinary incontinence

PhD candidate: Mislav Mikuš

Part of the thesis: Efficacy comparison between Kegel exercises and extracorporeal magnetic innervation in treatment of female patients with stress urinary incontinence

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

Affiliation: Department of Gynecology and Obstetrics, Clinical Hospital Center Zagreb; University of Zagreb School of Medicine

Introduction: Stress urinary incontinence (SUI) is defined as a complaint of inadvertent loss of urine occurring as a result of an increase in intraabdominal pressure, in the absence of a detrusor muscle contraction. It has been reported that SUI affects up to 35% of postmenopausal women, unfavourably affecting quality of life (QoL). The aim of this study is to directly compare the two most frequent conservative treatment modalities - extracorporeal magnetic stimulation (EMS) and Kegel exercise (KE).

Materials and methods: A randomized clinical trial was conducted at the Department of Obstetrics and Gynaecology, Clinical Hospital Center Zagreb, Croatia. Participants were women aged 18 to 65 years who have previously given at least one vaginal delivery (at least 12 months before joining the study), who present with symptoms of SUI lasting at least 6 months and without previous treatment for urinary incontinence. A questionnaires ICIQ UI-SF, ICIQ-LUTSqol and PGI were completed at enrolment (T0), at the end of the treatment (T1, 8 weeks) and during the follow-up period (T2 three months after treatment). The aim of those three questionnaires is to assess symptom distress, quality of life and patient's satisfaction with the treatment. The pelvic floor muscle function evaluated by a perineometer were performed at T0, T1 and T2 in order to have a more objective approach towards the symptom distress. Furthermore, every patient fulfilled bladder diary at T0, T1 and T2. Descriptive statistics measures (arithmetic mean, standard deviation, minimum and maximum values and total range) were used to show the distributions of the participants. Categorical variables were displayed as frequencies with their percentages. In order to calculate whether there are statistically significant differences initially, immediately after the treatment and 3 months after the treatment in ICIQ-UI-SF results between the two groups (KE vs. EMS), we have conducted mixed-model analysis of variance.

Results: Data collection is still in progress (expected to be finalized in June 2022). At the moment there are not any preliminary results available.

Discussion: To our knowledge, KE and EMS have not been previously compared directly in a randomized clinical trial setting. Although the mid-urethral sling procedure is considered the gold standard treatment for SUI, it may not be suitable for all patients, particularly in women with significant comorbidities, which are common in older age. Moreover, it can lead to postoperative voiding dysfunction, including urine retention in up to 36% of patients. Other, non-specific complications such as postoperative pain, wound infections and mesh erosion may permanently impair QoL. Most available conservative treatment approaches are designed to improve the function of pelvic floor muscles in order to maintain positive urethral closure pressure during an increase of intra-abdominal pressure. Our randomised trial will provide the evidence from a direct comparison of two common conservative treatments for SUI, using state-of-the-art clinical and research methodologies. The results will directly inform clinical decision making in caring for patients with SUI.

MeSH/Keywords: stress urinary incontinence; Kegel exercise; extracorporeal magnetic stimulation; quality of life

Poster code: R-02-05-052

Poster Title: Pancreastatin levels during vaginal delivery in women with gestational diabetes

PhD candidate: Ivka Djaković

Part of the thesis: Pancreastatin levels during vaginal delivery in women with gestational diabetes

Mentor(s): Assistant Professor Miro Bakula, MD PhD, Associate Professor Krunoslav Kuna, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Gestational diabetes is closely related to the metabolic syndrome, which is associated with dysregulation of the secretion of a number of hormones like insulin, glucagon, growth hormone, cortisol, and prolactin. Chromogranin A (CgA) is a hormone that affects the secretion of all these hormones and the premise of this study is that a disorder in the secretion of chromogranin A may be a common component of hormonal disbalance in metabolic syndrome and associated diseases. Cleavage of chromogranin results in several other peptides, one of these is pancreastatin. Pregnant women with metabolic syndrome have higher concentrations of CgA than healthy pregnant women. Therefore the concentration of CgA and pancreastatin in serum may be significant for the degree of endocrine and autonomic nervous systems dysregulation. All this corelates with development of complications during childbirth. The aim of this study was to compare the dynamics of changes in pancreastatin concentration during childbirth in healthy pregnant women and those with gestational diabetes.

Materials and methods: The trial was a prospective clinical trial. Pregnant women who were monitored in the maternity clinic and who gave birth in the Department of Obstetrics, Clinic for Women's Diseases and Obstetrics, KBC Sestre milosrdnice were examined. All pregnant women are secondigravide with single pregnancies for which vaginal delivery is planned and completed. Two groups were examined: 14 subjects with gestational diabetes and 15 healthy pregnant women. Pregnant women in the gestational diabetes group achieved satisfactory glycemia exclusively through a diabetic diet. Exclusion criteria are: firstgravide or multigravide, pregnant women with chronic inflammatory or autoimmune diseases (excluding autoimmune thyroid disease), drug addicts, women on antidiabetic therapy. Methodology and research material: Detailed anamnesis and status, anthropometric measurements and blood sampling were taken during hospitalization. The study collected blood samples at three points according to the following protocol: 1. before labor (venous blood, two tubes with coagulation activator of 6 ml), 2. during expulsion of the fetus (venous blood, one tube with coagulation activator of 6 ml) and 3. two hours after delivery (venous blood, one tube with a coagulation activator of 6 ml). In point 1, from the venous blood sample before the onset of labor, general biochemical and endocrinological parameters were determined, and in all points (1-3) pancreatatin and cromogranin were determined. Statistical analysis: In accordance with the characteristics of each variable, the data obtained were described by determining the appropriate mean value as well as the corresponding scatter of the results. After testing the normality of the distribution of numerical variables, the appropriate measurement of the central tendency and variability and the appropriate statistical-analytical procedures for testing the differences between the examined groups were applied. If the obtained distributions will not differ significantly from normal, parametric tests will be applied to analyze the statistical significance of the difference between the groups (ANOVA for repeated measurements). Otherwise, nonparametric tests (Friedman test) will be applied. A chi-square test and, if necessary, Fisher's exact test will be used to analyze the differences in the distribution of qualitative variables. The results will be interpreted at the 5% significance level.

Results: CgA concentrations decreased significantly and pancreastatin increased significantly when observing the both groups. There were no significant differences in CgA changes between the study groups. Also, there were no significant differences in pancreastatin concentration changes between study groups when RM ANOVA was performed on logarithmically transformed data.

Discussion: The results did not confirm our hypothesis. We received very valuable information that the level of chromogranin A decreases during childbirth, which may mean that it degrades more to its products, which is also pancreatatin. The probable reason for these results is the fact that all pregnant women with gestational diabetes criteria achieved satisfactory glycemia with a diabetic diet.

MeSH/Keywords: chromogranin A, pancreastatin, term pregnancy, gestational diabetes

Poster code: R-02-05-138

Poster Title: Waning vaccine-induced immunity against pertussis in children aged 6 to 18 years

PhD candidate: Vedran Stevanović

Part of the thesis: Seroprevalence of IgG antibodies against pertussis in children aged 6 to 18 years

Mentor(s): Professor Goran Tešović, MD PhD, Assistant Professor Oktavija Đaković Rode, MD PhD

Affiliation: University of Zagreb School of Medicine, University Hospital for Infectious Diseases Dr Fran Mihaljevic

Introduction: Pertussis is a vaccine preventable disease caused by still circulating Bordetella pertussis. Serological surveillance studies help us investigate the pertussis infection rate in the population by determining serum antibodies concentrations against pertussis toxin. Current childhood immunization strategies do not completely control the transmission of the organism and adolescents with pertussis continue to be a significant source of infection for unvaccinated or incompletely vaccinated infants who are in harm for developing severe disease.

Materials and methods: An ongoing single-center cross sectional seroprevalence study is conducted in regularly vaccinated children and adolescents in age range 6 to 18 years at the University Hospital for Infectious Diseases in Croatia from January 2021. Serum samples are collected and frozen at the study site until serological analysis. Statistically significant serum sample size will be achieved at 840 to 1080 samples for all age groups.

Results: Questionnaire was filled out for 670 regularly vaccinated children and adolescents in the age groups from 6 to 18 years. Out of 670 collected serum samples, 400 have been analysed. Preliminary results include 399 serum samples with low IgG concentration and 1 serum sample with high IgG concentration.

Discussion: Low IgG concentration in the observed population indicates waning vaccine-induced immunity against pertussis with no difference between inflicted immunization programs, and the absence of the B.pertussis circulation at the time of serum collection. Our proposed study will give an insight in seroprevalence of IgG-anti-PT in children and adolescents aged 6-18 years and estimate pertussis infection activity and compare it regarding inflicted immunization programs at the time.

MeSH/Keywords: waning vaccine-induced immunity, pertussis, children

Poster code: R-02-07-036

Poster Title: Changes in the incidence rate and prevalence of syphilis in people living with HIV in Croatia in the period from 2009. to 2019.

PhD candidate: Vanja Romih Pintar

Part of the thesis: Changes in the incidence rate and prevalence of syphilis in people living with HIV in Croatia in the period from 2009. to 2019.

Mentor(s): Associate Professor Davorka Lukas, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Syphilis is a sexually transmitted disease with observed increase in prevalence worldwide. In a person infected with HIV, co-infection with Treponema pallidum increases the likelihood of virus transmission and may adversely affect the natural course of HIV infection (temporary decrease in CD4+ lymphocytes and an increase in HIV viremia). Patients with syphilis co-infected with human immunodeficiency virus (HIV) may have an altered clinical presentation of syphilis (eg multiple or painful ulcerative lesions, atypical rash), faster progression of neurosyphilis as well as atypical serological response to treatment. In Europe, almost two thirds (66%) of newly diagnosed syphilis cases were recorded among men who have sex with men. In Croatia, this risk group is prevalent (more than 80%) among people with newly diagnosed HIV infection. The aim of this study was to determine the incidence and prevalence of syphilis and potential factors that could contribute to their increase in HIV positive men who have sex with men (MSM).

Materials and methods: We conducted a retrospective longitudinal cohort study including 676 HIV-infected males belonging to MSM population who were treated at University hospital for infectious diseases "Dr. Fran Mihaljevi" (UHID) in Zagreb, in the period from 2009. to 2019. The database of Croatian HIV cohort was examined, and we extracted demographic, clinical and treatment data for the syphilis cases. All cases with a diagnosis of syphilis were identified and reviewed for the clinical features and type of treatment. We describe our data with frequencies, median and first and third quartiles. The analysis was done in adults (18 years) who belong to the risk group of men who have sex with men (MSM) and who have not been in HIV care anywhere else before entering care at UHID.

Results: These are partial and preliminary results. Only 2 years of the 11-year longitudinal cohort study was calculated. We reviewed all newly diagnosed syphilis cases among HIV infected MSM in Croatia during years 2018. and 2019. There were 33 patients with 35 episodes of syphilis in 2018. and 45 patients with 47 episodes of syphilis in 2019. The viral load was < 50 copies/ml in 94% and 93% of patients in years 2018. and 2019. Median age of patients was 41 years and approx. 40% of patients was asymptomatic. More then 80% of patients were treated with pencillin (88.6% in 2018. and 80.9% in 2019.). The clinical presentation and treatment of syphilis is presented in Table 1.

Discussion: To our knowledge, this is the first population-based syphilis survey conducted among HIV positive MSM in Croatia. As the analysis of this retrospective longitudinal study is not complete, risk factors associated with increase in syphilis incidence and prevalence is not yet determined. We presume that the prevalence of syphilis in HIV infected patients entering care in Croatia was high for analyzed years. The results of the research suggest that targeted new preventive public health measures for the MSM population are needed. The frequency of routine screening for syphilis in the MSM population should be increased.

MeSH/Keywords: HIV, syphilis, incidence, prevalence, men who have sex with men

Poster code: R-02-07-162

Poster Title: Impact of increased intraabdominal pressure on ultrasound mesurement of the inferior vena cava diameter and central venous pressure estimate

PhD candidate: Mia Rora

Part of the thesis: Impact of increased intraabdominal pressure on ultrasound mesurement of the inferior vena cava diameter and central venous pressure estimate

Mentor(s): Associate Professor Radovan Radonić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Measurement of the diameter of the inferior vena cava (IVC) by ultrasound enables a fast and non-invasive assessment of the patients volume status. Based on the measured IVC diameter and collapsibility index of the IVC, the central venous pressure (CVP) can be approximately estimated. It is not known to what extent intra-abdominal pressure (IAP) changes the diameter and collapsibility index of the IVC.

Materials and methods: The research consists of two parts. The first is the observational part, which includes subjects with an indication for ascites drainage. The second is the experimental part, which involves subjects with artificially elevated IAP. In both groups of respondents the diameter and collapsibility index of IVC and CVP will be measured for different levels of intra-abdominal hypertension.

Results: Measurements were performed on twenty patients with ascites. The results, so far, have shown that increased intra-abdominal pressure leads to a decrease in the diameter of the IVC. Ascites puncture in these patients leads to a decrease in intra-abdominal pressure and an increase in the diameter of the IVC. The changes are more pronounced with the evacuation of the first two liters of ascites. Later evacuation of equal volume will change the intra-abdominal pressure and the diameter of the IVC to a lesser degree.

Discussion: The results of this research could indicate how different levels of elevated intra-abdominal pressure affect the diameter and collapsibility index of IVC. Based on the results this research will estimate the extent of the error in the classification of CVP among subjects with elevated IAT based on the ASE table.

MeSH/Keywords: inferior vena cava diameter, collapsibility index, intra-abdominal hypertension, central venous pressure

Poster code: R-02-08-027

Poster Title: The use of interleukin 6 and leukocyte cell population data for outcome prediction in immunocompetent and immunocompromised patients with sepsis

PhD candidate: Sara Šundalić

Part of the thesis: The use of interleukin 6 and leukocyte cell population data for outcome prediction in immunocompetent and immunocompromised patients with sepsis

Mentor(s): Assistant Professor Ana Vujaklija Brajković, MD PhD, Associate Professor Radovan Radonić, MD PhD

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

Introduction: Sepsis is a life-threatening condition and a medical emergency. Regardless of modern therapeutic approaches, sepsis mortality is high, from 20% to over 60%. Timely diagnosis and early interventions are crucial for disease outcome, especially in immunocompromised patients. Procalcitonin (PCT) has shown to be useful in early sepsis diagnosis and to measure therapeutic success, while interleukin 6 (IL-6) has shown to be a good predictor of outcome. Leukocyte cell population data (CPD) (based on fluorescence flow cytometry) is showing promising results in early diagnosis of sepsis and seems very cost-effective. PCT was investigated in immunocompromised patients, while research regarding IL-6 and leukocyte CPD in these patients is scarce, mostly retrospective and does not offer a separate analysis of this patient group.

Materials and methods: This research is designed as a prospective, observational, cohort study. It will be carried out in the Intensive care unit (ICU), Department of internal medicine, University Hospital Centre Zagreb, Croatia. Consenting adult patients hospitalized in the ICU because of sepsis will be included. During data analysis patients will be divided into two patient groups - immunocompetent and immunocompromised. At admission vital parameters will be noted, blood will be sampled for laboratory and microbiological analysis, APACHE II, SOFA and SAPS II scores will be calculated. Blood will be sampled within 24 hours of admission, then the third, fifth and seventh day. Complete blood count will be determined (including leukocyte CPD; immature granulocyte count, IG%, number of total reactive lymphocytes, RE-LYMP, antibody-synthesizing lymphocytes, AS-LYMP, quantified parameters of neutrophil activation (cytoplasmic granulation of neutrophils, NEUT-GI, and reactivity of neutrophils, NEUT-RI)), as well as routine biochemical parameters (including PCT), lactate and IL-6 levels. We aim to include 146 patients.

Results: For now, we can present purely descriptive data (having in mind that not all relevant information is gathered, or available, yet). Up till now, we have included 97 patients. The average age was 64,31 ± 15,6 years. There were 51 male patients (52,58%) and 46 female patients (47,42%). The included patients had the following SOFA, APACHE II and SAPS II scores (median score and interquartile range): median SOFA score 8, IQR 5 - 12; median APACHE II score 24, IQR 16 - 29 and median SAPS II score 47, IQR 35 - 62. From the 97 patients 30 (30,93%) were immunocompromised. The immunocompromised states include: 24 patients with solid tumors, 11 with hematologic malignancies, 3 had a solid organ transplantation and 4 patients underwent hematopoietic stem cell transplantation, while 6 had an autoimmune disease and 16 patients had chronic glucocorticoid therapy (some of these patients had more than one cause of immunocompromise). Positive blood cultures were found in 46 patients (47,42%). Gram-negative bacteria were isolated in 22 cases, gram-positive bacteria in 19 patients, while both gram-negative and gram-positive bacteria were found in 5 patients. The remaining 51 cases (52,58%) were classified as culture-negative sepsis.

Discussion: According to the dynamics witnessed in the past two years, we expect to finish this research (including the remaining 49 patients, data acquisition and data analysis) in the next 12 months. Hopefully, this study will bring new information regarding the use of IL-6 and leukocyte CPD for outcome prediction in patients with sepsis, including an immunocompromised patient group. Also, a comparative insight in the dynamics of IL-6 and leukocyte CPD between immunocompetent and immunocompromised patients with sepsis will be given.

MeSH/Keywords: sepsis, immunocompromised, interleukin 6, leukocyte cell population data

Poster code: R-02-08-055

Poster Title: Safety and efficacy of alteplase compared to heparin in patients with pulmonary embolism

PhD candidate: Nikolina Marić

Part of the thesis: Comparison of safety and efficacy of alteplase and heparin in patients with moderate risk pulmonary embolism

Mentor(s): Associate Professor Robert Likić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Optimal therapeutic strategy for patients with intermediate-risk pulmonary embolism is still a topic of debate. While the use of fibrinolytic therapy is recommended in high-risk patients, in intermediate-risk group it often poses a complex clinical dilemma and requires a careful weighing of risk-benefit ratio. So far, several studies with different types and doses of fibrinolytics in patients with intermediate-risk pulmonary embolism were done with mixed results. In all of them, heparin was co-administered with a fibrinolytic. The aim of this study is to compare safety and efficacy of alteplase with unfractionated heparin in patients with intermediate-risk pulmonary embolism using protocol in which alteplase is administered without concomitant use of heparin.

Materials and methods: In this prospective cohort study, we plan to compare alteplase to heparin in treatment of intermediate-high pulmonary embolism. Patients are eligible if they meet the following criteria: an age of 18 years or older, diagnosis confirmed with computed tomography, haemodynamic stability, right ventricular dysfunction on echocardiography or computed tomography, myocardial injury confirmed by positive biomarker troponin I, no signs of any bleeding, estimated low bleeding risk and obtained written informed consent. Patients are divided in two groups. Intervention group receives alteplase at a total dose of 100 mg without concomitant heparin during alteplase infusion. The control group receives intravenous infusion of unfractionated heparin according to the weight based Raschke protocol. The following parameters are determined for each group: laboratory tests, vital signs, use of vasoactive drugs, respiratory support, CPR, signs of bleeding, need for transfusion, length of ICU and hospital stay, mortality rate. Main end points of the study are mortality rate and clinical deterioration requiring escalation of therapy (vasoactive drugs, rescue fibrinolysis, endotracheal intubation, CPR). The main safety outcome is appearance of major bleeding defined by ISTH classification.

Results: The study is still in progress with 49 enroled participants, 36 in intervention and 13 in control group. The median patient age was 69,1±9,4 years, 54% were women and 46% men. The mean length of hospitalization was 7,65±4,25 days. Of 49 patients one expired due to a newly diagnosed malignant disease. One patient in the control group needed escalation of therapy for rescue fibrinolysis. No patients had intracranial bleeding. Major bleeding requiring blood transfusion emerged in one patient in intervention group and in one in control group. Patient in intervention group had muscle haemathoma (ISTH-SSC bleeding score 4), and the one in the control group had retroperitoneal haemathoma (ISTH-SSC score 4). The remaining data collected from the study is still insufficient for proper statistical analysis.

Discussion: Current recommendations for treatment of intermediate-high risk pulmonary embolism are not well defined. Decision to use thrombolytic therapy is made on a case by case basis evaluating and weighing risk for haemodynamic decompensation to risk of major bleeding. Our preliminary results suggest thrombolytic therapy in this category of patients could be a safe therapeutic option when used without concomitant heparin infusion.

MeSH/Keywords: pulmonary embolism, fibrinolytic therapy, heparin

Poster code: R-02-08-071

Poster Title: The assessment of pleural pressure change by measuring inferior vena cava diameter change with ultrasound in mechanically ventilated patients with acute respiratory distress syndrome

PhD candidate: Ela Ćurčić

Part of the thesis: The assessment of pleural pressure change by measuring inferior vena cava diameter change with ultrasound in mechanically ventilated patients with acute respiratory distress syndrome

Mentor(s): Associate Professor Radovan Radonić, MD PhD

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

Introduction: Acute respiratory distress syndrome (ARDS) is a clinical syndrome characterized by acute onset of bilateral lung infiltrates which lead to respiratory failure. It is a syndrome with high morbidity and mortality among patients with respiratory failure hospitalized in the intensive care unit (ICU). Mechanical ventilatory support is a mainstay of therapy, while balancing between adequate gas exchange and minimal iatrogenic lung injury. According to the principles of respiratory mechanics, the distending lung pressure is the difference between airway pressure (Paw), generated by the ventilator, and pleural pressure (Ppl), generated by the patient. Mechanically ventilated, sedated, but non-curarized patients with ARDS have spontaneous inspiratory efforts. While Paw can be easily read from the ventilator, Ppl cannot be directly measured. Esophageal manometry (EM) is the clinically available method for measurement of esophageal pressure (Pes), which is used as a surrogate for Ppl. Although not easily available, esophageal manometry remains the principal method for the estimation of Ppl. On the other side, ultrasound (US) is a feasible and available tool in the ICU. In our study we hypothesized that by measuring changes in the inferior vena cava diameter (VCI) with US it is possible to assess changes in pleural pressure (Ppl) in mechanically ventilated patients with ARDS who have preserved spontaneous inspiratory efforts.

Materials and methods: The study will include mechanically ventilated patients who fulfil the diagnosis of ARDS according to the Berlin definition and who are hospitalized in the Department of Intensive Care Medicine, University Hospital Centre (UHC) Zagreb. The study was approved by the Ethics committee of the UHC Zagreb. The inclusion criteria are: age 18 years, invasive mechanical ventilation, adequate sedation without neuromuscular blockade (NMB), obtained informed consent from the patient or next of kin/legal representative. Exclusion criteria are: previous NMB (within 24 hours), thrombocytopenia (<20x109/L), severe coagulopathy, esophageal varices, facial or skull base trauma, central venous pressure (CVP) >16 cmH2O, VCI thrombosis, extraluminal compression of VCI, ascites, pregnancy, neuromuscular diseases. Three repeated measurements of Ppl with esophageal manometry and three repeated measurements of VCI with ultrasound will be performed simultaneously on each patient during inspiration in two ventilatory modalities (intermittent positive pressure ventilation, IPPV and bilevel positive airway pressure, BiPAP), before and after NMB. In statistical analysis the dependent variable (Ppl) will be modeled in generalized linear models as a continuous, ordinal and binary variable. The main independent variable of interest (VCI) will be treated as a continuous variable.

Results: Up till now, 19 patients were diagnosed with ARDS, three of whom did not fulfil the inclusion criteria (due to high intraabdominal pressure, ascites or suboptimal US view) and were excluded from the preliminary data analysis. The average age of the included patients was 65.19 ± 13.37 years. There were 10 male (62.5%) and 6 female (37.5%) patients. Nine patients (56.25%) received vasoactive drugs (noradrenaline). For every patient we obtained six measurements of Ppl and VCI (3 in BiPAP and 3 in IPPV) before and six measurements after NMB. We observed a decrease in Ppl, as well as VCI diameter reduction during inspiration in the non-curarized patients. After NMB there was no significant Ppl or VCI.

Discussion: Our preliminary results show that we can determine the relationship between Ppl and VCI by simultaneous measurement of Ppl with EM and VCI with US. Thus, by assessing VCI with US we can have a better insight into the respiratory mechanics and the need for NMB in ARDS patients.

MeSH/Keywords: acute respiratory distress syndrome, respiratory mechanics, vena cava inferior, neuromuscular blockade

Poster code: R-02-08-074

Poster Title: Diagnosis of clinically significant portal hypertension by two-dimensional ultrasound elastography

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: Portal hypertension (PH) is a clinical syndrome characterized by a pathological increase in portal vein pressure with a key role in the prognosis of chronic liver disease. The gold standard for determining the severity of PH is to measure the hepatic vein pressure gradient (HVPG), which is an invasive and costly method. The aim of this research is to evaluate diagnostic performance of two-dimensional shear wave elastography (2DSWE) in comparison to Transient elastography (TE), as a ultrasound-based non-invasive method that might be potentially used for diagnosing clinically significant PH (CSPH) and high-risk esophageal varices (HRV).

Materials and methods: Prospectively collected data from 76 patients suspected of compensated advanced chronic liver disease (cACLD), who underwent HVPG measurement, esophagogastroduodenoscopy (EGD), transjugular liver biopsy, as well as liver stiffness measurement, (LSM) and spleen stiffness measurement (SSM) by 2DSWE and TE, were retrospectively analysed. HVPG served as the gold-standard method for the assessment of PH, and EGD as the gold-standard method for the assessment of esophageal varices (EV). The suspicion of cACLD was considered in patients with LSM10 kPa by TE, or morphological signs suggestive of cACLD on imaging with no history of liver decompensation. Diagnostic performance of LSM and SSM by 2DSWE alone, or in combination with platelet counts, was analysed with respect to their ability to correctly diagnose CSPH and HRV.

Results: Seventy-six (76) patients were included: 60/76 (78.9%) patients were male, mean age 62 years, body mass index 28.3 kg/m2, mostly with fatty alcoholic and non-alcoholic liver disease 51 (67.1%). Out of them 40 (52.6%) had significant CSPH (HVPG 10 mmHg) and 17 (22.4%) HRV. LSM performed better than SSM in diagnosing CSPH and HRV. For CSPH, AUROCs (0.926 vs. 0.866), optimal LSM cut-offs (20.1 kPa vs 20.2 kPa) and sensitivity/specificity (80.5%/94.3% vs. 77.5% /86.1%) were comparable for 2DSWE and TE. For ruling-in CSPH LSM cut-offs with 90% specificity were 20.1 kPa (2DSWE) and 27.7 kPa (TE). Ruling-out CSPH by 2DSWE (using LSM cut-off with90% sensitivity (13.5 kPa), in combination with platelets150x109/L), performed comparably to TE (using already established criteria of LSM<15 kPa and platelets150x109/L), with only 1/24 cases falsely classified as negative. AUROCs for CSPH were numerically lower for SSM than for LSM (0.877 2DSWE, 0.857 TE) with optimal SSM cut-offs (34.8 kPa 2DSWE, 43.5 kPa TE) and modest sensitivities/specificities (83.3%/81.5% 2DSWE, 89.3%/69.7% TE). Altogether, the presence of CSPH could be correctly classified in 54/74 (73%) patients by 2DSWE and 45/76 (59%) by TE using LSM and platelets count. For HRV AUROCs were similar (0.875 2DSWE, 0,851 TE) with similar optimal LSM cut-offs (19.3 kPa for 2DSWE, 21.8 kPa for TE) resulting in 100% sensitivity for ruling-out HRV by both methods, with modest specificity (68,4% 2DSWE vs. 71.1% TE). We additionally evaluated Baveno VI recommended approach using a combination of the optimal 2DSWE LSM cut-off (19.3 kPa) and the TE cut-off suggested by Baveno VI (<20 kPa), in combination with platelet count150x109/L. Application of this approach indicated that 29/74 (39.2%) patients meeting these criteria by 2DSWE, and 31/76 (40.8%) patients by TE could be ruled-out as HRV, without false-negative cases.

Discussion: The results of our study provide evidence for a good performance of LSM by 2DSWE as an additional and relatively new non-invasive diagnostic method for CSPH and HRV in patients with cACLD. On the other hand, SSM by either method (2DSWE or TE) was not found informative for identification of patients with CSPH. In conclusion, in the present cohort of patients with compensated chronic liver disease, LSM by 2DSWE demonstrated very good performance for diagnosing CSPH and ruling-out HRV, thus obviating the need for HVPG and EGD as more invasive methods in this indication.

MeSH/Keywords: cirrhosis liver; elastography; non-invasive tests; oesophageal varices; portal hypertension.

Poster code: R-02-09-031

Poster Title: Comparison of arterial stiffness in office and 24h measurements in treated hypertensive patients

PhD candidate: Vladimir Prelević

Part of the thesis: Blood vessel stiffness and target organ damage in treated hypertensive patients

Mentor(s): Academician Bojan Jelaković

Affiliation: University of Zagreb School of Medicine

Introduction: Estimation of arterial stiffness by measuring pulse wave velocity (PVW) has been included in international guidelines for assessment of general cardiovascular risk based on large observational studies and interventional clinical trials. In the last few years, a special attention has been given to a new method of measuring PVW, i.e. dynamic, 24-hour measurement of the pulse wave velocity, which provides data on circadian rhythm, night values, and variability, what is of huge importance, and is a counterpart to the phenomenon observed in the measurement of brachial arterial pressure - the phenomenon of white coat stiffness can be excluded. The aim was to determine the concordance of pulse wave velocity values as a measure of vascular stiffness determined by office one-time measurement and 24-hour continuous measurement, as a significant parameter for assessing overall cardiovascular risk in treated hypertensives. The Sphygmocor device was used for office measurements, and the Mobilograph for 24 hours. In addition to the pulse wave velocity, central arterial pressure was measured by both methods, office brachial pressure and heart rate.

Materials and methods: The pilot study included 33 consecutively treated patients with essential arterial hypertension over 18 years of age. The group consisted of 24 men and 9 women with a median age of 33 (range: 19-74). The average values of office-measured brachial pressure for the whole group were: 143 / 99mmHg (+/- 8.14), heart rate 80.15 / min (+/- 10.17), body mass index 19.82 kg / m² (+/- 3.43), office central arterial pressure 128.12mmHg (+/- 15.29), office pulse wave velocities 8.16 m / s (+/- 2.34), 24h central aortic pressure 118.36mmHg (+/- 14.74), 24h pulse wave velocities 6.73 m / s (+ / -2.15). There were 24 smokers, 3 of whom consumed alcohol, while 30 respondents had a positive family history.

Results: Comparing the office and 24h pulse wave velocity measurements, a statistically significant difference was found with p 0.0004 at the expense of the higher value measured by the office, while the measuring central aortic pressure also found a statically significant difference, p 0.0002, again at the expense of the office measured higher value.

Discussion: Central arterial pressure is a variable parameter and the effect of white matter in office measurements should not be forgotten. The pulse wave velocity also depends on a number of hemodynamic variable parameters, and the subjective experience of the person who measure. Measurement of central aortic pressure and pulse wave velocity during 24 h instead of just one moment could give a more objective and accurate picture of those significant markers of cardiovascular risk, but further research is needed on a larger number of subjects

MeSH/Keywords: 24h pulse wave velocity, arterial stiffness, target organ damage, arterial hypertension

Poster code: R-02-09-033

Poster Title: Echocardiographic changes of the right ventricle in patients with high-degree AV block after implantation of permanent single-chamber cardiac pacemaker

PhD candidate: Edita Pllana-Pruthi

Part of the thesis: Echocardiographic changes of the right ventricle in patients with high-degree AV block after implantation of permanent single-chamber cardiac pacemaker

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

Affiliation: University of Zagreb School of Medicine

Introduction: The use of single chamber pacemakers with the lead that is implanted in the right ventricle is known to induce atrio-ventricular, intraventricular, and interventricular dyssynchrony. This leads to the fall of the left ventricular function and therefore the fall of the right ventricular function. On the other hand, right ventricular function deterioration due to permanent right ventricular pacing, is not well established. So, we will try to acquire some new insights regarding the right ventricular function by tracing echocardiographic parameters during permanent right ventricular pacing.

Materials and methods: In this study, we will include 100 patients, aged from 18-85 years. All our patients will be diagnosed with second- and third-degree heart block (II- and III- degree heart block), which are due to receive single chamber pacemakers. They will have three echocardiographic scans. The first echocardiography scan will be done just before pacemaker implantation. The second and third echocardiography scans, will be done three months, and six months after pacemaker implantation, for the purpose of the follow up of echocardiographic changes.

Results: Due to the Covid-19 pandemic the research project was paused, therefore there are no results yet.

Discussion: We aim to give some new insights regarding right ventricle echocardiographic changes due to the permanent right ventricular pacing.

MeSH/Keywords: echocardiography, pacemaker, right ventricle.

Poster code: R-02-09-035

Poster Title: Prevalence of C4d in primary focal segmental glomerulosclerosis

PhD candidate: Nikola Zagorec

Part of the thesis: Prognostic role of C4d in primary IgA nephropathy and primary focal segmental glomerulosclerosis

Mentor(s): Professor Krešimir Galešić, MD PhD, Professor Danica Galešić Ljubanović, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Primary focal segmental glomerulosclerosis (pFSGS) is one of the most common causes of nephrotic syndrome in adults with high tendency of progression to end-stage renal disease (ESRD). Complement system seems to play role in pathogenesis of pFSGS wich is still unresolved. IgM and C3 could be related with worse outcome in FSGS. C4d is a split product od C4 and stabile marker of tissue complement activation via classical and lectin pathway. Due to unpredictable clinical course of pFSGS, we are still searching for novel prognostic factors. C4d is a potential candidate the role of which in primary FSGS should be clarified.

Materials and methods: This is a cohort study. Patients with biopsy proven primary FSGS, who are over 18 years of age, will be recruited from Hospital register of kidney biopsies at Department of Nephrology and Dialysis, Clinical Hospital Dubrava. All patients between October 2003 and October 2020 will be included except those who already had ESRD at the time of biopsy. Relevant data from medical history and histological data will be reviewed retrospectively. Every biopsy sample has already been analyzed by light (number of glomeruli, percentage of globally sclerosed glomeruli and percentage of interstitial fibrosis and tubular atrophy), immunofluorescent (deposits of IgA, IgG, IgM, C3, C1q, and light chains) and electron microscopy (location of immune deposits and podocyte foot process effacement). Immunochemistry (IHC) using monoclonal rabbit antibody for C4d (using detection system Ventana BenchMark Ultra system) will be done additionally on paraffin-embedded sections for every patient. Experienced nephropathologist will classify all samples into C4d positive and C4d negative group excluding those with insufficient sample for IHC analysis (less than one non-globally sclerosed glomerulus on IHC sample). All patients have been treated and followed as outpatients in our hospital.

Results: Sixty patients with pFSGS were identified by reviewing our register. Three of them were excluded from analysis, two because of histological findings of severe diabetic nephropathy and one due to insufficient sample for IHC analysis. Of 57 patients, there were 25 females and median age was 52 (range 20 77). Tip variant was the most common variant of pFSGS (21 patients, 37 %) followed by NOS (20, 35 %), cellular (12, 21 %), perihilar (2, 3.5 %) and collapsing (2, 3.5 %) variant. Average number of non-globally sclerosed glomeruli was 11.6 per sample (patient). Average number of C4d positive glomeruli was 7,4 per sample. Pattern of C4d positivity was segmental in almost all glomeruli and only in two glomeruli was global. Average number of focal segmental lesion was two per sample and all segmental lesions were C4d positive. Of 31 globally sclerosed glomeruli, 8 was C4d positive (25 %).

Discussion: We showed that all patients with pFSGS had glomerular C4d deposition, both in sclerotic and non-sclerotic distribution. All segmental sclerotic lesions were C4d positive. Two third of non-globally sclerotic glomeruli are C4d positive which means that C4d deposition is mostly related to non-sclerotic glomerular area. Previous theories presumed that deposition of components of complement system is passive due to their capture in glomerular sclerotic areas. Our finding showed that activation of complement system happened in glomeruli in patients with pFSGS independently of sclerosis formation or may precede it. Role of C4d in pathogenesis of pFSGS remains to be elucidated as well as correlation between intensity of C4d positivity and disease outcome.

MeSH/Keywords: complemet system, C4d, focal segmental glomerulosclerosis, immunochemistry

Poster code: R-02-09-039

Poster Title: Ultrasound assessment of diaphragm mobility in patients with systemic sclerosis with and without interstitial lung disease: analysis of preliminary results

PhD candidate: Anja Ljilja Posavec

Part of the thesis: Ultrasound assessment of diaphragm mobility in patients with systemic sclerosis with and without interstitial lung disease

Mentor(s): Assistant Professor Joško Mitrović, MD PhD, Nevenka Piskač Živković, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Interstitial lung disease is one of the most common causes of death in patients with systemic sclerosis (SSc). Patients with SSc often have gastroesophageal involvement. Results of the previous studies indicate that patients with some fibrosing lung diseases have decreased diaphragm mobility. Diaphragm is the main muscle in physiology of respiration and its mobility can be reduced in some chronic lung and autoimmune diseases. ILD in SSc is associated with esophageal involvement, albeit previous studies have shown conflicting results.

Materials and methods: To compare diaphragm mobility in patients with SSc with ILD to the patients without ILD.

Results: The study included 50 patients with SSc which were treated between 2021 and 2022 at the Department of Clinical immunology, rheumatology and allergology, University Hospital Dubrava and Department of Clinical immunology and rheumatology University Hospital Centre Zagreb, Croatia. Criteria for exclusion were heart failure, chronic lung disease of etiology not specific to the patients with SSc, neuromuscular disease or other autoimmune disease that could contribute to impaired diaphragmatic mobility. Mobility of the diaphragm during the respiratory cycle was evaluated with ultrasound. Amplitude of craniocaudal diaphragmatic mobility during normal and deep breathing was measured in M mode. Gastroesophageal symptoms such as heartburn, gastric content regurgitation, dysphagia, and epigastric pain were evaluated, as well as lung function tests- spirometry and lung diffusing capacity for CO were analyzed. ILD was evaluated on HRCT of the thorax by experienced radiologist.

Discussion: The median patient age was 62 years, ranging from 27 to 77 years. Percentage of patients with interstitial lung disease was 36%. Thirty-eight percent of the patients with ILD had decreased diaphragm mobility in deep breathing. Thirty-two patients did not have ILD, from which 9% patients had decreased diaphragmal mobility in deep breathing. Diaphragm mobility was significantly reduced in patients with ILD comparing to patients without ILD. Four of all patients were male (8%) of which all had ILD and one patient had reduced diaphragm mobility. Furthermore, of all patients with reduced diaphragm mobility only 10% had reduced values during normal breathing.

Acknowledgments: I would like to express my gratitude to my mentors, Joško Mitrović and Nevenka Piskač Živković. I would also like to thank to the Department of Clinical immunology and rheumatology University Hospital Centre Zagreb, Croatia.

MeSH/Keywords: systemic sclerosis, interstitial lung disease, ultrasound, diaphragm

Poster code: R-02-09-042

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

PhD candidate: Ivan Prepolec

Part of the thesis: Intra-procedural three-dimensional rotational angiography in cryoballoon ablation for 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: We designed a single-centre unblinded randomised clinical trial and recruited 101 patients (65.3% male, 59.3±11.5 years) with paroxysmal (92%) and early-persistent AF. Patients were randomized 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 being collected.

Results: Of all the patients recruited, 48 (47%) underwent 3DRA. In patients who received no imaging (non-3DRA group) one PV couldnt be isolated while in 3DRA group isolation of one PV could not be achieved in 3 patients (OR 3.47, 95% CI 0.35-34.51). Procedure time was significantly longer when 3DRA was performed (87.4±27.7 min compared to 69.9±23.0 in non-3DRA group, $p<0.001$). Total radiation dose (148.2±177.7 vs 387.7±203.8 mGy, $p<0.001$) and contrast administration (42.5±27.1 vs 133.8±26.8 ml, $p<0.001$) were significantly lower in the control group. Only minor complications were reported in both groups. Three patients developed large haematoma (2 in 3DRA group vs 1 in control group). In each group there was one incident of transient phrenic nerve palsy.

Discussion: 3DRA is a safe and efficient intra-procedural imaging method to guide CB ablation for AF. However, it significantly increases procedure duration, total radiation dose and contrast expenditure. In our trial it did not have any impact on the acute success rate of PV isolation. One-year follow up data is still being collected and will subsequently be presented.

MeSH/Keywords: atrial fibrillation, catheter ablation, angiography, cardiac imaging techniques

Poster code: R-02-09-043

Poster Title: The role of large blood vessel stiffness in cardiovascular risk assessment in patients with prehypertension and untreated hypertension

PhD candidate: Danilo Radunović

Part of the thesis: Estimated pulse wave velocity and cardiovascular risk in patients with prehypertension and untreated hypertension

Mentor(s): Academician Bojan Jelaković

Affiliation: University of Zagreb School of Medicine

Introduction: Introduction: Patients with prehypertension are a heterogeneous group in which not all individuals have the same cardiovascular (CV) risk, so attempts are being made to determine predictors that would allow additional classification within that group. Stiffness of large arteries, ie its measure used in clinical work, pulse wave velocity (PWV), is included in the guidelines as a useful predictor of clinical course. Some authors suggest its use in the classification of cardiovascular (CV) risk, which is especially important in patients with prehypertension (PHT) and patients with untreated hypertension. Since direct measurement is usually not possible to organize in regular work, the equation for estimating PWV (ePWV) has been validated and its role needs to be confirmed in various populations. Aim of the study is to determine the predictability of ePWV for general and cardiovascular mortality in the general population, and in groups of subjects with prehypertension and stage 1 arterial hypertension, and to analyze the strength of predictability in relation to traditional cardiovascular risk factors.

Materials and methods: Respondents from three large cohorts from three different projects using the same methodology will be included in this study. The first cohort consists of subjects who were followed for an average of 20 years (randomized representative sample of the adult population of Croatia), and the second cohort consists of subjects who were followed for an average of 13 years (randomized sample of the adult rural population of the continental part of Croatia). In the longitudinal part of the research, based on the analysis of these two groups, we will obtain data on the independent predictive value of ePWV for general and CV mortality in the general population, and in groups of patients with prehypertension and patients with AH in stage 1. The third group consists of subjects included from randomized representative sample of the adult population of Croatia. We will use the data of this third group in the cross-sectional part of the study in which we will analyze the association of ePWV with subclinical target organ damage (HLK- left ventricular hypertrophy, albuminuria, eGFR- estimated glomerular filtration) and with traditional CV factors (cardiovascular) risk in the general population. Laboratory analyses in all cohorts were done in one and the same laboratory using the same devices and methods. The estimated pulse wave velocity (ePWV) will be calculated using a validated equation. Statistical data processing will be done using SPSS statistical software.

Results: Preliminary results suggest that increased stiffness of large arteries, ie accelerated PWV increases the risk of left ventricular hypertrophy, myocardial ischemia, albuminuria, impaired renal and brain function. Estimated PWV (ePWV) shows a significant association with measured PWV (cfPWV) in patients with present cardiovascular risk factors. Estimated PWV also predicts cardiovascular mortality, non-fatal myocardial infarction and stroke, and hospitalization for ischemic heart disease, regardless of tradiotional risk factors. In addition, in various groups of subjects, and especially in patients with untreated arterial hypertension, estimated PWV showed a higher predictive value compared to traditional factors used in cardiovascular risk assessment.

Discussion: The estimated pulse wave velocity calculated by the validated equation is an independent predictor of total and cardiovascular mortality in patients with prehypertension and patients with untreated hypertension in stage 1 of arterial hypertension. The results of this study will contribute to the scientific discussion of the prognostic value of the estimated stiffness of large blood vessels for total and cardiovascular mortality. Results can affect the recommendations for the use of this method in everyday clinical work, especially when deciding to start drug treatment in patients with prehypertension.

MeSH/Keywords: large blood vessel stiffness, estimated pulse wave velocity, cardiovascular mortality, arterial hypertension, prehypertension

Poster code: R-02-09-048

Poster Title: Presepsin and glycoprotein YKL-40 in the early diagnosis of sepsis during the pre-engraftment phase of allogeneic haematopoietic stem cell transplantation - preliminary results

PhD candidate: Jakša Babel

Part of the thesis: Presepsin and glycoprotein YKL-40 in the early diagnosis of sepsis during the pre-engraftment phase of allogeneic haematopoietic stem cell transplantation

Mentor(s): Assistant Professor Nadira Duraković, MD PhD, Professor Dunja Rogić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Early diagnosis of sepsis after allogeneic hematopoietic stem cell transplantation (alloHSCT) is crucial because timely initiation of antibiotic treatment reduces mortality in such patients. Therefore, there is a need to find more accurate biomarkers of sepsis to assist clinicians in their daily practice.

Materials and methods: In this prospective, observational study we included consecutively all febrile patients, 18 years of age and older with signed informed consent, during the pre-engraftment phase of alloHSCT treatment. We analysed 17 consecutive febrile episodes (FE). Day of engraftment is defined as the first of 3 consecutive days with an absolute neutrophil granulocyte count 0.5 × 109 / L. (1) Fever is defined as axillary body temperature 38 ° C measured twice in a 1 h interval or one measurement 38.5 ° C. (2) Febrile episodes were stratified into two groups, sepsis and non-sepsis, according to the Sepsis-3 International Conference definition. (3) FE stratification was performed prospectively based on clinical, laboratory, radiological, and microbiological results (blinded to presepsin and YKL-40 values) with final classification at the end of FE. Serum presepsin and YKL- 40 concentrations were determined by the sandwich ELISA technique at the beginning of each febrile episode and every 48 hours during the first 7 days.

Results: We preliminarily analysed 17 febrile episodes (FE) in a total of 17 patients, of which 9 (53%) were men and 8 (47%) were women. The most common indication for alloHSCT was acute leukaemia in a total of 10 (59%) patients. The mean age of the patients was 49.8 years (SD 10.6). Out of 17 febrile episodes (FE) analysed, 4 FE (23.5%) were classified as septic and 13 (76.5%) as non-septic. One patient died due to septic shock on day 2. In the group of patients with sepsis YKL-40, and presepsin levels were higher compared to the patients without sepsis. The medians with interquartile ranges of YKL-40 (ng/L) on day 1, day 3, day 5, day 7 were 161.3 (140.2 207.1), 129.8 (99.9 142.0), 100.3 (92.15 169.3) and 121.8 (91.8 172.5) in septic patients. The respective values in patients without sepsis were 74.4 (46.2 - 88.3), 79.8 (43.3 - 110.4), 52.9 (35.3 71.9) and 55.8 (21.3 71.3), with P-values 0.004, 0.296, 0.057 and 0.039. The medians with interquartile ranges of presepsin (ng/L) on day 1, day 3, day 5, day 7 were 19.27 (17.96 20.06), 6.85 (6.64 9.86), 3.59 (3.01 - 3.73) and 2.08 (1.81 2.15) for septic patients and 3.23 (1.70 8.95), 2.48 (1.78 6.85), 1.43 (1.20 3.00), 1.03 (0.59 1.74) for non septic once respectively with P-values for differences 0.044, 0.179, 0.225 and 0.139.

Discussion: Faster and more accurate diagnostic tools are needed in clinical practice to identify febrile patients who are at increased risk of developing septic complications after alloHSCT. Our preliminary results show that YKL-40 and presepsin levels were increased in septic patients compared to non-septic patients during the first 7 days upon onset of a febrile episode, although a statistical significance was achieved for presepsin at day 1 and YKL-40 at days 1 and 7. Compared to previous studies of YKL-40 in infectious conditions, the measured concentrations in our subjects are slightly lower than reported in the literature, so for a more accurate conclusion about the value of YKL-40, it is necessary to analyse a larger number of samples. Presepsin concentrations in our patients are comparable to previous literature reports. Also, our results show that both YKL-40 and presepsin levels peaked 24 hours upon onset of a febrile episode in patients who developed sepsis which could make them more suitable for early diagnosis than usual biomarkers like CRP which levels peak during the first 48-72 h (4).

