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Contents

Preliminary research results

Basic medical sciences – preliminary research results

Poster Title: Comparison of osteoinductive efficacy of rhBMP2 and rhBMP6 on novel carrier consisting of autologous blood coagulum and synthetic ceramics in ectopic bone formation

PhD candidate: Natalia Ivanjko

Part of the thesis: Comparison of the dynamics of ectopic bone formation using bone morphogenetic protein 2 on a collagen sponge carrier and bone morphogenetic protein 6 in an autologous blood coagulum

Mentor(s): Academician Slobodan Vukičević

Affiliation: University of Zagreb School of Medicine

Introduction: Bone morphogenetic protein 2 (rhBMP2) on collagen sponge is a golden standard for treating open fractures and other severe bone injuries. However, this product showed numerous problems, mainly due to inadequate carrier – bovine collagen. The main problem of collagen as a carrier is the initial burst release of rhBMP2 which subsequently requires supraphysiological dosing to achieve a satisfactory osteogenic effect leading to the risk of ectopic bone formation and severe inflammation. Autologous blood coagulum (ABC) containing synthetic ceramics is a novel biocompatible carrier for BMPs. It promotes strong rhBMP6 binding to the plasma proteins within the fibrin meshwork, suppresses foreign body response and provides good biomechanical properties due to synthetic ceramics. The aim of this study was to compare osteoinductive efficacy of rhBMP2 and rhBMP6 on the same carrier, ABC with synthetic ceramics in rat subcutaneous assay, 14 days following implantation, when bone formation reaches its peak.

Materials and methods: In order to compare osteoinductive efficacy of rhBMP2 and rhBMP6, implants were prepared by mixing rhBMP2 or rhBMP6 in three different doses (5, 20 and 50 µg) with 500 µL of autologous blood and 100 mg of synthetic ceramics, in particle size range from 500 - 1700 µm. Sample size per group was 6. Newly formed bone was harvested 14 days after implantation. New ectopic bone was analysed on histology sections stained by Goldner. To visualize and quantify new ectopic bone, implants were scanned after explantation using 1076 SkyScan µCT device.

Results: MicroCT analyses of implants showed that rhBMP2 and rhBMP6 had the same osteoinductive efficacy (bone volume) when using doses of 5 and 20 µg, while in dose of 50 µg rhBMP6 was superior to rhBMP2. Histology results were aligned with results of microCT analysis, showing the most abundant bone in implats with 50 µg of rhBMP6.

Discussion: In this study we demonstrated that rhBMP6 is superior to rhBMP2 when using higher doses (50 µg); in doses of 5 and 20 µg rhBMP2 and rhBMP6 showed the same osteoinductive efficacy. Higher efficacy of rhBMP6 could be due to the resistance to noggin inhibition and affinity across the BMP type I receptors in comparison to BMP2 and BMP7. Also, novel carrier containing autologous blood coagulum combined with synthetic ceramic showed as a better carrier for rhBMP2 than collagen sponge in inducing new ectopic bone.

MeSH/Keywords: bone morphogenetic protein 2, bone morphogenetic protein 6, bone regeneration, synthetic ceramics

Poster code: R-01-01-039

Poster Title: A novel osteoinductive device comprised of rhBMP6 within autologous blood coagulum with bioceramics induces ectopic bone formation in rats and spinal fusion in rabbits and sheep

PhD candidate: Nikola Štoković

Part of the thesis: Ectopic bone induction by device composed of recombinant human bone morphogenetic protein 6 (rhBMP6), autologous blood coagulum and biphasic bioceramics

Mentor(s): Academician Slobodan Vukičević

Affiliation: University of Zagreb School of Medicine

Introduction: Osteoinductive device containing rhBMP6 within autologous blood coagulum (ABC) with bioceramic particles is a novel autologous bone graft substitute (ABGS) for a broad range of orthopaedic indications including spinal fusions and management of large bone segmental defects. Calcium phosphate (CaP) bioceramics are used as compression resistant matrices (CRM) due to their well-known osteoconductive properties. CaP bioceramics are available in a broad range of sizes and geometrical shapes as well as different chemical compositions (tricalcium phosphate-TCP, hydroxyapatite-HA, and biphasic ceramics (BCP) containing TCP and HA in various ratios). Posterolateral lumbar fusion (PLF) is a surgical procedure used for the treatment of degenerative diseases of the spine including degenerative disc disease, spondylolisthesis, spinal instability, and symptomatic scoliosis.