MeSH/Keywords: Bone Marrow Transplantation, Hematopoietic Stem Cell Transplantation, Sepsis, Chitinase-3-Like Protein 1, Presepsin, Biomarkers

Poster code: R-02-09-069

Poster Title: Impact of ibrutinib on expression of adhesion molecules, chemokine receptors and distribution of B clone in patients with B-cell chronic lymphocytic leukemia

PhD candidate: Marija Ivić

Part of the thesis: Impact of ibrutinib on expression of adhesion molecules, chemokine receptors and distribution of B clone in patients with B-cell chronic lymphocytic leukemia

Mentor(s): Associate Professor Ozren Jakšić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: B-cell chronic lymphocytic leukemia (B-CLL) is a lymphoproliferative disorder characterized by circulation of B lymphocytes between bone marrow, peripheral blood, and lymphoid organs. Accumulation of lymphocytes in certain organs is manifested by tumor distribution. Homing, proliferation, and apoptosis of lymphocytes is a consequence of the interaction with tumor microenvironment. Ibrutinib in addition to inhibiting Bruton's tyrosine kinase also has significant interactions with the microenvironment. Prior to reducing tumor mass ibrutinib may induce a significant redistribution of B lymphocytes from lymphoid organs to the peripheral blood. The aims of this study are to assess tumor mass and tumor distribution at the beginning and during the treatment with ibrutinib and to examine the association of tumor distribution with changes in the expression of chemokine receptors and adhesion molecules on neoplastic B lymphocytes in patients with B-CLL.

Materials and methods: Patients with B chronic lymphocitic leukemia received ibrutinib are included in this prospective, longitudinal, observational study. Inclusion criteria are indications for treatment according to the International working group for CLL. Exclusion criterion is active chemotherapy treatment of other malignant disease. Patients will be monitored for six months after starting of ibrutinib treatment and time points are beginning of the treatment, third and six months. At every time points for every patient will be determined total tumor mass (TTM1) and tumor distribution (TD2). CT will be performed at the beginning of treatment and in six months for every patient. Expression levels of surface molecules CXCR4, CXCR5, CD38 and CD49d will be determined by flow cytometry on malignant lymphocytes. The results will be quantified as the percentage of positive cells and medium fluorescence intensity ("Medium fluorescence intensity" - MFI) 1 TTM = TM1 + TM2 + TM3; TD=TTM/TM1; TM1= square root of the number of peripheral blood lymphocytes per nl; TM2 = the diameter of the largest lymph node according to the CT in cm; TM3 = vertical length of spleen according to the CT 13 cm

Results: We evaluated 15 patients (six female, nine male). Decrease in TTM is observed among 14 patients and in one patient TTM has increased after six months of ibrutinib therapy. In 13 patients (86%) complete remission has been achieved and there was no visible lymph node enlargement or splenomegaly on CT after six months of therapy. Median of TD at the beginning of the treatment was 0,35 (range 0,09-0,86) and 0,92 (range 0,7-1) at the end of sixth months of treatment. Imunophenotype analysis was performed at the beginning of the treatment for the molecule CXCR4 in peripheral blood (PB), lymph node (LN) and bone marrow (BM). Median MFI of CXCR4 in PB was 9466 (range 378817365), 7474 (range 2283-1586) in LN, and 4591 (range 2395-18350) in BM. Median MFI of CXCR4 in peripheral blood after three months of treatment was 11389 (range 614519350). The analysis MFI of molecules CXCR5, CD38 and CD49 is currently ongoing. Statistical analysis and preliminary results expected to be finalized over the next year.

Discussion: Ibrutinib induces the tumor distribution of malignant lymphocytes from lymphoid organs and bone marrow to the peripheral blood in the first months of the treatment. Tumor distribution has been insufficiently investigated but the possible mechanism is interaction of ibrutinib and tumor microenvironment. Interaction of surface molecules CXCR4, CXCR5, CD38, CD49d with chemokines and adhesion molecules play an important role in the disease pathogenesis. Investigation of the impact of ibrutinib on the expression of each molecule can contribute to understanding the pathophysiology of the disease, drug resistance and reveal a new treatment strategy.

MeSH/Keywords: B chronic lymphocytic leukemia, tumor distribution, ibrutinib, microenvironment, immunophenotyping

Poster code: R-02-09-075

Poster Title: The concentration of chitinase 3 like 1 protein (YKL 40) in serum of patients with acute pancreatitis as a potential novel biomarker of disease severity

PhD candidate: Nina Blažević

Part of the thesis: The concentration of chitinase 3 like 1 protein (YKL 40) in serum of patients with acute pancreatitis as a potential novel biomarker of disease severity

Mentor(s): Assistant Professor Tajana Pavić, MD PhD, Professor Dunja Rogić, MD PhD

Affiliation: University of Zagreb School of Medicine; University Hospital Center Sestre Milosrdnice, Department of Gastroenterology and hepatology; Clinical Hospital Center Zagreb, Department of Laboratory Diagnostics

Introduction: Acute pancreatitis (AP) is an acute disease of the pancreas with multiple etiologies and high mortality in severe cases. The severity of the disease is defined by the presence of organ failure and local complications. The management of patients with AP is complicated due to the difficult assessment of the severity of the disease on admission. There are several serum indicators of the disease severity (C-reactive protein (CRP), procalcitonin (PCT)), but none of them are accurate. YKL 40 (chitinase 3 like 1 protein) is a glycoprotein that plays a mayor role in inflammation and angiogenesis. The aim of this study is to determine the diagnostic accuracy of YKL 40 in the evaluation of AP severity that includes pancreatic necrosis or peripancreatic collections (PC).

Materials and methods: In this prospective study, we recruited 98 patients with AP who met rigorous inclusion and exclusion criteria at the University Hospital Center Sestre Milosrdnice from June 2020 until April 2022. After obtaining informed consent, patients had blood drawn for routine laboratory studies (including CRP and PCT) with additional 7 milliliters of venous blood for determining serum concentrations of YKL 40 on admission, 48 hours and 7 days after admission. Serum for YKL 40 concentration measurement was separated by centrifugation within 3 hours of collection, stored and transported to the Department of Laboratory Diagnostics at the Clinical Hospital Center Zagreb until thawed for analysis using ELISA assays. Revised Atlanta classification was used to determine the severity of the disease: mild, moderate or severe form. Abdominal ultrasound (US) was perfomed in all patients within 24h of admission. In those patients with clinically mild disease, US was repeated after 48 hours, and in all other patients abdominal MSCT was performed.

Results: Among all of the enrolled patients (N=98), we performed preliminary statistical analysis on 32 patients (10 male patients, 22 female patients). Chi square and Fisher exact test were used to compare discrete, and Mann Whitney U for continuous variables. Receiver operating characteristic curve was calculated to assess diagnostic ability of YKL 40 and CRP for severe form AP, pancreatic necrosis, and PC. Areas under the curve (AUC) were compared using the bootstrap method. Mean age was 64 SD 17 years (range between 35 and 87 years). 22 patients (0,69) had mild, 7 patients (0,22) had moderate and 3 patients (0,09) had severe form of the disease. Etiology of AP was biliary in 25 (0,78), alcoholic in 3 (0,13) and undetermined in 4 (0,09) cases. We have noticed more biliary AP in women (0,86 vs. 0,60) and more alcoholic AP in men (0,30 vs. 0,00), P 0.03. Men had more severe forms of the disease (0,30 vs. 0,00, P 0,01), possibly due to alcoholic etiology of the disease. 1 patient with mild form and 2 patients with severe form of the disease had necrosis of the pancreatic parenchyma (detected with MSCT imaging). Both YKL 40 and CRP are moderately good predictors for AP severity, with CRP being better predictor (AUC for YKL 40 0,67 CI 0,95 [between 0.47 and 0.80]; 0,84 CI 0,95 [between 0.47 and 0.80] and 0,25 CI 0,95 [between 0.0 and 0.52]; AUC for CRP 0,76 CI 0,95 [between 0.39 and 1.0]; 0,94 CI 0,95 [between 0.78 and 1.0] and 0,94 CI 0,95 [between 0.88 and 1.0] for the first, second and third measurement). In the context of pancreatic necrosis and PC, both YKL-40 and CRP are moderately good predictors, with YKL-40 being better predictor.

Discussion: According to our results, taking into consideration small sample size and consequently inadequate study power, further measurements are needed to determine whether YKL-40 and CRP alone or in a combination can predict severity of AP that involves necrosis or PC. We have shown that YKL-40 may me a good predictor for pancreatis necrosis and PC, which is reasonably explained by it's patophysiologic role in the tissue remodelling.

MeSH/Keywords: Acute pancreatitis, disease severity, YKL 40, pancreatic necrosis, peripancreatic collections

Poster code: R-02-09-086

Poster Title: Arteriosclerosis determined by kidney biopsy in comparison with signs of arteriosclerosis in other parts of the body

PhD candidate: Dino Kasumović

Part of the thesis: Comparison of arteriosclerosis of kidney arteries determined by kidney biopsy with signs of arteriosclerosis in other parts of the body

Mentor(s): Professor Krešimir Galešić, MD PhD, Professor Danica Galešić Ljubanović, MD PhD

Affiliation: University of Zagreb School of Medicine; Clinical Hospital Dubrava, Zagreb

Introduction: Changes of the arteries in the form of arteriosclerosis are directly related to certain cardiovascular (CV) risk factors and they are associated with certain CV diseases. Arterial changes in some parts of the body can be detected indirectly by using noninvasive diagnostic methods, such as Doppler ultrasound. They can also be directly detected pathohistologically, as in case of kidney biopsy. The aim of this study was to show how these changes correlate, depending on CV risk factors.

Materials and methods: Examinees were hospitalized patients in Clinical Hospital Dubrava, Zagreb, in whom a kidney biopsy was indicated and performed and so pathohistological diagnostics were made. For the analysis, the patients were divided in two main groups according to their CV risk level - low and the other moderate to very high, which was assessed by European Society of Cardiology SCORE 2 for high risk regions. Two arteriosclerotic changes were inspected in kidney biopsy specimens - fibromuscular intimal hyperplasia of the arteries and hyalinosis of the arterioles. They were categorized by their severity in two groups no changes or mild ones, and moderate or severe changes. Methods and parameters that were used to detect changes noninvasively in other arteries of the body were: increased intima-media thickness (> 0,9 mm) and/or presence of plaques in carotid arteries measured by Doppler ultrasound, mid low or too high ankle-brachial index (1,00 or >1,4) measured by Doppler ultrasound, increased aortic pulse wave velocity measured by oscillometric method and hypertensive and arterioslerotic changes of the retina inspected by ophthalmoscope. Presence of at least two out of four of these parameters was considered significant. The statistic analysis is descriptive in this stage of study.

Results: Total of 32 patients, 16 males and 16 females, participated. In the group with low CV risk and no or mild changes in kidney arteries there were 9 patients 77,7 % of them not having any vascular changes detected noninvasively. In the group with low CV risk and moderate or severe changes in kidney arteries there were 3 patients and two of them had two vascular changes detected noninvasively. In the group with higher CV risk and no or mild changes in kidney arteries there were 6 patients - 50 % of them having only one and 50 % of them two or three vascular changes detected noninvasively. Finally, in the group with higher CV risk and moderate or severe changes in kidney arteries there were 14 patients 71,4 % of them having two or three vascular changes detected noninvasively.

Discussion: The number of participants is still low (the main reason is two years of COVID pandemic that temporarily stopped the investigation). Nevertheless, in the group with low CV risk and milder changes in kidney arteries, the majority of patients were without any vascular changes measured noninvasively. On the other hand, in the group with higher CV risk and more severe changes in kidney arteries, the majority of patients had changes in at least two out of four vascular regions in the body. That is supporting the theory that the severity of arteriosclerosis in kidney arteries goes in hand with the presence of changes in vascular regions in other parts of the body measured noninvasively, considering the overall CV risk. Further analysis is to be done, like dependence of arterial changes in kidney from possible chronic kidney disease, which will be assessed by Sethi score, or the influence of specific CV risk factors on the overall pathology.

MeSH/Keywords: cardiovascular risk, arterial, vascular, change, kidney

Poster code: R-02-09-089

Poster Title: Effect of preventive extracorporeal photopheresis on early development of cardiac graft vasculopathy

PhD candidate: Mia Dubravčić

Part of the thesis: Effect of preventive extracorporeal photopheresis on early development of cardiac graft vasculopathy

Mentor(s): Associate Professor Boško Skorić, MD PhD

Affiliation: University of Zagreb School of Medicine; University hospital centre Zagreb

Introduction: Heart transplantation (HTx) is gold standard in terminal heart failure treatment. Main cause of death in late post-transplant period is cardiac allograft vasculopathy (CAV) with high incidence and minimal incidence reduction in last two decades. Early sign of CAV is coronary artery intimal thickening that can be determined by intracoronary imaging techniques (e.g. intravascular ultrasound IVUS, or optical coherence tomography - OCT). Extracorporeal photopheresis (ECP) is an immunomodulatory procedure that reduces graft rejection and possibly CAV. The aim of our study is to determine effect of extracorporeal photopheresis on coronary artery intimal thickening using optical coherent tomography and on graft rejection in first 12 months after heart transplantation.

Materials and methods: Our study is randomized controlled clinical trial in duration of 12 months. We plan to enroll patients after heart transplant who will be randomized in two arms intervention arm that will undergo 10 cycles of ECP in the first year after HTx in addition to standard immunosuppressive drug therapy, and a control arm that will receive only standard immunosuppressive drug therapy. We will perform two optical coherence tomographies (in first three months and 12 months after heart transplantation) to determine difference in intimal thickness and six myocardial biopsies to determine humoral/cellular rejection.

Results: From February 2018 till November 2021 we have enrolled 39 patients in our study, 32 male (82,1%). Fifteen patients (38,5%) were randomized to intervention arm. Out of those, 4 patients did not receive all 10 cycles of ECP due to various reasons (death, Covid-19 outbreak, renal insufficiency, severe myocarditis). Five patients died and did not finish the study, three in control arm (12,5%) and two in intervention arm (13,3%). Other primary and secondary outcomes are still being studied and are not yet available for further analysis.

Discussion: This is the first study to evaluate preventive effect of extracorporeal photopheresis on cardiac allograft vasculopathy compared to standard modern immunosuppressive drug therapy only, using optical coherence tomography. Future results may change the current clinical practice in allograft vasculopathy early diagnosis and prevention.

MeSH/Keywords: heart transplantation, extracorporeal photopheresis, graft rejection, vasculopathy

Poster code: R-02-09-090

Poster Title: Disease activity at the time of biopsy is not associated with higher risk of serious infections in patients with 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: Systematic lupus erythematosus (SLE) and lupus nephritis (LN) are associated with a higher frequency of serious infections compared to the general population which are in turn associated with adverse outcomes, morbidity and mortality. Very few studies have explored risk factors for infections in LN and there are limited data on the association of disease activity and serious infections in LN.

Materials and methods: We have conducted a retrospective cohort study to evaluate the prognostic significance of disease activity for serious infections in LN. Disease activity was assessed using the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K). Serious infections were defined as those that: (a) require intravenous therapy or (b) lead to hospitalization or (c) have resulted in death within 30 days from diagnosis.

Results: A total of 51 patients with biopsy-proven LN were followed up for 4.5±2.9 years (80% women, mean age at biopsy 38±14). Of these, 22 (43%) had at least one episode of serious infection with 4 patients having 2 episodes for an incidence of 5.7 infections per year of follow-up. Most common sites of infection were pneumonia (N=6), urinary tract infections (N=5), gastrointestinal infections (N=4) and skin infections (N=2). Five patients had sepsis with one progressing to septic shock, whereas two patients died. Disease activity was higher at the time of biopsy compared to the time of infection/up to one month prior to infection (15.4±6.3 vs. 11.3±5.5, p=0.001). When comparing patients who had at least one infection vs. those who did not have an infection, there was no difference in disease activity at the time of biopsy (18.0±1.0 vs. 15.5±6.5, p=0.36). SLEDAI-2K activity index at the time of biopsy was not an independent predictor of serious infection (OR 0.88 [0.72, 1.08]).

Discussion: Serious infections are common in LN with nearly half of the patients having at least one episode. SLEDAI-2K at the time of biopsy has not been shown to be an independent predictor of serious infections in our cohort of patients with LN.

MeSH/Keywords: Lupus nephritis, Infections, Disease activity

Poster code: R-02-09-107

Poster Title: The effect of dietary supplements for pregnancy on levothyroxine absorption

PhD candidate: Tomo Lucijanić

Part of the thesis: The effect of dietary supplements for pregnancy on levothyroxine absorption

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

Affiliation: University of Zagreb School of Medicine

Introduction: Hypothyroidism is a common disease that is successfully treated with the replacement of thyroid hormones in the form of levothyroxine. Levothyroxine is drug used in the first line of substitution therapy because it appropriately mimics physiological secretion of thyroxine (T4) and its peripheral conversion to triiodothyronine (T3). However, due to its narrow therapeutic index, and because it is administered at very low doses (g), it is particularly sensitive to the absorption phase interactions, which are due to its narrow therapeutic index, mainly clinically significant. In pregnancy, one of the goals is to maintain the values of thyroid stimulating hormone (TSH) in the lower part of the reference interval (below 2,5 mIU/L) for a proper child development. Also, due to increased nutritional needs during pregnancy, nutritional supplements are commonly used by pregnant women on their own initiative, but also often recommended by healthcare personnel. Reviewing the literature, we did not find high quality evidence to suggest the existence or to refute the interaction between dietary supplements for pregnant women and the absorption of levothyroxine. The aim of this study is to investigate this potential interaction between dietary supplements used in pregnancy and levothyroxine absorption in order to test the safety of the use of these preparations in pregnant women who are on replacement therapy with levothyroxine.

Materials and methods: This is a prospective randomized, double-blind, placebo-controlled clinical trial. The study would include subjects diagnosed with primary hypothyroidism over the age of 18 and on stable LT4 therapy for at least 6 months. Although the study is intended to examine dietary supplements for pregnant women, the said population will be excluded from the study for safety reasons. Given that the mechanism of the vast majority of LT4 interactions is based on the adsorption of levothyroxine on the interfering substance, we believe that potential differences in LT4 absorption between pregnant women and the study population should not affect the clinical interpretation of this study. According to the calculated required sample size, for 90% strength of the study, at least 37 patients will need to be included to determine clinically significant interference of these preparations with LT4 absorption. After inclusion, patients will be randomized into two groups at a ratio of 1: 1 - one group will take the selected dietary supplement for pregnant women for 8 weeks at the same time as LT4 which will be taken according to the recommended method in the Summary of Product Characteristics-SmPC with a glass of water), while the second tablet of the preparation intended for pregnant women will be taken in the evening - 12 hours after the morning dose. The second group of respondents will take a placebo tablet whose composition will be based on starch. The selected dietary supplement for pregnant women is Prenatal nutrients from Solgar. The preparation was selected from several other preparations based on the widespread use in the pregnant population of our region and beyond.

Results: Six subjects have been recruited. Of these, two completed the study protocol. Since the study is double-blind, we cannot know in what order they took the study supplement or placebo. In both patients at the first control there was an increase in TSH by 1.5 and 1.8 mIU / L, respectively. In both patients, TSH did not exceed the reference values, and by the last control it had fallen again by 1.3 and 1.9 mIU / L, respectively. The other 4 subjects expect the first control (2 months after inclusion in the study).

Discussion: As the earlier studies have shown, the absorbtion of levothyroxine is diminished when a person takes it with calcium or iron preparations. The study is double blind, so at this phase we can not know if the 2 subjects that finished the study protocol took the vitamin and mineral preparation at the time before the TSH has risen. If that is the case, it would confirm the earlier studies. I am eager to aquire new data from addiotional subjects to see the effects of the food supplements on levothyroxine absorption.

MeSH/Keywords: Hypothyroidism, Pregnancy, Levothyroxine, Dietary supplements

Poster code: R-02-09-120

Poster Title: Impact of previous chronic statin therapy on mortality in patients with acute myocardial infarction treated with primary percutaneous coronary intervention

PhD candidate: Nikola Kos

Part of the thesis: Impact of previous chronic statin therapy on mortality in patients with acute myocardial infarction treated with primary percutaneous coronary intervention

Mentor(s): Professor Diana Delić Brkljačić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Cardiovascular (CV) diseases, especially coronary disease, are the leading cause of mortality in developed countries and represent a significant burden on the health system. Initiation of statin therapy in the acute phase of myocardial infarction has been shown to reduce the risk of CV death, recurrent nonfatal MI, stroke, and the need for coronary revascularization. The role of statins in primary and secondary prevention of AIM is unquestionable. No randomized study to date has examined whether long-term statin therapy prior to acute myocardial infarction alone has had an impact on a more favorable long-term outcome than those not previously treated with statins before AMI. It is unclear whether prior chronic statin therapy has an impact on mortality in patients with acute myocardial infarction.

Materials and methods: The aim of the study was to determine whether previous statin therapy affects the outcomes of patients treated with primary PCI for AMI. A prospective, observational, non-intervetional cohort study will be conducted based on a case-series study. The expected number of patients that will be included is 1000. The study will include patients older than 18 years, treated for the first AMI with PCI at the Clinic for Cardiovascular Diseases in the period between 1.6.2011. and 1.1.2019. Patients will be monitored clinically for at least 12 months, and no later than June 1, 2021. Patients will be divided into two groups: group of patients who were chronically treated with a statin prior to AMI and the other group of patients who were not chronically treated with a statin prior to AMI. The register of the Diagnostic and Therapeutic Department for Invasive and Interventional Cardiology UHC Sestre milosrdnice, will be used as a database. From the mentioned register, patients will be included in the research. The case histories of the patients involved will be used as an additional source of data. Mortality data will be collected from available medical histories and from the database of the Registry of Deaths. In addition to general anamnestic and demographic data of patients, data on acute myocardial infarction, pharmacotherapy before and after the index event, echocardiographic parameters and laboratory findings. After division into the previously mentioned groups and subgroups, the previously mentioned outcomes will be observed and compared in patients.

Results: During the past year, further systematic review of the literature was conducted and further research on this topic has not been published. A systematic search of the above mentioned register was conducted, and so far a total of 670 patients have been included in thestudy. Of these, 230 patients was taking statins before myocardial infarction as a part of chronic therapy. Also, it was observed that despite statin intake, only 45% of patients' LDL values were adequate during admission (suggesting poorly regulated dyslipidiema in primary prevention). Atorvastatin (55%), rosuvastatin (32%) and simvastatin (13%) were predominant. Additional hypolipidemics (such as ezetimibe) were used in only 4% of patients. Modern drugs (PCSK9i) have not been used. Of all patients taking statins, only 35% used a moderately potent or very potent dose of a statin. Based on the above preliminary analysis, a statistically significant separation of the Kaplan-Mayer curve was observed between patients who were treated with a statin before acute myocardial infarction and those who were not. The outcome did not depend on the statin dose. Completion of patient involvement as well as completion of statistical analysis is estimated over the next year.

Discussion: The preliminary results are going forward hypothesis confirmation. Over the next year, it is planned to complete the inclusion of patients and statistical analysis of data.

MeSH/Keywords: acute myocardial infarction, statin, dyslipidemia, percutaneous coronary intervention

Poster code: R-02-09-167

Poster Title: Validation of equations for the renal function assessment in the obese population in relation to the Salazar-Corcoran equation and scintigraphic measurement of glomerular filtration

PhD candidate: Lana Gellineo

Part of the thesis: Validation of equations for the renal function assessment in the obese population in relation to the Salazar-Corcoran equation and scintigraphic measurement of glomerular filtration

Mentor(s): Assistant Professor Živka Dika, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Kidney function is usually assessed with the GFR CKD EPI formula, and there are also other formulas as MDRD, cystatin C and BIS2 for kidney function assessment in other subpopulations, but the gold standard is the use of radioisotope markers (ie 99mTc DTPA, iothalamate). For overweight patients there is currently no validated formula that could precisely assess kidney function the Salazar-Corcoran formula is in use but it needs to be validated. In the population of overweight patients the Salazar Corcoran formula is more precise for renal function determination than the CKD EPI formula or the de-indexed formulas. The aim is to validate the existing Salazar-Corcoran formula for overweight patients in comparison to the gold standard (radioisotope markers ie 99mTc DTPA, 125I iothalamate), and then to compare it to other formulas (GFR CKD EPI, cystatin C, MDRD, Cockroft Gault).

Materials and methods: There will be 100 patients included in the study with the BMI greater than 30 kg/m2, acquired from the Endocrinology and Nephrology Day care hospital units. Blood samples will be drawn and the radioisotope method in kidney scintigraphy will be used and the other renal function formulas calculated. 24 hour urine will be collected, kidney ultrasound performed. Statistical analysis will be done in SPSS Statistics.

Results: Preliminary results show that measured GFR correlates more with the Salazar-Corcoran formula and with the deindexed formulas than with the KDIGO recommended CKD-EPI formula in the obese population. The patient population is still too small to make subanalysis according to CKD stage and BMI subgroup.

Discussion: The results so far show a significant difference in eGFR when taking into account differences in obese population. Expected contribution of this study is accurate assessment of kidney function in this specific population group - the overweight patient, which will lead to better renal dosing of medication (thus avoiding subdosing), and also, better renal function assessment in preparing a patient for radiologic imaging with contrast and correct assessment of the right time to start dialysis treatment. Also, this accurate GFR estimation is important for accurate cardiovascular risk determination.

Acknowledgments: I thank professor Bojan Jelaković and my menthor dr. Živka Dika for guidance in my research.

MeSH/Keywords: obesity, kidney function, glomerular filtration, Salazar-Corcoran

Poster code: R-02-09-169

Poster Title: Relationship between hair transplantation and biopsychosocial determinants of health

PhD candidate: Ana Maletić

Part of the thesis: Relationship between hair transplantation and biopsychosocial determinants of health

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

Affiliation: University of Zagreb School of Medicine

Introduction: Androgenic alopecia is defined as a partial or total hair loss that affects between 50% and 80% of men. Previously published studies have proven an adverse effect of hair loss on self-image, which can lead to problems in personal and professional life. Androgenic alopecia can be prevented and delayed by a conservative treatment. However, significant restoration can only be achieved by hair transplantation, which is nowadays the most frequent aesthetic surgical procedure in men. The follicular unit extraction (FUE) method is the most advanced approach to hair transplantation. Our study aimed to examine the quality of life and psychosocial functioning of patients after FUE.

Materials and methods: Study is carried out in the Dr Maleti Polyclinic in Zagreb and Daruvar since May 2021, while the expected end of the study is December 2022. So far, more than 50 adult males were included in the study. Inclusion criteria are the presence of androgenic alopecia and informed consent for participation in the study. Enrolled patients fill out a questionnaire with personal data such as demographic data, clinical data, SF 36 self-reported measure of health questionnaire, Depression, Anxiety and Stress Scale (DASS-21), Diener subjective well-being scale, and Multidimensional Scale of Perceived Social Support (MSPSS). The questionnaires will be filled before hair transplantation and during control check-up one year after the surgery. So far, six patients filled questionnaire 1 year after FUE, therefore enabling preliminary estimation of patient satisfaction. Appropriate statistical software is used to determine the significance. The breakdown of the study subjects will be shown by descriptive statistics. Categorical variables will be shown as frequencies, along with percentages. T-test for dependent samples will be used to determine differences before and after the surgery. The 5% significance level will be applied.

Results: Among 52 patients enrolled in study six of them completed follow-up questionnaire, therefore enabling preliminary conclusion about satisfaction with quality of life after FUE. Preliminary results comprise data of six patients and calculated difference between two measured time points. SF-36 and DASS-21 questionnaires did not show any significant difference in the follow-up interval. Deiner scale revealed increased quality of life after FUE, detected by increased satisfaction for 41% and 33% shown by answers on 2 questions: My life is very close to what I consider as ideal and I am satisfied with my life. Based on preliminary findings, hair transplantation improves life quality and psychosocial status among male adults with androgenic alopecia.

Discussion: The research published to date fails to provide a definitive answer on how hair transplantation influences the quality of life. We expect to obtain verifiable insight into how hair transplantation influences the quality of life and psychosocial functioning of persons with androgenic alopecia. In this preliminary report we analysed perception of 6 patients that underwent FUE hair transplantation before the surgery and after one year. While SF-36 and DASS-21 questionnaires failed to prove satisfaction, while Deiner scale clearly revealed increased life quality after FUE. SF-36 is consisted of general questions and therefore not very specific questionnaire about physical and psychic condition of individual. Since FUE patients are mainly healthy and middle-aged individuals with good life conditions it was not expected that SF-36 will detect serious problems even before FUE. DASS-21 scale is created to detect even more worse conditions like depression and anxiety, therefore explaining lack of statistical significance before and after FUE. Further analyses of recruited patients after follow-up are mandatory to define expected increase in life quality after FUE.

MeSH/Keywords: alopecia, hair transplantation, FUE

Poster code: R-02-10-024

Poster Title: Relationship of immunohistochemical expression of MAGEA3 and CD86/CD163 positive intratumoral macrophages with prognosis of pancreatic ductal adenocarcinoma

PhD candidate: Goran Glavčić

Part of the thesis: Relationship of immunohistochemical expression of MAGEA3 and CD86/CD163 positive intratumoral macrophages with prognosis of pancreatic ductal adenocarcinoma

Mentor(s): Assistant Professor Mario Zovak, MD PhD, Petra Radulović, PhD, research associate

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

Introduction: Latest studies have shown an association between the expression of the MAGEA3 antibody as well as the number of TAM (tumor associated macrophages) with the prognosis and treatment outcomes of ductal pancreatic adenocarcinoma. The hypothesis is that the immunohistochemical expression of MAGEA3 and the number of CD163 positive M2 intratumoral macrophages is greater in ductal adenocarcinoma with a higher TNM stage, while the number of CD86 positive M2 macrophages and the M1/M2 intratumoral macrophage ratio is lower.

Materials and methods: A prospective research was conducted in which the archival materials of the Ljudevit Jurak Clinical department for pathology (UHC Sestre milosrdnice) were used together with all relevant patients clinical data obtained from BIS (Hospital information system) in 77 patients operated due pancreatic carcinoma. All the tissue blocks were analyzed to do a review and to estimate the presence of certain macrophages and antibodies. Descriptive and analytical statistical methods will be used in the statistical analysis of the results.

Results: After immunohistochemical analysis and data processing, all required information for 77 patients was collected. Statistical analysis of the results is underway. Images of the pathological analysis will be shown with special regard to differences in antibody expression and macrophage counts.

Discussion: Preliminary results of the analysis show support for the hypothesis. Further analytical tests will help reach a definitive conclusion. The TNM stage is still a very important prognostic factor as patients with higher grade disease were shown to have significantly shorter survival periods.

MeSH/Keywords: pancreatic adenocarcinoma, MAGEA3, M1 M2 macrophages, CD68, CD163, TAM

Poster code: R-02-10-063

Poster Title: The role of nEGFR and ABCG2 expression in malignant transformation of oral precancerous lesions

PhD candidate: Marko Tarle

Part of the thesis: Expression of epidermal growth factor receptor nuclei as a marker in premalignant and malignant changes of the oral cavity

Mentor(s): Associate Professor Ivica Lukšić, MD PhD, Assistant Professor Danko Müller, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Oral squamous cell carcinoma (OSCC) is usually diagnosed late and has a high mortality rate. It is imperative to prevent the onset of OSCC through prevention programs and to identify those patients with oral potentially malignant disorders (OPMD) who are at high risk for malignant transformation. The risk of malignant transformation depending on the degree of dysplasia is not reliable, and there is a need to identify molecular biomarkers in premalignant lesions that accurately indicate possible malignant transformation. The purpose of this study was to investigate the expression of nuclear EGFR (nEGFR) and stem cell marker ABCG2 in oral leukoplakia (OL) and oral erythroplakia (OE) and to assess their significance as prognostic biomarkers for malignant transformation.

Materials and methods: In this retrospective follow-up study, we included 50 patients with premalignant oral disorders, 31 with OL and 19 with OE, in whom we monitored the expression of nEGFR and ABCG2 in lesions with malignant transformation (MT) by immunohistochemical methods compared with lesions without malignant transformation (UT) over a follow-up period of 5 years and 3 months.

Results: 21 (42%) of 50 patients with OL and OE developed OSCC. We observed nEGFR expression in 8 of 29 (27.6%) patients with untransformed (UT) lesions and in 16 of 21 (76.2%) patients with malignant transformed (MT) lesions. ABCG2 expression was observed in 18 of 29 (62.1%) patients with UT lesions and in 16 of 21 (76.2%) patients with MT lesions. The malignant transformation was increased 12,84-fold (95% CI, 2.15 - 76.44, $p = 0.005$) in lesions expressing both ABCG2 and nEGFR. Expression of nEGFR is a strong indicator of MT (area under the curve, AUC = 0.77; $p < 0.001$, sensitivity, SE = 76.2%, specificity, SP = 72.4%), unlike ABCG2 expression (AUC = 0.62; $p = 0.15$, SE = 76.2%, SP = 41.4%), respectively.

Discussion: Determining the co-expression of the biomarkers nEGFR and ABCG2 in OL and OE may serve us to determine the risk of malignant transformation in OSCC.

MeSH/Keywords: oral leukoplakia, oral erythroplakia, oral cancer, nuclear EGFR, ABCG2

Poster code: R-02-10-070

Poster Title: Prognostic value of serum levels of interleukin-6 and interleukin-8 in dermal burn injuries among pediatric patients

PhD candidate: Rok Kralj

Part of the thesis: Prognostic value of serum levels of interleukin-6 and interleukin-8 in dermal burn injuries among pediatric patients

Mentor(s): Assistant Professor Rado Žic, MD PhD, research advisor, Associate Professor Stjepan Višnjić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: There are no objective methods that can assess depth of burn injuries in children apart from Laser doppler imaging. Studies in patients with severe burns have confirmed that interleukin 6 (IL-6) and interleukin 8 (IL-8) have a prognostic value in the prediction of sepsis and mortality. These two cytokines have also been isolated in significant amounts in burn blisters in dermal burns and it was confirmed that IL-8 is being synthetized in burned skin. We hypothesize that the relative decline of serum concentrations of IL-6 and IL-8 is negatively correlated to the length of the epithelialization of burn injuries.

Materials and methods: Measurement of serum concentrations of IL-6 i IL-8 and duration of epithelialization among children (between 0 an 18 years of age) with dermal burn wounds hospitalized in Children's Hospital Zagreb on the 3rd, 5th and 8th day after injury. The exclusion criteria are as follows: burn injury involving more then 20% of total body surface area, presence of areas with third degree burns, burn wound infection or infection from other foci, presence of a surgical condition unrelated to the burn injury or presence of a systemic disease which elevates serum concentrations of IL-6 and IL-8.

Results: We have collected samples of 23 patients who had injuries which fulfilled the eligibility criteria. Since we have arranged that we will measure serum concentrations of IL-6 and IL-8 once we collected samples of all of the planned 36 patients, we do not have any preliminary results.

Discussion: During the previous period we have struggled with the lack of hospital capacity for burn patients in Children's Hospital Zagreb because of the consequences of the earthquake and the fact that COVID patients took up a lot of beds intended for surgical patients. Consequently, some of the patients who were included in the study were treated on an outpatient basis rather than being admitted to the hospital. We believe that this has not jeopardized our results. Three patients who were included in the study were treated in hospitals outside Zagreb. That is because this patients were planned to be referred to Children's Hospital Zagreb but were not because of lack of capacity.

MeSH/Keywords: burns, pediatric, IL-6, IL-8, diagnosis

Poster code: R-02-10-158

Poster Title: The role of early physical therapy in postoperative hand function recovery in working-age patients with carpal tunnel syndrome

PhD candidate: Doroteja Caktaš

Part of the thesis: The role of early physical therapy in postoperative hand function recovery in working-age patients with carpal tunnel syndrome

Mentor(s): Assistant Professor Krešimir Martić, MD PhD, Frane Grubišić, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Carpal tunnel syndrome (CTS) is the most common entrapment neuropathy of the upper extremity. It is often a major cause of work incapacity, thus representing a significant portion of the socio-economic burden. Research on the effectiveness of postoperative physical therapy has shown a significant reduction in the recovery time of the hand function as well as return-to-work interval after applied physical therapy. As of yet, no standardized protocol for the use of physical therapy has been established.

Materials and methods: The study will include subjects with CTS divided into four groups: patients who received physical therapy (electrostimulation (ES) of the thenar muscles + kinesitherapy) on the 3rd and 14th postoperative day , and patients who will after proper education conduct kinesitherapy at home, also on the 3rd and 14th postoperative day. The recovery of hand function will be compared between these groups. Physical therapy will be conducted during 15 consecutive days. All subjects included will complete a standardized questionnaire for self-assessment of symptom severity and functional status, the Boston Carpal Tunnel Syndrome Questionnaire (BCTQ) as well as the functional status questionnaire - DASH (Disabilities of the Arm, Shoulder and Hand).

Results: We have obtained and translated Boston Carpal Tunnel Syndrome Questionnaire (BCTQ) as well as DASH functional status questionnaire. We have prepared tables for research data entry, as well as started to include patients in our research. A programe of physical therapy and kinesitherapy at home has been established . Up till now we have included 15 patients in our study.

Discussion: Due to Covid-19 pandemic conducting our research was very challenging. Since the situation with pandemic improved lately, we will be able to continue with our research and include more patient.

MeSH/Keywords: carpal tunnel syndrome, physical therapy

Poster code: R-02-10-165

Poster Title: Molecular characterization of human bocavirus detected in children with respiratory infection

PhD candidate: Maja Mijač

Part of the thesis: Epidemiological and clinical characteristics of acute respiratory infections in children's age with proved human bocavirus

Mentor(s): Professor Sunčanica Ljubin Sternak, MD PhD

Affiliation: University of Zagreb School of Medicine, Dr. Andrija Štampar Teaching Institute of Public Health

Introduction: Human bocavirus (HBoV) is relatively recently discovered parvovirus. HBoV 1 is associated with respiratory infections, while other three types (HBoV 2-4) are discovered in samples from gastrointestinal tract and their significance is yet to be established. HBoV is characterized by rapid evolution, both by mutation and recombination. Aim of this study is to investigate prevalence of HBoV genotypes and phylogeny of the circulating strains of HBoV in children hospitalized for acute viral respiratory infection.

Materials and methods: From May 2017 to March 2021, we investigated total of 957 patients younger than 18 years hospitalized for suspected viral respiratory tract infection from two Croatian hospitals: Children's Hospital Zagreb and General Hospital Karlovac, respectively. For respiratory virus detection, nasopharyngeal and pharyngeal flocked swabs from each patient were collected and investigated using multiplex PCR for 15 respiratory viruses. Samples positive for HBoV were analysed further with next generation sequencing (NGS). Viral DNA was re-extracted from positive samples and subjected to PCR amplification using Phusion polymerase (New Englands Biolabs), either by amplification of near-complete genome using primers B_fw and B_rev or combinations of primers generating overlapping fragments. After sequence analysis, phylogenetic analysis was performed. Nucleotide sequences of HBoV 1 strains were obtained from the GenBank and used to construct alignments and phylogenetic trees. Sequences belonging to other three viral genotypes (HBoV 2-4) were included in analysis. Evolutionary rate was estimated using MCMC approach implemented in Beast v1.8.2. Recombination events were assessed using Recombination Detection Program v4. Only full coding regions were considered for recombination analysis.

Results: In four-year period, total of 957 patients were screened for respiratory viruses and viral aetiology was proven in 739 (67.12%) patients. Human bocavirus was fifth most found virus, detected in 73 patients (7.63%), mostly in combination with other respiratory virus (codetection with other virus was present in 82.2 % samples). Out of 73 HBoV positive samples, 49 (67.1%) were available for further molecular analysis, of which 29 sequences could be successfully determined. Of those, amplification of nearly complete genome in one reaction was successful for five samples and others had to be amplified in three overlapping fragments. With this approach, we were able to sequence 24 samples and additional five samples contained two out of three genome segments. Only full coding regions were considered for estimation of rates of evolution. Calculated rates of evolution for HBoV1 were 10-4 and 10-5 substitutions per site and year. Recombination was not detected among sequences from this study.

Discussion: As expected, all sequenced samples belonged to HBoV 1 genotype, consistent with other studies showing HBoV 1 is primarily respiratory pathogen. Phylogenetic analysis showed that Croatian HBoV 1 sequences are closely related to strains isolated worldwide, there was no phylogenetic grouping based on mono- or coinfection cases or year of infection. The sequences are closely related, and no phylogenetic lineages could be distinguished with certainty. Although HBoVs are considered to be diverse and frequently recombinant pathogens, especially HBoV 2-4 which primarily replicate in gastrointestinal tract, recombination was not detected in the samples investigated in this study.

Acknowledgments: Work supported by Croatian Science Foundation project "New and neglected respiratory viruses in vulnerable groups of patients", [grant number IP-2016-06-7556 to S.LJ.S.] Special thanks to Anamarija Slović, Mirna Jurković and Dubravko Forčić for NGS.

MeSH/Keywords: human bocavirus, genotype, next generation sequencing, evolution, recombination

Poster code: R-02-16-078

Poster Title: The correlation between the presence of V600E mutation of BRAF gene and the response to treatment with iodine 131 in differentiated thyroid cancer patients with distant metastases

PhD candidate: Roko Granić

Part of the thesis: The correlation between the presence of BRAF V600E and TERT promotor mutation and the response to treatment with iodine 131 in differentiated thyroid cancer patients

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

Affiliation: University of Zagreb School of Medicine

Introduction: Thyroid cancer is one of the most common endocrine tumors and its high incidence puts a great strain on medical institutions especially in Croatia which is among top five European countries with the highest differentiated thyroid cancer (DTC) incidence. DTC metastasizes mostly to regional lymph nodes but distant metastases (M1) are also reported in 2-13 % of patients, mostly to lungs (papillary TC) and bones (follicular TC). These patients have the highest incidence of the recurrence of the disease and the highest percentage of radioiodine (RAI) refractory disease. Patients with high risk of the recurrence of the disease benefit the most from RAI (iodine 131) treatment. Despite recent scientific studies the presence of genetic mutations such as V600E mutation of BRAF gene in thyroid cancer and its influence on the disease course in DTC is not yet widely recognized or clinically accepted as a standard prognostic parameter. We hypothesize that the patients with high risk of the recurrence of the disease with V600E mutation of the BRAF gene will have poorer response to radioiodine therapy compared to the similar group of patients without that mutation.

Materials and methods: A subgroup of 31 DTC patients that presented with distant metastases (M1) have been selected. The response to RAI therapy was determined according to American Thyroid Associations 2015 Guidelines modified criteria for RAI refractory disease and was classified as: good response (disease control) or poor response (RAI refractory disease). DNA was isolated from formalin-fixed paraffin-embedded tumor tissue samples and assessed for presence of BRAF V600E mutation using competitive TaqMan polymerase chain reaction. Patients from aforementioned subgroup were analyzed for the presence of V600E mutation of BRAF gene.

Results: In the subgroup of 31 DTC patients that presented with distant metastases 15 pts. (48,39 %) had metastases to the lung, 9 pts. (29,03 %) had multiple metastatic loci, 3 pts. (9,68 %) to the bones, 2 pts. (6,45 %) to mediastinum, 1 pt. (3,23 %) to the liver and 1 pt. (3,23 %) to pharynx. 25 pts. (80,65 %) with distant DTC metastases had papillary thyroid cancer and 5 pts. (16,13 %) had follicular thyroid cancer. Most of the patients analyzed, 21 of them (67,74 %), had poor response to RAI therapy and finally proved to be RAI refractory. However, 10 pts. (32,26 %) showed good response to RAI therapy. Out of 31 patients with distant DTC metastases analyzed for V600E mutation of the BRAF gene 15 pts. (48,39 %) had BRAF V600E mutation and 16 pts. (51,61 %) did not have the mutation.

Discussion: In this subgroup of DTC patients that presented with distant metastases there were more patients with papillary thyroid cancer than those with follicular thyroid cancer that confirms the data from recent relevant publications. The sites of metastatic lesions also followed well established distribution patterns (DTC most frequently metastasizes to the lungs, then bones etc.). As RAI therapy is considered the golden standard in thyroid cancer treatment, and the response to RAI therapy might be used as a surrogate for long term outcome of the disease, the V600E mutation of BRAF gene status should probably be considered as the negative prognostic parameter in risk stratification of these patient. However, at this point of the investigation, we found no statistically significant correlation between patients BRAF V600E mutation status and poorer response to radioiodine therapy with iodine 131, therefore a greater sample of DTC patients with distant metastases will probably be required to obtain more accurate conclusions.

MeSH/Keywords: Iodine Radioisotopes; Prognosis; Proto-Oncogene Proteins B-raf; Thyroid Neoplasms; Metastases

Poster code: R-02-17-151

Poster Title: Corrected distance visual acuity prediction on a case-by-case basis

PhD candidate: Fanka Gilevska

Part of the thesis: Impact of the postoperative corneal density on the shape of the cornea after corneal cross linking procedure

Mentor(s): Associate Professor Smiljka Popović-Suić, MD PhD, Maja Bohač, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: The shape of the cornea described best by the single parameter, the ratio of the anterior radii curvature and posterior radii curvature (ARC/PRC). Monitoring the value of this ratio in correlation to COD is an objective indicator of the dynamic changes occurring within the cone itself and visual acuity after CXL

Materials and methods: Prospective, semi-randomized, partially masked, interventional clinical study performed in Sistina ophthalmology Hospital. Preoperative and on every scheduled visit, full ophthalmologic examination including corneal topography were performed. Following Pentacam maps were further analyzed: topometric/KC Staging, corneal densitometry and corneal pachymetry. Patients selected for surgical treatment adhered to the cross-linking procedure with the standard Dresden protocol, performed in the operating room and by the same surgeon. Manual epithelial scraping followed by application of Riboflavin drops and corneal UV-A radiation with wavelength 370nm and energy of 3mW, using UVA CXL lamp for the 30 minutes was performed. All data were stored in the Excel spreadsheet and analyzed to determine the significance of the changes in the CDVA, COD and ARC/PRC ratio preoperative during the one year postoperatively.

Results: In the treated group there were significant changes in the ARC/PRC ratio and COD over the year after CXL and correlations between ARC/PRC and COD at 12 months (r= 0.410 for 0-2ant, 0.458 for 0-2cent, 0.267 for tot ant, 0.303 for tot cent, 0.317 for tot, $p<0.05$, n=69). The change (d=preop value- postop value) in ARC/PRC was correlated with dCOD in the central part of the cornea at 6 &12 months postop (dARC/PRC & d0-2cent,r=0.248 at 6 months, r= r=0.412 at 12moths) and in the anterior part at 12months (dARC/PRC & d0-2ant,r=0.280). In treated group, significant difference between pre- and postop CDVA when compared with preop CDVA was significant at 6 (rs=0.367, n=53, p=0.014) and 12 (rs=0.398, n=45, p=0.029) months postop. A significant relationship between log CDVA and the ARC/PRC ratio was found in treated group at preop (rs= -0.388, n=53, p=0.004), and 1 (rs = -0.332, n = 53, p=0.015), 6 (rs = -0.422, n=53, $p<0.01$), and 12 months postop (rs= -0.464, n=45, p=0.006) In treated group, multiple linear regression revealed significant correlations between log CDVA at postop (y), log CDVA at preop (x1), and ARC/PRC ratio at preop (x2). The form of the associations between y, x1, and x2 at 12 months postop is: Eq1. y = 0.356x (2) 1 - 1.312x2 + 1.806 (r2 log preop CDVA = 0.494, $p < 0.001$;r2 preop ARC/PRC ratio = 0.203, p = 0.002

Discussion: ARC/PRC was significantly correlated with some markers of COD at 12 months postop which implies there was an ongoing reorganization in the anterior and central layers of the cornea within the central 2mm apical zone of the cornea postoperatively. Eqs.1 show the improvement in CDVA at 6 and 12 months postop was linked, on a case-by-case basis, to the preop ARC/PRC ratio and CDVA. For a preop CDVA of 0.2 and ARC/PRC ratio of 1.31, the predicted CDVA values at 6 and 12 months postop are 0.50 and 0.70. When the ARC/PRC ratio is 1.41, the predicted CDVA values are 0.46 and 0.51 respectively. Thus, the lower the ARC/PRC ratio at preop, the greater the estimated improvement in CDVA. The CXL treatment clearly improved CDVA with concomitant rise, not reduction, in the ARC/PRC ratio. Clearly, the CXL treatment disrupted the relationship between CDVA and the ARC/PRC ratio. The increase in the ARC/PRC ratio in the treated group likely resulted from a greater fattening of the anterior surface, with any change at the posterior surface.

MeSH/Keywords: Cross-linking, keratoconus, anterior radii curvature, posterior radii curvature

Poster code: R-02-18-025

Poster Title: Refractive error changes in Graves ophthalmopathy - myth or reality?

PhD candidate: Jasenka Petrović Jurčević

Part of the thesis: Influence of Graves' ophthalmopathy on the changes of the eye refractive power

Mentor(s): Assistant Professor Jelena Juri Mandić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Graves ophthalmopathy (GO) is an extrathyroidal manifestation of a systemic autoimmune disease affecting the tissues of the orbit. Literature on refractive error changes in GO is scarce. Through GO outpatient clinic we observed several things that were rationale for the study. Namely, patients with clinically active disease are more likely to seek help regarding visual disturbances during frequent check-ups than patients with non-clinically active disease. Patients with clinically active disease are more likely to complain about changed visual acuity even though they have neither keratopathy nor neuroopticopathy. Terefore, we considered that there might be a correlation between clinical activity score and refractive error, as well as clinical activity score and visual acuity.

Materials and methods: The study was prospective and observational, including 60 eyes of 30 patients with clinically active GO. All the patients were monitored and evaluated through a period of 36 months by the clinical activity score, the refractive error of the eye expressed by spherical equivalent, and visual acuity expressed by the logarithm of the minimum angle of resolution (logMAR). The study was performed at Referral Center for Graves ophthalmopathy at University Hospital Center Zagreb according to all the ethical standards. All the patients included in the study signed the informed consent and were treated according to 2021 European Group on Graves orbitopathy (EUGOGO) clinical practice guidelines for the medical management of Graves orbitopathy. Inclusion criteria was clinically active disease on the beginning of the study. Exclusion criteria were ophthalmic comorbidities that are not a consequence of the underlying disease; previous surgical procedures on the eye, eyelids, extraocular muscles, or orbit; amblyopia; refractive error greater than 6 diopters of myopia or hyperopia; keratoconus and keratoglobus. Descriptive statistics by months of observation were produced for all analyzed variables. In all statistical analyses, a significance level of 5% was considered statistically significant. The correlation among the observed VA, SE, and clinical activity score variables over the months was tested by the Spearman rank correlation. The differences in VA and SE over months were tested using RM ANOVA.

Results: The mean values of spherical equivalent and visual acuity through the observed period showed continuous fluctuation. Repeated measure analysis of variance showed statistically significant differences in visual acuity and spherical equivalent over the observed period. There was a statistically significant positive correlation between visual acuity and clinical activity score. The correlation between spherical equivalent and clinical activity score was also positive but not statistically significant.

Discussion: A decrease in the clinical activity score is either the result of a spontaneously resolving course of Graves ophthalmopathy or a consequence of treatment, so lowering in fluctuation of refractive error and improved visual acuity may be associated with a reduction in orbital inflammation. Refractive errors of the eye expressed through higher-order eye aberrations might more clearly correlate with clinical activity score.

Acknowledgments: None

MeSH/Keywords: Graves' ophthalmopathy, clinical activity score, refractive error, visual acuity

Poster code: R-02-18-029

Poster Title: Characteristics of Macula Hole patients and initial results of surgical treatment

PhD candidate: Marija Štanfel

Part of the thesis: Effect of Type of macular hole surgery on visual acuity improvement and quality of life

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

Affiliation: University of Zagreb School of Medicine

Introduction: Macular Hole is defect of the macular centre that strike full thicknes neural retina. It couses decreas of visual acuity, central scotoma, distortion or disruption of the image. It is seen in 1:5000 patients, twice more offten in women, mostly in 6th and 7th life decade. Surgical treatment has more than 85% succes regarding macular hole closing.

Materials and methods: Here we show demografic characteristics, preoperative ophthalmological features and anatomical results 4 months after the surgery. We use data from first year of trial, with patients that signed informed consent and finished all trial visits and tests. Of demografic characteristics we use sex and age. Preoperative visual aquity was measured using Snellen Chart. For contrast sensitivity testing Pelli-Robson Chart was used. Color sensitivity was measured using Ishihara chart. Dimension and grading od macular hole was performed using optical coherence tomography (OCT). Same was used for postoperative scaning.

Results: We included 9 patients with 9 eyes, 1 left and 8 right. There were 4 male and 5 female patients. Average age was 65 years, going from 44 to 81 years of age. Macular hole was 350 microns or bigger. Two had hyaloid membrane attached (gradus III) and 7 had hyaloid membrane detached (gradus IV). Visual acuity preoperative was 0,05 and less in 6 patients and 0,1 and more in 3 patients. All patients had positive Amsler testing, meaning they all had distortion of the image in affected Eye. Contrast and Colour sensitivity was mostly normal, but in few cases it was very low. Surgical treatment was performed. In 6 cases ILM peeling was performed and in 3 cases ILM flap was used. In 8 patients hole was closed after the surgery and in 1 case it was persistent. Postoperative visual acuity was maximum 0,2 in patients with preoperative 0,05 and less. Postoperative visual acuity was 2,5-3 times better in patients with preoperative visual aquity of 0,1 and more. All patients had positive Amsler testing 4 months after surgery, but lesser in intensity and seeing improvement.

Discussion: Our results correlate to those from literature, regarding at least 85% succesfull postoperative anatomical results. We see improved visual acuity, relating initial preoperative measurement. Contrast and Colour sensitivity testing results in this small sample do not correlate to initial visual acuity. For clear and definite prognostic factors we have to finish Trial on bigger sample.

MeSH/Keywords: macular hole, ILM peeling, ILM flap, quality of life, visual function

Poster code: R-02-18-103

Poster Title: High sensitive Troponin I values in patients with left- sided breast cancer undergoing hypofractionated adjuvant radiotherapy with concurrent anti-HER2 therapy

PhD candidate: Katarina Antunac

Part of the thesis: High sensitive Troponin I values in patients with left- sided breast cancer undergoing hypofractionated adjuvant radiotherapy with concurrent anti-HER2 therapy

Mentor(s): Professor Lidija Beketić-Orešković, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: In patients receiving antiHER2 therapy based on trastuzumab, cardiotoxicity has been reported, presenting as a decline in left ventricle ejection fraction. In patients undergoing adjuvant radiotherapy of left breast, cardiotoxicity has been reported as well, depending on the radiation dose on heart and its substructures: left ventricle (LV) and left anterior descending artery (LAD). AntiHER2 therapy and irradiation is given concomitantly. Troponin is being released by cardiac myocytes when exposed to injury. Hypothesis is that heart exposed to trastuzumab is more prone to radiation damage and that radiation damage is dependent on the radiation dose received by heart and its substructures. High sensitive Troponin I (hsTI) has been selected as an early marker of cardiotoxicity.

Materials and methods: Patients with left breast cancer undergoing hypofractionated adjuvant radiotherapy in parallel with antiHER2 therapy- trastuzumab, combination trastuzumab/pertuzumab or trastuzumab emtansine (T-DM1). Prescribed radiotherapy dose is 40.05 Gy in 15 fractions. Clinical target volume consists of left breast or left thoracic wall- depending on prior surgical procedure, with or without supra/infraclavicular/axillary lymph nodes- depending on pathohistological report. For every patient isodose plan is calculated, with regard to planned target volume dose coverage and doses on organs at risk- left lung, heart, LV, LAD and, in case of lymph node irradiation, spinal cord. Radiation doses are shown in form of dose- volume histograms (DVH). For heart, LV and LAD, evaluated parameters are mean and maximal dose (in Grays) and volume of organ receiving the dose of 2 Gy, 4 Gy, 5 Gy, V8 Gy, 10 Gy, 12 Gy, 16 Gy, 24 Gy, 38 Gy and 40 Gy (in percentage). hsTI is being measured prior to beginning of radiotherapy (day 1) and on the last day of radiotherapy, after 15th fraction has been delivered.

Results: Between February 15th and April 14th 7 patients (out of 60 planned) were included in research and completed treatment. Median patients age was 56 (range 46- 78). Five patients received primary systemic treatment; in 4 patients complete pathological response has been achieved. Five patients received anthracyclines, median time between last application of anthracyclines and radiotherapy was 208 days (range 31- 228 days). Two patients underwent breast conserving treatment, 5 patients had mastectomy, in one patient it was followed by reconstruction. During radiotherapy 6 patients were receiving combination of trastuzumab and pertuzumab and one patient was receiving T-DM1. Median of maximal heart dose was 37 Gy (range 24.9- 41.1 Gy) and median of mean heart dose was 1.9 Gy (range 1.4- 2.9). For LV median of maximal dose was 37.2 Gy (range 27.1- 41 Gy) and median of mean dose was 3 Gy (range 2.2- 5.5 Gy). Values for LAD are: median of maximal dose 16 Gy (range 10.3- 37.8 Gy) and median of mean dose 5.7 Gy (range 2.4- 7.5 Gy). In 3 patients elevation of hsTI value (defined as 30% increase from baseline value) upon completion of radiotherapy has been observed. So far no connection between increase of baseline hsTI value and mean or maximal dose on heart and its substructures has been found.

Discussion: Based on literature data, increase of hsTI is to be expected in about 50% of patients undergoing radiotherapy of left breast in parallel with antiHER2 therapy; we have observed it in 3 out of 7 patients (43%). Literature data for patients not receiving antiHER2 therapy concomitant with radiotherapy have shown connection between higher radiation doses for the whole heart and LV and troponin elevation. So far we could not find the connection between the rise of hsTI and mean or maximal dose on heart, LV or LAD, perhaps because of too small number of patients (7 out of 60 planned).

MeSH/Keywords: Left sided breast cancer, adjuvant radiotherapy, trastuzumab, pertuzumab, trastuzumab emtansine, radiation dose hypofractionation, Troponin I

Poster code: R-02-19-005

Poster Title: Predicting response to preoperative systemic therapy of HER2-positive breast cancer using a metabolomic approach

PhD candidate: Marija Križić

Part of the thesis: Predicting response to preoperative systemic therapy of HER2-positive breast cancer using a metabolomic approach

Mentor(s): Associate Professor Natalija Dedić Plavetić, MD PhD, Neven Žarković, PhD, research advisor

Affiliation: University of Zagreb School of Medicine

Introduction: For patients with HER2-positive breast cancer, neoadjuvant therapy (NAT) with dual anti-HER2 treatment combined with chemotherapy has become standard for a majority of patients. Since the pathological complete response (pCR) correlates with a better long-term treatment outcome, many studies are currently directed at detection predictors of response to NAT. Metabolomics is the field of research concerned with the analysis of small molecules (metabolites), and its approach has been used to determine possible biomarkers and key metabolic pathways in various types of cancer.

Materials and methods: This prospective study has used the metabolomic approach on plasma samples of 36 patients with HER2-positive breast cancer. The Ethics Committee approved the study design. All patients received NAT with dual anti-HER2 therapy (pertuzumab +trastuzumab) combined with chemotherapy (anthracyclines and taxanes). Serum samples were obtained before the first and last cycle of NAT. We use liquid chromatography- mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) methods to perform an untargeted metabolomic analysis. The SIMCA-P+ software was used for multivariate statistical analyses, including building up Principal Component Analysis (PCA) models and Partial Least-Squares Discriminant Analysis (PLS-DA) and Orthogonal PLS-DA (OPLS-DA) models. The metabolites for which the observed Variable Influence in Projection (VIP) for OPLS-DA models was higher than 1 were considered as statistically significant. For univariate statistical comparisons between groups, Students t-test or Mann-Whitney U test was performed, depending on the data distribution, followed by Bonferroni post hoc correction for multiple comparisons (p 0.050). Shapiro-Wilk test was used to check for normal distribution in the data set for each compound separately. Univariate statistical analysis was performed using MATLAB. In LC-MS, the resulting list of statistically significant accurate masses was annotated using the CEU Mass Mediator search tool in order to assign tentative metabolite candidates by matching their masses with different databases KEGG, HMDB, LipidMaps and Metlin. The biological role of each suggested compound was additionally evaluated to exclude the unrelated and impossible identification matches.

Results: The 36 of patients enrolled in our study, were divided in two groups according to NAT response: pCR group (n=20) and non pCR group (n=16). There is no significant difference in age, menopausal status, ER status, BMI, and clinical stage between the two groups (p>0.05). At this moment, LC-MS and GC-MS analysis are ongoing. We are expecting preliminary results in September 2022.

Discussion: The main goal of our study is to detect differences in the metabolic profile before NAT between the pCR and non pCR group with the aim of detecting the predictive biomarkers of the NAT response in HER2-positive patients.

MeSH/Keywords: HER2-positive, pathological complete response, metabolomics, neoadjuvant therapy

Poster code: R-02-19-172

Poster Title: The outcome analysis for the treatment of cartilage lesions in the knee using autologous cartilage graft derived from the harvesting of the nasal chondrocytes on a collagen scaffold

PhD candidate: Filip Vuletić

Part of the thesis: The outcome analysis for the treatment of cartilage lesions in the knee using autologous cartilage graft derived from the harvesting of the nasal chondrocytes on a collagen scaffold

Mentor(s): Assistant Professor Alan Ivković, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Articular cartilage injuries have a limited capacity for self-repair, and cannot be restored predictably by either conventional treatments or advanced therapies based on implantation of articular chondrocytes. Not properly treated, articular cartilage injuries are associated with pain and disability, and are known to double the incidence of degenerative joint disorders in the elderly. However, compared with articular chondrocytes, chondrocytes derived from the nasal septum have superior and more reproducible capacity to generate hyaline-like cartilage tissues, with the plasticity to adapt to a joint environment. Despite the known advantages of nasal chondrocytes, a direct comparison of the clinical efficacy of a mature graft (active ingredient is cells and matrix) vs an immature graft (active ingredient only cells) has not yet been described.

Materials and methods: The study was designed as a prospective, randomized and unblinded phase II study for the comparison of a therapy with a mature (N-TEC) versus a therapy with an immature graft (N-CAM). 30 patients between 18 - 65 years old with symptomatic full-thickness cartilage lesions (2-8 cm^2) of the knee treated in University hospital Sveti Duh will be enrolled in this trial. 15 patients will be treated with N-TEC and 15 with N-CAM therapy. The clinical follow-up was done at 6 weeks as well as at 3, 12 and 24 months to assess recovery. 12 and 24 months after treatment questionnaires were filled out by the patient to collect the clinical data. The primary outcome was therapeutic efficacy as assessed by the difference in KOOS score at 24 months postoperatively between treatment (N-TEC) and control (N-CAM) group. The secondary outcomes was KOOS at 12 months, Visual Analog Scale score, and EQ-5D score both at 12 and 24 months postoperatively as well as the incidence of postoperative adverse events.

Results: All 30 patients (median age 30 years, range 19-50 years) reached a 24-month follow-up. No serious adverse events or surgery-related complications were observed during the treatment and follow-up periods. One case failed and was reported as a treatment failure. Mean values of KOOS, VAS and EQ-5D improved significantly from before surgery to 24 months after surgery. When comparing groups, better improvement in KOOS scores was observed in patients treated with a mature graft.

Discussion: We have shown that the treatment of cartilage lesions in the knee, using autologous cartilage graft derived from the harvesting of the nasal chondrocytes on a collagen scaffold, is feasible and safe. Both techniques used in our study have demonstrated significant improvement in clinical outcomes at the 2-year follow-up. However, better clinical results assessed with objective and subjective KOOS scores at 2-year follow-up were found in the group treated with a mature graft. Consistent with the data from pre-clinical studies, the abundant extracellular matrix in mature grafts might have had a role in preserving the quality of the repair tissue and consequently influencing the clinical outcome.

MeSH/Keywords: Knee, cartilage lesion, nasal chondrocytes, scaffold

Poster code: R-02-20-038

Poster Title: Immunophenotypization of osteochondroprogenitor cells from subchondral bone samples from acetabulum and femoral head of patients suffering from primary osteoarthritis and secondary osteoarthritis of the hip caused by developmental dysplasia of hips: preliminary results

PhD candidate: Mihovil Plečko

Part of the thesis: Immunophenotyping of osteochondroprogenitor cells from subchondral acetabulum and femoral head of patients with primary and secondary hip osteoarthritis caused by developmental disorder

Mentor(s): Professor Domagoj Delimar, MD PhD, Assistant Professor Ivan Bohaček, MD PhD

Affiliation: Department of Orthopedics, University Hospital Centre Zagreb, and Department of Orthopedics, School of Medicine, University of Zagreb

Introduction: Osteoarthritis (OA) is a degenerative joint disease. The moleculaar mechanisms that cause OA are still unknown. When the cause of OA is not clear, it is defined as primary OA (pOA), while in cases of injury or known cause of OA, it is called secondary OA. One of important causes of secondary OA of the hip is developmental dysplasia of the hip (DDH-OA). There are currently no efficient treatment options for cartilage regeneration or cessation of OA progression. A significant number of studies suggest that the use of mesenchymal stem cells (MSCs) may provide good treatment results. However, there is a paucity of studies comparing these cell populations in patients with hip pOA and DDH-OA. Therefore, this study would be the first to describe the proportions of these cell populations in acetabular (Ac) and femoral head (Fh) subchondral bone samples from patients with hip pOA and DDH-OA.

Materials and methods: Samples were taken from waste tissue of patients undergoing total hip arthroplasty due to pOA or DDH-OA. Two samples were obtained from Ac, and two from Fh. Samples were obtained using OATS (Arthrex, Naples, Florida, USA). From one sample per location, cells were isolated from subchondral bone, stained with antibodies against osteochondroprogenitor markers (CD73, PDPN, CD146, CD164, GD2, CD140b, CD271, CD10) and analyzed by flow cytometry. Flow cytometry was performed on Attune (Applied Biosystems, Foster City, USA), while analysis was performed in FlowJo (FlowJo, Ashland, Oregon, USA). The second sample from each location was stored in formaldehyde, if there will be a need for immunohistochemical analysis in the near future.

Results: The study included 25 patients, 12 in DDH-OA group and 13 in pOA group. Of these, in pOA group 4 were male, while in DDH-OA group only 1 was male. The average age for pOA group was 65.1 (range 49-75), while in DDH-OA group it was 51 (range 35-65). When comparing BMI, duration of symptoms prior to surgery, visual analogue scale of pain and WOMAC score, there were no significant differences between these two groups. When comparing cells by osteochondroprogenitor markers, Ac propotion of CD164+ cells is significantly higher in DDH-OA group than in pOA group. Also, when comparing all samples together, there is a significantly higher proportion of cells that are CD164+ in the DDH-OA group, while CD10+ and GD2+ cell proportions seem to be significantly higher in pOA group. When comparing populations of Ac to Fh all together, there is a significantly higher proportion of CD271+ in Fh compared to Ac. However, all these results are preliminary, and still need to go through quality control processes and validated.

Discussion: The finding that CD164+ cells are in a higher proportion in samples for DDH-OA is a finding currently not published in the literature. Finding that CD271+ cells are in a higher proportion in Fh compared to Ac is confirming results of some studies already published in the literature. However, to the best of our knowledge, in this study Ac samples are analyzed appropriately for the first time. Nevertheless, it is still needed to do quality control and to confirm these results with other methods as well in order to discuss on the importance of hereby mentioned findings.

MeSH/Keywords: Osteoarthritis, Hip; Developmental Dysplasia of the Hip; Mesenchymal Stem Cells;

Poster code: R-02-20-044

Poster Title: Comparison of Two Different Local Modes of Tranexamic Acid Administration on Perioperative Blood Loss during Total Knee Arthroplasty

PhD candidate: Krešimir Crnogaća

Part of the thesis: Comparison of Two Different Local Modes of Tranexamic Acid Administration on Perioperative Blood Loss during Total Knee Arthroplasty

Mentor(s): Professor Domagoj Delimar, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Tranexamic acid is routinely used as a standard procedure in blood preservation strategies during total knee arthroplasty (TKA). Administration protocol that would lead to the best result in regard to blood loss still remains arguable. Local tranexamic acid administration has been demonstrated to have theoretical advantages in regard to the thromboembolic complications.

Materials and methods: Patients with osteoarthritis of the knee are included in the study. Exclusion criteria are tranexamic acid alergy, history of thromboembolic incidents, raised CRP levels and kidney failure. Eligible patients are interviewed and are obliged to take a medical questionnare. Afterwards they are randomised in 2 distinct groups. Group A follows a standard protocol of local tranexamic acid administation while test group B in addition to standrad administration gets an injection of 1 g of tranexamic acid intraarticularly 15 min before the start of the operation. Primary outcome is perioperative blood loss. Secondary outcomes are as follow, postoperative haemoglobin levels, CRP levels, need for blood transfusions and complications especially thromboembolic incidents.

Results: Total of 18 patients, with 9 in each distinctive group, so far are included in the study and analyzed. We found lower perioperative blood loss in the test group 650 ml +-30 ml vs 730 ml +- 50 ml in the standard group. In addition haemoglobin levels were higher in the test group (95 mg/dL +-15 mg/dL) vs 88 mg/dL +-15 mg/dL in the standard group. There was no difference in regard to CRP levels in both groups. One wound complication was observed in each of the groups. No adverse events associated with the tranexamic acid were reported.

Discussion: The aim of this prospective study is to analyze effectivenes of two different local regiments of tranexamic acid administration during TKA without the use of tourniquet.The hypothesis of this study is that perioperative blood loss will be lower in patients receiving new local regiment of tranexamic acid in which patients recieve intraarticular injection of tranexamic acid before the operation than in those receiving standard local regiment of tranexamic acid during TKA without the use of tourniquet.

MeSH/Keywords: total knee arthroplasty, tranexamic acid, tourniquet, blood loss

Poster code: R-02-20-102

Poster Title: The effect of tendon graft twisting on tensile properties on a human cadaveric tendon model

PhD candidate: Jure Serdar

Part of the thesis: The effect of tendon graft twisting on tensile properties on a human cadaveric tendon model

Mentor(s): Associate Professor Tomislav Smoljanović, MD PhD, Assistant Professor Ana Pilipović, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Ruptures of the anterior cruciate ligament (ACL) are among the commonest knee injuries. Managing these injuries includes surgical reconstruction using hamstring tendon grafts (semitendinosus et gracilis muscles). There is up to 20 % of bad outcomes of the operative treatment that require revision surgery. One of the reasons for bad outcomes following ACL reconstruction surgery is tendon graft failure due to rupture. Therefore, there is a need to improve the properties of the tendons used as transplants for ACL reconstruction. The twisting phenomenon is used in the technical sciences, such as mechanical engineering for rope production, to improve material properties. A rope consists of multiple threads that twist around their axis during production to make the rope load higher. As the tendon graft for ACL reconstruction also consists of multiple threads, the question that arises is whether tendon twisting can improve graft properties as well.

Materials and methods: This study was performed on fresh frozen human cadaveric tendons. From one cadaver right and left hamstrings tendons (semitendinosus and gracilis muscles) were harvested. Tendons from 12 cadavers were harvested which gave 12 pairs of semitendinosus and 12 pairs of gracilis tendons. These matched pairs of tendons were divided in two groups. In the examined group tendons were twisted along the entire length by 180 degrees and in the control group plain tendons were tested. Matched tendon pairs underwent tensile testing. The tensile testing was performed on a universal testing machine (Shimadzu AGS-X, Shimadzu Corporation, Japan) with a maximum force of 10 kN. After preconditioning which included cyclic loading for 10 cycles to 30 N specimens were tested to failure at the constant displacement rate of 5 mm/min. Maximum force before failure was recorded.

Results: Results showed significant difference ($p < 0.05$) between twisted and plain tendons grafts in semitendinosus group regarding maximum force value. Mean (±SD) maximum force for twisted tendons was 893.6 ± 148.3 N versus 571.3 ± 50.1 N for plain tendons. In gracilis group mean (±SD) maximum force for twisted tendons group was 637.3 ± 157.9 N versus 524.7 ± 82.6 N for plain tendons. Although mean maximum force was higher for twisted tendons in comparison with plain tendons, this difference was not statistically significant ($p > 0.05$).

Discussion: These preliminary results showed that tendon twisting during tendon graft preparation could improve graft properties in terms of better resistence on tensile load. We hypothesized that by twisting the tendon each part of the graft becomes equally tensed. Therefore tensile load is equally distibuted on each thread of the graft making the whole graft more ressistant on tensile forces. However, we still do not know at which angle of twisting this phenomena occurs. Therfore, in future studies we will examine tendon graft behaviour under various angles of twisting.

MeSH/Keywords: ACL reconstruction, ACL rupture, hamstrings graft, tendon graft failure, tendon graft strength, twiating tendon graft

Poster code: R-02-20-164

Poster Title: Analysis of gustatory function in chronic otitis media without cholesteatoma

PhD candidate: Mislav Malić

Part of the thesis: Analysis of gustatory function in chronic otitis media without cholesteatoma

Mentor(s): Associate Professor Mislav Gjurić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: It is well known that in chronic otitis media there is an impairment of gustatory function, however there is insufficient data on the effect of middle ear surgery on the impaired sense of taste. The aim of this research is to determine gustatory function in patients with unilateral chronic otitis media without cholesteatoma and compare the results with the gustatory function of the unaffected side, before and after surgery. Herby, we present out preliminary results.

Materials and methods: Patients are divided in two groups, first group (Group 1) is operated endoscopically without elevation of the tympanic membrane and without the chorda tympani manipulation, and the second group (Group 2) of patients had standard surgery that involves raising the tympanomeatal flap and manipulating the chorda tympani. Gustatory function testing was administered using taste strips test, before the surgery, one day after the surgery and three months after the surgery. Maximal score is 16. Total of 59 patients have been analysed by now.

Results: 54 successfully operated patients (of 59 91 %) have been included in this study. Our preliminary results show that there is a preoperative difference in taste function between healthy and disseised side (13.02 and 10.92 respectively). If we analyse all patients together, we can see that after the surgery there is decline in a function (9.66), which after the period of three months improves 11.29, but never reaches the function of the healthy side. However, when we analyse patients according to surgery groups, there is huge difference. Group 1 had taste function of 10.01 on the disseised side before the surgery (12.45 healthy side), which declined only to 9.72 after the surgery and improved to the 12.04 three months after the surgery, which is very close to the healthy side function. Group 2 on the other side, had taste function of the 11.36 before the surgery which declined to 9.81 after the surgery and improved only to 11.09 after three months, which is significantly lower than the function on the healthy side (13.09).

Discussion: Couple of conclusions can be made according to our preliminary results. First, we confirm to current literature data that there is impaired gustatory function on the side with chronic otitis media. Second, if we analyse all patients together, we can see that there is improvement in gustatory function after successful tympanoplasty, although not quite significant. Third and the most important, if we exclude chorda tympani surgical manipulation as a potential risk factor for full potential of the nerve recovery, we can see that in a group without nerve manipulation (Group 1), there is significantly better improvement in the gustatory function, almost to the results of the healthy side. This results are according to our hypothesis that surgical manipulation of the chorda tympani nerve influences the gustatory function.

MeSH/Keywords: Gustatory testing, otitis media, surgery, myringoplasty

Poster code: R-02-21-009

Poster Title: Correlation of GATA3, CK5/6 and p16 expression and overall survival in muscle-invasive bladder cancer

PhD candidate: Robert Terlević

Part of the thesis: Immunohistochemical expression of cytokeratin 5/6 and transcription factor GATA3 in muscle-invasive urothelial bladder cancer

Mentor(s): Professor Božo Krušlin, MD PhD, Goran Štimac, PhD, research advisor

Affiliation: University of Zagreb School of Medicine

Introduction: Muscle-invasive bladder cancer therapy choice could be influenced by the tumor molecular subtype. Currently well-defined subtypes are RNA based. Surrogate molecular subtypes based on immunohistochemistry are needed to make subtyping useful in routine work and facilitate future research.

Materials and methods: A retrospective single center series of 85 cases with localized disease was identified, and routine immunohistochemistry for GATA3, CK5/6 and p16 was performed on whole tissue blocks containing muscle-invasive disease. Surrogate molecular subtypes were defined. Patient information regarding treatment and survival was obtained from medical archives.

Results: The mean population age was 70 years (SD=8.7), and 73% were males. Conservative treatment (TUR with radiotherapy) was used in 45% of cases, while cystectomy with adjuvant chemotherapy was performed in 47%. Only 7% underwent neoadjuvant chemotherapy or primary chemoradiotherapy. GATA3 and CK5/6 expression segregated cases into broad luminal and basal subtypes respectively, while p16 expression was used to subclassify luminal cases into luminal papillary and luminal unstable types. When subtyped this way, no difference in survival was observed overall and within therapy groups. GATA3 expression was strongly associated with better survival across all treatment groups, while CK5/6 was linked to worse survival. No effect of p16 on survival was observed.

Discussion: No effect of p16 expression was observed on clinical outcome. When used together with GATA3 and CK5/6 in a combined immunohistochemical molecular subtype no difference in survival was observed. In our study GATA3 and CK5/6 expression was found to be an indicator of respectively improved and worse survival both overall and within conservatively treated and cystectomized patients.

MeSH/Keywords: pathology, urology

Poster code: R-02-23-007

Poster Title: Expression of P53 in mantle-cell lymphomas at diagnosis and in relapse-Preliminary research results

PhD candidate: Adriatik Berisha

Part of the thesis: Expression of P53 in mantle-cell lymphomas at diagnosis and in relapse

Mentor(s): Assistant Professor Snježana Dotlić, MD PhD, Professor Igor Aurer, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Mantle cell lymphoma (MCL) is a B-cell non-Hodgkin lymphoma (NHL) with a continuous tendency for relapses. It is molecularly characterized by the hallmark t(11;14)(q13;q32) leading to CCND1 (cyclin D1) over-expression (1). Recent data suggest that molecular alterations of TP53, leading to aberrant p53 protein expression, detectable by immunohistochemistry (IHC), occur in MCL and confer an unfavourable prognosis. It is unknown whether the frequency of these mutations increases in relapse.

Materials and methods: Bioptic samples of MCL patients obtained for diagnostic purposes, irrespective of the disease phase, will be identified in the database of the two participating pathology departments. Samples of patients with available clinical data will be used for this study. We plan to include at least 40 patients in the study. Data on demographic (age, sex), clinical data (disease stage, LDH, WBC, PS) will be extracted from patient files. All of the biopsy samples will be reviewed including the analysis of immunophenotype and histopathological variants of MCL (classic vs blastoid morphology). If needed, staining for Ki67 will be performed additionally. Data on treatment and outcomes (response, event-free survival (EFS), overall survival (OS)) will also be extracted from patient files. All bioptic samples will be stained for P53 expression with Immunochemistry (IHC).

Results: We have collected most of the biopsy samples and started testing and measuring the parameters, but our partial data collected so far are still in the process of statistical testing

Discussion: This study will generate new data on the prognostic importance of aberrant P53 expression in MCL, its correlation with classic prognostic factors and clarify whether the freqeuncy of these aberrations increases in later disease phases. Thus it will aid in elucidating the role of P53 aberrations in resistance to therapy and disease progression

MeSH/Keywords: Mantle cell lymphoma, P53, Ki67, relapse.

Poster code: R-02-23-123

Poster Title: Histological classification in predicting outcomes in HenochSchönlein purpura nephritis

PhD candidate: Nastasia Kifer

Part of the thesis: Histological classification in predicting outcomes in HenochSchönlein purpura nephritis

Mentor(s): Professor Marija Jelušić, MD PhD, Associate Professor Marijana Ćorić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Henoch-Schönlein's purpura (HSP) or IgA vasculitis (IgAV) is the most common vasculitis in childhood. Although the disease is most often self-limiting, the main prognostic factor is the development of nephritis (HSPN), which when occurs can lead to chronic kidney disease. While kidney biopsy has been a continued standard in determining the severity of HSPN, there is a wide selection of histologic classifications used in evaluating the severity of this disease. However, there are no studies comparing the performance of the most frequently used histological classifications and it remains unknown which one has the strongest association with the severity of HSPN.

Materials and methods: This research included patients diagnosed with HSP and HSPN based on EULAR, PRINTO and PRES criteria. Prospectively patients were included in the research for a period of two years. Demographic, clinical, laboratory, and medication administration records were maintained and renal biopsy findings of HSPN patients were analysed. The retrospective part of the project included the reanalysis of prior biopsy samples of patients treated for HSPN. Renal biopsy findings were examined using light microscopy, immunofluorescence, and electron microscopy analyses. Four classifications were used: ISKDC, Haas classification, Oxford classification, and SQC classification. The clinical outcome was defined through four categories (A-D), graded according to the modified classification of Counahan.

Results: The study included 67 patients collected retrospectively and prospectively over the course of two years. The SQC classification proved to be the best reducing the deviation (of the model-predicted outcome value from the observed value) by additional 7.38% (2 = 10.06, p = 0.007), followed by the Oxford classification which reduced by additional 5.01%, (2 = 6.83, p = 0.033), compared to the base model. ISKDC and Haas did not explain statistically significant reduced residual deviance of the base proportional odds logistic regression model, reducing the deviation by 4.14% (2 = 5.71, p = 0.058) and 3.11% (2 = 4.27, p = 0.118), respectively. Statistically significant quantity of residual deviances was explained with time from biopsy to outcome estimation (base model), by 6.79% of the total variance (2 = 10.25, p = 0.002)). When evaluating histologic variables independently, Oxford's parameters mesangial hypercellularity and tubular atrophy, as well as SQC's parameter cellular crescents did show statistically significant contribution in residual deviance reduction (by additional 8.55% (2 = 11.66, p = 0.002); 5.64% (2 = 7.69, p = 0.021); and 5.57% (2 = 7.59, p = 0.028) of total residual deviance, respectively.

Discussion: This study compares for the first time the four most frequently used classifications. We have determined that the SQC, followed by the Oxford classification are the best classifications to be used in renal biopsy analysis in patients with HSPN. While ISKDC and Haas are mainly analysing active variables, SQC and Oxford classifications are taking into account chronic parameters, enabling a better outcome prediction. However, when analysing individual variables, cellular crescents have shown a significant contribution, alongside mesangial hypercellularity and tubular atrophy, signaling that active variables should also be included in the estimation. By using the best histological classification for HSPN we believe it is possible to identify patients with unfavourable disease outcome which will enable the selection of the best treatment modality for each patient.

Acknowledgments: Part of Croatian Science Foundation project PURPURAPREDICTORS IP-2019-04-8822.

MeSH/Keywords: Henoch-Schönlein's purpura, HSPN, IgA vasculitis, IgAV, histological classification

Poster code: R-02-24-064

Poster Title: Radiomic-based pretreatment prediction of response to neoadjuvant chemotherapy in hormone receptor-positive, human epidermal growth factor receptor 2-negative breast cancer

PhD candidate: Lucija Kovačević

Part of the thesis: Radiomic features of dynamic magnetic resonance imaging of breasts in the assessment of responses to neoadjuvant chemotherapy in patients with breast cancer

Mentor(s): Associate Professor Maja Prutki, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Hormone receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2-) breast cancer patients rarely achieve residual cancer burden (RCB) class 0 or 1 at the histopathological analysis after neoadjuvant chemotherapy (NAC). Proper selection of candidates for NAC is necessary to avoid toxicity and delays in more effective treatment. This study aims to evaluate dynamic contrast-enhanced breast magnetic resonance imaging (DCE-MRI) radiomic features for the pretreatment prediction of HR+HER2- breast cancers insensitive to NAC, according to RCB class as the standard of reference.

Materials and methods: This IRB-approved retrospective study included 56 patients with HR+HER2- breast cancer treated with neoadjuvant chemotherapy who underwent pretreatment DCE-MRI at the University Hospital Center Zagreb between January 2015 and January 2021. Based on the RCB class, the patients were divided into two groups named good responders (RCB 0 and RCB 1) and poor responders (RCB 2 and RCB 3). Stratified random sampling was employed to develop train cohort (N = 44) and test cohort (N= 12). 1781 IBSI-compliant 3D volumetric radiomic features were extracted from each manually segmented breast cancer from the first post-contrast DCE-MRI sequence. To reduce model complexity, and avoid overfitting during the modeling step, feature ranking and selection were performed as a preprocessing step using a combination of various ranking schemes based on mutual information and the ReliefE (Feature Ranking in High-dimensional Spaces via Manifold Embeddings) algorithm. Furthermore, extensive state-of-the-art feature selection algorithms were employed together with machine learning algorithms for handling small sample sizes and imbalanced classes data to develop a neoadjuvant chemotherapy response classification model with good generalization performance. To internally validate and select our model, we employed 10 times repeated 3-fold cross-validation (CV) on the primary cohort, and the model with the best CV score was evaluated on the validation cohort.

Results: The results of a predictive model, which is based on a random forest algorithm using 25 selected features, showed the best overall classification performance, with an area under the receiver operating characteristic (ROC) curve value of 0.9 and an F1 score of 0.95 on the validation (test) cohort.

Discussion: Our preliminary results show that the response to neoadjuvant chemotherapy in patients with HR + HER2- breast cancer could be predicted based on imaging features from the first DCE-MRI sequence after contrast.

Acknowledgments: This work is supported by Croatian Science Foundation under the project IP-2019-04-3684 ''Biomedical imaging of breast carcinoma''.

MeSH/Keywords: breast cancer; magnetic resonance imaging; machine learning; neoadjuvant therapy; radiomics

Poster code: R-02-25-002

Poster Title: Neoadjuvant therapy impact assessment with contrast enhanced ultrasound in patients diagnosed with breast cancer - preliminary research results

PhD candidate: Iva Biondić Špoljar

Part of the thesis: Neoadjuvant therapy impact assessment with contrast enhanced ultrasound in patients diagnosed with breast cancer

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

Affiliation: University of Zagreb School of Medicine

Introduction: Contrast-enhanced ultrasound (CEUS) is a relatively new diagnostic method in the breast imaging field. The golden standard for monitoring the effects of the neoadjuvant therapy (NACT) in patients diagnosed with breast cancer is magnetic resonance imaging (MRI), which can be used to assess not only morphological analysis but also imbibition and the microstructure of the lesion. With a gray-scale ultrasound examination it is only possible to evaluate morphologic features of the lesion. By applying an ultrasound contrast agent composed of a suspension of gas micro-bubbles, which are retained in the blood vessels, it is possible to analyze the blood supply of the lesion. Neoangiogenesis is a feature of malignant lesions, and previous research and clinical practice have shown that NACT acts not only on the cells of the malignant lesion, but also on the blood vessels that supply it. The effects of and response to the therapy in the early phase of treatment can be noticed precisely in the change in the imbibition of the lesion, while morphologic changes usually follow later. The application of the sonographic contrast agent makes it possible to monitor the effects of the neoadjuvant therapy on malignant breast lesions with a simpler, more accessible and more affordable imaging method.

Materials and methods: The study is provided at the Department of diagnostic and interventional radiology of the University hospital Dubrava. For every patient is provided MRI and ultrasound imaging including CEUS, before the beginning of NACT, during the application and at the end of the therapy. All procedures are done on the same MRI and ultrasound machines with the use of the same protocols. For MRI procedures special breast coil is used. The imaging protocol consists of pre contrast T1 and T2 sequences, DWI and after the intravenous application of paramagnetic contrast agent post contrast sequences and generated subtraction images. For ultrasound examinations high frequency linear probe is used for B mode. After the analysis of the lesion in B mode ultrasound and optimization of the image, CEUS is done using SonoVue (Bracco, Milan, Italy) contrast agent. On both imaging techniques morphologic and kinetic parameters are analyzed including micro structure of the lesion parameters on MRI. All lesions were described according to the BIRADS lexicon. Before the therapy, small metal clip is placed in the lesion under the control of ultrasound for better tracking of the lesion, that is especially useful when complete response to therapy occurs.

Results: At the moment for 16 patients three scans of MRI and CEUS in different phases of therapy were performed, in total 48 MRI and 48 CEUS examinations. The final results of both imaging techniques were compared. The answer of the lesion to the therapy was described based on RECIST 1.1 criteria. The answer to the applied therapy was analyzed with both imaging methods. Statistical analysis was performed in R Studio 4.1.2. programme. The preliminary results of collected data show positive correlations between CEUS and MRI responses to the therapy (Kendall's Tau, $p=0.0009$, Spearman's rank, $p=0.0003$, $tau=0.5963$ and CohenKapp=0.5714).

Discussion: Preliminary results of the collected data show that CUES is a powerful imaging technique in follow up of patients diagnosed with breast cancer who underwent neoadjuvant chemotherapy. The advantages of CEUS and ultrasound contrast agent are availability, short scanning time and rare side effects. We are aware of disadvantage when there are multiple lesions in the breast, as in case of multicentric cancer, because with CEUS we can only follow one lesion at the time. In that case, MRI stands as a better choice. This study is still ongoing and more will be concluded after processing all collected data.

MeSH/Keywords: breast cancer, neoadjuvant chemotherapy, contrast-enhanced ultrasound, magnetic resonance imaging,

Poster code: R-02-25-040

Poster Title: Significance of chondrocyte survival analysis in postmortem interval assessment

PhD candidate: Anita Galić Mihić

Part of the thesis: Significance of chondrocyte survival analysis in postmortem interval assessment

Mentor(s): Associate Professor Davor Mayer, MD PhD, Assistant Professor Armin Alibegović, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Assessment of the time since death or postmortem interval (PMI) is one of the most frequently asked questions in forensic practice. It is important to determine whether the time of death is consistent with the alibi of a suspect because sometimes the time of death play a major role in court proceedings as the only evidence regarding the guilt of a suspect. Estimation of the time since death is based on postmortem findings such as body cooling, lividity, rigor mortis, supravital reactions, and others. But these parameters are applicable in the early postmortem period, while the determination of the late PMI is based primarily on a subjective assessment and is less precise due to the lack of objective methods. So every forthcoming parameter for the practical relevance in evaluating the PMI should fulfill the following criteria: the quantitative measurement, mathematical description, a declaration of the precision and the proof of precision on independent material. Previous studies reveled that the chondrocytes in human joints retain their viability for a prolonged period after the death of an individual depending on the ambient conditions. The highly dense structure of the knee cartilage protects the chondrocytes from the spread of microorganisms. The chondrocytes depend predominately on the nutrient diffusion from the abundant matrix and the synovial fluid. This makes their metabolism anaerobic and relatively resistant to oxygen starvation and acidosis. Therefore, cartilage could be a new parameter for PMI determination. This research will be the first longitudinal in corpore study that will analyze dynamics of the decrease in the proportion of viable chondrocytes in knee cartilage, under controlled conditions. The aim of the study is to determine the dynamics of the decrease in the proportion of viable chondrocytes successively excluded from deceased's knee in different time intervals after death. Standardize the method of sampling and processing the samples.

Materials and methods: Knee cartilage cylinders of 30 male bodies (age over 50 years) stored in refrigerators at a temperature of 8 ± 2 °C, at the ale City Cemetery in Ljubljana. The cartilage samples will be procured bilaterally from the knee joints. The articular surfaces, will be exposed by a wide transversal arthrotomy. Four osteochondral cylinders (macroscopically intact part, ICRS grade 017) will be removed with the osteochondral autograft transfer system from both femoral condyles (Ø 6 mm, depth 20 mm). Every osteochondral cylinder will be immediately stored in a 2 mL tube filled with Dulbeccos Modified Eagle Medium (DMEM). Every sample will be washed with DMEM solution, diced to 1x1 mm pieces, and enzymatically digested in 13-15 mL of collagenase II solution, kept for 1820 h at 37 °C. The degraded cartilage samples afterward will be washed through a cell strainer with 40 m pores. Cells with residual fluid will be centrifuged at 580 RCF (g) for 5 min. After centrifugation, the liquid will be discarded and 1 mL of DMEM will be added to the cells. The percentage of viable/non-viable chondrocytes will be determined by flow cytometry (FC) and automatic cell analyzer (CVA). The chondrocyte suspension for the CVA will be treated with trypan blue vital dye included in the kit for automatic dyeing (Vi-CELL XR, Beckman Coulter). The solution for FC will be centrifuged again and resuspended in 200 L Dulbecco's phosphate buffered saline, free of calcium and magnesium (DPBS). RedDot + 7AA-D (7-Aminoactinomycin D) will be used to mark viable/non-viable chondrocytes.

Results: No results.

Discussion: After the results.

MeSH/Keywords: knee cartillage, chondrocyte, time since death, flow citometry, cell viability analyzer

Poster code: R-02-27-094

Poster Title: Value of determination of 1,5 anhydroglucitol concentration in pericardial fluid in diagnosing diabetes mellitus postmortem

PhD candidate: Martina Tkalčić

Part of the thesis: Value of determination of 1,5 anhydroglucitol concentration in pericardial fluid in diagnosing diabetes mellitus postmortem

Mentor(s): Associate Professor Davor Mayer, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Diabetes mellitus, one of the worlds leading causes of morbidity and mortality, represents a major public health problem. Approximately one third of the cases are never diagnosed. Acute complications of diabetes, such as diabetic ketoacidosis and hyperglycaemic hyperosmolar coma, present an immense challenge in a forensic setting and as an autopsy finding because usual diagnostic tests for diabetes (blood glucose concentrations) are not reliable due to postmortem changes. Furthermore, postmortem changes that occur or environmental circumstances of a place where the body is found, can render the specimens inadequate for analysis or they can even be missing altogether. For that reason, there is a need for additional reliable specimens and stable quantifiable compounds with diagnostic significance. Recently studied 1,5 anhydroglucitol, an inert deoxy-form of glucose, which is regularly ingested by food, is one of such compounds. 1,5 anhydroglucitol binds competitively with glucose in kidney tubules and its levels are indirect indication of blood glucose levels. Since 1,5 anhydroglucitol is more sensitive to daily changes in blood glucose levels it provides a better insight into metabolic status of the deceased in shorter period prior to death than glycated haemoglobin. So far, pericardial fluid has been proven as a stable body fluid in which measured parameters are comparable to those in other specimens.

Materials and methods: 1,5 anhydroglucitol, glucose, acetone, glycated haemoglobin and ß hydroxybutyrate concentrations will be measured in femoral blood, urine, vitreous humor and pericardial fluid sampled at autopsies at Institute of Forensic Medicine and Criminalistics in Zagreb. Those parameters will be measured in two predefined groups of cases and controls based on their medical history of diabetes, information which will be obtained from the elected General Practitioner of the deceased. All the specimens will be taken by standard autopsy protocols. Analysis of the specimens will be done partially at the Toxicology Department at the Institute of Forensic Medicine and Criminalistics, School of Medicine, University of Zagreb, and partially at Department of Laboratory Diagnostics at University Hospital Centre Zagreb. Analysis of 1,5-anhydroglucitol will be performed by enzymatic method Diazyme 1,5 anhydroglucitol (1,5 AG) Assay.

Results: No results at this point.

Discussion: Discussion will be elaborated after results.