Materials and methods: In this study we conducted a series of animal experiments using rat subcutaneous implant assay and rabbit and sheep PLF models to investigate the biology of ectopic bone induction and to evaluate different formulations of osteoinductive devices. In the rat subcutaneous assay we compared the ectopic bone formation between osteoinductive devices containing bioceramics of different sizes (small (74-420 μm), medium (500-1700 μm), large (1000-4000 μm), and macroporous blocks) in five time points (7, 14, 21, 35 and 50 days) following implantation. Moreover, we evaluated how chemical composition of bioceramics and method of rhBMP6 application (added in blood or lyophilized on bioceramics) affects the amount of newly formed bone. In rabbit PLF model we evaluated the efficacy and safety of ABGS containing bioceramic particles in three different time points (7, 14, and 27 weeks after surgery). In this model we have compared ABGS with different chemical composition of ceramics as well as different methods of rhBMP6 application. We conducted a pilot sheep PLF experiment to demonstrate the efficacy of ABGS in larger animals. In all experiment the outcome was evaluated by microCT, histological and histomorphometrical analyses.

Results: ABGS induced bone formation at rat ectopic site. Endochondral bone formation was present in the peripheral parts of implants 7 days following implantation while 14 days following implantation the implants were completely ossified and bone along with bioceramics formed bone-ceramic structure (BCS). The amount of newly induced bone on day 21 after implantation was significantly higher in BCS containing small ceramic particles compared to large particles. Moreover, BCS with small particles contained dense trabecular network in between ceramic particles while ABGS containing medium and large particles resulted with pronounced cortical bone, bone on the surface of particles as well as inside the pores. The spinal fusion success rate was above 90% in all rabbit experiments (90,9% after 7 weeks and 100% after 14 and 27 weeks). Chemical composition of ceramics did not affect the amount of newly formed bone. However, the amount of CRM was significantly decreased in implants containing TCP particles due to its higher resorbability compared to HA. Importantly, there was no difference in the amount of newly induced bone among tested methods of rhBMP6 application neither in rat ectopic nor rabbit PLF model. Finally, the ABGS with bioceramics achieved successful spinal fusion in a pilot ovine PLF experiment.

Discussion: ABGS containing rhBMP6 in ABC with bioceramics is a novel therapeutic solution which was herein proven safe and efficacious in rat ectopic and rabbit PLF model. Following the successful outcome in these studies, this novel osteoinductive device is currently being tested in the ovine PLF model and due to its superior osteoinductive and osteoconcutive properties it might be a gamechanger in the bone regenerative medicine.

MeSH/Keywords: Bone morphogenetic proteins, Regenerative medicine, Tissue engineering, Bioceramics, Synthetic ceramics, Tricalcium phosphate (TCP), Hydroxyapatite (HA)

Poster code: R-01-01-044

Poster Title: Effect of bone morphogenetic protein 3 in bone tissue formation and regeneration

PhD candidate: Viktorija Rumenović

Part of the thesis: Effect of bone morphogenetic protein 3 in bone tissue formation and regeneration

Mentor(s): Igor Erjavec, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Bone morphogenetic protein 3 (BMP3) is the most abundant BMP in bone tissue. Members of this protein family display osteogenic activity, however the role of BMP3, in bone, is considered antagonistic to other BMPs. Thus far, BMP3-related research is insufficient and with shortcomings, especially its interplay and potential inhibition of osteogenic activity of other BMPs. The aim of this research is to explore the effect of the lack of BMP3 on bone formation and regeneration in Bmp3 knock-out (Bmp3-/-) mice as compared to wild type (WT) mice using in vitro and in vivo methods.

Materials and methods: Effect of BMP3 inhibition on BMP6 osteogenic activity was tested in bone-marrow mesenchymal stem cells (BMSCs) isolated from long bones. Differentiation and bone nodule formation was visualized by Von Kossa staining. To analyze the effect of BMP3 on in vivo bone formation, blood coagulum containing BMP6 was implanted in WT and Bmp3-/- mice axillary region. Implants were removed after 14 days and new bone formation was analyzed using micro-CT and immunohistochemistry. To establish the model and assess fracture repair, tibia fracture pilot study was performed using WT animals where successful bone callus formation and bone remodeling was seen after 21 days.

Results: In preliminary treatments, BMSCs from Bmp3-/- mice showed better osteogenic response to BMP6 due to the lack of BMP3. For in vivo experiments, ectopic bone formation in Bmp3-/- mice was increased compared to WT animals.

Discussion: Fracture model was successfully implemented on WT mice, resulting in consistent baseline for future experiments on Bmp3-/- mice. Further experiments with sufficient number of animals are required for more significant results.