MeSH/Keywords: 1,5 anhydroglucitol, pericardial fluid, forensic medicine, cause of death, diabetes mellitus

Poster code: R-02-27-128

Poster Title: The role of mobile teams in the process of functional recovery of people with severe mental illness

PhD candidate: Sarah Levaj

Part of the thesis: The role of mobile teams in the process of functional recovery of people with severe mental illness

Mentor(s): Associate Professor Martina Rojnić Kuzman, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: The community-based treatment for people with severe mental illness (SMI) is still at an early stage of development in Croatia, and it lacks specific and well-organized programs. With the aim of implementation and evaluation of the community-based service delivery model in Croatia, a partnership was established in the project "LaRge-scalE implementation of COmmunity based mental health care for people with severe and Enduring mental ill health in EuRopE" - RECOVER-E. This project provided an opportunity for people with SMI treated at University Hospital Centre Zagreb to receive care through newly implemented multidisciplinary community mental health teams (commonly named mobile teams), in addition to the current standard psychiatric treatment. The teams service delivery is focused on home visits to patients, and the interventions are patient-centered, with the purpose of achieving recovery goals. Considering that mobile psychiatric teams, based on the principle of flexible assertive community treatment, represent a novel approach to psychiatric treatment in Croatia, the overall aim of this study is to examine the effect of their services on the process of functional recovery of people with SMI. The specific aims include the comparison of 1) general functioning; 2) health-related quality of life; 3) number of admissions to inpatient psychiatric care and the number of days of inpatient treatment, between people with SMI receiving additional care provided by mobile teams and patients receiving only standard psychiatric treatment.

Materials and methods: This study was conducted at the Department of Psychiatry and Psychological Medicine at the University Hospital Centre Zagreb, as a part of the project RECOVER-E. It represents a prospective randomized clinical trial, in which participants were allocated to either the intervention group, which received care provided by mobile teams in addition to standard psychiatric treatment, or to the control group which received only standard psychiatric treatment. Eligible participants were patients with SMI aged 18 to 65 years. The total of 169 participants was included at enrollment 83 in the intervention group and 86 in the control group. Data collection and assessment were performed at enrollment, and after 12 and 18 months. Global functioning and health-related quality of life were assessed through self-assessment scales, while data on the number of admissions to inpatient psychiatric care and the duration of inpatient stay was collected from medical records.

Results: This study is currently in the phase of data analysing and statistical processing, and at the moment preliminary results are not available.

Discussion: We expect that the results of this research will contribute to the comprehensive evaluation of the newly implemented treatment model through mobile teams in Croatia. These findings could also serve as a basis for discussion in the dialogues on planned health policies, supporting the continuation of reforms towards the systematic development of community-based model of mental health services.

MeSH/Keywords: Recovery, severe mental illness, mobile teams

Poster code: R-02-29-113

Poster Title: The role of the inflammatory response and the severity of the disease as a clinical predictor for development of anxiety, depression and PTSD in patients hospitalized for COVID-19

PhD candidate: Dijana Lucijanić

Part of the thesis: The role of the inflammatory response and the severity of the disease as a clinical predictor for development of anxiety, depression and PTSD in patients hospitalized for COVID-19

Mentor(s): Professor Alma Mihaljević-Peleš, MD PhD, Nevenka Piskač Živković, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: The general objective is to investigate patients with criteria of a more severe clinical picture and expressed systemic inflammatory response to SARS-CoV-2 coronavirus infection and if they develop mental disorders, anxiety, depression, excessive stress and PTSD measured by DASS-21 and IES-R scales.

Materials and methods: This is an observational prospective study on a selected sample at two time points. . Subjects were included according to the following inclusion criteria: hospitalized patients giving informed consent, aged 18 years or older, both sexes, who tested positive for SARS-Cov-2, conscious, post-stabilization, and co-operative filling out questionnaires. Exclusion criteria are patients with impaired consciousness, poor physical condition, patients on a respirator or in an intensive care unit, who have more severe communication difficulties (dementia, deafness, intellectual disabilities), than previously diagnosed anxiety, depressive or ptsp disorders, who have physical diseases with accompanying psychological symptoms. The scales were applied at two time points: during hospitalization and 3 months after discharge.

Results: A total of 169 patients during hospitalization were analyzed, of which data were available for 92 after 3 months. The median age of the analyzed patients was 65 years, IKR (57/71). There were 105 (62.1%) men. On admission, most patients had a severe (134, 79.3%) or critical (17, 10.1%) form of COVID-19. The median Charlson comorbidity index was 3 points, IKR (2-4). Arterial hypertension was present in 101 (59.8%), diabetes mellitus 42 (24.9%), hyperlipoproteinemia 30 (17.8%), obesity 61 (36.1%), malignant disease 17 (10.1%) patients. 11 (6.5%) smoked and 7 (4.1%) patients consumed alcohol. The median CRPa was 72.75 mg / L, IKR (31.4-141.4). Median SII was 1741, IKR (822-3821). During hospitalization, the median IES-R score was 20 points, IKR (11-31.25). A total of 103 (60.9%) patients had no symptoms of PTSD, 27 (16%) had symptoms without a diagnosis, and 39 (23.1%) met the criteria for a diagnosis of PTSD. 3 months after discharge, 65 (70.2%) had no symptoms, 18 (19.6%) had no symptoms without a diagnosis, and 9 (9.8%) met the criteria for a diagnosis of PTSD. In patients with available data, a significant decrease in IES-R score was seen after 3 months (median 13 vs 20, P <0.001). During hospitalization, the median DASS21 score for depression was 14, IKR (822), for anxiety 8, IKR (220), and for stress 6, IKR (212.5). In patients for whom data were available after three months, a significant reduction in score was seen for all three measured parameters: depression (median 6 vs 12 points, P <0.001), anxiety (median 6 vs 7 points, P = 0.007), stress median 0 vs 6 points, P <0.001). Regarding depression, it was absent in 49 (29%), mild in 27 (16%), moderate in 47 (27.8%), severe in 18 (10.7%) and extremely severe in 28 (16.6%) patients during hospitalization, and absent in 57 (62%), mild in 14 (15.2%), moderate in 19 (20.7%), severe in 1 (1.1%) and extremely severe in 1 (1.1%) after 3 months. Regarding anxiety, it was absent in 74 (43.8%), mild in 11 (6.5%), moderate in 29 (17.2%), severe in 9 (5.3%) and extremely severe in 46 (27.2%) patients during hospitalization, and absent in 50 (54.3%), mild in 9 (9.8%), moderate in 17 (18.5%), severe in 9 (9.8%) and extremely severe in 7 (7.6%) patients after 3 months. Regarding stress, it was absent in 133 (78.7%), mild in 8 (4.7%), moderate in 12 (7.1%), severe in 13 (7.7%) and extremely severe in 3 (1.8%) patients during hospitalization, and absent in 89 (96.7%) and mild in 3 (3.3%) patients after 3 months.

Discussion: Meta-analysis found that the pooled prevalence of depression was 45% (95% CI: 37-54%, I2 = 96%), the pooled prevalence of anxiety was 47% (95% CI: 37-57%, I2 = 97%), and the pooled prevalence of sleeping disturbances was 34% (95% CI: 19-50%, I2 = 98%). They did not find any significant differences in the prevalence estimates between different genders; however, the depression and anxiety prevalence estimates varied based on different screening tools. Our results show there was no clear association with covid severity at admission but more detailed analyzes follow. We can tell that patients were more afraid and stressed during hospitalisation than later. There were mostly stronger fears in women. There was some association with crp, but for now we have not found corelation with ssi.

MeSH/Keywords: COVID-19; Depression; Anxiety

Poster code: R-02-29-119

Poster Title: The role of ubiquitin C - terminal hydrolase and protein S100 - B in differentiation of patients with epileptic seizures and psychogenic non-epileptic seizures

PhD candidate: Biljana Đapić Ivančić

Part of the thesis: The role of ubiquitin C - terminal hydrolase and protein S100 - B in differentiation of patients with epileptic seizures and psychogenic non-epileptic seizures

Mentor(s): Maja Živković, PhD, research associate, Associate Professor Željka Petelin Gadže, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Psychogenic non-epileptic seizures (PNES) are functional neurological disorders or a subtype of conversion disorder where an individual exhibits paroxysmal convulsive and / or behaviour symptomatology with changes of state of consciousness that resemble epileptic seizures but are not associated with changes in cortical activity. Video-EEG monitoring is the gold standard method for differentiating epileptic seizures (ES) from PNES. However, it has the limitations of high cost, low accessibility and long hospitalisation. Laboratory tests may provide a more accessible way in differentiating ES from PNES. Recently there has been increasing interest in the use of different biomarkers to help better understanding underlying mechanism of neurological diseases. Ubiquitin C-terminal hydrolase L1 (UCH - L1) and S100-B are considered as important biomarkers which release following neuronal and glial damages. Various experimental and clinical studies have shown increased serum and cerebrospinal fluid UCHL-1 and S100-B levels in patients with ES.

Materials and methods: Patients will be included in this study according to the following criteria: (1) patients with generalised ES and focal ES with evolution to bilateral tonic - clonic seizures and with normal brain MRI (30 patients), (2) patients with PNES with normal brain MRI who underwent video - EEG monitoring (30 patients) and control group of 30 healthy controls (healthy individuals without chronic therapy, without psychiatric comorbidities).

Results: So far, the study included 20 patients with PNES, of which 15 women and 5 men and 21 patients with epileptic seizures, of which 5 women and 14 men. The average age of patients with PNES is 25 years, and patients with epilepsy 30 years. The average blood sampling time is 1 hour for both PNEN and epileptic seizures. All patients admitted to our Department for video EEG evaluation had previously diagnosed epilepsy (focal seizures with evolution to bilateral tonic clonic seizures or generalised epilepsy).

Discussion: This study will be conducted in patients with ES and PNES with normal brain MRI, and a venous blood sample will be taken between 30 minutes to 3 hours, and studies with this methods were not published so far. At the moment we have 41 patient included in study from which 20 patients with PNES and 21 with epileptic seizures and median of blood sampling is one hour. Once we conduct our study with all patients and have results of ubiquitin C - terminal hydrolase and protein S100 - B we will be able to compare them and conclude if they could be used as new biomarkers for the diagnosis of PNES.

MeSH/Keywords: epilepsy, psychogenic non-epileptic seizures (PNES), Ubiquitin C-terminal hydrolase L1 (UCHL-1), Protein S100-B

Poster code: R-02-30-065

Poster Title: Multimodal evoked potentials in the assessment of fatigue in people with early relapsing-remitting multiple sclerosis

PhD candidate: Gorana Vukorepa

Part of the thesis: Multimodal evoked potentials in the assessment of fatigue in people with early relapsing-remitting multiple sclerosis

Mentor(s): Tereza Gabelić, PhD, research associate, Assistant Professor Magdalena Krbot Skorić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Fatigue is a common and extremely disabling symptom in people with MS (pwMS) for which there are no adequate biomarkers. The most commonly used in the evaluation of fatigue are rating scales among which psychometrically most roboust is Neurology Fatigue Indeks (NFI), and as neurophysiological marker evoked potentials (EP) are used.

Materials and methods: This is an observational, prospective, longitudinal study which plans to include 113 pwMS evaluated in daily and outpatient clinic of Department of Neurology diagnosed with relapsing-remitting multiple sclerosis (RRMS) according to revised McDonald criteria 2017. Every enrolled patient will do following tests and diagnostic work up: standard diagnostic work-up for pwMS, neurological exam quantified in Expanded Disability Status Scale (EDSS) form, rating scales: Neurology Fatigue Index-(NFI-MS), Fatigue Severitiy Scale (FSS) , Modified Fatigue Severity Scale (MFIS), evoked potentials: VEP, AEP, SSEP n.tibialis and SSEP n.medianus, VEMP. For all evoked potentials latencies and amplitudes will be determined as well as EP score. Level of fatigue measured by numerical value on NFI, FSS, MFIS will be correlated with following evoked potentials modalities: VEP , AEP, SSEP n.medianus , SSEP n.tibialis, VEMP as well as with sum of total EP pathology, that is with EP score.

Results: So far the study included fifty-four pwRRMS with duration of disease 3 years of which 11 men and 43 women with mean age of 31.87 ±7.40. Each subject fulfilled following fatigue rating scales: NFI, FSS and MFIS. Absolute values mean according to fatigue rating scales was NFI 11, FSS 24, MFIS 18 (Figure 1.)

Discussion: Further determination and correlation of fatigue level quantified with rating scales with results of multimodal evoked potentials is promising tool to objectify fatigue as one of the most disabling symptom of multiple sclerosis. Once we analyze all the raw data we will be able to determine the value of neurophysiological method of multimodal evoked potentials and EP score as a new, objective functional biomarker of fatigue in pwMS.

Acknowledgments: I would like to thank to Tereza Gabelić, MD, PhD, research associate and Associate Professor Magdalena Krbot Skorić, PhD for their support in design of this study

MeSH/Keywords: fatigue, multiple sclerosis, evoked potentials

Poster code: R-02-30-068

Poster Title: Clinical characteristics of the patients with epilepsy correlated to the level of physical activity - the first insights

PhD candidate: Filip Đerke

Part of the thesis: Epidemiological and clinical characteristics of patients treated for epilepsy in the Republic of Croatia

Mentor(s): academic Vida Demarin

Affiliation: University of Zagreb School of Medicine

Introduction: Epilepsy is a chronic neurological disease that affects 1% of the general population. People with epilepsy often have comorbidities such as cognitive and psychological problems, but also social difficulties. Although it is well known that physical activity has a protective effect on most chronic non-communicable diseases, people with epilepsy have, for protective reasons, often been discouraged from playing sports. Primarily on a fear that sporting activity may cause injuries in case they get an epileptic seizure while playing sports. In 2016, the International League Against Epilepsy published a consensus paper that recommends "safe sports activity".

Materials and methods: We conducted a prospective, cross-sectional study on 280 patients, where, in addition to the clinical characteristics of epilepsy, we examined the level of physical activity using a validated query (IPAQ-SF Croatian version). All patients are adults, have been diagnosed with epilepsy and have signed informed consent to participate in the study.

Results: The included criteria meet 279 respondents of whom 270 answered all questions correctly. The study involved 131 male and 139 female respondents, with an average age of 41 years. There is a statistically significant difference between the groups of patients with low intensity and the groups of moderate and high intensity of physical activity (PA) in relation to the clinical outcome of the disease (number of epileptic seizures related to the number of years from diagnosis). In general, in the group of patients with epilepsy who has a high level of PA, an average of 0.4 epileptic seizures per year are recorded, while in the group of low-intensity PA, an average of 1.8 is recorded.

Discussion: The current results support our hypothesis of a positive effect of PA on the clinical manifestation of the disease. However, to obtain a stronger conclusion and results, it is necessary to increase the sample of respondents and consider confounding factors such as anthropometric measures, the impact of antiepileptic therapy, the aethology of the disease and the age structure of the study group.

MeSH/Keywords: epilepsy, physical activity, IPAQ-SF, clinical characteristics

Poster code: R-02-30-076

Poster Title: Demographic and clinical characteristics of patients treated with Leksell gamma knife radiosurgery in UHC Zagreb since 2015

PhD candidate: Mirea Hančević

Part of the thesis: Value of cone beam computerized tomography angiography in treatment planning for Gamma Knife radiosurgery of intracranial arteriovenous malformations

Mentor(s): Jakob Nemir, PhD, research associate, Professor Ervina Bilić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Arteriovenous malformations (AVM) are congenital vascular anomalies constructed from abnormally developed blood vessels in which blood flows directly from arteries to veins without capillaries in-between that would slow the flow. This leads to risk of rupture and intracranial hemorrhage. Decision on AVM treatment is made after consideration of numerous risk factors. Treatment options include surgery, endovascular treatment and stereotactic surgery (SRS). Gamma knife SRS is a method that uses ionizing gamma radiation to damage tumor cells, it is highly conformal with minimal radiation into surrounding healthy tissue but it is dependent on the accuracy of images used in treatment planning. Higher obliteration rate is achieved by inclusion of the entire AVM nidus which makes exact AVM visualization essential. Cone-Beam Computed Tomography Angiography (CBCT-A) is an imaging technique with high soft tissue delineation and the possibility of providing details of AVM nidus angioarchitecture but its role in gamma knife SRS planning has not been extensively explored.

Materials and methods: Study will include 60 patients with arteriovenous malformation who were treated with Leksell gamma knife radiosurgery in Clinical Hospital Centre Zagreb. All patients had MRA, DSA and CBCT-A during radiosurgery treatment planning. For this study three independent examiners will, on existing images, localize and delineate AVM nidus using following imaging combinations: MRA, MRA+DSA, CBCT-A, CBCT-A+ DSA, CBCT-A+DSA+MRA. These 3D contours will be divided into three groups by examiners and five groups by imaging methods combination. Examiner inter-variability will be assessed for volume and localization of contours. Using dose-volumetric software tools in radiosurgery computer planning system a treatment plan will be created for all contours that will be characterized by standard dose-volumetric parameters. Using MRA+DSA as a reference imaging modality, differences in dose-volumetric parameters will be determined for other imaging modalities which will allow us to assess the impact of CBCT-A. Sum of intracranial dose burden for each patient will be determined by adding doses from CBCT-A, DSA and CBCT during position verification immediately before radiosurgery and radiosurgery itself.

Results: We present demographic and clinical characteristics of patients treated with Leksell gamma knife radiosurgery in UHC Zagreb since 2015. Out of 63 patients 39 were female and 24 male. Age at the time of treatment is presented in Figure 2 with average and median patient age being 40 years. If symptomatic, patient AVM presentation was most frequently with a rupture (26), followed by seizures (17) or headache (9). Only two patients were treated surgically prior to gamma knife surgery, however 24 were treated with embolization. Pre- treatment modified Rankin score (mRS) is presented in Figure 4.

Discussion: Population based studies have reported that half of the adults with a first presentation of brain AVM have intracranial haemorrhage of which 5-25% are fatal. Second most common initial presentation are seizures which occur in up to 30% of patients. Age at diagnosis varies with various authors reporting from 57% to 95% of patients being under 50 years old. Demographic and clinical characteristics of our patients do not seem to differ from previous reports. When looking at the rates of patients treated with embolization prior to SRS, our group appears to have higher percentage of these patients than usually reported. This might be reflecting initial AVM morphology and is something that we will explore further

MeSH/Keywords: AVM, stereotactic surgery, CBCT angiography, gamma knife

Poster code: R-02-30-080

Poster Title: Apnea-hypopnea index in REM stage of sleep versus non-REM is higher in PTSD patients than in general population

PhD candidate: Ana Marija Šantić

Part of the thesis: Apnea-hypopnea index in REM stage of sleep versus non-REM is higher in PTSD patients than in general population

Mentor(s): Assistant Professor Petrana Brečić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Currently among all psychiatric disorders there is most evidence on connection between sleep apnea and posttraumatic stress disorder (PTSD) meaning that there is increaed prevalence of sleep apnea in PTSD patients. The reason for this could be molecular and inflammatory changes in central nervous system, oxydative stress, as well as significant endocrinological changes observed in patients with PTSD. Also, sleep fragmentation, sympathetic nervous system hyperactivity leading to permanent state of hyperarousal increase the occurence of sleep apnea. In the pathogenesis of PTSD there is much evidence on significant role of REM sleep in initiation and maintenance of the disorder. Changes in rapid eye movement (REM) sleep in PTSD have been well documented. In recent years there is also increased knowledge on role of REM related sleep apnea (apnea-hypopnea index (AHI) significantly higher in REM than in non-REM sleep) which serves as an independent cardiovascular risk factor. The main goal of this thesis is to try to connect between the two, documented increased prevalence of sleep apnea in PTSD and role of REM sleep in PTSD patophysiology and to research the potential connection between REM related sleep apnea and PTSD.

Materials and methods: This is a pilot study on this area. We plan to enroll 80 patients in the research, 40 with PTSD and 40 without the disorder. The patients will be recruited among outpatient population referred to the Center for sleep disorders in Psychiatric Clinic Vrape for clinical evaluation. There will only be recruited patients with significant risk for sleep apnea measured by STOP Bang questionnaire. Upon coming to the Center anthropometric measurements (weight, height, neck circumference) will be taken, patients will fulfill standardized questionnaires (Epworth questionnaire, STOP Bang). All patients will undergo overnight polysomnography by standard protocol.

Results: This research was created on clinical observation that patients with PTSD might have increased rate of REM related sleep apnea, but to this day we do not have enough enrolled patients to present.

Discussion: Considering significant connection between changes in REM stage of sleep and PTSD it could be expected that treating REM sleep related disorders, out of which is REM related sleep apnea, could lead to improvement of PTSD symptomatology. Therapeutic interventions of apnea, including REM related sleep apnea, are nowadays very efficient, especially in the form of CPAP (continous positive airway pressure) treatment. Patients with PTSD also have higher than average cardiovascular risk, and REM related sleep apnea also is independently connected with increased cardiovascular risk so in cases of joined pathology one should probably initiate treatment for sleep apnea earlier.

MeSH/Keywords: Sleep apnea, PTSD, Sleep, REM, Heart Disease Risk Factors

Poster code: R-02-30-096

Public health and healthcare – preliminary research results

Poster Title: Nasopharyngeal pneumococcal carriage and serotype distribution among children and adolescents before the introduction of the 10-valent pneumococcal conjugate vaccine

PhD candidate: Nina Krajcar

Part of the thesis: Effect of 10-valent pneumococcal conjugate vaccine on nasopharyngeal pneumococcal germination and serotype distribution in children and adolescents

Mentor(s): Professor Goran Tešović, MD PhD

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

Introduction: Streptococcus pneumoniae (Sp) is a leading cause of mucosal and invasive bacterial infections in children. Prevalence of nasopharyngeal (NP) Sp carriage, as well as incidence of pneumococcal diseases declined after pneumococcal conjugate vaccines (PCVs) have been introduced worldwide. However, a so-called serotype replacement, a marked increase in NP carriage and occurrence of pneumococcal infections due to non-vaccine serotypes (NVTs), has been observed soon after PCVs introduction. In 2018 we conducted study on NP carriage in Croatian children prior to introduction of PCV into the Croatian National Immunization Program (NIP). The objectives of the study were to analyse serotype distribution and antimicrobial susceptibility of NP Sp isolates in healthy children and in children with upper (acute otitis media - AOM) and lower (community acquired alveolar pneumonia - CAAP) respiratory tract infections (RTI).

Materials and methods: A cross-sectional multi-centric study was conducted during 12 months (2018-2019) in the pediatric consulting-rooms of 4 primary health care centers in Zagreb and in emergency room of Pediatric Infectious Diseases Department at University Hospital for Infectious Diseases (UHID). We included 900 patients in total: 750 healthy children between 6 and 48 months old and 150 children (<18 years old) with AOM and/or CAAP. Samples (one nasopharyngeal swab or aspirate per study subject) were processed in microbiologic laboratory and Sp was identified by standard methods, optochin disc and bile solubility test. Susceptibility of Sp isolates were determined from minimal inhibitory concentrations (MICs) by the gradient test method and all isolates were serotyped by Quellung reactions with antisera.

Results: A total number of Sp isolates were 143 (19%) in the first group. The first group included 750 healthy children aged 6 months (113 subjects), 13-24 months (525 subjects) and children belonging to age group 36-48 months (112 subjects). In the second group we included and analysed 150 Sp isolates form children (<18 years old) treated for AOM/CAAP. A total number of Sp isolates in both groups was 293 and to date we serotyped 191 isolates. The most frequent serotypes were 11A (2% of isolates in group 1; 3% of isolates in group 2), 14 (4% of isolates in group 1; 20% of isolates in group 2), 15C (7% of isolates in group 1; 4% of isolates in group 2), 19A (0% of isolates in group 1; 5% of isolates in group 2), 19F (10% of isolates in group 1; 10% of isolates in group 2), 23A (8% of isolates in group 1; 4% of isolates in group 2), 23F (15% of isolates in group 1; 8% of isolates in group 2), 3 (10% of isolates in group 1; 8% of isolates in group 2), 4 (2% of isolates in group 1; 5% of isolates in group 2), 6A (10% of isolates in group 1; 8% of isolates in group 2), 6B (7% of isolates in group 1; 4% of isolates in group 2) and 6C (5% of isolates in group 1; 2% of isolates in group 2). Antimicrobial susceptibility of all Sp isolates to penicillin were 79%, to ceftriaxone 98%, to azithromycin 74% and to norfloxacin 99%.

Discussion: The initial results of the study show that main predominant serotypes are 14 (9.5%), 23F (6.8%) and 19F (6.5%), followed by serotypes 3 (5.8%), 6A (5.8%) and 6B (3%) and 19A (2%). These results are similar to previous data on seroepidemiology of invasive pneumococcal diseases in Croatia which ranked serotypes 14, 3 and 19A as the most prevalent in Croatian population. These data could be useful in prediction of effectiveness of newly implemented PCV10 in Croatian NIP and possible serotype replacement in years after the vaccine introduction.

MeSH/Keywords: Streptococcus pneumoniae, nasopharyngeal carriage, 10-valent pneumococcal conjugate vaccine

Poster code: R-03-01-161

Poster Title: Sociodemographic and clinical characteristics of women with newly diagnosed breast cancer

PhD candidate: Ivana Prga Borojević

Part of the thesis: Association of symptoms of anxiety and depression with work ability in women with breast cancer

Mentor(s): Associate Professor Darko Marčinko, MD PhD, Bojana Knežević

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

Introduction: Cancer diagnoses in the working-age population are increasing. Overall, 63.5% of cancer survivors return to work, with a mean duration of absence from work of 151 days. Being able to work is in the interest of both societies at large and the individual. For cancer survivors, return to work is a part of social recovery and a step toward improving their quality of life. Because most of the research on cancer patients´ work ability has been conducted in high-income countries, there is a need for research in low- and middle-income countries where factors affecting work ability may be diverse.

Materials and methods: This prospective study will be performed at the University Hospital Center Zagreb. The study group will include a total of 77 (110 at the first assessment point) women with newly diagnosed breast cancer (stage 0, I, II, or III), aged 20 to 60 years, employed at least six months at the time of diagnosis and with no personal history of psychotic disorders. The study group will be asked to complete the Work Ability Index questionnaire, State-Trait Anxiety Inventory, Beck Depression Inventory-II, European Organization for Research and Treatment of Cancer (EORTC) QLQ-C30 core questionnaire (Version 3) with the breast module (EORTC QLQ-BR23) at two assessment points: 1-3 months after breast cancer diagnosis and 12-15 months after the first data collection. Sociodemographic data and clinical data will be collected.

Results: The research is currently in the data collecting phase, first assessment started in May 2021 with 86/110 newly diagnosed women with breast cancer who met the inclusion criteria and fulfilled the above-mentioned questionnaires. This data is partially assessed. The median age was 49.50 (range 28-60). Most participants were married (67.44%), had children (80.23%), and had at least secondary education (54.65%). Almost all the participants (94.19%) were full-time employed at the time of diagnosis, 5.81% were part-time employed, and 61.68% were working morning shifts. Almost half of the participants, 41 (47.68%), were in tumor stage I. Thirty-one (36.05%) participants had breast-conserving surgery, while 47 (54.65%) had a mastectomy. Twenty-nine participants (33.72%) received neoadjuvant chemotherapy.

Discussion: As this study is still ongoing, further measurements and statistical analysis need to be done. This study will provide data on the association of symptoms of anxiety and depression with work ability in women with breast cancer. The results of this study can be used for the development of the return to work early risk assessment model for women with breast cancer. There is also a possibility of designing a return to work planning system with the support of occupational health specialists and active psychosocial support to influence the work ability of women with breast cancer and certainly their quality of life.

MeSH/Keywords: neoplasms, return to work

Poster code: R-03-02-018

Poster Title: Assessment of miRNA expression, cadmium and lead levels in placenta in association with maternal smoking during pregnancy

PhD candidate: Adrijana Dorotić

Part of the thesis: Assessment of miRNA expression in maternal and neonatal pairs in association with cigarette smoke exposure

Mentor(s): Professor Daria Pašalić, MD PhD, Tatjana Orct, PhD, research associate

Affiliation: University of Zagreb School of Medicine, University Hospital Sveti Duh, Institute for Medical Research and Occupational Health

Introduction: Toxic and harmful substances from cigarette smoke might affect the expression of miRNA, small non-coding regulatory RNA molecules. Maternal smoking habit during pregnancy has well evidenced impact on reproductive outcomes and offspring development. The aim of the study was to determine the association of maternal cigarette smoking with placental miRNA expression of 5 candidate miRNAs and amounts of toxic metals, Cd and Pb, in healthy postpartum women.

Materials and methods: The retrospective cross-sectional study included 72 postpartum woman (37 non-smokers and 35 smokers), previously recruited at the University Hospital Merkur (Zagreb, Croatia), within the scientific project Metalorigins coordinated by the Institute of Medical Research and Occupational Health. Personal data such as age, education, gestational week, smoking habits, etc. were collected by questionnaire in hospital. The whole placentas were collected and put in clean zip-lock polyethylene bags immediately after delivery, transferred to laboratory where trophoblast tissue were sampled and stored at -80C until miRNAs isolation and metals analysis. mRNAs enriched with miRNAs were isolated from placenta, transcribed to cDNA (using kits from Qiagen, Hilden, Germany) and candidate miRNAs, miR-1537, miR-190b, miR-16, miR-21, and miR-146a were quantified by RT-PCR using Custom miScript miRNA PCR array and miScript SYBR Green PCR Kit (Qiagen, Hilden, Germany). The concentration of Cd and Pb in placenta, were analyzed by ICP-MS. Differences between smokers vs. non-smokers were tested by Mann-Whitney U-test while Spearmans correlation coefficients was used to test the relationships between relative expressions of candidate miRNAs in placenta and maternal smoking habit (expressed as number of cigarettes smoked per day) as well as concentrations of Cd and Pb in placenta. Statistical analysis was done software package TIBCO StatisticaTM, (TIBCO Software, Inc., Palo Alto, CA, USA) at a statistical significance of 5% ($P<0.05$).

Results: Smokers had lower expression of miR-21 in the placenta than non-smokers ($P<0.001$). Negative weak correlations were found between the number of cigarettes smoked per day and miR-21 in placenta ($s=-0.348$). No differences were found for other analyzed miRNAs in placenta. The concentration of Cd and Pb expressed in g/kg wet weight of placental tissue were significantly higher in placenta of smokers than non-smokers 7.62 (10.5-5.28) vs 5.48 (4.39-7.34) respectively for Cd ($P=0.014$), and 2.56 (1.86-3.66) vs 1.44 (1.06-2.52) respectively for Pb ($P=0.002$).

Discussion: The results show that smokers have higher levels of Cd and Pb in placenta than non-smokers. Previous studies showed that Cd accumulated in placenta and only a portions of Cd passes the placental barrier while Pb easily pass placental barrier and its concentrations in the cord blood being 50-60% of the values in maternal blood. Different environmental pollutants, including cigarette smoking can induce changes in miRNA expression. These changes can altered expression of different genes. Downregulation of miR-16, miR-21 and miR-146a in placenta of smokers were previously notified. This is in agreement with our findings of lower expression of miR-21 in placenta of smokers vs. non-smokers. Our previously results showed that maternal smoking (expressed as the number of cigarettes smoked per day) correlated positively with miR-16 in maternal plasma and in this study we found negative correlation between maternal smoking and miR-21 in placenta. The results of this study suggest that maternal smoking might be associated with alteration for some placental miRNAs expression. We can, therefore consider that changes of miRNA expressions might be useful biomarker which determinates the consequences of smoking on changes in microNOME and accumulation of toxic metals in placental tissues.

Acknowledgments: This study was conducted within the research project funded by Croatian Science Foundation, grant HRZZIP-2016-06-1998.

MeSH/Keywords: microRNAs, cigarette smoking, prenatal exposure

Poster code: R-03-02-127

Poster Title: Dietary exposure to pyrethroid and organophosphate insecticides in Croatian population - preliminary results of the Zagreb region Total Diet Study

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, Ireland, School of Public Health "Andrija Štampar"

Introduction: Epidemiological studies and animal experiments indicate the potential adverse effects of exposure to pyrethroid (PYR) and organophosphate (OP) insecticides on sexual development in children, even at very low exposure levels. However, limited number of studies explored these potential risks during puberty, with inconsistent results. The main objective of the PhD thesis is to assess the risk of adverse effects of exposure to PYR and OP insecticides on sexual development in (pre)pubertal boys. The first phase of the study, preliminary presented here, is to assess insufficiently known exposure to PYR and OP insecticides in Croatian population.

Materials and methods: Within the frame of the PyrOPECh study (Exposure to pyrethroid and organophosphate insecticides in children risk assessment for adverse effects on neuropsychological development and hormonal status), a Total Diet Study for the city of Zagreb and Zagreb County was conducted. The sampling plan, based on national food consumption data, was designed to cover at least 95% of the target populations diet. The purchased products were prepared "as consumed" at the dedicated kitchen facility in Zagreb, in order to reflect practices in Croatian homes. The samples were homogenised, stored frozen at -20 °C, and shipped on dry ice to Backweston Laboratory Complex, the Department of Agriculture, Food and the Marine, Celbridge, Ireland, where the samples were analysed for residues of 288 pesticides and their metabolites. The samples were extracted by the standard miniLuke method and analysed using GC-High Resolution Accurate Mass Spectrometry and LC-QQQ (Quality Control: SANTE/11312/2021). Dietary exposure assessment for PYR and OP insecticides for the Croatian and European adults and the European adolescents, was performed by the EFSA DietEX Tool v. 1.0.

Results: Out of 509 analysed composite samples (corresponding to 1800 subsamples), pesticide residues were quantified in 29% of samples. Values above a maximum residue level (MRL) were found in 4% of samples (2.5% in fruit and vegetable (FV) samples; 9% in food samples of animal origin (AO)). The most frequently quantified insecticides were neonicotinoids and PYR (33% and 22% out of quantified insecticides, respectively), followed by carbamates and OP (12% and 9%, respectively). Out of 8 PYR and 4 OP quantified insecticides, 4 PYR and 3 OP are not approved in the EU. PYR were found in various fruits, pickled vegetables, chicken meat and processed pork and beef meat. OP were found in spring onions, lettuce and honey. In Croatian adults, mean dietary exposure to PYR insecticide residues ranged from 6.6E-07 to 3.0E-05 ug/kg body weight/day for FV and from 2.7E-05 to 4.1E-02 ug/kg bw/d for AO food, and for OP insecticide residues from 8.6E-06 to 4.5E-02 ug/kg bw/d for FV and honey. These values were within the range of estimates obtained for the EU adult and adolescent population.

Discussion: The results, in general, follow the pattern obtained through EU and national monitoring programmes for pesticide residues in food. It should be pointed out, however, that in contrast to TDS, a limited number of AO samples is covered by the monitoring programmes. A higher percentage of positive results was found in unprocessed compared to processed food (43% vs. 21%), due to peeling of food as well as dilution (e.g. boiling in water), thermal degradation and/or hydrolysis of pesticide residues. The occurrence of pesticides not approved in the EU could be the result of an import from third countries, but also by degradation of approved pesticide in the case of omethoate (dimethoates metabolite). The main source of dietary exposure to PYR insecticides seems to be meat, while for OP insecticides the main source is vegetables. Higher exposure to PYR from AO food compared to FV food is due to higher average residue levels in the meat samples compared to FV samples, as well as higher dietary consumption of AO food compared to FV foods in which the PYR residues were found.

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; insecticides; dietary exposure; total diet study

Poster code: R-03-02-147

Poster Title: Association of serum calprotectin with fitness indicators and biochemical markers in continuous dynamic monitoring of the top athletes during one competitive season

PhD candidate: Frane Bukvić

Part of the thesis: Association of serum calprotectin with fitness indicators and biochemical markers in continuous dynamic monitoring of the top athletes during one competitive season

Mentor(s): Professor Daria Pašalić, MD PhD, Professor Saša Janković, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Sport is present in people's lives nowadays, either in recreational or professional form and athletes have high demands from coaches, teammates and others. Due to overtraining and various injuries, it is important to know how to dose training, recognize the level of physical fitness of athletes and to provide them the time to recover from training.

Materials and methods: This is a retrospective study. 20 professional male athletes (waterpolo players, N = 20, middle age 21 years, range 15-31 years) were included in this study during one competitive season (September - May). Blood samples were taken and tests of physical fitness were measured four times during one competitive season (September, December, February and May). We have determined serum concentrations of calprotectin, CRP and myoglobin by immunoturbidimetric method, CK, AST and ALT activities with photometric UV method, cholesterol, HDL-cholesterol and LDL-cholesterolwith homogeneous enzymatic method and IL-6, cortisol and troponin by immunochemical method. Repetitive power, maximum power and static power tests were performed in analysing physical fitness.

Results: Our preliminary results of calprotectin measurements show a tendency to decrease calprotectin values during the competitive season in a group of 20 professional athletes (median values: I - 2.92 µg / mL; II - 2.35 µg / mL; III - 2.27 µg / mL; IV - 1.47 µg / mL (0,012)). Cortisol values were the highest in the middle of the season, during peak training level (median values: I - 214 nmol / L; II - 385 nmol / L; III - 376 nmol / L; IV - 351 nmol / L (0,050). Respondents had better physical fitness results at the beginning of the season than at the end of the season. For example, number of maximum pull ups until failure: median number I - 12,1; II 10,1; III 8,1; IV 6,8 ($p < 0.001$) or maximum number of sit ups in 60 seconds: median number I 68,150; II 62,150; III 54,150; IV 51,600 ($p < 0.001$).

Discussion: Our results show that professional athletes lead their body to overtraining during the season, which is manifested by a decline in fitness indicators and changes in the values of certain biochemical markers. Calprotectin, as a potentially new marker of fitness monitoring, shows a proportional tendency to decline together with fitness indicators. There is not much research on the topic of calprotectin as a fitness indicator. Figo et al., in a study of 9 healthy individuals, showed that calprotectin values showed a slight increase after continuous moderate physical activity compared to acute intense activity. Our further investigation will be focused to determine the usefulness and prognostic significance of determining the concentration of calprotectin and other biochemical markers in specific phases of physical fitness at different stages of the season. We also intend to compare and determine the relationship between biochemical markers at different stages of physical fitness during competitive season

MeSH/Keywords: calprotection, biochemical markers, physical fitness

Poster code: R-03-03-104

Poster Title: GPs knowledge/awareness about EMB and their attitudes towards clinical drug trials

PhD candidate: Maja Marković Zoya

Part of the thesis: GPs knowledge/awareness about EMB and their attitudes towards clinical drug trials

Mentor(s): Professor Vladimir Trkulja, MD PhD, Assistant Professor Ksenija Kranjčević, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Aim. To present preliminary data from the countries included in the project.

Materials and methods: Methods. Simple descriptives on included general practitioners (GPs) and patients from the included countries

Results: Results. In addition to Croatia, of the 11 invited South-Eastern European countries, 8 accepted participation and 5 actually participated (Bosnia and Herzegovina [Federation BiH and also Republika Srpska], Montenegro, Serbia, North Macedonia and Turkey); of the 10 invited Western European countries, 5 accepted participation and 3 actually participated (Poland, Portugal and Spain). Included GPs and their patients: i) Croatia 102 GPs (80 women [80%]/22 men) enrolled 463 patients. Total number of GPs in Croatia is 2.212 (78% women/22% men); ii) Federation BiH 20 GPs (17 women [85.0%]/3 men) enrolled 100 patients. Total number of GPs in Federation BiH is 746 (82.4% women/17.6% men); iii) Republika Srpska 20 GPs (19 women [95.0%]/1 man) enrolled 98 patients. Total number of GPs is 394 (77% women/ 23% men); iv) Montenegro 13 GPs (11 women [84.6%]/2 men) enrolled 56 patients. Total number of GPs in Montenegro is presently not known; v) Serbia 20 GPs (18 women [90%]/2 men) enrolled 100 patients. Total number of GPs in Serbia is 4358 (76% women/24% men); vi) North Macedonia 20 GPs (15 women [75%]/5 men) enrolled 99 patients. Total number of GPs in North Macedonia is 1525 (73% women/27% men); vii) Turkey 20 GPs (11 women [55%]/ 9 men) enrolled 100 patients. Total number of GPs in Turkey is 46843 (gender structure not known at the moment); viii) Poland 20 GPs (13 women [65%]/7 men) enrolled 93 patients. Total number of GPs is Poland is 35534. Gender structure is currently not known; ix) Portugal 20 GPs (12 women [60%] / 8 men) enrolled 198 patients. Total number of GPs in Portugal is 7768 (62.4% women); x) Spain 20 GPs (11 women [55%]/9 men) enrolled 94 patients. Total number of GPs in Spain is 35534 (54.0% women). Overall, questionnaires were completed by 275 GPs from Croatia and 7 other countries, and by 1404 of their patients.

Discussion: Discussion. Enrolled numbers of GPs and patients are very close to the planned minimal numbers (20 GPs per country except Croatia, where 100 were planned; 5 patients per GP, i.e., 20 per country except Croatia were 500 were planned). Although no systematic sampling was implemented, the gender structure of included GPs reflects the gender structure of GPs in the respective country. Considering the lack of systematic sampling and the fact that the numbers included are small fractions of the total number of GPs in larger countries (e.g.,Turkey, Poland, Spain) no sample weights will be assigned, but data will be analyzed as generated. This will limit generalizability (ie., projections to the country level), but potential differences within the sample are nevertheless valid. Conclusion. The extent of collected data is in line with realistic a priori expectations (on the minimum expected numbers). Data analysis is in progress.

MeSH/Keywords: South-Eastern Europe, Western Europe, GP's, attitudes

Poster code: R-03-04-049

Poster Title: Effect of short-term intervention using an integrated, individual and interdisciplinary approach in cardiovascular disease prevention on SCORE value

PhD candidate: Ino Kermc

Part of the thesis: Effect of short-term intervention using an integrated, individual and interdisciplinary approach in cardiovascular disease prevention on SCORE value

Mentor(s): Associate Professor Venija Cerovečki Nekić, MD PhD, Assistant Professor Jure Samardžić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Cardiovascular diseases are the leading cause of death in all developed countries of the world. In the Republic of Croatia, they are also the leading cause of overall mortality and despite the fact that Croatia is a Mediterranean country, the European Society of Cardiology defines Croatia as a country at high risk for cardiovascular disease. As preventive work is represented in the daily work of a family doctor, and cardiovascular diseases are the most common chronic diseases in the family doctor's office, there is a need to improve the preventive activities of family doctors in the prevention of cardiovascular diseases. The overall goal of this research is to develop an effective prevention model at the primary health care level for patients at risk for cardiovascular disease using an integrated care, individual and interdisciplinary approach

Materials and methods: The research will be in form of the prospective cohort study, with subjects aged 40 to 65 years with one or more risk factors for CVD development. Sampling will be convenient, consecutive. Every fifth patient meeting the inclusion and exclusion criteria, 17 per practice. The research would include 20 family medicine practices. All family physicians from institutions that have a multidisciplinary team available will be invited, and we will select those who can provide a suitable sample. The total number of respondents would be 340. Inclusion criteria: SCORE> = 5%, 40-65 years, without previous cardiovascular disease, able to participate in the intervention. Exclusion criteria: diabetics, patients with moderate or severe chronic kidney disease (GFR <60), pregnant women, patients with malignant tumors, patients with severe mental illness. The intervention would include an individual approach of the family doctor in the assessment of cardiovascular risk, conducting a motivational interview and creating an individual treatment and monitoring plan that would include the formation of an interdisciplinary team. An interdisciplinary team can consist of a psychologist, a nutritionist and a kinesiologist. The SCORE value is recorded 6 months before the start of the intervention, at the beginning of the intervention and after 6 months from the beginning of the intervention. Risk factors in accordance with the guidelines for the prevention of CVD are recorded at the beginning of the intervention and after 6 months from the beginning of the intervention. Risk factors which are going to be measured are: body weight, body mass index, waist circumference, hip circumference, number of cigarettes per day, blood pressure, blood glucose, total cholesterol, HDL, LDL, triglycerides, creatinine, physical activity measured by IPAQ questionnaire and quality of life as measured by the SF-36 questionnaire. Descriptive statistical methods and two-way t-test for paired samples using effect size as the initial parameter will be used for statistical data processing.

Results: At this moment the research is in its first phase 17 practices are defined, and the initial sampling has begun. Because of the limited capacities of our multidisciplinary team, we could not have started with all 17 practices at the same time. This way participants first selected in February would be in the intervention phase in August. We still do not have enough data to analyze but in the next months some of the participants will enter in the second phase of the research, the intervention. Then we can compare first SCORE value, measured at the start of the research and the second SCORE value, measured on the start of the intervention. That way we can see if only the measurement of the risk factors and the SCORE value two times in six months, without any other intervention, had any impact on the SCORE value.

Discussion: Only measuring the SCORE value two times in the six months could have an impact on participants behavior, or could motivate the physicians to prescribe certain medications, advise or conduct motivational interview. If that would be the case, and there would not be a difference between the last SCORE value, measured after the intervention and the one measured before then our intervention would not have any effect. We do not expect that, but we included that first, control, phase in our research just to be sure there is not any difference between the regular physicians work and the intervention.

MeSH/Keywords: primary health care, family medicine, prevention of cardiovascular diseases, interdisciplinary approach, individual approach, integrated care

Poster code: R-03-04-111

Poster Title: Physical activity, self-assessed health and quality of life of the elderly during the COVID-19 pandemic

PhD candidate: Nada Pjevač

Part of the thesis: Physical activity, self-assessed health and quality of life of the elderly during the COVID-19 pandemic

Mentor(s): Professor Mirjana Kujundžić Tiljak, MD PhD, Associate Professor Venija Cerovečki Nekić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: According to the definition of the World Health Organization, the elderly are aged 60 to 75, old are aged 76 to 90, and very old are people over 90. The coronavirus discovered in China in 2019 and was named SARS-CoV -2 (Severe Acute respiratory Syndrome Coronavirus -2). The disease caused by the SARS-CoV -2 virus is called COVID-19. According to existing data, the elderly and people with chronic diseases (hypertension, heart disease, diabetes, respiratory diseases, malignant diseases) have a higher risk of developing a more severe clinical picture, so hospital treatment is needed.

Materials and methods: Respondents will be citizens of the city of Zagreb aged 65 and over (500 will be patients of general practices in the city of Zagreb and 500 respondents will be residents of Homes for the elderly and infirm in the city of Zagreb).We will use these 3 questionnaires: Personal wellbeing index questionnaire for measuring the quality of life, Short form health survey SF-36- structured questionnaire with questions related to demographic characteristics and way of living, self-assessment of health, and International physical activity questionnaire IPAQ-International Physical Activity Questionnaire. And also, we will ask respondents questions related to COVID-19 disease.

Results: The study is currently in the phase of data collection. Until now, 420 participants were included, 320 participants are patients of general practices and 100 are residents of Homes for the elderly and infirm in the city of Zagreb.

Discussion: Assessing the impact of physical activity and the associated lower health self-assessments on the quality of life of the elderly during the COVID-19 pandemic will help define factors that reduce the daily activities of the elderly and have a negative impact on health and new health problems. The results of the research can be used to develop special programs to encourage physical activity in the elderly in extraordinary circumstances such as the COVID-19 pandemic.

MeSH/Keywords: physical activity, elderly people, Covid-19, quality of life, self-assessed health

Poster code: R-03-04-112

Research proposals

Basic medical sciences – research proposals

Poster Title: The effect of BPC 157 on surgically induced lumbar spine instability in the rat

PhD candidate: Stjepan Dokuzović

Part of the thesis: The effect of BPC 157 on surgically induced lumbar spine instability in the rat

Mentor(s): Professor Predrag Sikirić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Some aspects of spinal instability are a controversial topic even in the spine surgery literature today, particularly instability due to lumbar degenerative spinal stenosis and the precise effects of various surgical interventions on segmental stability. The dynamic spinal segment is a complex anatomical whole involving many different types of tissues working together smoothly. Most open surgeries on the posterior aspect of the spine necessitate some form of damaging the muscle tissue "in the way", as well as the removal of some bone, cartilage and/or i.v. disk tissue. The therapeutic effects of BPC 157 on many types of tissues in various contexts have already been thoroughly covered in the literature, including its effect on the healing of bone, ligament, tendon, myotendinous junction, and cartilage. The effect of BPC 157 on the spinal dynamic segment has not yet been evaluated, though it should follow from previous studies that there should be profound beneficial effects, which warrants investigation. A model of lumbar disk degeneration by means of instability through surgical facetectomy has already been described and will serve as the foundation for this experiment.