MeSH/Keywords: BMP3, bone morphogenetic proteins, ectopic ossification, osteogenesis, tibial fractures, mesenchymal stem cells

Poster code: R-01-01-074

Poster Title: Comparison of degenerative changes of articular cartilage in DDH-induced secondary OA and primary OA

PhD candidate: Tea Duvančić

Part of the thesis: Specifičnosti građe osteohondralne jedinice acetabuluma, neoacetabuluma i glave femura u bolesnika sa sekundarnom koksartrozom uzrokovanom razvojnim poremećajem kuka

Mentor(s): Professor Domagoj Delimar, MD PhD

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

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. Development of new, compositional MRI techniques has enabled early detection and quantification of biochemical changes in articular cartilage. T1 mapping allows glycosaminoglycan (GAG) composition characterization. By measuring T1 relaxation times, it is possible to quantify GAGs in the tissue, with higher values representing higher GAG content. Delayed-gadolinium enhanced MRI of cartilage (dGEMRIC), a T1 relaxation-time technique, uses a gadolinium contrast agent that accumulates in areas poor in glycosaminoglycans and allows indirect assessment of GAG content.

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 (n=5) and those with primary OA (n=5). Samples obtained from the DDH group were taken from acetabulum (A), neoacetabulum (NA), non-weight bearing part of the femoral head (NWB) and weight-bearing part of the femoral head (WB), and those obtained from the OA group from the acetabulum (A) and femoral head (F). Samples were washed in saline solution and scanned on a 7.0T micro-magnetic resonance imaging (u-MRI) machine immediately following the surgery. T1 relaxation times of cartilage were calculated. Samples were then immersed in 0,5mM gadolinium solution and scanned using delayed-gadolinium enhanced MRI of cartilage (dGEMRIC). To determine glycosaminoglycan content of cartilage, ΔR was calculated as (1-T1 relaxation time after gadolinium application) / (1-T1 relaxation time before gadolinium application). Results were compared between different anatomical regions and experimental groups.

Results: Mean T1 relaxation times after gadolinium application for A, NA, NWB and WB regions of the DDH group were 622.51, 590.49, 678.46 and 637.70 ms, respectively. Both acetabular regions of the DDH group had lower T1 relaxation times than the acetabulum of the OA group (632.58 ms). T1 relaxation time of femoral head of the OA group was 664.45 ms. Within both the DDH and the OA group, acetabular regions had lower T1 relaxation times compared to the femoral head regions of the same experimental group. The highest ΔR value was found at neoacetabulum, followed by acetabulum of DDH group and acetabulum of OA group. Femoral regions of both experimental groups had lower ΔR values than those of acetabular regions, with the lowest value found at the NWB of the DDH group, which implicates low glycosaminoglycan content.

Discussion: Lower T1 relaxation times of A, NA and WB regions of the DDH group compared to those of A and F of the OA group indicate that DDH-induced secondary OA causes more severe degeneration of cartilage than does primary OA. According to these results, acetabular cartilage is more degenerated than femoral cartilage in both primary and secondary OA, as indicated by T1 and ΔR values. Our results show that micro-MRI is a valuable method for the evaluation of biochemical changes of articular cartilage in OA.

MeSH/Keywords: Hip dysplasia, osteoarthritis, cartilage, micro-MRI, T1 mapping, dGEMRIC

Poster code: R-01-02-028

Poster Title: Cell cycle regulation by RB1 protein and genomic imprinting in the testicular development of men and rat

PhD candidate: Marta Himelreich Perić

Part of the thesis: Povezanost regulacije staničnog ciklusa RB1 proteinom s genomskim utiskivanjem u razvoju testisa čovjeka i štakora

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

Affiliation: University of Zagreb School of Medicine

Introduction: Prenatal development of the testes is a complex process, easily disrupted by environmental causes leading to male reproductive health disorders: infertility or testicular tumors. It has been shown in mice that the cell cycle quiescent phase is necessary for implementation of epigenetic changes, such as DNA de novo methylation. One of the main cell cycle regulators is the RB1 protein. The exact timing and connection of these changes has not been investigated throughoutly in men and rat testes.

Materials and methods: 35 archive human fetal formalin-fixed, paraffin-embedded samples were collected, as well as minimum of 6 fresh-frozen samples per stage from E14.5 to E20.5 in rat (Figure 1). Antibodies against RB1 (phospho S795 and 780) (1:50, ab47474 and 1:100, ab47763, Abcam, respectively) were incubated overnight at 4°C after deparaffinisation, antigen retrieval and serum blocking. The next day, after H2O2 application, a goat anti-rabbit, HRP-conjugated secondary antibody (1:1000, ab97051, Abcam) was kept for 1h on RT. 3,3'-diaminobenzidine-tetrahydrochloride (DAB) was used for signal staining, and hematoxylin for counterstaining. DNA was isolated in TE buffer (pH9) with 0.1 mg/mL of Proteinase K at 56°C for 24 h, heated for 10 min at 95°C to inactivate Proteinase K, spun and the supernatant was then frozen at -20°C. DNA concentration and quality were measured with the NanoDrop ND-2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Variable volumes of unpurified isolated genomic DNA was used for bisulfite conversion by EpiTect Plus DNA Bisulfite Kit (#59124; Qiagen), including a clean-up step with no necessity for prior purification of DNA.

Results: Immunohistochemically stained slides showed clear, nuclear signal of both antibodies, located both within the tubule and in the interstitium. The signal intensity of RB1 phospho 780 varied from mild to strong in both compartments and the signal of RB1 phospho 795 was mostly mild and slightly cytoplasmatic (Figure 2). The power-analysis showed that the collected number of samples is sufficient for the statistical analysis. Mean isolated DNA concentrations and the variable amounts of genomic DNA for bisulfite sequencing are shown in Figure 3. DNA concentrations from fresh-frozen and FFPE tissues were relatively high and satisfactory for further analysis.

Discussion: The aim of this study is to perform an analysis of sample and method quality. It was concluded that the methods used fulfill the requirements for further analysis of collected samples. The study results could help elucidate the origin of male reproductive health disorders derived from the fetal period.

MeSH/Keywords: testis, development, RB1, methylation, isolation

Poster code: R-01-02-144

Poster Title: Cell Energy Phenotype of Patients' Osteosarcoma Stem Cells Treated in vitro with Ascorbic Acid

PhD candidate: Marijana Šimić Jovičić

Part of the thesis: The Effects of Ascorbic Acid on Metabolism of Osteosarcoma Stem Cells Grown from Patients' Tumour Tissue Samples

Mentor(s): Professor Vladimir Trkulja, MD PhD, Associate Professor Inga Urlić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Osteosarcoma (OS) is a malignant mesenchymal tumour whose cells produce osteoid. It most commonly affects children and adolescents. It shows wide genetic diversity and histological heterogeneity. Limited results of OS treatment indicate the need for new therapeutic methods. OS cancer stem cells (OS-CSCs) are considered to be responsible for the disease relapse because they have ability to self-renew and developed mechanisms of anticancer drugs resistance. Given the mechanisms of resistance, it is difficult to kill them selectively, but their metabolism has been recognized as a potential target of therapeutic action. According to previous in vitro studies, ascorbic acid (AA) induces apoptosis of OS-CSCs by oxidative effect and by inhibition of glycolysis. This study will examine the impact of AA on the metabolism of OS-CSCs isolated from tumour tissue samples obtained during diagnostic biopsy from three patients, with the aim of better understanding the biological phenomenon of the selective action of AA on OS-CSCs.

Materials and methods: The second generation of cell lines from 3 patients (OS-CSCs), HEK293, hMSC and U2OS cell lines respectively were seeded at the density of 6x104 cells/well and treated with AA for 24 hours in a concentration ranging from 1 µg/mL to 30 µg/mL in triplicates for the calorimetric MTT assay. IC50 (inhibition concentration where 50% of the cells are dead) was calculated. Bioenergetic profiling was performed using Seahorse XFe24. OS-CSCs, HEK293, hMSC, U2OS cell lines and parental tumour cells respectively were plated 48 hours before the assay at the cell density of 6x104 cells/well in 100 µL of appropriate culture medium. 24 hours prior to measuring, cells were exposed to 1 mM and 10 mM AA, while controls were left untreated. Seahorse XF Cell Energy Phenotype Test Kit was used and the assay was run following the manufacturer's instructions. The assay results were analysed using Wave Desktop software.

Results: IC50 of HEK293, U2OS, hMSC, parental cells and OS-CSCs derived from patients treated with AA ranged from 11.46 mM to 25.63 mM. Treatment with 1mM AA did not show any changes in metabolic phenotype in all cell lines. Response of different cell types to 10mM AA treatment can be divided into two groups. A shift toward glycolytic phenotype was observed in U2OS cell line and parental cells and OS-CSC from patient 3. Patient 3-derived parental and OS-CSC also have IC50 value in the lower range of AA concentrations (11-16mM). A decrease in aerobic metabolic potential was observed in hMSC, HEK and parental cells and OS-CSC from patients 1 and 2. Patient 1 and 2-derived cells are less sensitive to AA with IC50 value in the higher range (18.5-20 mM AA).

Discussion: OS-CSCs can potentially use both glycolysis and oxidative phosphorilation, and can turn to glycolysis in unfavourable conditions. The differences in the results of the studies of the metabolic profiles of OS-CSCs have several explanations that should be kept in mind when conducting similar studies. One proposed explanation is the plasticity of these cells and the potential influence of experimental conditions. Another cause may be the lack of phenotypic homogeneity and precision in defining OS-CSCs and the different techniques used to isolate them. The third factor is the microenvironment because the metabolic status of OS-CSCs differs in normoxic, hypoxic, and metastatic sites, and tumour stroma plays a role too. AA treatment may reduce the metabolic activity of the tumour cells, possibly due to its pro oxidative effect regardless of the metabolic pathway. To complete the study, it remains to see the significance of this decrease in metabolic activity in the context of cell viability, and to confirm the pro oxidative effect of AA by measuring levels of reactive oxygen species.