Hypothesis: BPC 157 alleviates the effects of lumbar instability induced surgically by interlaminectomy and bilateral facetectomy in a rat model.

Aims: To show that BPC 157 provides benefits in the clinical performance of the test subjects, and that the degenerative downstream changes will be lessened and clearly visible in radiographic images and on histological analysis.

Materials and methods: Female Wistar rats roughly 4 months of age are used in the experiment. All of them undergo the same procedure in intraperitoneal anesthesia - an interlaminectomy and bilateral facetectomy at the mid lumbar (L3/4) level, thereby causing spinal instability. They are further separated into subgroups depending on the treatment to be given (drinking water, BPC 157 in a microgram dosage, or BPC 157 in a nanogram dosage), as well as time period until MSCT evaluation and sacrificing. Time periods selected for MSCT and histological evaluation include 1, 2, 4, 6, and 12 months from the day of surgery. Each subgroup consists of 6 animals - 6 animals in each of 5 time periods receiving one of 3 treatments, bringing the total animal count up to 90. Measurable clinical parameters are gathered along the way during the animals' lifespans, including gait analysis as described in previous studies. Upon sacrificing, lumbar spines are harvested and used for histological analysis of muscle tissue changes and intervertebral disk degeneration. Radiographic analysis will consist of measuring the local kyphotic angle and observing degenerative changes on i.v. disks and bony endplates. Histological findings are to be graded according to defined grading systems from the histopathology literature.

Expected scientific contribution: It is expected that the known beneficial effects of BPC 157 will work in concert on multiple types of tissues as presented by the rat spinal instability model, providing a potential pharmacological treatment to an otherwise surgical issue.

MeSH/Keywords: Rat lumbar spine, lumbar spine instability, BPC 157, rat gait analysis

Poster code: T-01-03-066

Poster Title: The effect of pentadecapeptide BPC 157 on alcohol-induced lesions of breast and sourrounding tissue in rats

PhD candidate: Mariam Samara

Part of the thesis: The effect of pentadecapeptide BPC 157 on alcohol-induced lesions of breast and sourrounding tissue in rats

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

Affiliation: University of Zagreb School of Medicine

Introduction: The effect of alcohol by direct application to the gastric mucosa and the consequences of direct contact of alcohol with the cell is described in Andre Robert's work on cytoprotection. According to the paper, the cytoprotective effect would be to antagonize these lesions. One of the agents that has been shown to be a potential essential mediator of Robert's cytoprotection is the stable gastric pentadecapeptide BPC 157, which has shown direct endothelial protection, anti-inflammatory activity - most likely due to its angiomodular effect, and high healing potential. Mastitis, a synonym for breast inflammation, regardless of etiology, which can be infectious or non-infectious, has been the focus of numerous studies. Therefore, seeing the previously mentioned effects of pentadecapeptide BPC 157, and given the currently existing models of inflammation that are complicated, expensive and focus almost exclusively on the effect on milk secretion and quality, the idea was to transfer the model shown in gastric tissue to mammary gland and adjacent tissue.

Hypothesis: Application of 96% ethanol to the mammary gland and surrounding rat tissue will create a lesion that will correspond to mastitis model that will be comparable to human mastitis due to pathohistological features, and the use of pentadecapeptide BPC 157 will reduce the resulting tissue lesions.

Aims: The aim of this study was to create a non-lactation model of mastitis by direct application of ethanol, to antagonize lesions with pentadecapeptide BPC 157 - to define the effect on inflammation and the connection with the NO system

Materials and methods: In this study, 12-week-old female albino rats with 200g body weight, randomly divided into groups, will be used. Each group contains 6 animals, and there are control groups, groups to which the pentadecapeptide BPC 157 or NOS substrate / blocker was administered intraperitoneally. Different intervals were done, intervals of 15min, 30min, hour, week and 2 weeks. The total number of experimental groups will be 8, and the total number of animals in the experiment will be 240. 0.1 ml of 96% ethanol will be administered subcutaneously to the mammary region with a 30G insulin injection, followed immediately by various agents intraperitoneally (0.9% NaCl solution, pentadecapeptide BPC 157, NOS substrate L-arginine, NOS blocker L-NAME) . To assess the lesions, I used the same indicators used in existing models - clinical images, macroscopic and microscopic changes (pathohistological analysis).

Expected scientific contribution: Since the existing models of mastitis are complicated and expensive, the idea was to create a model of inflammation that is fast, reproducible, cheap, and effective. In addition, existing models throw the main focus on milk secretion and quality, and not so much on the effect on the mammary gland and surrounding tissue.

MeSH/Keywords: ethanol, cytoprotection, pentadecapeptide BPC 157, alcohol-induced lesions, mammary gland

Poster code: T-01-03-115

Poster Title: Effect of BPC 157 on multiorgan failure syndrome induced by high-dose megestrol acetate in rats

PhD candidate: Branimir Krtalić

Part of the thesis: Effect of pentadecapeptide BPC 157 on multiorgan failure syndrome induced by high-dose megestrol acetate in rats

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

Affiliation: University of Zagreb School of Medicine

Introduction: Pentadecapeptide BPC 157 is the synthetic variant of the active endogenous peptide fragment previously isolated from the human gastric juice. Trials have shown it to have a safe profile, LD1 dose has not been achieved in either human cells or animal models, and clinical trials found no toxicity. It has shown an organoprotective effect in rats in various conditions, e.g. occlusive states (e.g. Budd-Chiari syndrome), alcohol, lithium, or isoprenaline intoxication etc. All of the above share similar pathophysiological characteristics: aortic hypotension, widespread veinous and arterial thrombosis, intracranial hypertension, tissue oxidative stress, caval and portal hypertension, ECG disturbances, end-organ damage etc. Megestrol acetate (MA) is a synthetic progestagen used to treat cancer or AIDS-related anorexia, or as a palliative treatment for advanced breast cancer. Some studies have shown that MA increases the risk of deep vein thrombosis, possibly dose-dependently. Considering that other substances such as lithium, alcohol and isoprenaline cause the above mentioned syndrome, there might be a similar response to high dose MA in rats, which might also be counteracted by BPC 157.

Hypothesis: BPC 157 will counteract the multiple organ dysfunction syndrome caused by high doses of MA in rats: systemic arterial and venous thrombosis, aortic hypotension, portal and caval hypertension, intracranial hypertension and multiple internal organ lesions.

Aims: The primary aim is to prove that high doses of MA injected in rats will cause the syndrome that includes: systemic arterial and veinous thrombosis, aortic hypotension, portal and caval hypertension, intracranial hypertension and multiple internal organ lesions; the consequences of which shall be attenuated or prevented by BPC 157. Other aims include determining: the macroscopic and pathohystological differences in heart, lungs, GI tract, and brain between the groups; the ECG differences between the groups; hemodynamic changes in the groups.

Materials and methods: 4-month-old female Wistar rats weighing 200-250 g will be used in this trials, whilst maintaining good laboratory practice and upholding ethical norms. The animals will be anesthetized using thiopenthal 40 mg/kg and diazepam 10 mg/kg. After opening the abdomen, 80 mg/kg of MA will be injected into the inferior vena cava with a needle. In accordance to previous studies, the following shall be obtained: an ECG, a recording of the brain after craniotomy, a recording of the abdominal organs and vessels (superior mesenteric artery, superior mesenteric vein, portal vein (PV), inferior vena cava (ICV)), an invasive measurement of blood pressure in the ICV, saggital sinus (SS), PV, and the abdominal aorta (AA). After euthanasing the animals using double dose of anesthetic, the weight of the thrombi in ICV, SS, PV and AA shall be measured and the visceral organs, heart, lungs and the brain shall be extracted for pathohystological analysis using an appropriate scoring system. The animals will be divided into following groups: control (peritoneal shower with saline after 30 or 60 minutes, otherwise drinking water during the 24, 48 or 72 hours following the procedure); 1st therapeutic group (peritoneal shower with 10 ng/kg BPC 157 after 30 or 60 minutes, otherwise drinking 10 ng/kg BPC 157 during the 24, 48 or 72 hours following the procedure); 2nd therapeutic group (peritoneal shower with 10 mcg/kg BPC 157 after 30 or 60 minutes, otherwise drinking 10 mcg/kg BPC 157 during the 24, 48 or 72 hours following the procedure).

Expected scientific contribution: Expected scientific contribution is to prove the protective effect of BPC 157 in the model of multiorgan failure induced by MA in rats, which would suggest its potential clinical application in similar syndromes occuring in humans.

MeSH/Keywords: BPC 157, megestrol acetate, Multiple Organ Dysfunction Syndrome, Wistar rats

Poster code: T-01-03-141

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

PhD candidate: Ivan Krezić

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

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

Affiliation: University of Zagreb School of Medicine

Introduction: Huntingtons disease (HD) is an autosomal neurodegenerative disease associated with severe degeneration of basal ganglia neurons, especially the striatum, and characterized by motor, cognitive, and psychiatric disorders and has no existing cure for the disease.

Hypothesis: Pentadecapeptide BPC 157 prevents the onset and progression of HD, and reduces the degree of damage in the HD model caused by 3-nitroproprionic acid (3-NP).

Aims: Demonstrate the neuroprotective effect of pentadecapeptide BPC 157 on a model of 3-NP induced HD.

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

Expected scientific contribution: In this study, we will demonstrate the protective effect of BPC 157 on preventing and reducing damage of the brain in the animal model of 3-NPK-induced HD and its potential for clinical use in HD to halt disease progression.

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

Poster code: T-01-03-160

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

PhD candidate: Hrvoje Bilić

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

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

Affiliation: University of Zagreb School of Medicine

Introduction: Recent studies have shown an association between inflammasomes and genes encoding inflammasomes components with autoimmune diseases. Myasthenia gravis (MG) is a neurological autoimmune disorder while the NLRP3 inflammasome is the most researched inflammasomes until now.

Hypothesis: The incidence of SNPs rs3806265 and rs35829419 of the NLRP3 gene is higher in patients with myasthenia gravis when compared to healthy subjects.

Aims: The general aim of this study is to examine the incidence of SNPs rs3806265 and rs35829419 of the NLRP3 gene in patients with myasthenia gravis and healthy subjects. Specific aims include the following: 1. to examine whether there is a difference in the methylation status of the promoter region of the NLRP3 gene in patients with MG compared to healthy subjects, 2. analyse the clinical characteristics of patients with MG, 3. assess whether there is a difference in the incidence of SNPs rs3806265 and rs35829419 in individual subgroups of patients with MG (classified on the basis of clinical characteristics).

Materials and methods: The study will include a sample of 125 patients diagnosed with MG and a control group of 75 subjects. 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.

Expected scientific contribution: A more frequent occurrence of certain SNPs and altered methylation status of the NLRP3 gene promoter region in patients with MG compared to the general population may shed a new light on the mechanisms underlying autoimmune deregulation in MG.

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

Poster code: T-01-05-106

Poster Title: The cellular expression of molecular participants of the Wnt signaling pathway, LEF1, beta catenin, GSK 3beta and DVL1 in the development and progression of human retinoblastomas

PhD candidate: Leon Marković

Part of the thesis: The cellular expression of molecular participants of the Wnt signaling pathway, LEF1, beta catenin, GSK 3beta 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 a neuroectodermal intraocular malignant tumor that affects children. Although its incidence is low, it is the most common primary intraocular malignancy in children. Familial cases of retinoblastoma have shown that there is a genetic basis for the development and progression of this tumor. However, the genetic changes responsible for this tumor have only partly been elucidated in recent decades. The Wnt signaling pathway is one of the basic cellular signaling pathway essential for proper embryonic development, but is also involved in the tumorigenesis of numerous human tissues. The key executive molecules in this signaling, which begins in the extracellular space and ends in the nucleus, are Dishevelled, catenin, LEF1, GSK- 3 and N-myc. There are several studies showing that the Wnt signaling pathway is involved in the etiology of retinoblastoma, however the roles of individual genes and proteins in the formation and progression have not been sufficiently investigated.

Hypothesis: The expression of molecular participants in the Wnt signaling pathway (LEF1, DVL1, beta catenin, and GSK 3beta) is increased in human retinoblastoma cells relative to normal retinal cells.

Aims: The aim of the study is to determine the level and localization of expression of Wnt signaling pathway participants (LEF1, DVL1, beta catenin and GSK 3beta) in retinoblastomas and healthy human retina, the relationship between cellular expression in retinoblastoma and normal retina cells and to investigate the correlation between the levels of cellular expression in retinoblastomas and clinical and histopathological subgroups of retinoblastoma patients.

Materials and methods: Samples of 20 human retinoblastomas and 20 healthy human retinal tissue will be collected 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 . To determine the cellular localization of the components of the Wnt signaling pathway and to determine the level of their expression, the method of immunohistochemistry will be used. Next, the immunostained tissue samples will be analyzed using the semiquantitative method of color index, the so-called IRS (from eng. immunoreactivity score).

Expected scientific contribution: Detection of molecular changes of Wnt signaling, important in the development and progression of retinoblastoma, may represent potential diagnostic and prognostic markers and an attractive future strategy for the treatment of retinoblastoma.

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

Poster code: T-01-08-083

Poster Title: Regenerative effect of oral mucosa stem cells on hypoxia damaged neurons in vitro

PhD candidate: Paula Stančin

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

Mentor(s): Associate Professor Dinko Mitrečić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Human oral mucosa is potentially a new source of stem cells that can be used in regenerative medicine.

Hypothesis: Oral mucosa stem cells (hOMSC) improve the recovery of neurons damaged by hypoxia.

Aims: The aims of this study are to establish a standardized procedure for isolating stem cells from human oral mucosa tissue, to find markers that define hOMSC, to investigate hOMSC potential to differentiate into nerve cells and to see how hOMSC act on neurons damaged by hypoxia.

Materials and methods: Based on the approval obtained from the Ethical Board of the University of Zagreb School of Medicine this research starts with biopsy of oral mucosa tissue from volunteers that signed informed consent. Obtained tissue will be washed in a medium consisting of streptomycin, penicillin and amphotericin B, cut into small pieces and then immersed in the mixture of collagenase and dispase overnight to help separate lamina propria from epithelium which will be used in further research. Small pieces of lamina propria will be 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. After cells growing from the pieces of tissue have been noticed they will be detached using 0.25 % trypsin and seeded in new plates. In order to find the markers that best describe these cells, the expression of neural crest markers (Slug), embryonic stem cell markers (Oct4, Sox2) and markers we believe will help us in accurate characterization: CD40 and CD166 will be investigated. Marker expression analysis will be performed using RT-PCR and Western blot methods, and visualization by immunocytochemistry where cells will be labeled with fluorescent secondary antibodies and observed on a confocal microscope. Since we assume that hOMSCs originate from the neural crest, it is necessary to investigate their potential for differentiation into neurons. Cells will be kept in a medium of different combinations of supplements: -NGF, BDNF and antibiotics to encourage differentiation into neurons. In order to compare and monitor the course of differentiation, the cells will be fixed 1st, 3rd, 5th, 7th and 14th day. For the purpose of analysis, immunocytochemistry will be performed with markers of differentiation of the nervous system: nestin, DCX, Map2, Tub. The final step in this research will be to investigate the impact of oral stem cells on neurons damaged by hypoxia. The neurons will be obtained by differentiation from hOMSC. In order to investigate the presumed effect of oral stem cells on neurons we will have culture of neurons exposed to hypoxia for 12, 24 and 48h. To one group of neurons will be added hOMSC, to second one conditioned medium from hOMSC, to third one exosomes isolated from hOMSC and last group will be control group. To induce hypoxia, we will use a Memmert incubator with 1% oxygen. In all the above groups, we will monitore: a) cell survival (Live / Dead essay), b) degree of damage (LDH test) and c) morphological parameters, branching and length of neurons (semi-automated protocol developed in our group). In this way, we will get an answer as to how exogenously added oral stem cells act on damaged neurons and whether their positive effect requires direct contact with damaged cells or whether their products present in the medium are sufficient.

Expected scientific contribution: This research will characterize a new population of stem cells that has potential to be used in regenerative medicine. If it is shown that cells isolated from the oral mucosa can contribute to the recovery of neurons damaged by hypoxia, this will open new ways of researching therapeutic procedures in the treatment of stroke and other neurodegenerative diseases.

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

Poster code: T-01-08-098

Poster Title: Development of cerebral cortex and neurodegenerative changes in Down syndrome on human cerebral organoids model

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): Associate Professor Dinko Mitrečić, MD PhD, Assistant Professor Ivan Alić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Development of the human cerebral cortex includes many different cells and complex processes which occur during the long embryonal, foetal, and postnatal periods. Cortical region is also the site of the largest evolutionary differences between rodents and humans and the reason why animal models are not credible to study the human brain. Human cerebral organoids present a useful, three-dimensional in vitro model which are already being used to study human brain development as well as different neurodegenerative and many other disorders. In this study we will explore the possibility of modelling human brain corticogenesis and neurodegeneration in Down syndrome by using the in vitro model of isogenic cerebral organoids generated from induced pluripotent stem cells (iPSCs), which are reprogrammed from the fibroblasts of a person with constitutional mosaicism for Down syndrome, meaning that the reprogrammed cell lines are separately cloned to obtain both trisomic (T21) and disomic iPSCs lines (D21).

Hypothesis: Trisomy of the chromosome 21 leads to impaired development and neurodegenerative changes in the in vitro model of cerebral organoids.

Aims: The general aim of this study is to describe the development of cerebral cortex in people with Down syndrome and their isogenic controls by using the in vitro model of cerebral organoids. To specify, we will describe the development of all six layers of the cerebral cortex on the isogenic cerebral organoids and compare the neurodegenerative changes of the cerebral cortex on the isogenic cerebral organoids. The events observed during the differentiation of the isogenic cerebral organoids will be compared to those which occur during the development of embryonal and foetal brain in people with and without Down syndrome.

Materials and methods: T21 and D21 iPSCs lines will be used to generate cerebral organoids by using the already established protocol to explore the occurrence of developmental and neurodegenerative changes during the development of the human cerebral cortex in people with Down syndrome. iPSCs will be cultivated on ultra-low attachment 96 well plates until the formation of embryoid bodies (EB). After the neural induction of EBs, they will be embedded in Matrigel droplets and transferred to dishes containing organoid differentiation medium. Cerebral organoids will be analysed on the 30, 50, 70 and 100 days in vitro and compared with foetal human brains. Immunocytochemistry, immunohistochemistry, and Western blot will be performed by using the specific markers to describe the development of the cerebral cortex and neurodegenerative changes of the in vitro model of cerebral organoids. The same markers will be used on the post mortem human brain tissue. We will also separately generate neural stem cells (NSCs) from the same T21 and D21 iPSCs lines. These NSCs will be used for the differentiation of neurons so we will be able to analyse and compare the disomic and trisomic neurons. All samples will be analysed with the confocal laser microscope Olympus FV 3000. For quantification and colocalization calculations we will use Imaris software, and statistical analysis of the obtained data will be performed based on the normality of data distribution.

Expected scientific contribution: The results of this study will help with understanding the full potential of this in vitro model of human corticogenesis and neurodegenerative disorders in people with Down syndrome.

MeSH/Keywords: induced pluripotent stem cells, cerebral organoids, Down syndrome, corticogenesis, neurodegeneration

Poster code: T-01-08-130

Poster Title: Robot-assisted foramen ovale puncture during radiofrequency thermoablation of the Gasser's ganglion in patients with trigeminal neuralgia

PhD candidate: Anđelo Kaštelančić

Part of the thesis: Robot-assisted foramen ovale puncture during radiofrequency thermoablation of the Gasser's ganglion in patients with trigeminal neuralgia

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

Affiliation: University of Zagreb School of Medicine

Introduction: Trigeminal neuralgia is a condition characterized by the appearance of strong, paroxysmal, radiating pain that affects one or more innervation branches of the trigeminal nerve of one side of the face, rarely both, and is most often provoked by external factors and stimuli. Due to the chronicity and intensity of pain, the quality of life is greatly impaired and an increased suicide rate has even been observed in these patients compared to the general population. Trigeminal neuralgia is treated with medication, surgery or radiosurgery (Gamma-knife). Surgical techniques include microvascular decompression of the trigeminal nerve and percutaneous rhizotomy methods. Percutaneous methods are based on the "free-hand" technique of cannulating the foramen ovale using points determined on the face (Hartel's landmarks) and using X-rays to verify the position. Approaching from a point on the face that is about 10 cm away from the foramen ovale, which is 3x6 mm in size, is a challenge so percutaneous techniques require education and experience. There are 3 ways to act on the Gasser's ganglion by percutaneous methods: radiofrequency thermoablation, balloon compression or glycerol ablation. Despite the challenge of "free-hand" percutaneous technique, there are only a dozen papers in the literature that describe the different uses of standard neuronavigation systems in performing percutaneous surgery in trigeminal neuralgia. Today, robotic systems are increasingly used in neurosurgery whether it is intracranial or spinal surgery. For several years now, biopsies of intracranial tumor lesions have been successfully performed at the Dubrava University Hospital with the help of the RONNA robotic system.

Hypothesis: Robot-assisted foramen ovale puncture enables successful and safe radiofrequency thermoablation of the Gasser's ganglion.

Aims: Evaluation of the accuracy and safety of robot-assisted radiofrequency thermoablation of the Gasser's ganglion in patients with trigeminal neuralgia.

Materials and methods: A prospective study of robot-assisted radiofrequency thermoablation of the Gasser's ganglion will be conducted at the Dubrava University Hospital Dubrava for 2-3 years in about 20 patients with trigeminal neuralgia and in whom percutaneous rhizotomy is indicated. Each patient will be familiar with the characteristics of the procedure and will sign an informed consent and consent to participate in the study. Prior to that, the permission of the ethics committee for the said research will be obtained. Neuroradiological imaging will be performed preoperatively to identify the foramen ovale. Based on the preoperative images, the most adequate trajectory of the rhizotomy electrode will be planned also based on a standard technique using Hartel's landmarks. After trajectory planning the foramen ovale will be canulated with the help of a robotic neuronavigation system with standard rhizotomy electrode used at the Dubrava University Hospital during the percutaneous radiofrequency thermoablation of the Gasser's ganglion. A control neuroradiological scan will be performed to verify the location of the rhizotomy electrode tip. Evaluation of each step from preoperative imaging, foramen ovale determination, trajectory planning, robot-assisted rhizotomy and control imaging to locate the electrode tip will be performed. For each performed procedure, the localization of the electrode tip determined by control imaging will be compared with the target point determined by the initial imaging in order to determine the accuracy and safety of robot-assisted radiofrequency thermoablation of the Gasser's ganglion.

Expected scientific contribution: Demonstrate the possibility of performing robot-assisted percutaneous rhizotomy of the Gasser's ganglion with the aim of increasing the success of the operation and reducing the complication rate.

MeSH/Keywords: trigeminal neuralgia, Gasser's ganglion, rhizotomy, radiofrequency thermoablation, robot, robot-assisted radiofequency thermoablation

Poster code: T-01-08-132

Poster Title: Sphingolipidomic profiling of malignant and benign brain tumors

PhD candidate: Mia Jurilj

Part of the thesis: Sphingolipidomic profiling of malignant and benign brain tumors

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

Affiliation: Department of Neurosurgery, University Hospital Center ''Sestre milosrdnice'', Department of Medical Chemistry, Biochemistry and Clinical Chemistry, School of Medicine, University of Zagreb

Introduction: Central nervous system (CNS) tumors are a heterogenous group of neoplasms which originate from brain and spinal cord tissue and from surrounding structures. Sphingolipids are a class of amphipathic lipids especially abundant in neural tissue where they participate in numerous cell processes. It is known that different kinds of brain tumors show aberrant glycosphingolipid (GSL) metabolism and expression. Therefore, some of the GSLs can be used as therapeutic targets of specific tumor specimen.

Hypothesis: We expect to find an altered sphingolipidomic (SL) profile in brain tumors; in malignant brain tumors we expect the strongest expression of simple GSL species and the highest variability of ceramide species, in benign tumors we expect these alterations to be milder, while we expect the SL profile of peritumoral tissue (PT) to have resemblance to the healthy brain tissue of the corresponding neuroanatomical region. We also expect the SL profiles of patients' serums to correlate with these changes in benign and malignant brain tumoral tissue and to differ from the SL profiles of the healthy control-group serums.

Aims: The general aim is to analyze the SL profile in benign and malignant brain tumor tissue samples and the corresponding peritumoral tissue (PT). The specific aim is to analyze the SL profile in benign and malignant brain tumor blood samples (serums) and in the healthy control group serum samples. Finally, to correlate the results between the SL profile of the tumor tissue, PT and serums of the patients with benign and malignant tumors with/to serums of healthy individuals.

Materials and methods: Participants will be the patients who have undergone an open-surgical procedure of removing benign and malignant brain tumors and a control-group will be the patients who have undergone a lumbar-discectomy procedure and have no previous medical history of tumor pathology. Twelve benign tumor tissue samples (such as meningioma grade I) and twelve malignant tumor tissue samples (such as diffuse high-grade glioma) will be taken alongside with the surrounding peritumoral tissue. Twenty-four serums from the patients with tumors and twenty-four serums from the control-group patients will be taken as well. Complex and simple GSLs from tumoral and peritumoral tissue homogenates and serums will be isolated and analyzed using several methods such as high performance thin-layer chromatography (HPTLC), mass spectrometry (MS) and liquid chromatography mass spectrometry coupled systems (LC-MS). The HPTLC will be used for qualitative analysis and determination of the relative proportions of GSL species. The MS and LC-MS will be used for detection, structural characterization and quantification of particular GSL species. Using the MS alongside with the chromatography techniques makes it possible to determine a specific SL profile in different kinds of brain tumors and correlate it with patients' blood-profiles.

Expected scientific contribution: We expect to define a specific SL profile in tumoral and peritumoral brain tissue in correlations with the results from the blood-profile of patients with tumors and of healthy individuals. The obtained results could be used for a better understanding of the GSL biochemical changes occurring in CNS tumor cells and surrounding tissue, CNS tumor pathology and metabolism. Potentially, the results could be used for a specific brain tumor marker development.

MeSH/Keywords: glycosphingolipids, brain tumors, ceramides, thin-layer chromatography, mass spectrometry, tumor markers

Poster code: T-01-08-155

Clinical medical sciences – research proposals

Poster Title: Comparison between pericapsular nerve group (PENG) block and intrathecal morphine application 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: Intrathecal application of morphine prolongs analgesia after total hip arthroplasty (THA) performed under spinal anesthesia, however it is often accompanied by side effects that can hinder early postoperative mobilization. Regional nerve blocks also provide analgesia after THA, yet can produce motor blockade of the lower extremity impeding early mobilization. The pericapsular nerve group (PENG) block targets sensory branches passing bellow the iliopsoas muscle tendon between the anterior inferior iliac spine and iliopectineal eminence towards the anterior hip capsule having the potential of providing a motor sparing analgesic block of the hip.

Hypothesis: Compared to intrathecal morphine administration ultrasound guided pericapsular nerve group block can provide good analgesia with less opioid related side effects and preserved lower extremity motor function in patients undergoing THA.

Aims: General aim: To compare the impact of pericapsular nerve group block to intrathecal morphine administration on postoperative analgesia, motor function and occurrence of opioid related side effects in patients undergoing THA under spinal anesthesia. Specific aims: 1. Measure pain at rest and with active hip flexion using a numerical rating scale at 4, 6, 12, 20, 24 and 48 hours after surgery 2. Determine maximum patient reported pain intensity during the first 48 postoperative hours 3. Measure needs for supplemental parenteral morphine analgesia (in milligrams) and cumulative morphine consumption during the first 48 postoperative hours 4. Evaluate quadriceps muscle motor function by straight leg raise test at 4, 6, 12, 20 and 24 hours postoperatively 5. Monitor and note pruritus, postoperative nausea and vomiting and hypotension occurrence

Materials and methods: After providing written informed consent sixty adult patients with osteoarthritis undergoing primary THA under spinal anesthesia with levobupivacaine will be included in a prospective randomized double blinded study. Exclusion criteria are: contraindications for drugs and procedures included in the study protocol, high risk for perioperative morbidity (ASA Physical Status Classification IV), preoperative use of strong opioid analgesia, pregnancy and substance abuse. Patients in which the surgical plan has been changed after randomization or in case of violation of study protocol will be excluded from the study. Randomization and study drug preparation will be performed by a nurse not involved in patient care, data gathering or outcome evaluation. Group allocation will be kept in sealed opaque envelopes until all data is gathered. The outcome assessor will be blinded as to which group received which intervention until statistical analysis is completed. All patients will receive spinal anesthesia with 15mg levobupivacaine. One group of patients will receive ultrasound guided PENG block with 20ml 0.5% levobupivacaine and 2mg (0.5ml) dexamethasone and intrathecal placebo while in the control group a sham block will be performed and 100 micrograms (0.5ml) of morphine administered intrathecally. The intrathecal placebo and sham block will consist of normal saline (0.9%NaCl) administered at volumes equivalent to the active comparators. Pain at rest and during hip flexion as well as morphine consumption will be measured at multiple time points during the first 48 postoperative hours and compared among groups. Side effects of morphine therapy as well as quadriceps motor function recovery will also be monitored.

Expected scientific contribution: Replacing intrathecal morphine administration with pericapsular nerve group block could provide good analgesia after total hip arthroplasty with less opioid related side effects facilitating early postoperative rehabilitation.

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

Poster code: T-02-01-061

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

PhD candidate: Zrinka Šafarić Oremuš

Part of the thesis: Immunomodulatory Effects of Dexmedetomidine on Cardiopulmonary Bypass Induced Systemic Inflammatory Response in Patients with Aortic Stenosis

Mentor(s): Assistant Professor Igor Rudež, MD PhD, Assistant Professor Vlatka Sotošek Tokmadžić, 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 systemic inflammatory response and post-perfusion syndrome in the first 24 hours following surgery. There are numerous studies that focus on reduction of systemic inflammatory response associated with CPB, but to date there are no clear and applicable guidelines. Dexmedetomidine is 2 adrenergic agonist that beside its sedative effect has proven effective in modulation of systemic inflammatory response.

Hypothesis: The 2 adrenergic agonist dexmedetomidine can reduce systemic inflammatory response and improve patient outcome after open heart surgery with use of cardiopulmonary bypass.

Aims: Primary aim of this thesis is to determine if intraoperative dexmedetomidine use can elicit clinically significant effect on improving aortic valve replacement patient outcome. Specific aims are: 1. Measuring changes in concentration of inflammatory cytokines: tumor necrosis factor alpha (TNF-) and interleukin 6 (IL-6) in peripheral blood intraoperatively and in early postoperative period. 2. Measuring changes in concentration of inflammatory parameters: C reactive protein (CRP) and procalcitonin, determining infection rate and incidence of sepsis in postoperative period 3. Estimation of renal function in postoperative period by measuring hourly diuresis, urea and creatinine concentration, estimation of glomerular filtration rate using Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI) and diuretic use 4. Determining incidence of postoperative delirium by application of Confusion Assessment Method (CAM) questionnaire 5. Influence of dexmedetomidine on consumption od anaesthetic and analgetic medication in perioperative period

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- 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 will be made by measuring levels of urea, creatinine, glomerular filtration rate daily, and hourly diuresis and diuretic consumption during ICU stay. Incidence of postoperative delirium will be estimated by daily and on demand application od CAM questionnaire. 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 ICU protocol.

Expected scientific contribution: Intraoperative dexmedetomidine use could prove to be simple and effective intervention that could improve overall patient outcome by reducing inflammation, infection, improving renal and cognitive function and reducing anesthetic and analgetic consumption in perioperative period.

MeSH/Keywords: dexmedetomidine, aortic valve stenosis, systemic inflammatory response, TNF-alpha, IL-6

Poster code: T-02-01-116

Poster Title: Pharmacological treatment of early pregnancy loss

PhD candidate: Mate Milas

Part of the thesis: Comparison of efficacy and safety of different pharmacological treatment protocols in women with early pregnancy loss

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

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

Introduction: It is estimated that early pregnancy loss affects around 15% of pregnancies. Treatment can be expectant, pharmacological or surgical. Considering pharmacological treatment, leading international gynecologic/obstetric societies recommend misoprostol with estimated efficacy of around 70%, but a combination of mifepristone and misoprostol seems to be more effective. We aimed to compare efficacy and safety of sequential mifepristone-misoprostol protocol to misoprosol alone in the treatment of early pregnancy loss.

Hypothesis: Sequential administration of mifepristone (oral) and misoprostol (vaginal) is more effective than misoprostol (vaginal) alone in treating missed abortion in the first 13+6/7 weeks of pregnancy in terms of complete uterine evacuation without increasing the incidence of complications or side effects.

Aims: Primary outcome: incidence of complete uterine evacuation based on the clinical signs and ultrasound finding of the empty uterine cavity (antero-posterior endometrial thickness <15 mm) 21 days after treatment completion. Secondary outcomes: incidence of complications and side effects, duration of vaginal bleeding, total misoprostol dose used, hemoglobin level difference at the end and the beginning of the treatment, quality of life measured by EQ-5D-5L questionnaire, treatment satisfaction measured by CSQ-8 questionnaire

Materials and methods: Women diagnosed with early pregnancy loss will be randomized (1:1) to sequential mifepristone-misoprostol (tested treatment) or to misoprostol alone treatment (control). Primary outcome is incidence of complete uterine evacuation assessed at 21 days after treatment completion.

Expected scientific contribution: This thesis proposal was rejected at a public hearing by the expert committee because its members were of the opinion that the topic, even though methodologically well designed, would not have enough scientific contribution because it is already "known" that the mifepristone/misoprostol combination is superior to misoprostol alone. On the contrary, the recommendations of the two most relevant professional societies in the field are contradictory: a) American College of Obsetricians and Gynecologists (ACOG) in 2018. states that mifepristone may be offered (as an adjunct to treatment with misoprostol alone). The text formulated in this way is certainly different from the way in which a strong recommendation is expressed (eg "We recommend"); b) National Institute for Clinical Excellence (NICE) from UK in 2021. explicitly recommends that mifepristone should not be used in this indication (as an 'addition' to misoprostol); primarily vaginal and alternatively oral misoprostol is recommended. After careful re-evaluation of all the evidence published so far in the literature we ound the following: 1. There are nine RCTs published so far which all used different treatment protocols of both mifepristone/misoprostol combination and misoprostol alone, different routes and dosages, and also different time points and criteria for the evaluation of successfulness of the treatment. 2. There are two network meta-analyses published so far which are based on direct as well as indirect comparisons of the two treatments with all the other possible treatments for early pregnancy loss. The first one from 2019. found that there is no statistically significant difference between the two treatments regarding complete uterine evacuation either by direct, indirect or combined comparisons. The second one (which was a Cochrane network meta-analysis from 2021.) found that by direct comparison mifepristone/misoprostol combination was superior to misoprostol alone but both indirect and combined comparisons found no statistically significant difference between the treatments. The totality of evidence is therefore very inconsistent and heterogeneous which is reflected in the recommendations of the leading societies mentioned above. Considering everything written above and respecting the decision of the expert committee, we propose a trial that will retrospectively compare all the different pharmacological protocols used so far in the treatment of early pregnancy loss in Clinical Hospital Merkur from May 2015. onwards (misoprostol alone and different mifepristone/misoprostol combination regimens) which will contribute to further evaluation of relative efficacy of different pharmacological treatment protocols.

MeSH/Keywords: early pregnancy loss, mifepristone, misoprostol

Poster code: T-02-05-057

Poster Title: A comparison of personality traits in women with diminished and normal ovarian reserve

PhD candidate: Ana Šešerko

Part of the thesis: A comparison of personality traits in women with diminished and normal ovarian reserve

Mentor(s): Professor Marina Šprem Goldštajn, MD PhD, Professor Liborija Lugović Mihić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Patients with diminished ovarian reserve represent distinctive challenge in the field of infertility treatment. It has been known that stress perception can be different among different people and depending on personality traits there are different stress coping mechanisms that could influence neuroendocrine changes. Studies that would compare personality traits and salivary melatonin and cortisol level between women with diminished and those with normal ovarian reserve could help in better understanding of etiology of diminished ovarian reserve.

Hypothesis: Personality traits of women with diminished ovarian reserve differ from personality traits of women with normal ovarian reserve.

Aims: The aim of this study is to determine if there is a difference in personality traits between women with diminished and women with normal ovarian reserve. Also, this study aims to determine if there is a difference in salivary cortisol and melatonin level between women with diminished and women with normal ovarian reserve. If there is a relation of specific personality traits to salivary cortisol or melatonin level will be examined.

Materials and methods: This prospective study will include 40 patients with diminished ovarian reserve and 40 patients with normal ovarian reserve. The study will be conducted in the Department for gynecology and obstetrics at UHC Zagreb. During initial assessment of infertility, personality traits will be determined. Salivary melatonin and cortisol level will be determined during stimulated IVF cycle on the day of betaHCG administration.

Expected scientific contribution: This study will be the first to explore and evaluate the role of personality traits and, salivary melatonin and cortisol, in women with diminished ovarian reserve. Determining relation of specific personality traits to salivary cortisol and melatonin level, may lead to better understanding of the mechanism responsible for diminished ovarian reserve, which can eventually result in better outcome of infertility treatment in women with diminished ovarian reserve.

MeSH/Keywords: infertility, ovarian reserve, personality, cortisol, melatonin

Poster code: T-02-05-149

Poster Title: Involvement of molecules of GABA signaling pathway in mucus secretion and inflammation in human endocervical mucosa

PhD candidate: Adam Vrbanić

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

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

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

Introduction: Gamma-aminobutiric 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 presence of molecules of GABA signaling pathway in human endocervix, yet have not clarified their role.

Hypothesis: Expression of molecules of GABA signaling pathway in mucosa of human endocervix is associated with the expression of mucins in endocervical mucosa, estradiol blood concentration and expression of inflammatory markers in endocervical mucosa.

Aims: The aim of this research is to investigate the association of expression of molecules of GABA signaling pathway in mucosa of endocervix with the expression of mucins in endocervical mucosa, estradiol blood concentration and expression of inflammatory markers in endocervical mucosa assuming there is a link between the expression of molecules of GABA signaling pathway with mucus secretion and inflammation in endocervix.

Materials and methods: A prospective observational cohort study will be conducted on female patients of Department of gynaecology 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, puerperas, smokers, women with a malignant gynaecological disease, or those with 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. First set of samples will be collected in early follicular phase (expected low blood estradiol) and the second set will be taken in pre-ovulational phase (expected high blood estradiol) of menstrual cycle within 3-6 months. Every woman will be submitted to gynaecological ultrasound to confirm adequate phase of menstrual cycle. Also, a Pap smear will be taken. Blood sample will be used to establish estradiol level 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).

Expected scientific contribution: This research will try to bring new, so far unknown data of the correlation between the expression of molecules of GABA signaling pathway and expression of mucins and immunological response markers in the uterine cervix and the concentration of estradiol in blood to try to clarify the role of GABA in the uterine cervix.

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

Poster code: T-02-05-150

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): Neven Papić, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: COVID-19, a pandemic caused by the highly pathogenic coronavirus SARS-CoV-2, became the world's leading public health problem. Identified risk factors, among others, include nonalcoholic fatty liver disease (NAFLD) which is the most common chronic liver disease with simple steatosis as one of the conditions. According to published, NAFLD is a possible risk factor for SARS-CoV-2 infection and the development of a more severe clinical forms of COVID-19. In the pathogenesis of COVID-19, a key role is played by so-called "cytokine storm". Existing data suggest that the severity of COVID-19 disease is associated with higher levels of inflammatory mediators, including cytokines. In patients with NAFLD, inflammatory response is basally altered, characterized by so-called "chronic low-grade inflammation" with enhanced cytokine expression. Despite the links between inflammatory responses, the correlation between NAFLD and COVID-19 progression at the cytokine level has not been investigated, raising the question of whether NAFLD patients are more susceptible to severe forms of COVID-19 due to immune system changes.

Hypothesis: Patients with hepatic steatosis and severe COVID-19 have higher serum concentrations of proinflammatory cytokines than patients without hepatic steatosis.

Aims: Main aim: To determine the profile of serum cytokine concentrations in the immune response of patients with severe COVID-19 with and without hepatic steatosis. Specific aims: to determine the presence and prevalence of hepatic steatosis in hospitalized patients with severe COVID-19; to analyze the clinical presentation, course and outcome of COVID-19 in patients with and without hepatic steatosis; to compare serum cytokine profile concentrations in patients with COVID-19 with and without hepatic steatosis; to analyze the association of serum cytokine concentrations with other laboratory, clinical and demographic parameters in patients with COVID-19 with and without hepatic steatosis;

Materials and methods: A prospective, observational study will be conducted in the University hospital for infectious diseases "Dr. Fran Mihaljevi Zagreb for 1 year on 120 subjects (60 with hepatic steatosis and 60 without hepatic steatosis). The study will include adult patients with severe COVID-19 requiring hospital treatment. Exclusion criteria are 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, admission to the intensive care unit within the first 24 hours, death within 48 hours of admission, corticosteroid therapy intake before admission. All patients will be screened for steatosis by ultrasound during hospitalization. Routine demographic and clinical parameters will be collected as well as laboratory findings. Patients will be monitored daily during hospitalization and clinical parameters will be collected. Blood sample will be taken on admission to determine serum cytokine profile using multiplex technology. Data will be analyzed using descriptive and analytical methods.

Expected scientific contribution: Since the characteristics of the immune response to SARS-CoV-2 infection in patients with hepatic steatosis have not been described so far, this study will characterize the specifics of serum cytokine concentrations in patients with COVID-19 and hepatic steatosis. Identification of cytokines associated with severe clinical response specific to this patient population will define new biomarkers of disease severity, and thus possibly open new research questions with the aim of developing new individual therapeutic options for timely therapeutic intervention to prevent disease progression to multiorgan damage.

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

Poster code: T-02-07-010

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: Pre-exposure prophylaxis for the prevention of HIV infection in men who have sex with men 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.

Hypothesis: Bacterial sexually transmitted diseases (STDs) incidence in the second year of PrEP use in men who have sex with men (MSM) in Croatia is higher than STDs incidence in the first year of PrEP use.

Aims: The primary aim is to compare incidences of STDs in the first and the second year of PrEP use. Also, we will assess differences in sexual behaviour and STDs risk among participants who use PrEP daily, intermittent, combined (daily and intermittent alternately), and with interruptions.

Materials and methods: This is 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 will be MSM 18 years old who use PrEP.

Expected scientific contribution: This study will assess impact of PrEP on STDs incidence during the first two years of follow-up. It will provide insight into the sexual behavior of PrEP users in Croatia and contribute to direct prevention methods. The study will assess so far unexplored differences between persons who use PrEP daily, intermittent, combined, and with interruptions. It will also assess differences in risk of STDs among MSM and men who have sex with both men and woman.

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

Poster code: T-02-07-105

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, there has been a significant reduction in the number of HIV related deaths. The life expectancy of people living with HIV 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 results and short messages via a mobile application.

Hypothesis: We expect that the level of patient activation measured by the PAM questionnaire will be high before inclusion and will be maintained at a high level throughout the time of using the EmERGE mHealth application.

Aims: To determine whether the use of the mHealth application affects the active role in self-care of HIV infection.

Materials and methods: This research is a longitudinal prospective cohort study in which we started to include participants during the EmERGE project (Horizon 2020). The rest will be new entrants, making total of 550 participants in intevrention group. The control group will be patients who do not use the application (total of 100 participants). Criteria for inclusion in the study are: over 18 years of age, possession of a smartphone and informed consent. All participants are patients at University Hospital for Infectious Diseases "Dr. Fran Mihaljevi". We will measure the self-assessment of activation and the degree of responsibility for our own health by the Patient Activation Measure questionnaire - PAM. 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.

Expected scientific contribution: The study will supplement the scarce data on longitudinal monitoring of the level of activation of people living with HIV. The ultimate goal is that the results of this research contribute to the improvement of the model of care for people living with HIV.

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: T-02-07-110

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

PhD candidate: Juraj Jug

Part of the thesis: Arterial stiffness and R wave in aVL lead as additional 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

Introduction: In patients with hypertensive urgency (HU, blood pressure >180 and/or 120mmHg without hypertension mediated organ damage [HMOD]), PWV and R wave in aVL ECG lead could be key factors for a more accurate cardiovascular (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.

Hypothesis: Arterial stiffness and R wave amplitude in the aVL ECG lead, as measures of unrecognized CV damage, are significantly higher in patients with HU than in patients with arterial hypertension and healthy individuals.

Aims: To determine if patients with HU 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 will be conducted in 2022 on at least 300 subjects divided into three equal groups (patients with HU, hypertensive patients, and healthy persons) using 24-hour ambulatory blood pressure monitor (ABPM) findings. Additional three groups will be created depending on a night blood pressure change (dipper 10%, non-dipper 0-10% and inverse dipper <0%). SCORE 2 (Systematic coronary risk evaluation) risk will be calculated in all participants. PWV will be measured with an Agedio® B900 oscillometric device. The ABPM and ECG devices used will be BTL Cardiopoint®. The amplitude of the R wave in the aVL ECG lead will be measured and the voltage ECG criteria of LVH (Sokolow-Lion and Cornell) calculated. Exclusive criteria: hypertensive emergency (existence of acute HMOD [heart, brain, blood vessels, kidney, lungs, eyes]), CV event in past medical history, incomplete medical record, age <40 and >70 years, BMI>50kg/m2, cigarette smoking in the last 10 years, atrial fibrillation, diabetes, chronic kidney disease (G3b and higher), pregnancy, immunosuppressive, corticosteroid and/or biological therapy, chemotherapy in the last 5 years, life expectancy <6 months. Variables will be analyzed by the Student t-test, the Mann-Whitney U-test, MANOVA, and multivariate regression analysis.

Expected scientific contribution: This research shows additional ways of detecting patients with existing CV damage in family practice aiming for more successful CV risk stratification and prevention.

MeSH/Keywords: arterial hypertension, electrocardiography, hypertensive urgency, vascular stiffness

Poster code: T-02-09-008

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

PhD candidate: Petra Meliš

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

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

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

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

Hypothesis: Parenteral administration of semaglutide leads to a decrease in intestinal iron absorption in patients with type 2 diabetes.

Aims: General objective: The overall aim of this study was to determine whether parenteral administration of semaglutide resulted in decreased intestinal iron absorption in patients with type 2 diabetes using an oral iron absorption test. Specific objectives: 1. To determine whether there is a gender difference in intestinal iron absorption when using semaglutide in patients with type 2 diabetes. 2. To determine whether serum ferritin values affect the degree of iron absorption before performing an oral iron absorption test when using semaglutide in patients with type 2 diabetes.

Materials and methods: This prospective study would include patients with type 2 diabetes who were selected by an endocrinologist-diabetologist and treated at the outpatient clinic of the Department of Endocrinology, Diabetes, Metabolic Diseases, and Clinical Pharmacology, University Hospital Dubrava in Zagreb. It is expected that 50 subjects will be included in this study and each subject would be own control. The study will include patients aged 40 to 64 years with nonregulated type 2 diabetes in whom therapy with semaglutide is planned. An oral iron absorption test would be performed on day 0 of the study and 14 weeks after starting the therapy with semaglutide. Data will be analyzed using descriptive and analytical methods.

Expected scientific contribution: This research contributes to our additional knowledge and understanding of the pharmacokinetics and pharmacodynamics of parenteral administered semaglutide and directs us to a closer analysis of the interactions that may occur.