MeSH/Keywords: Osteosarcoma, cancer stem cells, tumour cell metabolism, ascorbic acid

Poster code: R-01-03-091

Poster Title: Pathophysiological changes of the gastrointestinal tract in a rat model of sporadic Alzheimer's disease

PhD candidate: Jan Homolak

Part of the thesis: Patofiziološke promjene probavnog sustava u životinjskim modelima Alzheimerove i Parkinsonove bolesti

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

Affiliation: University of Zagreb School of Medicine

Introduction: The gastrointestinal tract and the brain-gut axis are involved in the etiopathogenesis and progression of Alzheimer's disease (AD) by promoting metabolic dysfunction and inflammation. Failure of the gastrointestinal barrier accompanied by the breach of intestinal microorganisms, amyloid β, and proinflammatory mediators have been reported in animal models of familial AD, however, the role of the gastrointestinal has so far never been examined in non-transgenic models attempting to recapitulate pathogenetic processes driving neurodegeneration in ~95% of AD patients. The aim was to explore pathophysiological changes of the gastrointestinal tract and the brain-gut incretin axis in the intracerebroventricular streptozotocin-induced (STZ-icv) rat model of sporadic AD.

Materials and methods: Two separate cohorts of control and STZ-icv-treated rats (3mg/kg) were subjected to acute intracerebroventricular administration of either glucagon-like peptide-1 (GLP-1) or glucose-dependent insulinotropic polypeptide (GIP) 1 month after the STZ-icv treatment. Chronic effects of STZ-icv, acute effects of incretin antagonists, and their interaction were explored by analyzing plasma lipid peroxidation (TBARS) , superoxide dismutase (SOD) and nitrocellulose redox permanganometry (NRP), and duodenal and ileal TBARS, NRP, SOD, catalase activity and low molecular and protein sulfhydryls. The effects of STZ-icv on the gastrointestinal epithelial cell turnover were analyzed by morphometry and multiplex fluorescent signal amplification of caspase-3.

Results: Redox homeostasis is shifted toward a pro-oxidative state in the STZ-icv duodenum, but not in the ileum in comparison with the controls. Oxidative stress in the STZ-icv duodenum is accompanied by the decreased villus length/crypt depth ratio, epithelial cell flattening, and decreased expression and activation of epithelial caspase-3. Treatment-treatment interaction analysis indicates STZ-icv might affect the functioning of the brain-gut incretin axis.

Discussion: Pathophysiological changes of the gastrointestinal tract in the STZ-icv rat model of AD speak in favor of redox dyshomeostasis with a pro-oxidative shift and impaired epithelial cell turnover and apoptosis contributing to the dysfunctional gastrointestinal barrier that might promote systemic and central inflammation. Dysregulation of the brain-gut axis in the STZ-icv rats is characterized by resistance of gut to a central inhibition of GLP-1 and GIP receptors.

MeSH/Keywords: Alzheimer disease; streptozocin; incretins; oxidative stress

Poster code: R-01-03-135

Poster Title: Effect of pentadecapeptide BPC 157 on psoriasis model in rats

PhD candidate: Marija Šola

Part of the thesis: Učinak primjene pentadekapeptida BPC 157 na modelima psorijaze: imiquimodom inducirane u štakora i na HaCaT stanicama

Mentor(s): Professor Predrag Sikirić, MD PhD, Assistant Professor Nevena Skroza, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Pentadekapeptide BPC 157 was used in numerous laboratory rat animal models with proven cytoprotective and organ protective properties. In different wound therapy (incisional/excisional wound, deep burns, diabetic ulcer, alkali burns) fast and complete tissue restitution was achieved. BPC 157 was found to rapidly stimulate the expression of various genes involved in the healing process of the skin and mucosal lesions in these models. The lethal dose has not been determined, there are no side effects. Psoriasis is a chronic, relapsing, immune-mediated skin disease pathogenetically driven by proinflammatory cytokines of which: TNF-alpha, IL-12, IL-17, IL-23 play a key role. Since BPC 157 has shown a significant anti-inflammatory effect in several studies, the aim of this study is to show that it has the same role in the regression of inflammatory skin lesions such as those in psoriasis. An experimental model of psoriasis in laboratory animals is easily feasible, using 5% imiquimod topically.

Materials and methods: The experiment is done on male Wistar Albino rats. Animals are divided into groups of 6. Psoriatic-like lesions are induced in all animals applying 5% imiquimod creme topically on shaved skin on their backs for 7 consecutive days. On 7th day treatment starts using BPC in cream or neutral creme in control groups. Animals are treated and observed daily, video documentation was made. Animals are treated until the sacrifice day according to schedule. The severity of skin lesions is measured clinically using a modified version of PASI score.

Results: In the BPC-treated group faster regression of psoriasis-like skin lesions was observed.

Discussion: BPC 157 is well-known peptide used in various experimental models without any side effects of its use, with proven effects on the NO-system, and excellent results in wound healing. Psoriasis is a rather common immune-mediated skin disease driven by proinflammatory cytokines. TNF-alpha, IL-12, IL-17, IL-23 play a key role in the pathogenesis of psoriasis. Since the use of BPC 157 topically successfully neutralizes the effects of imiquimod and induces regression of „psoriasis-like" lesions the question is whether BPC has an impact on one of the above-mentioned cytokines. It is yet to be explored which mechanism is the key to its beneficial effects in treating „psoriasis-like" models.

MeSH/Keywords: BPC 157, imiquimod, psoriasis

Poster code: R-01-03-139

Poster Title: Association between sperm genome damage, chromatin maturity and glycan composition in male infertility

PhD candidate: Tihana Marić

Part of the thesis: Povezanost životnih i okolišnih faktora, kvalitete sjemena i N-glikana sjemene tekućine u muškaraca

Mentor(s): Associate Professor Ana Katušić Bojanac, MD PhD, Aleksandra Fučić, PhD, research advisor

Affiliation: University of Zagreb School of Medicine

Introduction: Recently, male infertility is becoming an increasing health problem and it affects around 7% of men worldwide. Condition has very heterogeneous background and it may originate from congenital or acquired diseases, genetic abnormalities but also as a result of exposure to different environmental stressors. Complexity of involved molecular mechanisms require a multiparameter approach, especially in the introduction of novel biomarkers. Thus, glycan composition and its association with other semen parameters could provide a promising biomarker in investigation and diagnostics of male fertility due to its specific association with many different diseases. The aim of this study was to investigate sperm genome damage, chromatin maturity and glycan composition between fertile and infertile men.

Materials and methods: The study included group of 49 fertile and 50 infertile men that underwent routine semen analysis by CASA system, where sperm count, motility, viability and morphology after Giemsa staining were assessed. Sperm genome damage was determined by DNA fragmentation assay with GoldCyto Sperm DNA kit on CASA system. Sperm chromatin maturity was evaluated by staining of fixed sperm with aniline blue staining that binds the remaining histone content indicating sperm immaturity. Total protein N-glycans from seminal plasma were isolated from samples and analyzed by ultra-performance liquid chromatography. Mann-Whitney test was used for all comparisons together with Bonferroni's correction.

Results: As expected, sperm parameters differ significantly between fertile and infertile men indicating separation of the groups based on basic clinical parameters in this preliminary analysis. Sperm genome was also significantly disrupted in the infertile group that has higher DNA fragmentation percentage. There was no significant difference between the groups in histone levels after aniline blue staining of sperm samples. Separation of isolated N-glycans from seminal plasma total proteins revealed 37 unique glycan peaks in all samples. Statistical analysis of the preliminary results also revealed that the pattern of seminal plasma glycan peaks (SPGP) showed that SPGP14, significantly differed between fertile and infertile men (p=0,00001). Another glycan peak, SPGP28, showed the association with sperm histone levels only in infertile group, although this was not confirmed after Bonferroni's correction.

Discussion: The differences in sperm parameters, count, motility, morphology and viability indicated a separation of the fertile and infertile group. Infertile patients also had significantly higher percentage of sperm DNA fragmentation implying more damaged genome, which was expected and in accordance with other published studies. Preliminary analysis also showed different pattern of SPGP14 values between fertile and infertile group, supporting our hypothesis that seminal plasma protein N-glycosylation differs between the groups and has a significant contribution to male fertility. An association between histone levels and SPGP28 was observed only in infertile men. This is another preliminary result that indicates potential importance of plasma protein N-glycosylation alternations correlated to other parameters of sperm quality, in this case maturity. Further investigations on larger cohorts could identify more glycans as potential infertility biomarkers. Moreover, their association with other sperm parameters such as DNA fragmentation or histone levels could serve as additional indicator of fertility status in men.