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

Poster code: T-02-09-011

Poster Title: SGLT2 inhibitors in patients with diabetes and HFpEF

PhD candidate: Zrinka Planinić

Part of the thesis: The effect of sodiumglucose cotransporter type 2 inhibitors on left atrial strain in patients with type 2 diabetes mellitus and heart failure with preserved ejection fraction

Mentor(s): Assistant Professor Jozica Šikić, MD PhD

Affiliation: University of Zagreb School of Medicine, University hospital "Sveti Duh"

Introduction: The prevalence of heart failure is constantly increasing and there is a growing number of patients with heart failure and preserved ejection fraction (HFpEF) that has complex pathophysiology. There is still no evidence that certain therapy is effective in reducing cardiovascular outcomes for HFpEF patients. SGLT2 inhibitors are the first oral hypoglycemic agents to show reduction in heart failure hospitalizations for patients with reduced ejection fraction regardless of diabetic status, and there are ongoing clinical trials investigating their potential benefit for HFpEF as well. Left atrial function is important in pathophysiology and prognosis of HFpEF. Reduced left atrial strain (LAS) measured by 2D speckle-tracking echocardiography is a very sensitive early marker of diastolic dysfunction and impaired left ventricle relaxation and therefore can be used for evaluating the effectiveness of potential novel treatment options in HFpEF.

Hypothesis: SGLT2 inhibitors improve left atrial strain as a marker of diastolic dysfunction in patients with type 2 diabetes mellitus and heart failure with preserved ejection fraction.

Aims: General aim: to investigate the effect of empagliflozin on LAS in patients with T2DM and HFpEF. Specific aims: to compare the effect of empagliflozin on LAS and global longitudinal strain of the left ventricle, to investigate the effect of empagliflozin on left atrial volume index (LAVI) and corrected QT interval (QTc).

Materials and methods: It is a unicentric prospective observational study that will be conducted in Department of cardiovascular diseases in University hospital Sveti Duh on patients with confirmed diagnosis of T2DM and HFpEF (LVEF50%) that are started on empaglifozin 10 mg daily, with previously signed informed consent and approval of Ethics Committee of University hospital Sveti Duh. Inclusion criteria: patients with stable HFpEF (NYHA I) with no signs of manifest heart failure in the past 6 months and T2DM with no history of previous use of SGLT2 inhibitors. Exclusion criteria: known allergy or hypersensitivity to any SGLT2 inhibitors; age <30 or >85 years; heart failure with reduced ejection fraction (EF<50%); heart failure with recovered EF; acute decompensated heart failure (NYHA II-IV); type 1 DM; insulin treated DM; eGFR-om < 45 ml/min/1,73 m2; BMI > 45 kg/m2; prior or current use of any SGLT2 inhibitors or GLP-1 receptor agonists; current corticosterioid therapy; uncontrolled hypertension; prior history of atrial fibrillation or ablation; myocardial infarction, coronary artery bypass graft surgery, or other major cardiovascular surgery, stroke or transient ischemic attack in past 3 months prior randomization; active malignant disease; history of acute or chronic pancreatitis; suboptimal echocardiographic imaging. Medical history, clinical examination, blood tests (complete blood count, liver and renal function, glucose, HBA1c, c-peptid, creatin kinase, high sensitive troponin I, NTpro-BNP, acidum uricum, ferrum), electrocardiogram (ECG), 24-hour Holter ECG and transthoracic echocardiography (TTE), including 2D speckle-tracking echocardiography, will be performed in all patients before starting empaglifozin 10 mg daily and 6 months after randomization. Number of patients determined with paired samples t-test with power of 80% and level of significancy of 0.05 is 34.

Expected scientific contribution: SGLT2 inhibitors improve LAS which can be beneficial in patients with T2DM and HFpEF. As a very sensitive early marker of diastolic dysfunction, LAS can be used for evaluating the effectiveness of potential novel treatment options in HFpEF.

MeSH/Keywords: heart failure, diabetes mellitus, SGLT2 inhibitors, left atrial function

Poster code: T-02-09-014

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

PhD candidate: Ana Jordan

Part of the thesis: Red cell distribution width as a predictor of outcome in hospitalized COVID-19 patients

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

Affiliation: University of Zagreb School of Medicine

Introduction: Coronavirus disease 2019 (COVID-19) is an acute respiratory disease with a high rate of hospitalization and mortality. In addition to the available multivariate prognostic models, additional parameters are needed to more reliably assess the risk of hospitalized COVID-19 patients. We believe that the reliability of existing prognostic models would be higher if red blood cell distribution width (RDW) is added as an additional parameter.

Hypothesis: Correcting prognostic models by adding RDW increases their prognostic value.

Aims: General aim of the study: Estimate RDW at admission and clinical deterioration within hospital which includes any of the following: the need for high flow oxygen therapy (HFOT), ventilation support (non-invasive ventilation, invasive mechanical ventilation or extracorporeal membrane oxygenation (ECMO) or death. Specific aims of the study: 1. Estimate of increasing confidence in prognostic models (Modified Early Warning Score , 4C Mortality Score) by adding RDW. 2. Estimate the relationship of RDW/platelet ratio with the outcome of COVID-19 treatement.

Materials and methods: An observational study will include consecutive patients with COVID-19 who were hospitalized at the Clinical Hospital Dubrava based on standard criteria. All diagnostic and therapeutic procedures are part of standard protocols and none will be conducted specifically for the purposes of the research. Data collected from 1 November 2020 to 1 December 2021 will be taken into account. With regard to the goal (to assess the prognostic value of RDW in patients hospitalized for COVID-19 in terms of stratification of risk of deterioration / adverse outcome) inclusion criteria are: age over 18 years and hospitalization in the respiratory or respiratory intensive care center of the Clinical Dubrava Hospital due to infection COVID-19 disease. Patients who require immediate mechanical ventilation or HFOT, or O2 flow> 10 L / min will not be included in the study. The primary outcome of interest is the time from admission to the complex outcome: death during the subject hospitalization or the need for treatment in the intensive care unit (ICU) or the need for HFOT, or the need for mehanical ventilation, or until the censored time (time to discharge from hospital as cured " or " recovered "). Secondary outcomes of interest are the time from admission to each element of the complex outcome, or to the censored time. The main independent variables of interest are: RDW and Mortality Warning Score (MEWS) and 4C Mortality score. It is expected that between 2,500 and 3,000 respondents will be included in the research. The data will be analyzed in two phases. In the first phase, all respondents will be taken into account. Primary and then all secondary outcomes will be analyzed by Cox regression analysis.

Expected scientific contribution: Evaluation of the prognostic value of red blood cell distibution width (RDW) could increase the reliability of the risk assessment for immediate adverse outcomes in COVID-19 patients in whom hospitalization is indicated but the severity of the disease is still moderate. This is potentially important both at the level of each individual patient and at the level of system organization.

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

Poster code: T-02-09-021

Poster Title: The effect of BNT162b2 and Ad26.CoV2.S coronavirus vaccines on the parameters of hemostatis and inflammation

PhD candidate: Iva Ivanko

Part of the thesis: The effect of BNT162b2 and Ad26.CoV2.S coronavirus vaccines on the parameters of hemostatis and inflammation

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

Affiliation: University of Zagreb School of Medicine

Introduction: In 2020 several coronavirus vaccines have been placed on the global market as an answer to the global health threat, using mRNA and recombinant vector technique. Soon after the vaccine releasement individual cases of thromboembolic events with or without thrombocytopenia have been assigned to the vaccination causing scepsis and concern amongst individuals, predominantly in non-scientific community. The prothrombotic nature of coronavirus disease 2019 (COVID-19) has been well observed and documented, explained by the endothelial damage and cytokine release syndrome. The question about potential etiologic connection between coronavirus vaccine and thromboembolic events remain.

Hypothesis: Coronavirus vaccines BNT162b2 and Ad26.CoV2.S do not have significant effects on the parameters of hemostasis and inflammation.

Aims: The general aim of this prospective clinical study is to define potential effects of the coronavirus vaccines BNT162b2 and Ad26.CoV2.S on the parameters of hemostasis and inflammation. Specific goals are to determine: the difference in the parameters of hemostasis and inflammation depending on the type of vaccine; the dynamic of the parameters depending on the specific time point and to analyze the relationship of investigated parameters with general characteristics of the subjects (age, gender, BMI, arterial hypertension).

Materials and methods: The study will include subjects age 18-65 years vaccinated with mRNA vaccine BNT162b2 and vector Ad26.CoV2.S vaccine according to the vaccination program issued by Ministry of Healthcare of the Republic of Croatia. Total number of subjects is 160 (80 in each vaccine group). Every subject will be provided with the informed consent. The study has been approved by the Ethic committee of the Sestre milosrdnice University Clinical Centre and Medical centre Centar where the vaccination is to be performed. The laboratory parameters will be investigated before 1st vaccination and 7 and 14 days after 1st and 2nd vaccination for the subjects in the mRNA group. In the vector-based vaccine group the parameters will be investigated before vaccination and 7 and 14 days after. The exclusion criteria are active malignant disease, recent major operation (one month before vaccination), thromboembolic incident 3 months prior to the vaccination, dialysis, peripheral artery disease, diabetes requiring insulin therapy, active immunologic disorder, puerperium, pregnancy, oral contraceptive therapy, hemophilic disorders, immune thrombocytopenia and anticoagulant therapy. All the laboratory parameters obtained by the venepuncture will be investigated at the Department of Clinical Chemistry at the University Hospital Center Sestre milosrdnice. The parameters of interest are thrombin-antithrombin complex, prothrombotic fragment 1+2, D-dimer and fibrinogen, prothrombin time and activated partial thromboplastin time, C reactive protein, endothelin, interleukine-6 and antibodies to platelet-activating antiplatelet factor 4. Plasma and serum form previously centrifugated blood specimens will undergo the process of deep freezing and stored until the moment of analysis. The principal investigator will participate in the laboratory diagnostic workup. The data collected by this study will be processed and presented using relevant statistic tests with descriptive analysis, data distribution test, parametric and nonparametric tests as needed.

Expected scientific contribution: The raising number of vaccinated population and the severity of the disease itself demands a new insight and scientifically provided data on the influence of vaccines on hemostasis and inflammatory system. This study will help to shed light on this still sparsely investigated area.

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

Poster code: T-02-09-047

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, University Hospital for Tumors, University Hosipital Centre Sestre Milosrdnice, University Hospital Centre Zagreb

Introduction: Locally advanced rectal adenocarcinoma is a challenge to treat and today the standard approach is neoadjuvant therapy (radio- or chemoradiotherapy) followed by surgery. Despite multimodal treatment only 20% of patients achieve a complete pathological response, while about 30% are resistant. There are no standard molecular predictive biomarkers to treatment response.

Hypothesis: High expression of CD133, CD44, PD-L1 and HNE in rectal adenocarcinoma is associated with worse response to neoadjuvant treatment.

Aims: The aim of this study is to evaluate the expression of CD133, CD44, PD-L1 and HNE in rectal adenocarcinoma.

Materials and methods: A retrospective study will be conducted on a cohort of about 100 rectal adenocarcinoma patients divided in 2 subgroups -patients in whom neoadjuvant radiotherapy or chemoradiotherapy was performed. The expression of abovementioned biomarkers will be compared between biopsy and operative samples, between 2 subgroups of patients and in relation to the pathological response.

Expected scientific contribution: This research could define a subgroup of patients with rectal adenocarcinoma in whom we do not expect a good response to neoadjuvant treatment.

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

Poster code: T-02-09-050

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

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

Introduction: In addition to the somatic consequences of acute coronary syndrome (ACS) (intolerance to exertion, incapacity for work, symptoms of chronic heart failure, angina pectoris, the manifestation of various arrhythmias, etc.), the development of a whole range of psychosomatic and mental disorders is also possible already in the early subacute and chronic phases of the disease, and if these mental disorders are not actively treated in a timely fashion they can contributed to unwanted outcomes and increased mortality in this group of patients. ACS is associated with chronic stress, anxiety, and depression and can be a trigger for later development of posttraumatic stress disorder (PTSD) with an average prevalence rate of 12% in patients with ACS. Several studies have shown that patients with symptoms of PTSD associated with ACS, especially if untreated, have increased mortality and higher rates of myocardial reinfarction.

Hypothesis: PTSD after ACS is associated with a higher incidence of adverse cardiovascular events.

Aims: General objective is to the determine the association of PTSD in patients after ACS who are in inpatient cardiac rehabilitation with adverse cardiovascular events (reinfarction, recurrent percutaneous coronary intervention, coronary artery bypass grafting, hospitalization due to heart failure, cerebrovascular outcome, death) on a sample of patients rehabilitated in the largest cardiorehabilitation center in Croatia. Specific objectives: 1. Analysis of social and behavioral factors, results of clinical and laboratory tests and their possible interactions in patients with PTSD in relation to the control group. 2. Analysis of autonomic system imbalance in patients with PTSD after ACS. 3. Incidence of PTSD after ACS in patients undergoing inpatient cardiac rehabilitation. 4. Quality of life in patients with PTSD after ACS.

Materials and methods: Subjects are patients after ACS who are in a cardiac rehabilitation program. Based on a PTSD self-assessment questionnaire and clinical psychiatric interview, a group of patients diagnosed with PTSD will be formed. Among subjects who are not diagnosed with PTSD, based on clinically and medically relevant stratification variables, will be selected ones to match those diagnosed with PTSD in order to allow comparability of the two groups. A combined analysis will be performed which, given the specific limitations of the sample, will be contextually interpreted in an unconventional way.

Expected scientific contribution: The provided data will represent the first data of this kind extracted on the rehabilitation sample in the Republic of Croatia, enabling focus on the subpopulation of these patients in terms of precision medicine (identification of a special group of patients) and give new opportunities for specific therapeutic and rehabilitation approaches in order to improve the quality of life and reduce the overall mortality of such a vulnerable group of patients.

MeSH/Keywords: acute coronary syndrome, posttraumatic stress disorder, adverse cardiovascular events, cardiac rehabilitation.

Poster code: T-02-09-067

Poster Title: The association of glycemic variability and incidence of large-for-gestational-age neonates in pregnant patients with type 1 diabetes melltius using continuos glucose monitoring

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 melltius using continuos glucose monitoring

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

Affiliation: University of Zagreb School of Medicine

Introduction: Diabetes mellitus type 1(DMT1) in pregnancy increases risk for adverse pregnancy outcomes including development of large-for-gestational-age neonates (LGA). So far, it is not clear if increased glycemic variability (GV) is associated with LGA. Continuos glucose monitoring (CGM) devices are the optimal method for assesing GV.

Hypothesis: Increased GV in pregnancy with DMT1 is associated with higher incidence of LGA neonates.

Aims: The aim of the study is to define association between GV parameters and incidence of LGA neonates in pregnant patients with DMT1.

Materials and methods: This is a prospective observational cohort study of patients with DMT1 who are using CGM devices during pregnancy. Patients are included in the study during the first trimester of pregnancy using precisely defined including criteria; age between 18-35 years, body mass index < 25kg/m2, HbA1c < 8.0%, signed informed consent, duration of DMT1 > 1 year, CGM device users (FreeStyle Libre Flash Glucose Monitoring System, Abott) > 3 months, sensor data captured > 70%. Excluding criteria are twin pregnancy, HbA1c in the second and third trimester > 8.0%, weight gain > 20 kilograms by the end of the pregnancy, sensor data captured < 70%, significant differences in glucose values measured from the CGM device and capillary blood. The data is being collected in the first, second and third trimester (hospital visits, clinical exam, laboratory diagnostic). The CGM data is analysed using LibreView software. The parameters of GV are divided into two groups. Short-term GV parameters (glucose fluctuations within the day and between the days): the percentage coefficient of variation (%CV), standard deviation of the mean blood glucose (SD), interquartile range (IQR), J-index, MODD (the mean of daily differences). Short-term GV parameters will be measured for every pregnancy trimester. Long-term GV parameters (glucose fluctuations in the period of several months, from visit-to-visit): %CV and SD of mean HbA1c across trimesters, %CV and SD of fasting plasma glucose across trimesters. The main pregnancy outcomes include: development of LGA neonates, adverse pregnancy outcomes for the fetus (abortion, death, congenital malformations, shoulder dystocia, intensive care unit admission, neonatal hypoglycemia), adverse pregnancy outcomes for the mother (preeclampsia, eclampsia, intensive care unit admission). Other outcomes include weight gain at the end of the pregnancy, neonatal birth weight and length, sex, the week of delivery, neonatal birth percentile, preterm delivery, Apgar score. Statistical analysis of the data will be performed in the programme IBM SPSS Statistics 21. The association between GV parameters and pregnancy outcomes will be assesed through logistic regression analysis. The power analysis for logistic regression (with alpha statistical significance of 0.05 and power of 0.80) showed that sample size needed for the study is 82 participants (G*Power, version 3.1.9.4.).

Expected scientific contribution: Glycemic variability as a possible new therapeuthic goal in DMT1 pregnancies.

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

Poster code: T-02-09-072

Poster Title: The effect of comorbidity at diagnosis on organ damage in patients with systemic lupus erythematosus

PhD candidate: Ivana Ježić Vukičević

Part of the thesis: The effect of comorbidity at diagnosis on organ damage in patients with systemic lupus erythematosus

Mentor(s): Ivan Padjen, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Systemic lupus erythematosus (SLE) is a chronic disease with the possibility of affecting any organ and causing irreversible damage. There are insufficient data on the impact of comorbidities at the time of diagnosis of SLE on organ damage.

Hypothesis: Patients with SLE who developed organ damage during the follow-up period have "higher" Charlson comorbidity index (CCI) at the time of diagnosis than patients who did not develop organ damage during the follow-up.

Aims: To analyze the effect of comorbidities expressed by the Charlson comorbidity index (CCI) at the time of diagnosis of SLE on organ damage in patients with SLE.

Materials and methods: The retrospective study will include patients with SLE treated at the Referral Center for SLE and Related Diseases. We will compare CCI, disease activity and other relevant parameters between patients who developed organ damage over their follow-up and patients who did not develop damage.

Expected scientific contribution: The proposed research should improve the understanding of the impact of comorbidity at the diagnosis of SLE on the development of organ damage in patients with SLE.

Acknowledgments: None

MeSH/Keywords: Systemic lupus erythematosus, damage, comorbidity.

Poster code: T-02-09-082

Poster Title: Serum phospholipase A2 levels in patients with familial hypercholesterolaemia as a possible predictor of personalized cardiovascular risk.

PhD candidate: Dražen Perica

Part of the thesis: Serum phospholipase A2 levels in patients with familial hypercholesterolaemia as a possible predictor of personalized cardiovascular risk.

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

Affiliation: University of Zagreb School of Medicine

Introduction: Familial hypercholesterolemia (FH) is the most common metabolic inherited autosomal dominant disease. The disease occurs due to gene variation for the LDL receptor, ApoB or PCSK9. The disease is manifested by elevated LDL-cholesterol from birth, which leads to accelerated atherosclerosis and, consequently, increased morbidity and mortality from cardiovascular diseases. The diagnosis is made clinically, using the Dutch lipid clinic network criteria (DLCN) and / or genetic analysis. Phospholipase A2 linked to lipoprotein (Lp-PLA2) comes from a large family of phospholipase enzymes, which are involved in many pro-inflammatory processes. Lp-PLA2 is a highly specific factor in vascular injury and the formation of the first atherosclerotic lesions.

Hypothesis: Patients with familial hypercholesterolemia have higher phospholipase A2 activity than patients with acquired dyslipidemia.

Aims: Determine the level of Lp-PLA2 activity in patients with FH. Also measure intima media thickness (IMT) and determine whether Lp-PLA2 is a potential new indicator of early atherosclerosis and as such a predictor of personalized cardiovascular (CV) risk.

Materials and methods: We will compare Lp-PLA2 activity in patients with FH and in patients with acquired dyslipidemia and measure IMT in both groups. We will include a total of 120 respondents (60 per group) who are treated at the Department of Metabolic Diseases, University Hospital Center Zagreb.

Expected scientific contribution: If an association was found between Lp-PLA2 and IMT (in patients with FH) as predictors of atherosclerosis, it could be used as a factor in calculating peresnalized CV risk, which offers the possibility of determining Lp-PLA2 in clinical practice as a quick and easy method. Also in the future there is the possibility of using Lp-PLA2 for early diagnosis of patients with FH as well as a factor in monitoring responses to therapeutic interventions.

MeSH/Keywords: familial hypercholesterolemia, phospholipase A2, atherosclerosis

Poster code: T-02-09-084

Poster Title: Impact of Sodium-Glucose Cotransporter 2 inhibitors on chromogranin A concentration in patients with 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 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, with an increasing incidence and high mortality rate, especially in patients with reduced left ventricular ejection fraction (HFrEF). A novelty in the current guidelines is the introduction of Sodium-Glucose Cotransporter 2 (SGLT2) inhibitors in the first line of HFrEF therapy. This study would be the first to examine the effect of SGLT2 inhibitor therapy on chromogranin A, a biomarker of heart failure other than natriuretic peptides, in patients with HFrEF.

Hypothesis: SGLT2 inhibitors could reduce chromogranin A levels in patients with HFrEF.

Aims: To determine the impact of SGLT2 inhibitors on chromogranin A concentration in patients with HFrEF. Secondary, to determine whether there is in patients with HFrEF before and after the introduction of SGLT2 therapy significant correlation between chromogranin A values and NT-proBNP levels, functional status of patients assessed by SF-36 questionnaire, and echocardiographic parameters of left ventricular systolic and diastolic function, as well as estimated pulmonary circulation pressures.

Materials and methods: The study will be prospective. Patients diagnosed with HFrEF who are treated at the Department of Cardiovascular Diseases, Clinical Hospital Dubrava will be included in the study. Using power analysis, the planned total number of patients is 40. All patients will 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 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; patients 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. Exclusion criteria will be 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 30 ml/min/1,73 m2. In the initial assessment, patients will complete an SF-36 questionnaire. A blood sample will then be taken from each subject for planned laboratory analyses: determination of chromogranin A (basal chromogranin A; maximum 2 weeks after the introduction of SGLT2 inhibitors) and NT -proBNP. After blood sampling, each subject will perform a 6-minute walk test and then have an echocardiographic examination of the heart to assess parameters of left ventricular systolic and diastolic function (EFLV, GLS, left and right ventricular volumes, left ventricular filling pressures), as well as estimated pulmonary circulation pressures. After 6 months, the same procedure as the initial one will be repeated.

Expected scientific contribution: The scientific contribution of the study would be the confirmation of the hypothesis, which would indicate chromogranin A as an alternative marker of heart failure, especially when assessing responses to SGLT2 inhibitor therapy in patients with HFrEF. Chromogranin A as an alternative marker of heart failure is a potentially available and reproducible marker for more frequent determination in daily clinical work, which would facilitate the treatment of patients with HFrEF.

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

Poster code: T-02-09-087

Poster Title: Electrical isolation of superior vena cava in the invasive treatment of paroxysmal atrial fibrillation

PhD candidate: Vedran Pašara

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

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

Affiliation: University of Zagreb School of Medicine

Introduction: Pulmonary vein isolation (PVI) is the standard of care for patients with atrial fibrillation (AF), but medium-term success rates are far from ideal. Therefore, new methods are being developed, with emphasis on ectopic foci outside the pulmonary veins. However, there is no clear evidence that additional lesions, other than standard PVI, would result in better outcomes.

Hypothesis: The isolation of superior vena cava (SVC) and PVI for paroxysmal atrial fibrillation using fourth-generation cryoballoon reduces the incidence of AF recurrence in comparison with conventional PVI.

Aims: The main goal of this study is to evaluate the effect of additional SVC isolation by fourth-generation cryoballoon (in addition to standard PVI) during conventional paroxysmal AF ablation on the incidence of AF recurrence over a one-year follow-up. It also aims to assess and compare the main procedural characteristics (procedure duration, fluoroscopy time, contrast dose, etc.) in two different ablation strategies (PVI vs PVI+SVC isolation), complication rate in the two groups, and predictive factors for AF recurrence after ablation.

Materials and methods: We designed an unblinded, randomized clinical trial and will recruit 130 patients with paroxysmal AF. All patients in whom PVI is indicated are eligible for this study and will be randomized to a group without SVC isolation and one with SVC isolation in addition to standard PVI. Exclusion criteria are the presence of significant structural heart disease, significant left atrium dilatation, the presence of thrombus in the left atrium, severe renal failure, and other significant noncardiac comorbidities. The inability to understand informed consent or reluctance to participate is also an exclusive criterion. All patients will undergo standard PVI procedure with additional SVC isolation in patients randomized for SVC isolation. Post-procedural follow-up will include telemetry monitoring until discharge from the hospital, standard transthoracic ultrasound to rule out possible complications, and outpatient follow-up after 3, 6, and 12 months including clinical examination and 24-hour Holter monitoring.

Expected scientific contribution: This research will contribute to the understanding of the role of additional ablation lesions, ie additional cryoapplications, in the invasive treatment of paroxysmal AF (in addition to conventional PVI). It will evaluate the value of additional SVC isolation with fourth-generation cryobalon in the invasive treatment of paroxysmal AF, and its impact on the incidence of AF recurrence compared to conventional PVI.

MeSH/Keywords: atrial fibrillation, pulmonary vein isolation, superior vena cava isolation, cryoballoon

Poster code: T-02-09-088

Poster Title: Cardiometabolic risk and arterial stiffness in recreational and competitive master athletes

PhD candidate: Petra Vitlov

Part of the thesis: Cardiometabolic risk and arterial stiffness in recreational and competitive master athletes

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

Affiliation: University of Zagreb School of Medicine

Introduction: Notwithstanding the well-known protective effects of regular physical activity on cardiovascular risks, intensive exercise can paradoxically lead to sudden cardiac death and life-threatening arrhythmias. Sudden cardiac death is the leading cause of sport- and exercise-related mortality and a serious public health problem. High levels of physical activity are associated with lower all-cause mortality, lower cardiovascular mortality and lower prevalence of several known malignancies. Arterial stiffness has proven to be a predictor of cardiovascular events that is independent of other risk factors. The incidence of metabolic risk factors also increases with age.

Hypothesis: Athletes aged 35 and older engaged in predominantly high static level sports could have a higher cardiometabolic risk and a higher arterial stiffness compared to athletes engaged in high-intense dynamic resistance sports.

Aims: The aim of this research is to define differences in cardiometabolic risk and arterial stiffness in recreational and competitive athletes with regard to the volume of exercise and type of resistance in sports.

Materials and methods: This study contains a prospective research of so far healthy athletes/subjects aged 35-64, who engage in sports recreationally (4h training per week, purpose of the activity is leisure) or competitively (6h training per week, better individual fitness level, purpose of the activity is competition). Athletes who so far in their medical history do not have obvious heart disease will also be included in the research. As will the athletes who suffer from so far unrecognised (so-called silent) cardiovascular and/or metabolic diseases detected during initial examination. Subjects of the study will be divided by gender and then subdivided, in accordance with 2020 ESC Guidelines on sports cardiology and exercise in patients with cardiovascular disease, with regard to minimal volume of exercise (in recreational and competitive athletes) and type of resistance in sports (depending on whether it is a predominantly static or dynamic resistance sports). After having signed an informed consent form, the subjects will fill out a physical activity self-assessment questionnaire. Furthermore, the subjects arterial stiffness will be noninvasively measured using an arteriograph and their blood samples will be taken, from which previously agreed standard metabolic risk parameters will be extracted. The same procedure involving all the subjects will be repeated after six months provided that they still engage in sports.

Expected scientific contribution: Expected scientific contribution of this research would be to define the connection between cardiometabolic risk and arterial stiffness in recreational and competitive master athletes. This could lead to an improvement of healthcare of so far sparsely researched group of athletes by using simple and non-invasive methods, to detection of athletes with high cardiometabolic and vascular risk and to a possible timely prevention of unwanted sports-related cardiovascular events.

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

Poster code: T-02-09-092

Poster Title: Whole genome sequencing and clinical relevance of M.xenopi subtypes in non tuberculous mycobacterial pulmonary disease

PhD candidate: Goran Glodić

Part of the thesis: Whole genome sequencing and clinical relevance of M.xenopi subtypes in non tuberculous mycobacterial pulmonary disease

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

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

Introduction: Nontuberculous mycobacterial pulmonary disease (NTM-PD) is increasingly recognized as an important opportunistic infection in humans. The pathogenic potential of different nontuberculous mycobacterial (NTM) species differs across geographic regions. Pulmonary disease caused by M.xenopi (MX-PD) is associated with an increased morbidity and mortality compared to other NTM in cohorts from Western Europe and Canada. Preliminary data suggest a lower clinical relevance and mortality rate of patients with MX-PD in Croatia.

Hypothesis: The incidence of clinically significant lung disease caused by M.xenopi varies depending on the genotype of the causative agent.

Aims: To perform whole genome sequencing (WGS) of the available M.xenopi isolates, to determine the clinical relevance of M.xenopi and different M.xenopi subtypes, and to describe the clinical characteristics and mortality of MX-PD patients.

Materials and methods: All Croatian residents with M. xenopi isolates from 2006 to 2022 will be included in the study, with follow up until 31.12.2023. Clinical and radiological data will be used to determine the clinical relevance of the isolates according to the current ATS/ERS/ESCMID/IDSA clinical guidelines. Based on previous sequencing studies of NTM we aim to do WGS on a minimum of 36 M.xenopi isolates 12 patients with MX-PD and 24 patients with clinically insignificant colonisation. WGS and data analysis will be performed in collaboration with Radboud University Medical Center, Nijmegen, the Netherlands. Samples of previously isolated M.xenopi strains will be prepared for DNA isolation by heat inactivation. DNA isolation from heat inactivated samples will be done using InstaGene matrix in combination with bead-beating. DNA integrity will then be measured on a TapeStation 2200 and concentration determined using a Qubit fluorometer. Library preparation will be achieved using the Illumina Nextera DNA Flex kit according to manufacturers instructions. Sequencing will be performed on a Illumina NextSeq 500 to generate 2x 150bp paired-end sequence reads. For de novo genome assembly, sequence reads will be assembled using SPAdes software; QC parameters will include genome size, N50 length, number of contigs and scaffolds, and GC-content. The assembled genomes will be first annotated using Prokka. Phylogenetic analyses and phylogeny construction will be performed using the BreSeq variant caller and PhyML software, respectively. To identify genotype-phenotype correlation, we will first test correlations between phylogenetic position and clinical phenotype. In a second stage, to confirm these findings and detect possible virulence factors, we will apply a homoplasy-based association test.

Expected scientific contribution: The results will improve our understanding of the pathogenicity of M. xenopi and help clinicians in deciding whether to treat or monitor patients with MX-PD. Patients with M.xenopi subtypes of a lower pathogenic potential could avoid prolonged antibiotic treatment with possible toxic side effects.

MeSH/Keywords: Mycobacterium xenopi, nontuberculous mycobacteria, whole genome sequencing

Poster code: T-02-09-101

Poster Title: Relation between intracranial artery calcification and major adverse cardiovascular events in hemodialyzed patients

PhD candidate: Danilo Gardijan

Part of the thesis: Relation between intracranial artery calcification and major adverse cardiovascular events in hemodialyzed patients

Mentor(s): Vedran Premužić, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Intracranial arterial calcification (IAC) has been associated with every chronic kidney disease (CKD) stage and worsens with progression to end-stage renal disease(ESRD). There are only a few large studies on IAC and their impact on CV mortality in HD patients with opposing results, while the factors influencing calcifications are still not yet known.

Hypothesis: Intracranial artery calcification in patients on hemodialysis is associated with a higher rate of major adverse cardiovascular events(cardiovascular death, myocardial ischemia, stroke).

Aims: The main aim of this study is to detect associations between intracranial artery calcification in hemodialysis patients with major adverse cardiovascular events. We will also explore associations of IAC with laboratory parameters, arterial stiffness markers of large arteries, atherosclerosis markers of conduit arteries, duration of hemodialysis treatment, and analyze IAC score in the diabetic subgroup of HD patients.

Materials and methods: All patients in the study will be patients from hemodialysis unit of the Internal medicine department and stroke patients of the Neurology department, University hospital Zagreb. In this prospective, observational, longitudinal study, 80 hemodialysis patients who had a CT-scan of the head will participate. Data on medical history and medication will be collected from hospital documentation. Patients will be selected if they have been on chronic HD for at least three months. Exclusion criteria are: acute stroke at the time of performing CT-scan, atrial fibrillation or other chronic arrhythmias and significant hemodynamic instability during the dialysis. As a control group for assessing IAC, we will analyze CT scans from 80 diabetic patients who had an acute stroke. Two cohorts of patients will be age and sex matched . Follow-up period is 48-month for every patient after head CT- scan or till the time of death. For every patient following data will be collected: demographic data, anthropometric measures, habits, history of the disease, medication data, complete blood count with differential, head CT. All CT examinations will be analyzed by two experienced radiologists who will be blinded for patient's clinical data. Calcium scoring will be done using semiquantitative method where 0 point will be given to arteries with no calcification and 1 point to arteries with any calcification. For HD patients additional data will be collected, 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. Mortality and clinical data of patients will be collected during regular hemodialysis procedures 2 or 3 times per week, from the hospital information system and the Croatian institute of public health. Statistical analysis will be performed using SPSS software. A p value <0.05 will be considered significant.

Expected scientific contribution: Results of the study will show association between intracranial artery calcifications and major adverse cardiovascular events, and also with stiffness and atherosclerosis of large and conduit arteries. This could be useful for the prevention of such adverse events and prognosis in hemodialysis patients.

MeSH/Keywords: Arterial stiffness, Calcifications, Major adverse cardiovascular events(MACE), Hemodialysis, Intracranial arteries

Poster code: T-02-09-108

Poster Title: Heart mass and muscle strength in Croatian elite football players - Hand grip test as a part of echocardiographic evaluation of elite football players

PhD candidate: Matija Marković

Part of the thesis: Heart mass and muscle strength in Croatian elite football players

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

Affiliation: University of Zagreb School of Medicine

Introduction: Volume and pressure workload leads to heart enlargement, and athletic heart can develop in competitive sport. Both echocardiography and electrocardiography are used in sudden cardiac death risk stratification among athletes, as there are similarities between athletic heart and different cardiomyopathies. Professional bodybuilders have thicker and heavier hearts than ice hockey players, and there are differences in heart mass between different sport types. Football is a combined-type sport, and there are also differences between heart sizes among different pitch roles. Increased heart mass is associated with training and gross muscle mass, but in cardiomyopathies heart mass is also changed. Distinguishing between athletic heart and structural heart diseases associated with sudden cardiac death is often challenging. It is unknown whether there is an association between hand grip test power and heart dimensions measured by echocardiography. Hand grip test is a simple method of evaluating general muscle power.

Hypothesis: We hypothesize that heart size and volume dimensions in male elite football players corelate with hand strength. If there is correlation between hand grip test and heart mass, it is expected that stronger athletes will have increased heart muscle regardless of body surface area, body weight or height.

Aims: Primary study aim is to test correlation between general muscle power measured by a hand-grip manometer/dynamometer and heart size, volume, and myocardial thickness measured by echocardiography. Secondary aims are the following: To describe and define the normal echocardiographic parameters among male elite Croatian football players. To describe and define normal hand grip strength among Croatian elite football players. To corelate hand grip strenght and specific pitch position and compare between clubs rankings. To describe echocardiographic changes among different pitch positions among Croatian elite football players, and different clubs rankings.

Materials and methods: This trial is going to evaluate 300 professional football players for hand grip test by a standard protocol including both hands and perform a standard echocardiography exam. After primary data collection we will do a correlation analysis between hand grip strength and heart size, volume and mass measured and calculated by echocardiography. In a secondary analysis we will analyze and evaluate standard echocardiographic parameters among Croatian elite football players, compare them among different pitch positions and among different clubs. In the primary analysis after the hand grip test we will collect the following parameters by echocardiography: Interventricular septum and posterior wall thickness, end-diastolic and end-systolic left ventricular diameter; as well as Deveraux calculated ventricular mass. In the secondary analysis we will collect and analyse the following parameters: End-diastolic basal diameter of right ventricle, left atrium and aorta, ejection fraction biplane, tissue doppler velocities of mitral and tricuspid anulus, M-mode excursion of mitral and tricuspid anulus, and acceleration time in pulmonary artery.

Expected scientific contribution: For the first time we will directly try to correlate whether the heart mass and dimension are connected with hand grip test. This type of correlation if proven could help in better future preparticipation evaluation of elite athletes; and one could use it to distinguish among enlarged hearts due to high muscle strength or evaluation for hypertrophic cardiomyopathy should be done instead. In long term one could validate whether there is a strength cut-off value bellow which there should be no mass increase.

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

Poster code: T-02-09-114

Poster Title: Association of solubile ACE2 enzyme with fatty liver index in patients with metabolic syndrome

PhD candidate: Matej Nedić

Part of the thesis: Association of solubile ACE2 enzyme with fatty liver index in patients with metabolic syndrome

Mentor(s): Assistant Professor Ana Gudelj Gračanin, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Hyperactivation of the renin-angiotensin system mediates detrimental metabolic effects in metabolic syndrome. The fatty liver index is singled out as the most reliable discriminant variable in patients with metabolic syndrome. ACE2 enzyme plays a central role in the production of angiotensin (1-7) which mediates opposite and beneficial metabolic effects. In studies, ACE2 is associated with cardiovascular outcomes and overall mortality

Hypothesis: In patients with metabolic syndrome and elevated fatty liver index, soluble ACE2 values are elevated

Aims: To establish an association between serum levels of ACE2 and fatty liver index in patients with metabolic syndrome. To investigate the relationship of interleukin-6 and hs-CRP with soluble ACE2 and distinguish serum ACE2 levels in patients with metabolic syndrome taking ACE inhibitors compared with patients not taking them

Materials and methods: The study group includes patients with metabolic syndrome based on the NCEP-ATP III definition treated at the Department of rheumatology, allergology and immunology Clinical Hospital Dubrava. After obtaining informed consent, participants will complete a standardized questionnaire. Anthropometric measures (height, weight, body mass index (BMI), waist circumference, waist to hip ratio), sociodemographic data, and serum samples will be taken during the initial examination and fatty liver index will be calculated. Waist circumference will be measured midway between the lowest rib and cristae iliacae. Hip circumference will be measured over the widest part of the gluteal region. The fatty liver index will be calculated according to the formula: (e0.953 × logs (triglycerides) + 0.139 × BMI + 0.718 × logs (GGT) + 0.053 × waist circumference 15.745) / (1 + e0.953 × logs (triglycerides) + 0.139 × BMI + 0.718 × logs (GGT) + 0.053 × waist circumference 15,745) × 100. The inclusion criteria of the target population are the diagnosis of metabolic syndrome based on NCEP-ATP III definition with a control group of participants who do not meet these criteria. According to the NCEP-ATP III definition, metabolic syndrome is defined as the presence of 3 of these risk factors: Waist circumference -Man > 102 cm -Woman > 88 cm Triglycerides 1.7 mmol / L HDL-cholesterol -Man <1.0 mmol / L -Woman <1.3 mmol / L Blood pressure > 130/85 mmHg (or therapy for arterial hypertension) Fasting glucose 6.1 mmol / L Exclusion criteria: age under 18, established diagnosis of liver cirrhosis, inflammatory diseases (active infection, autoimmune diseases), diagnosis of malignant disease, heart failure, recent surgery. The laboratory part of the study, including the determination of serum levels of ACE2 and interleukin 6 will be determined at the Clinical Institute for Laboratory Diagnostics of the Dubrava Clinical Hospital. Serum ACE2 and IL-6 values will be determined by ELISA immunoenzyme assay and biochemical parameters by a biochemical analyzer. Serum ACE2 levels will be divided into quartiles (Q1 <15 ng/mL, Q2 15-28 ng/mL, Q3 28-40 ng/mL, Q4> 40 ng/mL), and a comparison of the variables between the control group and the test group will be done using a multivariate permutation test, with $p < 0.05$ considered statistically significant. To estimate the association between individual parameters, a parametric or non-parametric test will be used to determine associations depending on data distribution. The optimal value of serum ACE2 level that statistically significantly determines discrimination between individual groups will be determined using ROC curve analysis. According to the power analysis, a sample of at least 30 participants is sufficient to achieve statistical significance

Expected scientific contribution: Characterization of the fatty liver index as a marker of renin-angiotensin system dysregulation will contribute to the identification of patients at increased risk and to whom a higher level of preventive measures needs to be directed

MeSH/Keywords: soluble ACE2 enzyme, fatty liver index, metabolic syndrome

Poster code: T-02-09-118

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, University Hospital Merkur

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). Different studies have demonstrated higher levels of inflammatory cytokines (IL-6, IL-8, IL-10, CCL2) in patients with alcoholic liver cirrhosis. The exact mechanism in which PNPL3 polymorphism leads to faster disease progression and worse outcomes is unknown.

Hypothesis: G allele carriers of PNPLA3 rs738409 gene will have higher levels of inflammatory cytokines compared to wild type (CC) in patients with alcoholic liver cirrhosis.

Aims: GENERAL AIM Investigate the association between PNPLA3 (rs738409) polymorphism and inflammatory cytokines in patients with alcoholic liver cirrhosis. SPECIFIC AIMS 1. Determine the concentration of cytokine markers of inflammation interleukins (IL)) IL-1, IL-6, IL-8, IL-10, IL-12p70, IFN-, MCP1, TNF- in patients with alcoholic liver cirrhosis 2. Determine which inflammatory cytokines are associated with the G allele. 3. Compare the association of inflammatory cytokines between patients with alcoholic liver cirrhosis and the control group. 4. Compare the association of inflammatory cytokines between patients with alcoholic liver cirrhosis and patients with hepatocellular carcinoma in the setting of alcoholic cirrhosis

Materials and methods: This is a prospective observational study, with study population of 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 will consist of patients without liver disease and without acute or chronic inflammatory disease, who will 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 proves malignant, the specified control patient will be excluded from the study. Inclusion criteria 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. Exclusive criteria liver disease of etiology other than alcoholic. Exclusive criteria for the control group of patients liver disease, acute or chronic inflammation, malignant tumor. Expected duration of the research is 2 years (2021-2023). The study will include patients with alcoholic cirrhosis, who are on the list for liver transplantation. Liver disease as well as HCC will be histologically verified during the transplant procedure. All patients in the study will be previously informed about the research and sign an informed consent. The research will be conducted in cooperation with the Clinical Hospital Merkur and the Department of Physiology, Faculty of Medicine, University of Zagreb. Sample storage, DNA isolation and determination of gene polymorphism will be performed in the Laboratory for Molecular Immunology at the Croatian Institute for Brain Research. Based on this analysis, subjects will be divided into 3 groups based on different genotypes (CC, CG and GG alleles). Flow cytometric analysis will determine the concentration of cytokine markers of inflammation using a commercially available kit.

Expected scientific contribution: G allele carriers of the PNPLA3 polymorphism have a higher risk of developing hepatocellular carcinoma. One of the suggested theories is that inflammation leads to the development of HCC but inflammatory mediators have not yet been established. This research will determine the association between the PNPLA3 rs738409 polymorphism and inflammatory cytokines and thus contribute to a better understanding of development of HCC in patients with alcoholic liver cirrhosis.

MeSH/Keywords: PNPLA3, liver cirrhosis, inflammatory cytokines

Poster code: T-02-09-124

Poster Title: Subpopulations of peripheral blood monocytes and monocyte-response cytokines in patients with chronic graft-versus-host disease

PhD candidate: Antonija Babić

Part of the thesis: Subpopulations of peripheral blood monocytes and monocyte-response cytokines in patients with chronic graft-versus-host disease

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

Affiliation: University of Zagreb School of Medicine

Introduction: Chronic graft-versus-host disease (cGVHD) is a major late complication in patients after allogeneic hematopoietic cell transplantation (allo-HCT). Its immunopathogenesis is characterized by three phases: 1) early inflammation and tissue damage; 2) immune system dysregulation and chronic inflammation and 3) aberrant tissue repair and fibrosis. Given the very heterogeneous and complex clinical presentation of cGVHD, the search for potential disease biomarkers has been an area of intensive research. Monocytes are cells of innate immunity that, according to the expression of two key cell surface markers - CD14 and CD16 - are divided into three subpopulations: classical, intermediate, and non-classical monocytes. Individual monocyte subpopulations, as well as monocyte-related cytokines, have different biological functions in phagocytosis, inflammation, angiogenesis, wound healing, coagulation, T-cell activation, antigen presentation, and their role has been investigated in various diseases. To date, few studies of monocytes in cGVHD have shown no differences in the proportions of individual monocyte subpopulations in patients with cGVHD with the inconsistency in findings of cytokine secretion between monocyte subsets. Chemokines and cytokines secreted by other immune cells have been shown to play a role as potential biomarkers in the diagnosis of cGVHD, but there are no data on the monocyte response-related cytokines and their predictive value for the development of cGVHD.

Hypothesis: Subpopulations of monocytes and monocyte response cytokines in peripheral blood correlate with the occurrence and clinical features of chronic graft versus host disease.

Aims: To determine a correlation between the occurrence and clinical features of cGVHD with the percentage and the absolute number of monocyte subpopulations, as well as with the concentration of monocyte response cytokines in peripheral blood of newly diagnosed cGVHD patients and patients with previously known cGVHD.

Materials and methods: Adult patients aged 18 to 65 years who were treated with allo-HCT at the Department of Hematology, Clinical Hospital Centre Zagreb after routine processing by the multidisciplinary cGVHD team, will be included in the study. The patients will be divided into three groups with at least 29 patients in each group: patients with newly diagnosed cGVHD, patients with previously known cGVHD, and a control group of patients after allo-HCT who haven't developed cGVHD. Peripheral blood monocytes will be labeled with appropriate monoclonal antibodies, processed on BD FACSLyric flow cytometer, and analyzed using BD FACSuite software. A microsphere immunoassay technology (multiplex bead array, LEGENDplex, BioLegend) will be used to measure the concentration of 13 different plasma cytokines: MCP-1, IL-17A, CX3CL1 (fractalkine), IL-12p70, TNF-, IL-6, IL-4, IL-10, IL-1, CCL17 (TRAC), IL-23, IFN- and CXCL10. Depending on the distribution of values, the differences between groups will be analyzed by one-way analysis of variance or with the Kruskal-Wallis tests and appropriate post hoc tests. Appropriate correlation coefficients (Pearson's or Spearman's coefficients) between individual clinical and laboratory values will be calculated. An appropriate regression model (binary logistic regression) will assess the multivariate impact of previously significant variables on the clinical picture of the disease. All P values less than 0.05 will be considered statistically significant.

Expected scientific contribution: To provide additional insight into the potential role of monocytes and monocyte-related cytokines in the immunopathogenesis of specific changes in cGVHD, as well as to determine their potential predictive value for the development and course of cGVHD.

Acknowledgments: This work was supported by the Croatian Science Foundation project IP-2016-06-8046 entitled "New biomarkers for chronic Graft-versus-Host disease".

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

Poster code: T-02-09-134

Poster Title: Estimated arterial stiffness and cardiovascular risk in chronic kidney disease

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

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.

Hypothesis: Arterial stiffness is an independent predictor of cardiovascular, cerebrovascular, and renal outcomes regardless of age-adjusted definition of chronic kidney disease.

Aims: The aim of this study is to determine whether the predictability of ePWV for fatal and non-fatal CV, cerebrovascular and renal outcomes is independent of CKD defined by guidelines as eGFR <60 mL /min/1.73m2 or age-adjusted definition of CKD. The specific aims are to analyse risk factors for ePWV depending on the stage of CKD. Determine the predictability of all traditional CV risk factors and ePWV for total and CV mortality, and for individual fatal and non-fatal outcomes. Analyse the causes of death depending on ePWV. Determine incidence of ePWV-dependent morbidity in subjects classified as subjects with CKD and without CKD.