MeSH/Keywords: male infertility, sperm, DNA fragmentation, histone levels, N-glycans

Poster code: R-01-04-075

Poster Title: Cell cycle arrest and differentiation of leukemia cells by activating Checkpoint kinase 1

PhD candidate: Barbara Tomić

Part of the thesis: Signalni mehanizmi i metaboličke promjene tijekom diferencijacije i proliferacije leukemijskih stanica

Mentor(s): Professor Dora Višnjić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Lack of differentiation is a hallmark of acute myeloid leukemia (AML), making differentiation therapy a promising treatment strategy. Several novel differentiation targets have been recently identified, including dihydroorotate dehydrogenase (DHODH), one of the key enzymes in de novo pyrimidine synthesis. Our previous study demonstrated that 5-aminoimidazole-4-carboxamide ribonucleoside (AICAr), a precursor in purine biosynthesis and a widely used activator of AMP-activated kinase (AMPK), promotes differentiation, inhibits proliferation, and induces S-phase arrest in monocytic U937 cells. Furthermore, AICAr-mediated effects were abrogated by the addition of either nucleosides or uridine suggesting that AICAr might interfere with pyrimidine synthesis. Additionally, it is known that depletion of nucleotide pools initiates the DNA damage signaling pathway and cell cycle arrest through activation of the ataxia telangiectasia and RAD3-related (ATR)/checkpoint kinase 1 (Chk1). This study is aimed to determine the effect of AICAr on ATR/Chk1 activation, to define the effect of AICAr on pyrimidine synthesis, to compare the effects of AICAr and DHODH inhibitor brequinar, and to test for the role of ATR/Chk1 in AICAr-mediated effects by using pharmacological inhibitors and siRNA transfection.

Materials and methods: U937 cells were incubated in the presence of AICAr (0.1, 0.2, 0.5 mM), brequinar (0.1, 0.5 µM), nucleosides (1x), uridine (30 and 300 µM) and pharmacological inhibitor of ATR/Chk1, Torin2 (100 nM). The expression of differentiation markers CD11b and CD64 and DNA content for cell cycle analysis were determined by flow cytometry (FACS Calibur) and FlowJo software. Uridine monophosphate (UMP) and orotate levels were measured by liquid chromatography/tandem mass spectrometry (LC/MS/MS) analysis. siRNA transfections were performed using siRNA targeting Chk1 (Dharmacon) and NeonTM transfection system (Invitrogen). Total cell lysates were analyzed for the level of Ser-345-p-Chk1, Tyr-15-p-Cdk1 and β-actin by Western blot. Statistical analysis was performed using Student's t-test ($p < 0.05$).

Results: AICAr induced arrest of U937 cells in the S-phase of the cell cycle, and Western blot analysis revealed that AICAr increased the level of Ser-345-phosphorylated Chk1, which is a marker of Chk1 activation. Both effects were abolished by addition of either nucleosides or uridine. Both AICAr and brequinar decreased the level of UMP, but only AICAr significantly increased the level of orotate, consistent with inhibition of UMP synthesis at a step downstream of DHODH. The activation of Chk1 was observed in the presence of brequinar, and the increase in the level of Ser-345-phosphorylated Chk1 in response to AICAr, brequinar and their combinations followed the same pattern as the expression of differentiation markers and S-phase arrest. Pharmacological inhibition of ATR/Chk1 pathway by Torin2 prevented differentiation and cell cycle arrest in response to AICAr and brequinar. Transfection of U937 cells with siRNA targeting Chk1 decreased the level of Chk1 and significantly reduced the effects of AICAr and brequinar on the expression of differentiation markers and cell cycle arrest. AICAr and brequinar increased the level of inhibitory Tyr-15-phosphorylation of cyclin-dependent kinase 1 (Cdk1), suggesting that Cdk1 inactivation is an important downstream target of Chk1 in cell cycle restriction.

Discussion: Results of this study demonstrate that AICAr and DHODH inhibitor brequinar induce cell cycle arrest and differentiation of U937 cells by activation of DNA damage checkpoint kinase Chk1 induced by pyrimidine depletion. There are many concurrent clinical studies testing DHODH inhibitors to establish their safety and efficiency in AML patients, even though the exact mechanism of their action is not completely elucidated. We propose that activation of the DNA damage pathway is a critical mediator of AML differentiation in response to pyrimidine depletion.

MeSH/Keywords: acute myeloid leukemia, cell cycle, differentiation, metabolism

Poster code: R-01-04-079

Poster Title: RASSF1 and CAV1 gene expression in prostate tissue biopsies

PhD candidate: Lucija Škara

Part of the thesis: Metilacija nestanične DNA gena RASSF1 i CAV1 u krvi i ejakulatu bolesnika s rakom prostate