Materials and methods: This prospective observational study will include 2058 subjects from the Endemic nephropathy in Croatia epidemiology, diagnosis and etiopathogenesis scientific research project. Study subjects were followed for an average of 12 years (2005-2020). At the starting point, all subjects signed a written informed consent and completed an extensive questionnaire containing information on personal and family history, were clinically examined, and a fasting blood sample and morning urine sample was taken. From the completed questionnaire and clinical examination, we obtained data on morbidity and anthropometric data that are important for the assessment of CV risk. Laboratory samples are marked with a unique identification code to eliminate laboratory and research bias. Laboratory processing was performed to determine all traditional CV risk factors (glucose, lipids, urate). GFR was estimated using the CKD Epi equation, but also by a de-indexed equation to obtain absolute GFR value. In people with CKD eGFR will also be calculated using the MDRD equation. CKD will be defined using guideline-accepted definition using a unique cut-off value of 60 mL/min/1.73 m2 and using an age-adjusted CKD definition that uses eGFR limits for under 40 years eGFR <75, for subjects between 40-65 years GFR <60, and for those over 65 years GFR <45. The ePWV values will be calculated using the validated equation. Total risk will be calculated using accepted risk scores (Heart Score and Framingham risk score). From the archive of family medicine doctors, and from the register of deaths of the Croatian Institute of Public Health, we will obtain data on fatal and non-fatal CV, cerebrovascular and renal outcomes. Statistical processing will be done in collaboration with our foreign partner and leading expert in this field Stephan Laurent (INSERM, Institut National de la Santé et de la Recherche Médicale).

Expected scientific contribution: This will be the first study that could show how the estimated arterial stiffness contributes to total CV, cerebrovascular and renal risk in CKD. Second, our results would contribute to the introduction of an age-adjusted definition of CKD into clinical work. In conclusion, based on the results of the proposed study, we could propose ePWV as a useful prognostic marker of total CV, cerebrovascular and renal risk.

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

Poster code: T-02-09-137

Poster Title: Proteomic profile of patients with compensated advanced chronic liver disease and clinically significant portal hypertension

PhD candidate: Frane Paštrović

Part of the thesis: Proteomic profile of patients with compensated advanced chronic liver disease and clinically significant portal hypertension

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

Affiliation: University of Zagreb School of Medicine

Introduction: Portal hypertension is the leading cause of complications in patients with advanced chronic liver disease including the development of ascites, gastroesophageal varices, portal encephalopathy and hepatorenal syndrome. These complications begin to develop when hepatic vein pressure gradient (HVPG) exceeds 10 mmHg (considered as clinically significant portal hypertension, CSPH). Hence early identification of patients with CSPH is of high importance. As HVPG measurement is invasive procedure, with limited availability, non-invasive tests represent an attractive diagnostic alternative. Several studies have been published using proteomics and other -omics approaches in the field of hepatology and they provided additional insight in the development of liver diseases. Nevertheless, no reliable serum biomarker for the presence of CSPH has been identified so far.

Hypothesis: There is a difference in proteomic profiles of serum samples between cACLD patients with and without CSPH. Proteins with different expressions among these groups might potentially be used as biomarkers for diagnosing CSPH in patients with cACLD.

Aims: We aim to compare proteomic profiles of cACLD patients with and without CSPH. We expect to identify proteins, biological processes and related pathways that differ between those two groups.

Materials and methods: Patients with confirmed cACLD (based on liver biopsy findings of bridging fibrosis or cirrhosis), with available results of HVPG measurements and collected serum samples are considered eligible for this study. These patients were recruited from the cohort that was involved in the scientific research project Non-invasive approach in the diagnostics of portal hypertension testing and comparison of existing methods and discovery of new serum markers (HePortoNew). For the purpose of that project patients with suspicion of having cACLD based on liver stiffness measurement above 10kPa and/or morphological features of advanced chronical liver disease, without a history of liver decompensation, were referred to HVPG measurement and transjugular liver biopsy, and serum samples were collected and stored. From the initial cohort of 76 patients that met the inclusion criteria for HePortoNew study, 28 were excluded from the further analysis (22 did not have bridging fibrosis or cirrhosis, in 3 patients serum samples were inadequate, and 3 patients had liver neoplasm). Therefore, 48 patients were considered eligible for the current study. Serum samples from these patients were pooled based on HVPG result into groups without (HVPG<10 mmHg, N=18) and with CSPH (HVPG10, N=30). We plan to purify the serum pools using HiTrap heparin sepharose columns and analyze the pools using Liquid Chromatography-Mass spectrometry. Samples will be analyzed in triplicates and proteins identified with at least one peptide (identified by at least a 7 amino acid sequence) will be considered relevant for analysis. With these settings the acquired false detection ratio is below 1%. Functional enrichment analysis will be conducted using FunRich 3.1.3 analysis tool.

Expected scientific contribution: We expect to reveal the difference between the proteomic profiles of cACLD patients with and without CSPH. Furthermore, by analyzing the difference in the represented biological processes, protein expressions and their interactions, we aim to identify potential novel serum biomarker candidates of CSPH as tipping point markers of disease progression which will shed new light on understanding the pathogenesis of portal hypertension development and possibly identify new potential therapeutic targets.

MeSH/Keywords: Liver cirrhosis; portal hypertension; proteomics; biomarkers

Poster code: T-02-09-139

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.

Hypothesis: Based on serum uric acid levels measured immediately before and repeated after ablation (PVI), it is possible to identify patients at increased risk of recurrence of FA and/or other atrial tachyarrhythmias.

Aims: The primary outcome is to examine whether patients at increased risk of recurrence of FA and/or other atrial tachyarrhythmias can be identified based on multiple measured serum uric acid concentrations within 12 months after treatment of PVI. Secondary outcomes are the observation of the relationship between serum uric acid concentration and changes in left atrial dimensions/function or recurrence of FA and/or other atrial tachyarrhythmias.

Materials and methods: We are planning to conduct a single-centre, non-randomized, observational, prospective cohort study. Our goal is to enroll 60 consecutive patients with symptomatic paroxysmal AF between 18-75 years who were scheduled for an initial PVI using a focal radiofrequency or 2nd-generation cryoballoon ablation. Patients with contraindication to anticoagulation, enlarged left atrium (LA PLAX >55mm ili LAVI >34ml/m2), history of myocardial infarction or stroke/TIA in the last 3 month before inclusion, significant valvular pathology, acute infectious disease, active malignancy, systemic connective tissue disease, alcohol abuse, gout, diuretics in chronic therapy (loop of Henle's, thiazide) and breastfeeding or pregnant women were excluded. During index visit complete standard work-up will be done - past medical history, clinical status, lab exams (including uric acid concentration in serum), ECG, transthoracic/transesophageal ultrasound. During the 12-month follow-up period, we plan visits every 3 months, when all initial variables will be re-measured and all outcomes monitored. Concerning statistical analysis, generalized linear mixed model (GLMM) for repeated measurement of a binary dependent variable with fixed (basal) and time-varying covariates will be used.

Expected scientific contribution: This is the first study to prospectively monitor the dynamics of serum uric acid concentration in patients after PVI and its relationship to the risk of recurrence of FA and/or other atrial tachyarrhythmias. The study will help assess the importance of serum uric acid levels as a potential risk indicator.

Acknowledgments: The study was conducted with no grant support.

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

Poster code: T-02-09-152

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): Assistant 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.

Hypothesis: Increased incidence of cardiovascular events in individuals with elevated lipoprotein (a) level is associated with the presence of single nucleotide polymorphisms rs10455872 and rs798220 LPA gene

Aims: Investigate the association of gene variations, single nucleotide polymorphisms rs10455872 and rs3798220, with serum lipoprotein (a) levels and their impact on cardiovascular risk

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. Blood for genotyping of these polymorphisms will be taken from each subject, and in selected cases with high lp (a) values, sequencing of the next generation for the lp (a) gene will be performed

Expected scientific contribution: Finding high-risk variations in the lp (a) gene for cardiovascular disease with timely intervention and treatment of patients could prevent or delay cardiovascular events and associated mortality

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

Poster code: T-02-09-153

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

PhD candidate: Tea-Terezija Cvetko

Part of the thesis: Predictive Value of Right Atrial Strain on Funcional Capacity in Non High Risk Pulmonary Embolism Patients

Mentor(s): Assistant 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 PE. We hypothesized that right atrial (RA) reservoir function, represented by peak RA systolic strain, could be used as a predictive marker for functional outcome in non high risk PE patients. All patients with diagnosed non-high risk PE will undergo TTE within 24h of proven PE. Functional outcome will be determined using 6 minute walk test (6MWT) after 3 and 6 months. We hypothesise that patients with reduced peak RA systolic strain will have reduced 6MWT.

Hypothesis: Reduced right atrial strain (represented by peak right atrial systolic strain) is a predictive marker of long term functional outcome in non high risk pulmonary embolism patients.

Aims: The aim of the study is to evaluate right atrial strain in non high risk pulmonary embolism patients and determine its predictive value for functional outcome. We also aim to evaluate standard right atrial echocardiographic measures in non-high risk patients; conduit function and contractile function of right atrium in non high risk pulmonary embolism patients; and right ventricular strain in non high risk pulmonary embolism patients.

Materials and methods: This will be an observational, non-interventional, multicenter, cohort study. The duration of the study will be 36 months: 24 months of enrollment and 12 months of follow-up (6 months per patient). The inclusion criteria are: patients older than 18 years with non high risk pulmonary embolism (hemodynamically stable) who signed the informed consent form. All patients will be evatuated according to protocol for PE (history, physical examination, ECG, echocardiography, CT pulmonary angiogram, troponin I, NTproBNP). Echocardiography will be performed within 24h of the diagnosis. Apart from standard echocardiography protocol, we will measure right atrial (reservoir, conduit and contractile function) and right ventricular strain. 6 minute walk test will be perfomed after 3 and 6 months according to American Thoracic Society protocol. After the test patient will be asked to rate their perceived dyspnea from 1 to 10 (1 no dypnea, 10 heavy dyspnea). For the analysis of data logistic regression will be applied, for 4 independent variables a sample of 80 patients is needed.

Expected scientific contribution: The predictive value of right atrial function assessed by echocardiography speckle tracking in patients with acute non-high risk PE has never been evaluated. To the best of our knowledge, this study will be the first evaluating RA systolic strain in patients with non high risk PE and using it as a predictive value for functional outcome. We believe this study could be a cornerstone in the field of potential rehabilitation for PE patients.

MeSH/Keywords: right atrial strain, right ventricular strain, pulmonary embolism, 6 minute walk test

Poster code: T-02-09-159

Poster Title: Value of Anti-Nuclear Antibodies Titre Dynamics as a Predictor of Systemic Lupus Erythematosus Relaps in Anti-dsDNA Positive Patients

PhD candidate: Darija Čubelić

Part of the thesis: Predictive value of anti-nuclear antibodies titre dynamics as a predictor of systemic lupus erythematosus relapse in anti-dsDNA positive patients

Mentor(s): Assistant Professor Miroslav Mayer, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Antinuclear antibodies (ANA) are routinely detected in systemic lupus erythematosus (SLE) and represent a potential biomarker of activity. There is controversy over the importance of ANA titer levels in measuring current and predicting future disease activity.

Hypothesis: ANA titer dynamics could be used as predictor of systemic lupus erythematosus relaps in anti-dsDNA positive patients.

Aims: To investigate the value of ANA titer dynamics as predictor of systemic lupus erythematosus relaps in anti-dsDNA positive patients.

Materials and methods: The prospective cohort study will include 100 consecutive patients with SLE. Clinical and laboratory findings will be collected after 0, 6 and 12 months. The impact of significant changes in ANA titer on disease activity expressed by ECLAM and SLEDAI indices and expert opinion will be analyzed.

Expected scientific contribution: To identify the possible role of ANA titer dynamics in predicting disease relapse, which may improve the therapeutic approach.

MeSH/Keywords: systemic lupus erythematosus, antinuclear antibodies, titre, predictor of relapse

Poster code: T-02-09-163

Poster Title: Retraction of the supraumbilical skin after laser-assisted liposuction / lipolysis

PhD candidate: Ivonne Žgaljardić

Part of the thesis: Retraction of the supraumbilical skin 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 greatest aesthetic deformities of the abdominal wall today. A significant degree of laxity is successfully treated with abdominoplasty with umbilical transposition. There is no quality solution for mild to moderate degrees of laxity. They can be treated with abdominoplasty with umbilical transposition that results in a vertical scar of the excised umbilicus located below the newly created umbilical position or reverse abdominoplasty resulting in an unacceptably visible scar at the junction of the chest and abdominal wall. Previous work on laser-assisted liposuction / lipolysis speaks in favour of thermal injury to the reticular dermis and subdermal connective tissue due to heating to a temperature of 48-50 ° C. The result is an inflammatory response and stimulation of collagen and elastin production and ultimately skin tightening over 3 months.

Hypothesis: Retraction of the supraumbilical skin after laser-assisted liposuction / lipolysis of the abdomen is significantly lower in elderly patients, as well as in patients with high body mass index, lower skin elasticity and the presence of stretch marks.

Aims: The main objective is to evaluate supraumbilical skin retraction during laser-assisted liposuction / lipolysis by measuring the reduction in the distance from the upper umbilical edge to the jugulum at intervals of seven days, three weeks, and three months after surgery. Also, the objective is to determine the association of vertical retraction of the supraumbilical skin with a decrease in circumferential supraumbilical abdominal circumference three months after surgery and to determine the relationship between age, sex, body mass index, skin elasticity and the presence of stretch marks with reduced distance between jugulum and upper umbilical margin three months after the surgery.

Materials and methods: Patients with mild or moderate supraumbilical skin laxity undergoing elective laser-assisted liposuction / lipolysis will be included in the prospective study. Excluded from the study are persons with significant supraumbilical skin laxity who are candidates for abdominoplasty with umbilical transposition, women who undergo simultaneous breast reduction and those who become pregnant at the end of the measurement period. All patients with previous abdominal surgery are also excluded from the study. Prior to the procedure, height, weight, body mass index, age, sex, skin elasticity, as well as the presence or absence of stretch marks in patients will be recorded. The distance from the jugulum to the upper umbilical edge will be measured (due to difficult palpation of xyphoids in localized supraumbilical lipodystrophies and the inability to accurately map its position on the skin of the wall, the distance from the jugulum to the navel is taken to make a more accurate measurement). Abdominal circumference will be measured at the level of the navel and five centimetres above the navel. These measurements will be performed before the procedure, as well as at intervals of seven days, three weeks and three months after the procedure, and preoperative measurements will serve as a control group.

Expected scientific contribution: Upon completion of the study, it is expected that it will be possible to determine the degree of retraction of the supraumbilical skin, i.e., the upper umbilical edge. Also, we expect that it will be possible to determine which patients can expect a good aesthetic result in terms of correction of laxity of the upper abdomen and correction of the appearance of hanging or sad navel after laser-assisted liposuction / lipolysis with regard to age, sex, body mass index, skin elasticity and the presence of stretch marks.

MeSH/Keywords: lipectomy, lipolysis, elasticity.

Poster code: T-02-10-026

Poster Title: Masseter and temporalis muscle activity impairment in Rheumatoid Arthritis (RA) of temporomandibular joint (TMJ)

PhD candidate: Kaltrina Kryeziu

Part of the thesis: Masseter and temporalis muscle activity impairment in Rheumatoid Arthritis (RA) of temporomandibular joint (TMJ)

Mentor(s): Assistant Professor Emil Dediol, MD PhD, Assistant Professor Mergime Prekazi-Loxha, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Rheumatoid arthritis RA is a chronic disease of which the etiology is not fully known. It is well known that the temporomandibular joint TMJ may be affected by rheumatic disease. It is important to assess, if the disease can lead to changes in function of masticatory muscles and how this affects function of the lower jaw. Rheumatoid arthritis causes alterations in muscle function, inflammation in the joint capsule, painful symptoms, and resorption of the head of the mandible. Abnormalities of the temporomandibular joint TMJ in patients with RA are well recognized, but their incidence as reported in the literature varies greatly, from 5 to 86%.4 2.5:1 is the female to male ration, and the disease most frequently occurs between ages 35 and 45 years.

Hypothesis: Masseter and temporalis muscles function is changed due to the effect of rheumatoid arthritis on temporomandibular joint.

Aims: To compare the masseter and temporalis muscles activity in women with and without rheumatoid arthritis of the temporomandibular joint.

Materials and methods: This study will include a total of 40 women patients at the Department of Rheumatology, University Clinical of Kosovo in Pristina, that have been diagnosed with Rheumatoid Arthritis of TMJ, Stage II and III of disease with duration of disease 5-10 years and 40 healthy women without rheumatoid arthritis will be a control group. All cases will be matched subject-to-subject by age and body mass index (BMI). Included in the first group of the study will be 40 female patients in the age group of 35-60. To evaluate TMJ function, a questionnaire that was developed by Fonseca in 1992, will be filled in for RA group. A 3D Orthopantomogram (TYP D3437 Sirona) will be the standard tool to diagnose rheumatoid arthritis of TMJ. Both groups of patients will undergo measurements of muscle activity by electromyography and T-scan III for bite force. Measurements of muscle function will be done on the both side of face. Maximum mouth opening of the patients and control group will be measured with a ruler in centimeter. In addition, erythrocyte sedimentation rates, C-Reactive protein, rheumatoid factor levels and Anti-CCP of patients will be performed.

Expected scientific contribution: This study could be a good initial step for further studies to open a new line of investigation in this important aspect of prevention of RA long-term complications.

MeSH/Keywords: masseter muscle, temporalis muscle, muscle activity, rheumatoid arthritis, temporomandibular joint.

Poster code: T-02-10-073

Poster Title: Breast skin innervation after subcutaneous mastectomy and implant-based breast reconstruction

PhD candidate: Jana Leskovar

Part of the thesis: Breast skin innervation after subcutaneous mastectomy and implant-based breast reconstruction

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

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

Introduction: Only a few studies have been examining sensation after subcutaneous mastectomy and implant-based breast reconstruction so far. The results have shown that breast sensation was significantly impaired, even though native breast skin envelope was spared. Moreover, the roles of factors suggested to affect sensation such as skin thickness and breast volume are yet unexplored.

Hypothesis: Smaller breasts with thicker mastectomy skin flaps have better tactile sensation.

Aims: Aims of this research are to examine the effects of reconstructed breast volume and mastectomy skin flap thickness on breast sensation (primary aim); to assess the impairment of somatosensory and sympathetic innervation after implant-based breast reconstruction; to evaluate the biomechanical features of mastectomy skin flaps and their association with breast sensation; to describe changes in thickness of skin layers upon impaired innervation.

Materials and methods: Patients who underwent unilateral nipple-sparing mastectomy and primary implant-based breast reconstruction due to breast neoplasm meet the inclusion criteria. Exclusion criteria are: chest wall radiotherapy, breast oedema (diagnosed by ultrasonography), peripheral or central nervous system disease affecting sensory perception, diabetes mellitus, systemic skin disease or breast skin changes, and systemic corticosteroid treatment during the previous three months. Examinations will take place at the earliest six months after breast reconstruction at University Hospital Centre Zagreb. Each participant will undergo reconstructed and contralateral healthy breast testing at a single time point. The examination will consist of several sections: anthropometric breast measurements, quantitative sensory testing (touch, cold, warm), ultrasonography, durometry, and thermography. Three anthropometric breast measures will be used to calculate breast volume utilizing the BREAST-V® predictive formula (diepflap.it Medical Software). The distance between center of the nipple and the most caudal point of the inframammary fold divided by three will be used as a reference measure to locate the testing regions in each breast. Tactile perception thresholds, mastectomy skin flap hardness and temperature at the breast surface will be determined in the eight regions (inner and outer superomedial, superolateral, inferomedial and inferolateral). Cold and warm perception thresholds and ultrasonographic features including thicknesses of mastectomy skin flap, dermis and epidermis will be determined across the four regions (one per breast quadrant). Primary outcome will be statistically evaluated by multiple regression analysis. Minimum sample size for test power 0.8 and 0.05 is 26 subjects.

Expected scientific contribution: to provide the first findings on the effects of breast volume and mastectomy skin flap thickness on breast sensation after implant-based breast reconstruction; to bring forth novel clinical evidence on the interactions between biomechanical skin features, skin innervation and sensation.

MeSH/Keywords: Breast Implants; Mastectomy, Subcutaneous; Peripheral Nerve Injuries; Sensation; Skin

Poster code: T-02-10-091

Poster Title: Influence of local glyceryl trinitrate application on surgical wound healing

PhD candidate: Lucija Brkić

Part of the thesis: Influence of local glyceryl trinitrate application on surgical wound healing

Mentor(s): Petar Matošević, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Wound healing is a process that depends on many factors. Certain ones may be manipulated (e.g., asepsis principle, regular dressing), and some parameters are independent of the physician (e.g., patient status, comorbidities affecting healing) and also dictate the pace of healing (e.g., delayed healing in patients with diabetes). NO (nitric oxide) has long been used in treating cardiac patients, but also to treat other diseases, such as anal fissures.

Hypothesis: This study hypothesizes that glyceryl trinitrate will be an effective NO donor in the area of the operative incision and will promote faster and less painful healing by increasing oxygen and circulation levels.

Aims: The study aims to determine whether glyceryl trinitrate has a positive effect on the healing of surgical wounds. Specific objectives are: Do patients have: 1. faster healing of the surgical incision, 2. less postoperative pain, and 3. less infection

Materials and methods: This is a prospective, two armed, triple blind, placebo controlled randomized trial. Participants will be patients treated at the Clinic of Surgery, University Hospital Center Zagreb, Department of Oncological Surgery, electively admitted and operated on with the classical approach for the diagnosis of malignant neoplasms of the colon and rectum. The active substance is nitric oxide. The single dose of glyceryl trinitrate in this study will be 0.8mg (two pushes). One push from a distance of 10 centimetres will be applied to each side of the surgical incision. From the second postoperative day (first dressing) to the sixth postoperative day, two pushes will also be applied. Early assessment and noninvasive tests begins the second postoperative day, once a day during dressing for a total of 5 days.

Expected scientific contribution: In 2017, the average cost of treating patients operated on for malignant diseases of the colon and rectum at the University Hospital Center Zagreb was HRK 20,519.97. One pack of Nitrolingual costs HRK 38,57 in pharmacy retail. If this research proves that the patient has faster healing of the surgical incision, less pain, and lower infection rate, it is concluded that such a patient could spend fewer resources (analgesia, antibiotics, additional costs in case of wound infection, etc.), therefore this research, in case of positive results and implementation in clinical practice, would also be a measure of savings. In case of positive results, the research would open a completely new field of research in surgery. If such a cheap intervention can speed up wound healing, lower the infection rate and reduce the subjective feeling of pain, it is adequate to hypothesize that there are even better methods that quickly and effectively increase the quality of treatment and reduce costs. Further research would answer questions such as enhanced healing in specific cases (e.g., chronic wounds in diabetics who have a microcirculatory defect), dose adjustment, and the general range of topical applications of glyceryl trinitrate in surgery.

MeSH/Keywords: abdominal surgery, glyceryl trinitrate, wound healing

Poster code: T-02-10-154

Poster Title: Ethical issues of health professionals related to end-of-life decision-making in patients with malignancies

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; University Hospital Center Zagreb; University Hospital Center Sestre milosrdnice; Clinical Hospital Dubrava; Clinical Hospital Merkur; Clinical Hospital Sveti Duh; University Hospital Center Split; University Hospi

Introduction: Treatment of patients in advanced stages of oncological and hemato-oncological diseases involves making end-of-life decisions which is a balance of medical, ethical, psychosocial and social issues. End-of-life decisions include withdrawal and withholding of therapeutic and diagnostic life-support procedures. Most guidelines state that withdrawing and withholding therapy is legally and ethically equal, but such decisions represent one of the greatest ethical challenges in clinical practice and can result in moral harm of physician.

Hypothesis: Ethical attitudes of health professionals about the treatment of patients with malignancies at the end-of-life differ with regard to age, gender, length of service, level of education and the department in which they are employed.

Aims: General aim is to examine and compare ethical attitudes towards the treatment of end-of-life patients in health professionals in oncology and hemato-oncology wards. The first specific aim is through focus groups identify and examine ethical issues faced by physicians and nurses involved in the care of end-of-life patients in oncology and hemato-oncology wards in tertiary institutions in the Republic of Croatia. The second specific aim is through an anonymous questionnaire investigate the ethical attitudes of doctors and nurses involved in the care of patients at the end of their lives in oncology and hemato-oncology wards in tertiary institutions in the Republic of Croatia. Additional aim is to identify similarities and differences in the ethical attitudes of health professionals with regard to age, gender, length of service, level of education and the department in which they are employed and compare them with the ethical attitudes of health professionals in other countries.

Materials and methods: Focus groups are a qualitative type of research in which a smaller group of participants (4-12) similar in hierarchical and educational status and experience discuss a particular topic and freely express their own opinions and views with the guidance of a professional person moderator (research leader plus psychologist / psychiatrist). The focus group will explore the ethical issues faced by health professionals, participant will share their experiences, explain how they function, communicate and participate in a team and how they deal with ethically questionable situations. The collected data will be analysed by qualitative methods, free text analysis. Quantitative research will be conducted through an anonymous questionnaire, using a double-translated questionnaire constructed by Grosek et al. to examine the attitudes of medical staff in the care of end-of-life patients in intensive care units. Similarities and differences in ethical attitudes with regard to age, gender, length of service, level of education and the department in which they are employed will be determined. About 60% of the total number of health workers employed in these departments is expected to respond.

Expected scientific contribution: For the first time, ethical attitudes in health care workers related to decision-making in the end-of-life patients in oncology and hemato-oncology wards in tertiary institutions (in the Republic of Croatia) will be analysed and the obtained data could be used for the development of professional guidelines.

MeSH/Keywords: palliative care; end of life; decision making; ethical issues

Poster code: T-02-15-144

Poster Title: Diastolic left ventricular dysfunction estimated with radionuclide ventriculography(ERNA) as a predictive factor of cardiotoxicity after anthracycline chemotherapy in women with breast cancer

PhD candidate: Ivan Jakšić

Part of the thesis: Diastolic left ventricular dysfunction estimated with radionuclide ventriculography(ERNA) as a predictive factor of cardiotoxicity after anthracycline chemotherapy in women with breast cancer

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

Affiliation: University of Zagreb School of Medicine

Introduction: Anthracyclines remain an important drug for the treatment of many solid tumours, including breast cancer. However, its use is primarily limited by the development of irreversible left ventricular dysfunction (chemotherapy related cardiac dysfunction, CTRD). Current monitoring for CTRD relies on the quantitative and reproducible assessment of left ventricular ejection fraction (LVEF) either by equilibrium radionuclide angiocardiography (ERNA), gated blood pool SPECT (GBPS), or echocardiography. This method of monitoring does not allow for the detection of CTRD until left ventricular dysfunction occurs. Therefore, novel method of detecting cardiotoxicity before the onset of CTRD would allow treatment with cardioprotective drugs to prevent CTRD. Low PFR (peak filling rate) as a parameter obtained by nuclear medicine imaging method ERNA may provide important predictive information identifying individuals who might benefit from cardioprotective therapy.

Hypothesis: Low peak filling rate on ERNA scan between cycle 1 and cycle 2 of anthracycline based chemotherapy will predict higher rate of chemotherapy related cardiac dysfunction (CTRD).

Aims: The primary research aim of this study is to determine early (within 6 weeks of chemotherapy initiation) imaging based (ERNA) predictor of left ventricular systolic dysfunction or heart failure at one year in women with breast cancer receiving anthracycline-based chemotherapy. The secondary research aim of this study is to determine clinical differences in the development of left ventricular systolic dysfunction or heart failure at one year, in women with breast cancer receiving anthracycline-based chemotherapy.

Materials and methods: This will be an prospective, observational study of women undergoing anthracycline-based chemotherapy for breast cancer. A group of patients on anthracycline therapy will be selected from the group of breast cancer patients treated at Departement of Oncology and Nuclear Medicine in University hospital center Sestre milosrdnice. PFR will be deteminated with radionuclide ventriculography, diagnostic nuclear medical imaging method, at 3-time points during the study (within 6 weeks of each other). It is important to note that the ERNA images are acquired from one radiotracer injection. Next ERNA imaging will be repeated in the time interval between the end of the first cycle and beginning of the second cycle of chemotherapy. In addition, a final assessment of left ventricular function will be made using ERNA imaging between 15 and 18 months after the initiation of chemotherapy t). The images will CTRCD is defined as a fall in LVEF >5% with symptoms of heart failure or a fall of >10% in asymptomatic patients to an LVEF <50%. We will use the ERNA derived LVEF for determining the primary outcome.

Expected scientific contribution: The results of this study will improve the knowledge of the nuclear imaging method - ERNA which predicts risk of anthracycline mediated cardiotoxicity and may change management and improve clinical outcomes.

MeSH/Keywords: radionuclid venticulography, breast cancer, myocard scintigraphy, anthracyclines

Poster code: T-02-17-142

Poster Title: Concentration of calprotectin in the aqueous humor of patients with the pseudoexfoliative syndrome

PhD candidate: Andrej Pleše

Part of the thesis: Concentration of calprotectin in the aqueous humor of patients with the pseudoexfoliative syndrome

Mentor(s): Sanja Masnec, PhD, senior research associate, Professor Biljana Kuzmanović Elabjer, MD PhD

Affiliation: University of Zagreb School of Medicine, University Hospital "Sveti Duh"

Introduction: Pseudoexfoliative syndrome (PEX) is an age-related systemic microfibrilopathy that affects eye tissue. The pathogenesis of PEX is not yet fully understood but is probably multifactorial and related to aging and genetics. Calprotectin is a heterodimer protein and is expressed in neutrophils and monocytes. During inflammation, it is actively released from these cells and plays a key role in modulating the inflammatory response by stimulating the recruitment of other inflammatory cells and by inducing the secretion of proinflammatory cytokines. Because calprotectin is released at the site of inflammation, calprotectin may serve as an early marker of recognition of inflammatory conditions of various tissues and organs, and thus could potentially be an indicator of the presence of inflammation in the aqueous humor in the case of PEX.

Hypothesis: The concentration of calprotectin in the aqueous humor is higher in patients with PEX and PEX glaucoma compared to patients without PEX.

Aims: The general aim is to prove the presence of calprotectin in the aqueous humor of patients with the pseudoexfoliative syndrome. The specific aims are: to determine whether there is a difference in the concentration of calprotectin in the aqueous humor of patients with cataracts complicated by PEX from a patient with cataracts without PEX, to determine the difference in calprotectin concentration in the aqueous humor between patients with PEX and glaucoma and patients with PEX without glaucoma and determine whether there is a correlation in the concentration of calprotectin in aqueous humor and serum in each individual subject in all groups.

Materials and methods: Subjects in this cross-sectional study are patients over 50 years of age undergoing phacoemulsification cataract surgery divided into three groups: Patients with PEX without glaucoma, patients with PEX and secondary PEX glaucoma, and a control group consisting of patients without PEX. The inclusion of patients in this study will be performed during a preoperative examination for cataract surgery. The group of subjects with PEX without glaucoma will consist of subjects with proven PEX and excluded glaucoma. Subjects who will form a group with controlled secondary PEX glaucoma will be subjects who have previously been diagnosed with secondary PEX glaucoma and who are therefore regularly monitored and are now referred for cataract surgery during this clinical trial. The control group will consist of patients of appropriate age and sex, without PEX, and who underwent cataract surgery. Sampling will be done on the day of cataract surgery. Samples of aqueous humor and serum will be taken and analyzed in the laboratory where the concentration of calprotectin in the samples will be determined by the immunoturbidimetric method. The plan is to include a minimum of 40 patients in each of these three groups, which represents the optimal sample size so that the strength of statistical tests is at least 80%. The study would take up to 24 months. The collected quantitative data will be processed and analyzed by descriptive statistics measures (central tendency measures, scatter measures, etc.) and inferential statistics tests depending on the distribution of data according to the Kolmogorov-Smirnov test: test for testing properties of independent properties (t-test for independent samples or Mann-Whitney U-test) and data correlation test (Pearson correlation or Spearman rank correlation).

Expected scientific contribution: This is the first study of calprotectin as an inflammatory biomarker in the aqueous humor of patients with PEX. Evidence of the presence of calprotectin suggests that inflammation is a factor that may play an important role in the etiopathogenesis of the disease and its conversion to pseudoexfoliative glaucoma which opens new spaces and ideas for research and thus therapeutic approaches.

MeSH/Keywords: Calprotectin, Pseudoexfoliation syndome, Aqueous humour, Serum, Cataract

Poster code: T-02-18-022

Poster Title: Atherosclerosis markers and Diabetic Retinopathy in patients with type 2 Diabetes Mellitus

PhD candidate: Albina Krasniqi

Part of the thesis: Association of Atherosclerosis markers with the presence and severity of Diabetic Retinopathy in patients with type 2 Diabetes Mellitus

Mentor(s): Assistant Professor Snježana Kaštelan, MD PhD, en: znanstveni savjetni, Professor Gani Bajraktari, MD PhD

Affiliation: University of Zagreb School of Medicine

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

Hypothesis: Diabetic retinopathy is associated with most conventional risk factors of atherosclerosis (diabetes, smoking, dyslipidemia, arterial hypertension) and recently used new atherosclerosis markers (CIMT, ABI, CACs, central arterial stiffness, inflammatory markers).

Aims: The aim of the study is to evaluate the relationship of atherosclerosis markers with the presence and severity of diabetic retinopathy in patients with type 2 Diabetes Mellitus.

Materials and methods: This study is designed as a prospective clinical study which will be undertaken at the Univeristy Clinical Center of Kosovo. A total of 100 consecutive patients with type 2 Diabetes Mellitus without known cardiovascular disorders and without previous cardiovascular events with demographical, anthropometric, clinical and biochemical data will be included in the study. After the approval of the ethics board committee, patients that meet the inclusion criteria of the research will be informed about the study and their consent will be obtained. Atherosclerosis will be assessed using imaging biomarkers as Carotid intima media thickness (CIMT), Ankle brachial index (ABI), Coronary artery calcium score(CACs) by CT angiography and Central arterial stiffness. In addition to imaging biomarkers of atherosclerosis we will also evaluate inflammatory markers (including hs- CRP and IL-6). Diabetic retinopathy will be evaluated using digital retinal photography. According to retinopathy status, based on The International Clinical Disease Severity Scale, patients will be divided into three study groups: patients without DR (stage 1), patients with mild and moderate nonproliferative DR (stages 2 and 3) and patients with advanced DR (stages 4 and 5). For statistical analysis differences between groups will be analysed using the unpaired Student t test following the analysis of variance, whereas the one-way ANOVA with Bonferroni correction will be performed to compare continuous variables between three study groups. The Chi-square test will be used to compare the categorical variables. P values < 0.05 will be considered statistically significant. Pearson correlations will be performed to identify simple correlations between variables.

Expected scientific contribution: Our study will be added as the evidence that will indicate whether atherosclerosis is associated with diabetic microvascular complications and will show the best markers to evaluate this association. Diabetic Retinopathy is also often diagnosed in advanced stages of the disease so we believe that by evaluating the atherosclerosis markers that will best correlate with its presence and severity we will help in earlier diagnosis and treatment of the disease.

MeSH/Keywords: Atherosclerosis, Diabetes Mellitus type 2, Diabetic retinopathy, CIMT, ABI, CACs, central arterial stiffness, IL-6, hs-CRP

Poster code: T-02-18-051

Poster Title: Patterns of myopia development dynamics 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 occurs on a pandemic scale in the world and is one of the major public health and socioeconomic problem. It affects almost 30% of the world's population, and the prevalence is expected to increase to 50% with a 10% share of high myopia associated with blindness. In 2050, myopia is thought to be the leading cause of blindness. Myopia is defined as a refractive error in which parallel rays of light intersect in front of the retina in the eye with relaxed accommodation. Currently, there are no data on the incidence and prevalence of myopia as well as the sex and age distribution of myopia in children and adolescents in the Republic of Croatia. Despite numerous attempts, so far no method has proven effective for the prevention, treatment, and cure of patients. Globally, there is no information on successfully implemented strategies to slow myopia progression in children and adolescents. Recent research suggests that cycloplegia is the gold standard in determining refractive eye status in children and adolescents.

Hypothesis: The patterns of development dynamics of primary myopia in children and adolescents in Central and Southeast Europe can be determined in the multi-year follow-up period.

Aims: To determine the patterns of development dynamics of primary myopia in children and adolescents of Central and Southeast Europe in the multi-year follow-up period since diagnosis. To determine the stratification criteria for the analysis of the distribution of primarily low and primarily high myopia in children and adolescents of Central and Southeast Europe.

Materials and methods: The study included data of patients diagnosed and monitored under the diagnosis of primary myopia in pediatric ophthalmological clinics of the University Eye Department of the University Hospital Sveti Duh since the introduction of electronic medical records through the hospital information system Spp, during the period from 30.1.2007. till 31.12.2021. The analysis of myopia variables will be performed by complex methods of data analysis on several conceptual levels in combination with inductive and machine learning methods. Inductive systems will be performed multiple times and validated by randomly selected subsets of data in the ratio of 70:30, of which 70% are randomly selected respondents who will be a subset for performing classification rules of decision-making, the so-called learning subset, and 30% are randomly selected respondents who will represent a subset of data for the validation of derived classifiers, the so-called testing subset. The data will be validated multiple times based on the idea of a medically meaningful result, aiming for reproducibility and replicability. Statistical predictive modeling will analyze whether the observed parameters are predictors of the dynamics of myopia in children and adolescents and the role of possible expected complex, latent variables in this prediction, ie new complex properties derived from available data.

Expected scientific contribution: Developmental patterns and specific properties of myopia will be defined; age and sex distribution of children and adolescents with primary myopia; the share of patients with low and high primary myopia in the total number of patients with primary myopia. The results of a complex analysis of knowledge discovery in terms of the idea of precision medicine will be presented, as well as a methodological guideline for the approach to the analysis of complex systems recorded through electronic health records.

MeSH/Keywords: myopia, child development, adolescent development, retinoscopy

Poster code: T-02-18-085

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, Associate Professor Marijana Ćorić, MD PhD

Affiliation: University of Zagreb School of Medicine

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

Hypothesis: The BAP1, HDAC-2, and -4 expressions are associated with a higher metastases incidence and shorter UM patients' survival.

Aims: The study's objective is to determine the existence of primary UM tumor cells subpopulations which show the BAP1, HDAC-2, and -4 expression and the relationship between the expression of these markers with metastases, overall and disease-free survival.

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. 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.

Expected scientific contribution: In this study, the correlation between the expression of BAP1 and histone deacetylases of class I and II (HDAC -2 and -4) in UM samples will be examined for the first time. In addition, the study aims to examine the possible existence of a predictive BAP1 value for HDAC amplification or whether BAP1 expression may be a predictor of therapeutic response to HDACI. Furthermore, studying the relationship between the expression of HDAC-2 and -4 with clinical and pathological features of UM could contribute to the evaluation of the use of HDACI in patients with UM.

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

Poster code: T-02-18-109

Poster Title: Comparison of the efficacy of bevacizumab and brolucizumab in the treatment of neovascular senile macular degeneration

PhD candidate: Dalibor Opačić

Part of the thesis: Comparison of the efficacy of bevacizumab and brolucizumab in the treatment of neovascular senile macular degeneration

Mentor(s): Associate Professor Nenad Vukojević, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Age-related macular degeneration (AMD) is characterized by progressive degeneration of the macula, the central part of the retina, which gradually leads to central vision loss. AMD can be classified as early, intermediate, or late-stage based on clinical features, which may include yellow macular deposits (druses), pigmentation abnormalities, retinal pigment epithelial atrophy, and exudative choroidal neovascularization. In 2004, the era of anti-vascular endothelial growth factor (anti-VEGF) drugs began with the approval of pegaptanib in the United States. Intravitreal anti-VEGF drugs are today the first line of treatment for neovascular AMD. Anti-VEGF drugs approved for neovascular AMD include ranibizumab, aflibercept, and brolucizumab. Bevacizumab, which is not approved for this indication, is a good and generally accepted treatment option due to its low cost and comparable efficacy. Brolucizumab, the latest agent in clinical use, has been designed to graft complementary region determinants of a new anti-VEGF-A antibody onto a human single chain antibody fragment. Due to higher molar concentration and higher solubility, more brolucizumab molecules come in the usual volume of intravitreal injection than molecules of other anti-VEGF agents. A preclinical study showed that brolucizumab was 2.2 times more available to the retina and 1.7 times more to the retinal pigment epithelium than ranibizumab. There is no data in the literature comparing the efficacy of brolucizumab and bevacizumab in the treatment of newly diagnosed patients with neovascular AMD.

Hypothesis: New anti-VEGF drug brolucizumab is more effective in treatment of neovascular AMD compared to bevacizumab in the same treatment regimen.

Aims: Test the efficacy of the new anti-VEGF drug brolucizumab in the treatment of previously untreated patients with neovascular AMD compared to bevacizumab, also in previously untreated patients. Test the assumption that brolucizumab will show better efficacy in treatment in patients who have not shown a satisfactory response to bevacizumab after the previous three months of treatment.

Materials and methods: Prospectively, 3 groups of patients in whom neovascular AMD was detected will be monitored. The first group of patients will be treated with intravitreal administration of bevacizumab at a standard dose for up to 3 months. Optical coherence tomography (OCT), fluorescein angiography (FA) and logMAR charts will be evaluated before each drug administration. OCT will determine the degree of macular edema and its progression or regression, reflecting the efficacy of the drug. LogMAR charts will assess the corrected visual acuity, ie its improvement or deterioration. After 3 months, patients in whom no improvement in visual acuity has been achieved and / or in whom macular edema has progressed will be isolated. These patients will represent a new group to be further treated with brolucizumab, also controlled by OCT and logMAR charts to assess treatment success. The remaining patients in whom bevacizumab has achieved good results will be further treated with the same drug and monitored by OCT and visual acuity testing with logMAR charts before each administration. A third group of patients will initially be treated with brolucizumab, and monitored before each administration with OCT and corrected visual acuity testing. The expected follow-up time of all three groups of patients is one year.

Expected scientific contribution: The study will determine the effectiveness of treatment with the new anti-VEGF drug brolucizumab in previously untreated patients with neovascular AMD. The success of treatment will be compared with the results of treatment with bevacizumab in previously untreated patients. A comparison of the two groups will answer the question of whether and to what extent the success of treatment with brolucizumab is more significant, which has not been investigated so far. Patients who have not responded to previous bevacizumab treatment after three months of treatment will continue to be treated with brolucizumab.

MeSH/Keywords: brolucizumab, bevacizumab, neovascular AMD, anti-VEGF

Poster code: T-02-18-143

Poster Title: Ocular morphological and biometric characteristics in Alport spectrum disorders

PhD candidate: Mira Knežić

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 of Zagreb School of Medicine

Introduction: Alport syndrome is a genetically heterogeneous disease due to mutations in COL4A3, COL4A4 or COL4A5 genes coding for alpha3alpha4alpha5 type IV collagen, one of a major components of glomerular basement membrane, basement membrane of the organ of Corti and several ocular basement membranes (Bowman's and Descemet's membrane, lens capsule, internal limiting membrane and Bruch's membrane). The most common ocular abnormalities in Alport syndrome are dot-and-fleck retinopathy and anterior lenticonus.

Hypothesis: Different types of mutations in COL4A5, COL4A4 or COL4A3 genes manifest with different ocular morphological and biometric characteristics in Alport spectrum disorders.

Aims: General aim of the study is to investigate ocular morphological and biometric characteristics in Alport spectrum disorders. Specific aims are to compare ocular morphological and biometric characteristics between patients and controls and between subgroups of patients with identified mutation in the COL4A5, COL4A4 or COL4A3 gene depending on the type of mutation.

Materials and methods: A cross-sectional study will be conducted. Patient data from Croatian Science Foundation's project Genotype-phenotype correlation in Alport syndrome and thin basement membrane nephropathy database will be reviewed. Patients aged 18 years with identified mutation in COL4A3, COL4A4 or COL4A5 gene will be enrolled in the study, at least 15 patients (30 eyes) in each subgroup. At least 15 patients (30 eyes) age, sex, and objective refraction matched controls will be enrolled too. A comprehensive eye examination will be performed, including best-corrected visual acuity (ETDRS charts, logMAR), slit-lamp examination, Goldmann aplanation tonometry, and dilated fundus examination. Objective refraction will be assessed using autorefractor (Righton Speedy-K Autorefractor Keratometer, Right Group, Tokyo, Japan) after instillation of 1% tropicamide (Mydriacyl®, Alcon Laboratories Inc., Geneva, Switzerland) in both eyes three times at 15-minute intervals to achieve maximal mydriasis and cycloplegia. Qualitative and quantitative (endothelial cell density, mean cell area, coefficient of variation, and percentage of hexagonality) analysis of the corneal endothelium will be performed using specular microscopy (CEM-530, Nidek, Gamagori, Japan). Optical biometry (IOLMaster 700®, Zeiss, Oberkochen, Germany) will provide data on axial length, keratometry, white-to-white distance, anterior chamber depth, and lens thickness. Scheimpflug imaging (OCULUS Optikgeräte GmbH, Wetzlar, Germany) will provide data on corneal topography and pachymetry. Ultra-wide field Optomap® color and fundus autofluorescence imaging will be obtained (California, Optos, Marlborough, MA, USA). Macular thickness, subfoveal choroidal thickness, and retinal nerve fiber layer thickness will be measured by SPECTRALIS® HRA + OCT device (Heidelberg Engineering, Heidelberg, Germany). Optical coherence tomography angiography will be performed on the same device. Categorical variables will be expressed as absolute and relative frequencies. The distribution of a categorical variable will be tested using chi-sqaured, and, if necessary, Fisher's exact test. The Kolmogorov-Smirnov test will examine if numerical variables are normally distributed. Numerical variables will be expressed as mean with standard deviation in the case of distributions following the normal, and in other cases as median and interquartile range. The differences of the normally distributed numerical variables between independent groups will be tested by analysis of variance (ANOVA), and in other cases by Kruskal-Wallis ANOVA test. Significance level will be set to 5%.

Expected scientific contribution: The results of this study may have important diagnostic, prognostic, and therapeutic significance in multidisciplinary monitoring and treatment of these patients.

MeSH/Keywords: Alport syndrome, type IV collagen, dot-and-fleck retinopathy, anterior lenticonus

Poster code: T-02-18-166

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, Vesna Bišof, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: There has been a steady increase in the incidence of colorectal cancer (CRC) in the population under the age of 50. Compared to CRC in elderly population (over 50 years) there is a poorer treatment outcome. Overexpression of polo-like kinase 1 (PLK1) has been found in CRC and is associated with poorer survival.

Hypothesis: The expression of polo-like kinase 1 is higher in patients with metastatic colorectal cancer younger than 50 years at diagnosis than in patients aged 50 years and older and is associated with a poorer treatment outcome.

Aims: General objective is to examine the expression of PLK1 protein in patients with metastatic CRC of younger age (under the age of 50 at the time of diagnosis) compared to elderly (at diagnosis aged 50 years and over). Specific objectives are to determine the association of PLK1 expression with overall survival (OS) in patients with mCRC in both groups, to determine the association of PLK1 expression with time to the first progression of the disease (TTP) in both groups, to examine the existence of differences in PLK1 expression and clinical and pathohistological characteristics (KRAS / BRAF and MSI tumor status) of patients with mCRC younger and older age groups.

Materials and methods: This retrospective study will include patients diagnosed with metastatic colorectal cancer at the ibenik-Knin County General Hospital in the period from January 1st, 2010, to December 31st, 2018. Archival tissue samples taken from the primary tumor (formalin-fixed paraffin-embedded tumor tissue) will be used. For each patient the certain clinical data, morphological characteristics and molecular status of the tumor will be collected by searching the medical history. The expression of PLK1 protein will be determined by standard immunohistochemical (IHC) staining on tumor tissue samples using a specific antibody. IHC staining results will be interpreted based on the IHC score where nuclear and cytoplasmic positivity of tumor cells will be considered. For IHS score the percentage of reactive cells will determine with a scale of 0-4 (0 = no response, 1 = less than 25% of cells, 2 = 25 - 50% of cells, 3 = 51-75% of cells and 4 = more than 75%). Staining intensity will be determined with a scale of 0-3 (0 = no reaction, 1 = weak reaction, 2 = medium intensity reaction, 3 = strong intensity reaction). Patients will be divided into two groups; the first group will contain patients of younger age (at diagnosis younger than 50 years), and the second group will contain patients of older age (at diagnosis aged 50 or more). The sample size in the study will be a minimum of 140 patients (two groups of 70 patients). The sample size was calculated based on comparison of PLK1 between the younger patient group and the elderly group with a significance level = 0.05 and a test power of 1- = 0.8. 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.

Expected scientific contribution: 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: T-02-19-045

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

PhD candidate: Ivan Levaj

Part of the thesis: Biomechanical study of a quadruple gracilis tendon graft for the anterior cruciate ligament reconstruction

Mentor(s): Associate Professor Ivan Bojanić, 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 although ACL reconstruction is considered one of the most successful reconstructive procedures in orthopeadic surgery there is always a room for potential improvement. One of the most important prerequisite for successful ACL reconstruction is choosing a graft that restores the biomechanical properties of the native ACL and has a minimal donor site morbidity. Quadruple hamstring tendon autograft is the most common choice for ACL reconstruction and it is usually constructed of semitendinosus and gracilis tendons, both folded in double fashion. Advancements in technology and development of new operative techniques enable surgeons to use shorter grafts for ACL reconstruction thus utilising only semitendinosus tendon for constructing a quadruple hamstring graft. However, semitendinosus muscle has a protective and stabilizing role of the knee joint acting as an ACL agonist and is very important knee flexor and internal rotator of the leg. Therefore, the search for an ideal autograft, the removal of which will not lead to a significant disruption of biomechanics and knee function, is still ongoing. One such potential graft that could be sufficient in length and strength to reconstruct the ACL is a construct made of a quadruple gracilis tendon.

Hypothesis: A quadruple gracilis tendon graft has comparable biomechanical properties with standard quadruple hamstring grafts and is therefore suitable for anterior cruciate ligament reconstruction.

Aims: To compare the biomechanical properties of a quadruple gracilis tendon with the biomechanical properties of a quadruple semitendinosus tendon and a quadruple hamstring graft made of double-folded semitendinosus and gracilis tendons.

Materials and methods: Removal of gracilis and semitendinosus tendons from total of thirty cadavers (sixty knees) was performed at the Department of Forensic Medicine and Criminology, Faculty of Medicine, University of Zagreb and health institutions where organ harvesting was performed within the organ donation program of the Croatian Ministry of Health. Available tendons will be divided into two separate groups with fifteen matched pairs in each group. In the first group we will compare quadruple gracilis tendon graft with quadruple hamstring graft composed of double-folded semitendinosus and gracilis tendons. In the second group we will compare quadruple gracilis tendon graft with graft made of quadruple semitendinosus tendon. Dimensions of each tendon and appropriate constructed graft will be measured and compared. Biomechanical testing of grafts will be performed on a device for testing static mechanical properties of materials (Shimadzus AGS X with maximum force of 10kN) and each grafts will be subjected to standard preconditioning protocol followed by a load-to-failure test. Primary measurements will include maximum load and elongation at failure and in regard to cross sectional area relevant biomechanical properties of each graft will be calculated. Statistical analysis of the obtained data will be performed and tensile properties between grafts within matched groups will be compared.

Expected scientific contribution: If the research confirms the hypothesis that the quadruple gracilis tendon graft is sufficient for the reconstruction of the anterior cruciate ligament of the knee, the need for harvesting semitendinosus tendon for this procedure will be eliminated. This will preserve a structure that has a very important function in maintaining the biomechanical relationships of the knee and therefore will potentially lead to better clinical outcome, shorter rehabilitation period and lower risk of postoperative complications.

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

Poster code: T-02-20-046

Poster Title: Value of tourniquet use in elbow arthroscopy

PhD candidate: Igor Knežević

Part of the thesis: Value of tourniquet use in elbow arthroscopy

Mentor(s): Associate 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.

Hypothesis: The volume of postoperative bleeding after an elbow arthroscopy procedure without the use of a tourniquet will be less than the volume of postoperative bleeding after an elbow arthroscopy procedure when a tourniquet was applied.

Aims: We aim to investigate what is the impact of tourniquet use on the volume of postoperative bleeding following elbow arthroscopies. We will also explore the difference in intraoperative visualisation, the volume of postoperative bleeding, the intensity of postoperative pain, outcome results as well as the rate of complications between the groups of patients who underwent elbow arthroscopy with or without the use of a tourniquet.

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 group would consist of patients who underwent elbow arthroscopy without the use of a tourniquet, while the second 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. Duration of the procedure, tourniquet time, mean blood pressure during the procedure as well as the volume of irrigation solution used, would be noted. Shortly after the procedure, a surgeon would grade the intraoperative visualisation on a numeric-analogue scale and following the instruction published by Johnson et al. 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.

Expected scientific contribution: Our research could establish that an elbow arthroscopy can be safely and efficiently performed without the use of a tourniquet. Therefore, there might be a possibility of shifting trends in elbow arthroscopy technique in favour of abandoning tourniquet use similar to changes made in technique of knee and ankle arthroscopy. It could also allow more complex and prolonged procedures to be performed.

MeSH/Keywords: arthroscopy, elbow, tourniquet, bleeding

Poster code: T-02-20-126

Poster Title: Expression and prognostic value of PRAME antigen 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. Previous studies on PRAME in breast carcinoma were mainly based on RT-PCR detection, with immunohistochemical studies limited to polyclonal antibody results.

Hypothesis: Expression of PRAME antigen is higher in triple negative and HER2 positive breast carcinoma in contrast to other surrogate subtypes of breast carcinoma and it is correlated with other prognostic and predictive factors of breast carcinoma.

Aims: The overall aim of this study is to evaluate PRAME antigen expression in different molecular subtypes of breast cancer and its correlation with other prognostic and predictive factors. Specific aims are 1. To determine the expression of PRAME antigen in invasive breast cancer; 2. To determine the value of PRAME expression in different molecular subtypes of breast cancer; 3. To determine the correlation of PRAME antigen with histomorphological characteristics of invasive breast cancer and other prognostic factors as well as survival rates (Overall Survival (OS), Disease Free Survival (DFS), Progression Free Survival (PFS), Event Free Survival (EFS)); 4. To assess the prognostic significance of PRAME expression in early breast cancer and in metastatic breast cancer; 5. To explore the potential role of PRAME expression in breast cancer before the neaoadjuvant treatment in the prediction of response to neoadjuvant therapy (RCB category).

Materials and methods: This retrospective study will include 180 patients who were diagnosed with invasive breast cancer (NST histological subtype) on core needle biopsy at Clinical Department of Pathology and Cytology ,University Hospital Centre Zagreb and have available archived tissue samples. Based on the immunophenotype of invasive breast cancer patients will be divided into five groups (Luminal A like, Luminal B like, Luminal B HER2 positive, HER2 positive and triple negative breast carcinoma), with 36 patients in each group. All data relevant for the research will be obtained from informatic system at University Hospital Centre Zagreb (age of the patient, pathohistological report, oncological treatment, DFS, EFS, PFS, OS). Expression of PRAME antigen will be 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 will be performed in consensus by two pathologists. Expression of PRAME will be 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 will be used as positive control for PRAME reaction (nuclear PRAME expression).

Expected scientific contribution: This study will assess the potential prognostic and predictive significance of PRAME antigen expression in breast cancer as well as its role in everyday clinical practice.

MeSH/Keywords: breast cancer; PRAME antigen; immunohistochemistry

Poster code: T-02-23-012

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

PhD candidate: Martina Held

Part of the thesis: Analysis of HMGB-1, RAGE, protocadherin and Gd-IgA1 in patients with IgA vasculitis

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 with still unknown etiopathogenesis and without valid biomarker in routine clinical use that would indicate the possibility of developing the most important complication - IgA vasculitis nephritis (IgAVN). For this purpose, the following potential biomarkers will be examined: galactose-deficient immunoglobulin A1 (Gd-IgA1), high mobility group box 1 (HMGB1), receptor for advanced glycation end products (RAGE) and protocadherin 1 (PCDH1).

Hypothesis: Elevated levels of Gd-IgA1, HMGB1 and PCDH1 and decreased levels of RAGE in serum and urine of patients with IgAV compared to the control group, indicate active disease and are a predictor of IgAVN.

Aims: Primary aim is to determine the levels of Gd-IgA1, HMGB1, RAGE and PCDH1 in the serum and urine of children with IgAV and to compare with the control group. Secondary aims are to determine the level of Gd-IgA1, HMGB1, RAGE and PCDH1 in the serum and urine of children with IgAV at the beginning of the disease and after 6 months from diagnosis and to compare the levels of these biomarkers in relation to the group of children with IgAVN and also to determine the degree of correlation between the levels of Gd-IgA1, HMGB1, RAGE and PCDH1 and the severity of the clinical picture, selected laboratory parameters and disease activity.

Materials and methods: This prospective study include patients diagnosed with IgAV according to EULAR/PRINTO/PRES criteria from 3 tertiary pediatric rheumatology centers of the Republic of Croatia (University Hospital Center Zagreb, Children's Hospital Zagreb and University Hospital Center Split) in the period from January 2020 to October 2023. The control group consists of children who by age and sex do not differ from children with IgAV, have no clinical or laboratory signs of inflammatory disease, and in whom due to other (non-inflammatory) diseases venous blood and urine will be taken as part of routine diagnostic process. Demographic, clinical and laboratory data on subjects as well as data of medicine administration will be recorded. Samples of venous blood, urine and stool will be collected at the disease diagnosis and at the six months follow up visits. In addition to laboratory tests in venous blood and urine samples of all subjects at the beginning of the disease or at diagnosis, the level of Gd-IgA1, HMGB1, RAGE and PCDH1 will be determined, and the same determination will be repeated only in patients with IgAV after 6 months. Levels of Gd-IgA1, HMGB1, RAGE and PCDH1 in serum and urine samples will be measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions, and will be determined at the Clinical Institute for Laboratory Diagnostics in University Hospital Center Zagreb. The minimal sample size for determining the differences between groups is estimated on 140 subjects (70 IgAV and 70 control subjects).

Expected scientific contribution: Determination of serum and urine biomarkers (Gd-IgA1, HMGB1, RAGE and PCDH1) in children with IgAV will contribute to better monitoring of disease activity and early assessment of the risk of renal impairment.

Acknowledgments: Croatian Science Foundation project IP-2019-04-8822.

MeSH/Keywords: IgA vasculitis; IgA vasculitis nephritis; biomarker

Poster code: T-02-24-093

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

Mentor(s): Zrinjka Mišak, PhD

Affiliation: Children's Hospital Zagreb; 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 celiac disease.

Hypothesis: The composition of the bacterial intestinal microbiota is not associated with the development of active disease in children with celiac disease.

Aims: We will 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 will include children diagnosed with active celiac disease, their healthy siblings and healthy peers. Patients will be recruited in Childrens Hospital Zagreb. Celiac disease will be diagnosed according to current ESPGHAN guidelines in children younger than 18 years of age. Our sample size will consist of 20 children per group, in accordance with the latest studies and guidelines of determining sample size in intestinal microbiota research. The fecal samples will be taken before the diagnosis, and after the induction of remission with gluten-free diet. Control groups will also provide fecal samples. All the fecal samples will be frozen at the temperature of -80°C. The analysis of the intestinal microbiota will be performed by fecal DNA extraction from a 150 mg stool sample and by PCR amplification of 16S ribosomal RNA. Afterward, we will analyse the polymorphisms of the length of the terminal restriction fragments. Terminal restriction fragments will be joined by OTU (operational taxonomic units), according to Local Southern Method GeneMapper 3.7 software (Thermo Fisher Scientific, USA). Assignment of OTU to bacterial taxonomy will be done according to RDP (Ribosomal Database Project) 16S rRNA database. We will use the Shannon Wiener diversity index to compare the diversity of the OTUs obtained between the study groups. Cluster analysis will be performed using BioNumerics software (Applied Maths Belgium), 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 (UPGMA). The difference in the distribution of taxonomic units between patients and the healthy controls will be analyzed by PERMANOVA (permutational multivariate analysis of variance) analysis.

Expected scientific contribution: 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: T-02-24-122

Poster Title: Professional and ethical positions of medical doctors and nurses regarding end of life treatment of critically ill children treated in PICU

PhD candidate: Filip Rubić

Part of the thesis: Professional and ethical positions of medical doctors and nurses regarding end of life treatment of critically ill children treated in PICU

Mentor(s): Professor Ana Borovečki, MD PhD, Professor Jurica Vuković, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Some patients that are treated in paediatric intensive care units are at the end of their lives. Decisions regarding their treatment are related to questions of introducing new therapeutic and diagnostic procedures as well as withholding and/or withdrawing those procedures. In these situations physician often rely on their interpretation of good medical practice thus ethical issues regarding those decisions arise. To date similar researches regarding pediatric population in Croatia have not conducted.

Hypothesis: The hypothesis of this research is that professional and ethical attitudes of health care providers (nurses, nurses with higher education, specialist doctors) working in paediatric intensive care units in tertiary care centres in the Republic of Croatia are related to their age, gender, years of service and level of education.

Aims: The aim is to determine and analyze the professional and ethical attitudes of health care professionals employed in pediatric intensive care units in the Republic of Croatia on the treatment of patients at the end of life. Additionally we will examine the attitudes of health care professionals (doctors, nurses) in institutions at the tertiary level of health care in the Republic of Croatia on the treatment of patients at the end of life by using a questionnaire. With that instrument we will interpret the differences in attitudes in relation to age, gender, level of education, occupation, subspecialty and years of service. We will compare the obtained results with relevant data from surveys conducted in other countries and determine which sociodemographic and clinical variables predict certain attitudes about the treatment of patients at the end of life.

Materials and methods: Our research will be conducted in several phases. The first phase includes searching the literature and writing a review article related to the research conducted so far in this area. In the second phase, research is conducted in focus groups. One group consists of specialist doctors employed in pediatric intensive care units (appropriate sample), and the other group of nurses employed in pediatric intensive care units (appropriate sample). The research is conducted in institutions at the tertiary level of health care in the Republic of Croatia as follows (KBC Zagreb, KBC Split, KBC Rijeka). Data will be analyzed by methods of qualitative data analysis. The third part of the research refers to the retrospective survey that examines attitudes and opinions of health care professionals using standardized and validated instrument - a double-translated questionnaire "Ethical attitudes in deciding on limiting therapeutic life support procedures in patients at the end of life in intensive care units" constructed by Grosek et al. which was adapted for the second phase of the research. In this phase, a pilot study includes health professionals doctors, senior nurses and nurses employed in pediatric intensive care units. Data will be analyzed and compared to similar researches in other countries.

Expected scientific contribution: Experiences and attitudes of health professionals in the Republic of Croatia (model for transition countries) on end-of-life decisions in paediatric intensive care units will be systematically examined. A research model that would contribute to the evaluation of end-of-life decisions in intensive care units in transition countries will be tested and validated. The research could contribute to the improvement of the quality of health care and the development of guidelines that would help health professionals in their daily work.

MeSH/Keywords: Paediatric critical care, paediatric intensive care, decision making, withdrawing treatment, withholding treatment, end-of-life, palliative care

Poster code: T-02-24-173

Poster Title: Craniometric analysis of sexual dimorphism of the modern Croatian population using MSCT images

PhD candidate: Elvira Krešić

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

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

Affiliation: University of Zagreb School of Medicine

Introduction: The basis of medico-legal research on human remains is identification, the first step of which is gender assessment. Although the cranial sexual dimorphism, in terms of the existence of a physical difference of the skull between the sexes, is known, it has never been established for the modern croatian population. In the past, skeletal collections were the only source of forensic anthropological measurements, but today, with virtual anthropology, we can bypass the shortcomings of skeletal collections.

Hypothesis: Measurements of the skull of the modern Croatian population will show sexual dimorphism.

Aims: GENERAL: Development of population-specific craniometric standards for sex assessment of the modern Croatian population using MSCT images and 3D models. SPECIFIC: 1. To determine whether the sexual dimorphism of individual measures is statistically significant 2. Determine whether total dimorphism is statistically significant 3. Creating a virtual database of MSCT images and 3D models

Materials and methods: In this retrospective research, a total of 400 archival MSCT head examinations (200 adult men and 200 adult women) recorded at KBC Split and KBC Zagreb will be used.

Expected scientific contribution: The general goal is to develop population-specific craniometric standards for sex assessment of the modern Croatian population using MSCT images and 3D models. The specific objectives are to determine the dimorphism of individual measures, to determine the total dimorphism.

MeSH/Keywords: Sexual dimorphism, virtual anthropology

Poster code: T-02-25-015

Poster Title: Ultra-low-dose chest and abdominal computed tomography in the follow up of the patients with lung cancer during the therapy

PhD candidate: Ivana Kuhtić

Part of the thesis: Sensitivity and specificity of ultra-low-dose chest and 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: Due to longer survival because of new modalities of treatment in patients with lung cancer and the need for more frequent follow-up CT scans, it is important to determine the best protocol that will be effective in providing relevant data for further therapy and reducing ionizing radiation as negative effects to a minimum. Today, the possibilities of newer modalities of sophisticated therapy such as immune and targeted therapy are opening up, which surely allow longer survival for lung cancer patients. In this method of treatment, more frequent monitoring of the therapeutic effect is needed, due to their side effects and the possibility of changing the available lines of therapy in time. Computerized tomography (CT) of the thorax and abdomen with the application of a contrast agent in the vein phase is the gold standard and a common diagnostic method that raises the suspicion of lung cancer and determines the preliminary TNM stage of the disease according to ESMO (European Society For Medical Oncology) guidelines. It is also the gold standard for monitoring responses and diagnosing side effects of the therapy. CT as a diagnostic method has advantages such as precision, painlessness and speed, but it is also necessary to take into account the negative sides, which are the use of contrast agents and the exposure of patients to ionizing radiation that has its long-term consequences.

Hypothesis: The sensitivity and specificity of CT examination performed according to the ultra-low-dose radiation protocol is not significantly reduced compared to the full dose radiation CT protocol which is the current gold standard in the detection of response to oncological therapy of primary lung tumor and the detection of distant metastases and their response to therapy.

Aims: The general objectives are to demonstrate that the specificity and sensitivity of an ultra-low-dose CT scan are not significantly reduced compared to a full-dose CT scan and can be used for routine monitoring of treatment effects in patients with lung cancer and to reduce the cumulative radiation dose received in patients during follow up. The specific objectives are to demonstrate that the sensitivity and specificity of ultra-low-dose CT examination are sufficient to monitor the response to oncological therapy of the primary lung tumor, to detect metastases and to monitor their response and also detect the side effects of therapy.

Materials and methods: This prospective, single-center study will include at least 120 patients diagnosed with any type of lung cancer. After informed consent, a regular, follow up CT examination will be performed, followed by an ultra-low-dose CT scan. For the purposes of the study, each patient will receive a control number that will be added to each of the examinations performed (regular, standard full-dose CT scan and associated ultra-low-dose CT protocol). First, all ultra-low-dose CT scans will be checked, which will subsequently be compared with the corresponding standard full-dose CT scan under the same control number (current gold standard). CT scans performed according to the standard protocol and the ultra-low-dose protocol will be independently analyzed by two radiologists and the reading results for different imaging protocols will be compared, as well as the degree of agreement between radiologists using adequate statistical methods.

Expected scientific contribution: If the sensitivity and specificity of the CT examination according to the ultra-low-dose protocol are not significantly reduced in the monitoring of lung cancer patients, this would reduce the cumulative dose of radiation, including the possibility of developing long-term consequences in patients who now have a prolonged time of active monitoring of the disease, due to more adequate therapy and extended life expectancy.

MeSH/Keywords: Lung cancer, control, monitoring of therapy, ultra-low-dose CT protocol

Poster code: T-02-25-019

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

PhD candidate: Nikolina Jurjević

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

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

Affiliation: University of Zagreb School of Medicine

Introduction: Since its introduction, CT colonography has rapidly evolved by virtue of imaging industry innovations. Data from the literature show that CT colonography has high sensitivity and specificity for detection of colorectal cancer and large polyps in symptomatic and asymptpomatic individuals. During last decade, radiation dose administered during CT colonography has been a topic of debate. Therefore, more studies are evaluating the feasibility of preserving image quality and polyp detection using new low dose protocols.

Hypothesis: Additional dose reduction, using newly adapted low dose and ultra low dose computed tomography colonography protocols will not diminish image quality and diagnostic confidence for 3D interpretation.

Aims: To assess diagnostic performance of newly adapted low dose (LD) and ultra low dose (ULD) computed tomography colonography (CTC) protocols, with dose reduction, in colon polyp detection and possibility of application in practice.

Materials and methods: We will conduct a study on Rando-Alderson phantom, to adapt new LD and ULD protocols. Radiation dose will be measured on phantom using thermoluminescent (TL) dosimeter. Then, in clinical research, 100 patients will undergo obtained LD and ULD protocols. Obtained protocols will be compared in terms of radiation dose and effective dose, image noise, image quality and polyp detection. Image quality assessment will be obtained with double-blind interpretation by three radiologists.

Expected scientific contribution: With confirmation of significant radiation dose reduction while maintaining image quality using low dose and ultra low dose CT colonography protocols, CT colonography can become first choice for early detection of colorectal cancer.

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

Poster code: T-02-25-136

Poster Title: Expression of TIGIT, LAG-3 and PD-L1 immune ligands 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 surgical and oncological treatment. Considering MIBC as an immunogenic tumor, new therapeutic option has been recently developed - immunotherapy that targets signaling points in the tumor microenvironment and revitalizes antitumor immunity, which led to changes in MIBC treatment paradigm.

Hypothesis: The expression of TIGIT, LAG-3 and PD-L1 immune ligands is higher in the group of molecular luminal subtypes of muscle-invasive bladder cancer, compared to the molecular group of the basal subtype.

Aims: In this study, we plan to investigate the PD-L1 checkpoint expression as well as the new potential biomarkers TIGIT and LAG-3 in the molecular subtypes of MIBC.

Materials and methods: A cross-sectional prospective study is planned. The material will be obtained from the archives of the Clinical Institute of Pathology and Cytology Ljudevit Jurak in the Clinical Hospital Center Sestre milosrdnice by random selection of 60 patients who underwent transurethral resection of the bladder tumor from 01.01.2016. to 31.12.2021. in the Clinic of Urology of the same institution. Clinical and pathological data, patient characteristics, treatment outcomes and survival will be monitored during one year. Standardized morphological analysis of resection tissue will be performed to confirm the histological type of the tumor, muscle invasion and molecular subtypes. Following immunohistochemical analysis will be performed in 30 samples of luminal and 30 of basal MIBC group. Anti-TIGIT (ABCAM BLR047F), LAG-3 Sigma-Aldrich HPA013967 and PD-L1 Ventana (SP142) clones will be used for immunohistochemical analysis of the investigated ligands. Immunohistochemical response to all antibodies will be determined semiquantitatively, determining the percentage of positive epithelial tumor cells and inflammatory cells in tumor and peritumoral stroma. Statistical analysis will be performed and appropriate parametric and non-parametric tests will be applied according to the obtained data. Differences in the expression of TIGIT, LAG-3, and PD-L1 ligands between subgroups of bladder cancer will be analyzed.

Expected scientific contribution: The results of this study could contribute to a better understanding of the MIBC immune context and the possible isolation of a patient subset who could benefit from immunotherapy.

MeSH/Keywords: Muscle-invasive bladder cancer, Tumor microenvironment, Immunohistochemistry, Biomarkers, TIGIT, LAG-3, PD-L1.

Poster code: T-02-28-030

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.

Hypothesis: Subjects with the first episode of schizophrenia differ from subjects with an early course of schizophrenia in cognitive deficits and reduction of fronto-temporal cerebral blood flow.

Aims: To examine and compare regional cerebral blood flow, cognitive functions, and psychopathology in subjects with the first episode of schizophrenia and with the early course of schizophrenia. To examine the interrelationship of changes in cognitive functions, regional cerebral blood flow, and psychopathology in both groups of subjects.

Materials and methods: This cross-sectional study will include 100 subjects of both sexes aged 19 to 30 who are being treated at the Psychiatric Clinic of University Hospital Centre Sestre Milosrdnice. The estimated duration of the research is at least one year. Subjects will be 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 will be drug naive. Subjects with an early course of schizophrenia will be drug free for at least three months prior to actual treatment. Socio-demographic and anamnestic data will be examined by a structured questionnaire. The severity of the clinical picture will be assessed using the Schizophrenia Positive and Negative Symptoms Scale (PANSS), cognitive functions will be measured using a 5-KOG cognition screening test, and rCBF will be scanned using SPECT with Tc-99m-HMPAO. The same regions of interest in both cerebral hemispheres will be determined for each subject, and the deviation of activity of standard SPECT rates for certain age will be calculated for each region. Statistical analysis will be performed using SPSS software. Sociodemographic, clinical characteristics, cognitive functions and rCBF will be presented with descriptive statistics. The Kolmogorov-Smirnov test will be used to estimate the normality of the distribution of continuous values. Differences in quantitative values will be assessed by the t-test or the Mann-Whitney U test. Differences in categorical variables will be analyzed by X2 test or Fisher's exact test. The correlation coefficients between changes in regional cerebral blood flow and cognitive functions will be calculated, and regression models will assess the multivariate effect of individual variables on cognition.

Expected scientific contribution: Insight into the changes and association of cognitive functions and brain activity in early schizophrenia could contribute to the identification of the pathophysiological status of the patient. The proposed research implies a wider application of cognitive tests for the purpose of early detecting cognitive dysfunction as well as the implementation of SPECT in routine clinical practice. These diagnostic tools could diagnose abnormal patterns of brain activity in the regions responsible for cognitive functions in a timely manner, assess the response to treatment, and enable the development of personalized cognitive therapeutic interventions.

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

Poster code: T-02-29-117

Poster Title: Art therapy as a treatment method for depression in adults: a randomized controlled trial

PhD candidate: Ivan Barun

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

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

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

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. One of the possible treatments for depressive disorders is art therapy.

Hypothesis: Art therapy in the ambulatory care in adult population suffering from depressive disorders brings to greater reduction of the symptoms of depression and anxiety compared to the ambulatory care alone.

Aims: To asses if art therapy in addition to ambulatory care brings to greater reduction of symptoms of depression and anxiety in adult population suffering from depressive disorders than ambulatory care alone.

Materials and methods: In a randomized controlled trial experimental group of 26 adults with depressive disorders will be assigned to 12 weekly art therapy sessions in addition to ambulatory care, and control group will include 26 adults with depressive disorders in ambulatory care alone. Intervention in form of group art therapy will be carried out once per week during twelve week period with each session lasting for 120 minutes. The sessions will be carried out by the same art therapist. Art therapy intervention carried out will be psychodinamically oriented. In the control group only ambulatory care will be carried out, involving only psychopharmacotherapy and supportive conversation. Participants in the intervention group will not take part in any other specific forms of therapy during the trial. Participants in both experimental and control group will be subjected to the same battery of standardized test in the beginning of the study, at the end of the period of twelve weeks when the intervention will be conducted, and after twelve weeks after the end of the last intervention. Depression will be evaluated with Beck Depression Inventory (BDI-II) and Hamilton Rating Scale for Depression (HRSD), while anxiety will be evaluated with Beck Anxiety Inventory (BAI) and Hamilton Rating Scale for Anxiety (HRSA).

Expected scientific contribution: According to literature review, the effect of art therapy on reduction of symptoms of depression and anxiety in adult population suffering from depressive disorder has not yet been show. Considering the heterogeneity of art therapy interventions, further studies of high levels of evidence are needed for validation of interventions. Furthermore, according to the results of the study, a specific art therapy programme will be designed with the goal of improving mental health of this population.

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

Poster code: T-02-29-146

Poster Title: Exploring psychological personality traits in female patients with obesity and anorexia nervosa

PhD candidate: Filip Mustač

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

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

Affiliation: Department of Psychiatry and Psychological Medicine, University Hospital Centre Zagreb (PhD student: University of Zagreb School of Medicine)

Introduction: Patients with obesity and anorexia nervosa are an important and unavoidable problem in today's medicine practice. However, the importance of pharmacological therapy is given to complications of the disease, and the importance of psychological factors in these diseases is often neglected.

Hypothesis: Female patients with obesity have a more pronounced dimension of temperament novelty seeking, emotional abuse in childhood and using the defense mechanism of displacement than those with anorexia nervosa, as well as a less pronounced dimension of harm avoidance, vulnerable narcissism and internal shame.

Aims: The aim of this study is to compare similarities and differences in psychological personality indicators in female patients with obesity and anorexia nervosa.

Materials and methods: This is a cross-sectional study involving females ages 18-50 divided into two clinical groups: obese patients and patients with anorexia nervosa. Data collection will be done through the custom created general data questionnaire and psychological instruments assessing the temperament dimensions in terms of novelty seeking and harm avoidance, usage of displacement as a defense mechanism, vulnerable narcissism, internal shame, uncertain reflective functioning, and childhood emotional abuse.

Expected scientific contribution: The results of the study are expected to contribute to a deeper psychological characterization of these disorders and point out the importance of recognizing psychodynamic phenomena present.

MeSH/Keywords: obesity, anorexia nervosa, personality traits, shame, narcissism

Poster code: T-02-29-156

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

PhD candidate: Lucija Čondić Jurjević

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

Mentor(s): Professor Srđana Telarović, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Parkinson's disease (PD) is the second most common neurodegenerative disease (after Alzheimer's disease). Common symptoms include movement disorders, speech disorders and the development of cognitive disorders. In addition to medical treatment, art therapy plays an important role in the treatment of PD.

Hypothesis: Art therapy plays an important role in slowing down the progression of PD and improving the quality of life of patients.

Aims: GENERAL OBJECTIVE: To assess the positive effect of different forms of art therapy on treatment outcomes and quality of life of patients with PD (through observation and listening of art and also through personal artistic-creative expression). SPECIFIC OBJECTIVES: 1. Analyze the improvement of motor skills and mobility of patients 2. Investigate the impact on improving cognitive abilities, raising self-confidence, connecting with the community and improving the overall quality of life 3. Investigate the role of uric acid in neurodegeneration and neuroprotection of PD and the relationship with the effects of art therapy

Materials and methods: The study will analyze the effect of two forms of art therapy in patients with PD (art-graphic expression and music therapy). Two groups of 30 patients with PD will participate in the study. Inclusion criteria are: patients who have met definite inclusion criteria according to relevant PD diagnostic guidelines, Hoehn and Yahr disease stage 2-3, MMSE test result 23, age 80 years, and exclusion criteria are: severe chronic diseases that would prevent the arrival and participation of subjects in the study (cardiovascular diseases, diseases of the locomotor system, etc.), stroke and other neurological diseases, previous participation in art therapy workshops, color blindness, hearing disorders, hyperuricemia , age 80 years. One group will, in addition to drug therapy, undergo cycles of art therapy (art-graphic expression and music therapy) during three years, and the control group will remain exclusively on drug therapy. Respondents, together with their family members and carers, will be informed about the entire survey, in accordance with all ethical principles, and they will sign an informed consent. All patients will have their uric acid levels determined at the beginning, during and at the end of the study. All participants will be continuously evaluated by completing validated questionnaires and scales (Mini-Mental State Examination (MMSE), Beck Depression Inventory Test II (BDI-II), Measuring Health-Related Quality Of Life (HRQOL), Unified Parkinson's Disease Rating Scale-part III-Motor Scale(UPDRS-III), Pittsburg Sleep Quality Index (PSQI), Rosenberg Self-Esteem Scale (RSES), SF-36 Questionnaire i The Parkinson's Disease Questionnaire (PDQ-39). Descriptive indicators (mean values and dispersion measures) will be calculated for all dependent variables (scale results and uric acid level). The normalities of the distributions will be tested by the Kolmogorov-Smirnov test, and the coefficients of symmetry and flatness of the distribution will be calculated. To determine the effects of treatment, analysis of variance for repeated measurements will be applied, and conclusions will be made at the usual level of significance of 5% ($p < 0.05$).

Expected scientific contribution: Examining the effect of art therapy on treatment outcomes and quality of life of patients with PD and, for the first time, the development of a new algorithm for the application of art therapy in these patients and its implementation in the health care system.

MeSH/Keywords: Art therapy, Parkinson's disease, Quality of life

Poster code: T-02-30-003

Poster Title: Sonoelastography in the assessment of skeletal muscle rigidity

PhD candidate: Vanja Vojnović

Part of the thesis: Sonoelastography in the assessment of skeletal muscle rigidity

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

Affiliation: University of Zagreb School of Medicine; Department of Neurology, Dubrava University Hospital, Zagreb, Croatia; Department of Diagnostic and Interventional Radiology, Dubrava University Hospital, Zagreb, Croatia

Introduction: To this day, there is no specific diagnostic or laboratory test finding that can diagnose definitive Parkinson's disease (PD) during patients lifetime. This diagnosis is made primarily on the basis of clinical findings (British Brain Bank Diagnostic Criteria). The only definitive diagnosis of PD is postmortem finding of Lewy bodies in the basal ganglia. Therefore, nowadays a lot is expected from PD biomarkers; we hope they could diagnose PD during lifetime, even as early as in preclinical or premotor stage. Recently, sonoelastography has also been mentioned as a possible PD biomarker. Our aim is to investigate if shear-wave elastography (SWE), as an objective non-invasive diagnostic tool, can be reliable in assessing and quantifying muscle rigidity in patients with PD and parkinsonism.

Hypothesis: Muscle rigidity will affect the change in muscle elastographic values.

Aims: Our aim is to show that SWE can quantify the skeletal muscle rigidity in patients with PD and parkinsonism, assess the progression of the disease, and thus be useful in planing an individualized approach to medical treatment of each PD patient.

Materials and methods: The study group will include 50 adult patients diagnosed with PD or parkinsonism. The inclusion criteria will be age 18 years old, and the diagnosis of PD or parkinsonism according to UK Brain Bank Diagnostic Criteria. Exclusion criteria will be other neurological diseases or conditions that may cause muscle rigidity (e.g. motor neurone disease, muscular dystrophy or neuropathy). The control group will include 50 adults (age 18 years old), of the same age and sex distribution as in the study group, without any disease or medical history that could affect their muscle tone. Prior to the ultrasound examination, motor Unified Parkinson's Disease Rating Scale score (UPDRS-III) will be determined for each subject, by a subspecialist in neurodegenerative diseases. The ultrasound examination will be performed by a radiologist with experience in musculoskeletal ultrasound, on an ultrasound machine Aixplorer Mach 30 (SuperSonic Imagine, France), in SWE mode, with a high-frequency L18-5 probe. We will compare the elastographic values of m.biceps brachii in patients with PD and parkinsonism to those in healthy subjects. Within the study group (group with PD and parkinsonism), we will compare elastographic values of symptomatically dominant hand (hand particularly affected by rigor) and symptomatically non-dominant hand (hand less affected by rigor). In the affected group, we will also conduct research on patients without clinically manifested muscle rigidity (according to the UPDRS-III scale) and see if there are any deviations in their elastographic values as well. The radiologist will not know the UPDRS-III score of the subjects. The correlation between the UPDRS-III scores and the obtained elastographic values will be expressed by the Spearman rank correlation coefficient. In the statistical analysis of the results, Bartlett's test of Homogeneity of Variances and the Shapiro-Wilk test of normal distribution will be performed first. In the case of normal distribution and homogeneity of variance, the t - test will be used to compare these groups. In case of deviation from the above, and despite the transformations, the Mann-Whitney U Test or the Kruskal Wallis Test will be used. The significance level will be determined at $p < 0.05$.

Expected scientific contribution: If our hypothesis is confirmed, we will show that sonoelastography is useful for objective assessment of muscle rigidity in patients with PD and parkinsonism, and therefore useful for planning an individual approach to treatment of each patient.

MeSH/Keywords: Parkinson's disease, parkinsonism, muscle rigidity, sonoelastography, individual treatment approach

Poster code: T-02-30-004

Public health and healthcare – research proposals

Poster Title: Diabetes mellitus and COVID19

PhD candidate: Jelena Dimnjaković

Part of the thesis: Blood Glucose Lowering Drugs and COVID19 Outcomes A Retrospective Cohort Study

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

Affiliation: University of Zagreb School of Medicine

Introduction: Blood glucose lowering drugs are known to have anti-inflammatory effects which may affect outcomes in diabetes patients with COVID-19 infection.

Hypothesis: In diabetes mellitus patients, blood glucose lowering drugs have a positive effect on COVID-19 outcomes.

Aims: Primary aim of the study is to ascertain effects of blood glucose lowering drugs on COVID-19 outcomes (positivity, hospitalization and death rates) between groups in diabetes mellitus patients in Croatia. Specific goals are to determine the incidence of COVID-19 positivity, hospitalizations and death rates between groups in diabetes mellitus patients, to determine the effect of specific glucose lowering drugs on COVID-19 positivity incidence and hospitalizations and death rates in diabetes mellitus patients between groups, to compare effect of blood glucose lowering drugs on COVID-19 outcomes between groups.

Materials and methods: National diabetes registry (CroDiab) data will be linked with COVID-19 positivity database, COVID-19 vaccination database, data of primary health care providers, hospital data and mortality database. Patients will be matched for important covariates i.e. demographic and clinical factors. In Croatia, according to CroDiab, in year 2020, there were 310 212 diabetes mellitus patients. The study will include data from registry patients with a cut off date of December 31st 2021, only patients older than 18 years of age will be included. Characteristics of the cohort will be described i.e. age, sex, COVID19 vaccination status, diabetes type, ACE inhibitors therapy, COVID19 positivity rate, COVID19 hospitalizations rate, COVID19 death rates, comorbidities such as cancer, arterial hypertension, ischemic heart disease, cardiomyopathy, cerebrovascular diseases, chronic diseases of lower respiratory tract, other chronic obstructive lung diseases, chronic kidney diseases. Patient data will be divided into groups according to blood glucose lowering drugs and according to if the patient is taking monotherapy or drug combination. For each group, COVID-19 incidence, COVID19 hospitalization rates and COVID19 mortality rates will be determined. Between groups, patients will be paired for age, sex and COVID19 vaccination status, at minimum. Data will be analyzed in Statistical Package for the Social Sciences (SPSS) software, descriptive statistics and parametric and non parametric tests will be used as appropriate. Propensity score matching will be used for patient pairing. Regression methods will be used to determine predictors.

Expected scientific contribution: This study can be the basis for conducting randomized controlled trials, can lead to update of clinical guidelines for COVID19 treatment and can contribute to use of real world data for obtaining new drug indications. This is the first study that includes a whole diabetes population of a country.

MeSH/Keywords: diabetes mellitus; COVID-19; Glucagon-Like Peptide-1 Receptor, metformin, pioglitazone, insulin, Cohort studies, registries, medical outcomes

Poster code: T-03-02-054

Poster Title: Determinants of coordination, integration and continuity of care for preschool children with complex needs

PhD candidate: Iva Lukačević Lovrenčić

Part of the thesis: Determinants of coordination, integration and continuity of care for children with chronic medical conditions in the Republic of Croatia

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: Preschool children with complex needs have "multidimensional health and social care needs in the presence of a recognized medical condition or where there is no unifying diagnosis". The current care system is marked by a large number of different stakeholders and is burdened with infrastructural, organizational, technical-administrative, and socio-economic challenges. To improve the coordination, integration, and continuity of care for preschool children with complex needs, in addition to ensuring the quality and quantity of available resources of formal and informal care, their applicability, interrelationship, and adaptation to the specifics of the child's needs and professional and legal practice must be achieved.

Hypothesis: By analyzing publicly available data, official publications, and experiences of stakeholders in the process of care for preschool children with complex needs, it is possible to identify determinants related to improving coordination, integration, and continuity of the process of care for preschool children with complex needs.

Aims: The general aim of the research is to identify the determinants and elements of the care process crucial for improving the coordination, integration, and continuity of care for preschool children with complex needs. Specifically, this research will identify already existing determinants of the care process for preschool children with complex needs by searching publicly available data and official publications; examine the experiences of pediatricians, parents/caregivers, representatives of NGOs, and representatives of the social care system on the applicability and interrelationship of previously recognized determinants and identify new determinants; analyze the relationship between determinants and elements of the care process.

Materials and methods: This research is based on a qualitative methodological approach. A search of electronic bibliographic databases (MEDLINE, Scopus, Web of Science Core Collection) using MeSH terms 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; and an analysis of selected relevant Croatian and international publicly available data and official publications will be performed. Using the method of document analysis, the current state of the care system for preschool children with complex needs will be mapped. The focus group method was chosen as the data collection method for the second part of the research. Focus group participants include representatives of NGOs, parents/caregivers of preschool children with complex needs, primary and hospital-based pediatricians, and representatives of the social care system. Purposive sampling and the snowball method will be used. The minimum expected number of participants is 24, the final number being based on the point of theoretical saturation. Focus groups will be held and recorded using digital video communication platforms. Recordings will be transcribed, and transcript analysis will be performed using the method of thematic analysis and constant comparison. The planned duration of this research is 12 months.

Expected scientific contribution: This research will contribute to the identification and understanding of the determinants of coordination, integration, and continuity of care for preschool children with complex needs and enable better anticipation of necessary changes in the organization of the care system. The results of the research will be useful in international studies in the field of health care organization, primarily through the European Observatory on Health Systems and Policies Health Systems and Policy Monitor.

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: T-03-02-097

Poster Title: Knowledge and attitudes of primary paediatric and school medicine nurses towards measles vaccination of preschool and school children

PhD candidate: Dubravka Pavlović

Part of the thesis: Geography and occupational characteristics as predictors of knowledge and attitudes towards measles vaccination of preschool and school children among primary paediatric and school medicine nurses

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

Affiliation: University of Zagreb School of Medicine

Introduction: Measles vaccination is a successful intervention that has prevented more than 23.2 million deaths in the last twenty years, but despite the availability of vaccines, measles outbreaks are still occurring worldwide. In Croatia, vaccination of preschool and school children against measles is mandatory and vaccination is carried out by primary paediatric and school medicine teams. Although vaccination is mandatory, some counties do not achieve the recommended vaccination coverage. Many studies showed that the parents interactions with health professionals (HCPs) is very important factor for shaping parental attitudes toward vaccination. A survey conducted among HCPs in northern Croatia found that nurses were significantly more hesitant about vaccinations than doctors. While the majority of HCPs express confidence in the benefits of measles vaccination, some still raise concerns and doubts. As nurses play important role in communicating with parents there is a need to better understand and assess their knowledge and attitudes toward measles vaccination of preschool and school children.

Hypothesis: In regions with higher measles vaccination coverage, primary paediatric and school medicine nurses have a higher level of knowledge and more positive attitudes towards vaccination of children against measles. In regions with higher measles vaccination coverage primary paediatric and school medicine nurses with longer length of service have more knowledge and more positive attitudes towards vaccination of children against measles.

Aims: The aim of this study is to investigate and assess the knowledge and attitudes towards measles vaccination of preschool and school children among primary paediatric care and school medicine nurses.

Materials and methods: A cross-sectional survey will be conducted on a sample of primary paediatric and school medicine nurses (N = 430). All nurses working in primary paediatrics (N = 283) and school medicine (N = 157) in the Republic of Croatia will be included in the research, with an expected response rate of 60%. For the purposes of this research, counties will be grouped into four statistical regions according to the Eurostat classification. The research will be conducted by quantitative (survey) and qualitative (in-depth interviews) methods. The online survey composed of sections exploring demographic and occupational characteristics of participants, vaccine knowledge and attitudes towards measles vaccination will be based on questionnaire developed by group of Italian experts. The questionnaire will be translated and pilot-tested for clarity prior use. All the primary paediatric and school medicine nurses will be invited to participate in collaboration with Croatian Chamber of Nurses and professional societies of the Croatian Association of Nurses. Distributions of qualitative variables will be presented in contingency tables, and compared with appropriate statistical and analytical procedures (Chi-Square test, Fisher test). Distributions of quantitative variables will be tested for normality by the Smirnov-Kolmogorov test and, appropriate statistical-analytical procedures (ANOVA, and alternatively the Kruskal Walis test) will be applied in the description and analysis. Of multivariate analysis, logistic regression will be applied. The results will be interpreted at least 5% significance level. The in-depth interviews on a sample of respondents from the regions that have achieved the highest and lowest vaccination coverage against measles will used as a data collection tool in the qualitative part of the research. The data will be analysed by the method of thematic analysis.

Expected scientific contribution: Findings from this research will contribute to the development of evidence based and context-specific future interventions for eliminating or reducing factors impacting vaccine confidence and acceptance among parents.The combination of quantitative and qualitative approach as well as multidimensional analytical approach will enable identification of factors that were not identified in previous research.

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

Poster code: T-03-02-135

Poster Title: Determinants of Self Perceived Health and Health Inequality of the Elderly in Croatia

PhD candidate: Vesna Štefančić Martić

Part of the thesis: Determinants of Self Perceived Health and Health Inequality of the Elderly in the Republic of Croatia

Mentor(s): Associate Professor Danijela Štimac Grbić, MD PhD, Ana Ivičević Uhernik, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Demographic trends show a constant growth of proportion of elderly people (65 +) in the population of Croatia, strongly influencing the socioeconomic and behavioural determinants of health. Ageing is a multiple challenge and opportunity to improve the health of the elderly aimed at a longer and healthier life. The aim of the research is to investigate the data obtained from the European Health Interview Survey (EHIS) wave 3 and to find the connection between socioeconomic, demographic and behavioural determinants of health and health of the elderly in the Republic of Croatia as grounds for health inequality, analyse public health policies and other frameworks and propose recommendations and measures for the creation and improvement of public policies and interventions related to the elderly.

Hypothesis: Socioeconomic and behavioural determinants of health significantly impact health of elderly in Croatia.

Aims: General aim: To research the connection between socioeconomic, demographic and behavioural determinants of health of elderly in Croatia Specific aims: (1) to research the impact of socioeconomic determinants (level of education, employment, income, social support, level of urbanisation of the place of living) on health, health inequalities and health self-assessment of elderly in Croatia; (2) to research the impact of demographic determinants (gender, age, marital status, number of members of the household) on health of elderly in Croatia; (3) to research the impact of life habits (smoking, alcohol consumption, physical activity, nutrition) on health of elderly in Croatia; (4) to analyse the relations between socioeconomic, demographic and behavioural determinants of health of elderly in Croatia, by taking into account regional distribution and health self-assessment; (5) to asses which determinants have the greatest impact on health of elderly, by taking into account regional distribution and health self-assessment; (6) to analyse public policies, research and frameworks in the area of health of elderly in Croatia and other EU countries, to suggest the recommendations and measures for the creation and improvement of public policies and interventions related to health improvement of elderly in Croatia.

Materials and methods: EHIS 3 has been implemented in Croatia in 2019. The institution in charge of implementation is the Croatian Institute of Public Health (CIPH) in cooperation with the Croatian Bureau of Statistics and the Ministry of Health. Health Interview Surveys (HIS) or any other population surveys are widely accepted instruments that can provide comparable data on health statistics related to personal population statistics. EHIS consists of four modules: background variables, health status, health care utilisation, determinants of health. It is implemented every five years in all the EU Member States at the same time. The research sample consists of 1907 elderly participants (34.9% of all participants), aged 65 years or more in Croatia. In the statistical analysis, descriptive methods will be used (proportion calculation, proportion calculation with Hi square test for the relevance score) and for the correlation analysis the univariant and multivariant logistic regression. IBM SPSS programme version 27 (CIPH licence) will be used for the statistical analysis.

Expected scientific contribution: Based on the results of this research, the pivotal healthcare issues and their determinants can be identified, for which there is a need for preventive activities. The results, recommendations and measures that will derive from this research could strongly influence the further development of public health policies as well as the improvement of health and health inequalities reduction of elderly in Croatia.

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MeSH/Keywords: elderly, social determinants, health inequalities, Croatia

Poster code: T-03-02-148