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

Affiliation: University of Zagreb School of Medicine

Introduction: Prostate cancer (PC) is the most common cancer diagnosed among men in Croatia. PC diagnosis is established on prostate biopsy which is performed due to positive digital rectal examination and elevated prostate-specific antigen (PSA) levels. PSA is not cancer specific and is increased in other conditions (e.g. benign prostatic hyperplasia (BPH), prostatitis). At the end for definitive PC diagnosis tissue biopsy is required. This invasive approach confirms cancer in approximately one third of patients which means that most patients is unnecessary biopsied. Moreover, it identifies indolent PC that needs no intervention and thus leads to cancer overdiagnosis. Other disadvantages are risk of potential complications, limited tissue access, false-negative biopsy results and lack of information about intratumoral heterogeneity. Those disadvantages are not present in new and minimally invasive diagnostic approach - liquid biopsy. Patients with PC have significantly higher concentrations of cell-free DNA (cfDNA) in blood and ejaculate which originates from disintegrated tumor and tumor-adjacent cells and makes liquid biopsy potential source of PC biomarkers. More than 50 genes are hypermethylated in PC, including RASSF1 and CAV1 genes. RASSF1 is tumor suppressor, which plays an important role in DNA damage repair and apoptosis. Caveolin-1 (CAV1) is important for caveolae formation, signaling molecules organization and cholesterol homeostasis. General aim of our study is to determine potential of cfDNA methylation of RASSF1 and CAV1 genes in blood and semen as biomarker to distinguish between PC and BPH, but our first step is to examine protein expression in tissue.

Materials and methods: Formalin-fixed, paraffin-embedded samples of prostate needle biopsy had been collected from 47 patients with PCa and 62 patients with BPH. Paraffin embedded tissues is used for immunohistochemical staining with antibodies raised against RASSF1 (HPA040735, 1:200) and CAV1 (HPA049326, 1:2500). Protein localization and expression was analyzed semi-quantitavely by morphometric analysis.

Results: Prostate epithelial cells do not express CAV1 protein neither in PCa nor in BPH. Stromal prostate cells in BPH have significantly higher expression of CAV1 than PC stromal cells. Immunohistochemical assessment of RASSF1 expression showed significantlly higher expression in BPH epithelial and stromal cells than in PC cells (Fig. 2 and 3).

Discussion: When looking overall signal, CAV1 and RASSF1 are more expressed in BPH tissue. Furthermore, relation of expression of the two genes between epithelial and stromal tissue is consistent in both prostate pathologies. Since it is not the case that expression of observed genes is higher in epithelial cells of one pathology while expression in stroma higher in another pathology, CAV1 and RASSF1 are worth of further research. We expect that their expression is regulated by methylation and, subsequently, that methylation of CAV1 and RASSF1 in cfDNA could be a good marker for distinguishing PC and BPH minimally invasively. It remains to confirm weather their expression is regulated by methylation which is our next step.

MeSH/Keywords: Prostate cancer, benign prostatic hyperplasia, RASSF1, CAV1, cell-free DNA, methylation, biomarker

Poster code: R-01-05-040

Poster Title: Protein expression of LGALS3 and APC in prostate cancer

PhD candidate: Irena Abramović

Part of the thesis: Cell free DNA methylation of APC and LGALS3 in blood and ejaculate of patients with prostate cancer

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

Affiliation: University of Zagreb School of Medicine

Introduction: Prostate cancer (PCa) represents the most common cancer among Croatian men, as in the rest of the world. DNA methylation is being investigated as a new PCa biomarker, especially due to the lack of prostate-specific antigen specificity and sensitivity. Numerous solid tumors show specific DNA methylation patterns, among them PCa, where changes in genomic DNA methylation in the promoter region of the APC and LGALS3 genes were detected. As a part of this thesis, protein expression of APC and LGALS3 was analyzed in PCa tissue, surrounding "healthy" tissue, and benign prostate hyperplasia (BPH) tissue; in order to later correlate it with genomic DNA methylation of the same genes.

Materials and methods: In 47 PCa and 62 BPH formalin-fixed, paraffin-embedded (FFPE) biopsy samples, APC and LGALS3 protein expression was analyzed by immunohistochemistry with antibodies raised against LGALS3 (ab76245, 1:1000) and APC (NBP2-15422, 1:750), as well as in 27 radical prostatectomy FFPE tissue samples of included PCa patients that were subjected to it. Morphometric analysis of the investigated genes for protein expression was performed by two pathologists. Staining signal was noted subcellularly (nuclear, cytoplasmic, or membranous in tumor cells (TC)) and histologically (prostate epithelium and stroma, BPH, PCa, and tumor surrounding "healthy" tissue). Staining percentage was scored from 0 to 5: 0 (negative TC), 1 (>1- ≤10% positive TC), 2 (>10%-≤25% positive TC), 3 (>25%- ≤50% positive TC), 4 (>50%-≤75% positive TC), and 5 (>75% positive TC). The intensity of staining was assessed (none-low-medium-high). Semi quantification of protein expression was expressed by the immunoreactivity score (IRS) which was calculated by multiplying staining percentage (0-5) and intensity of staining (0-3) creating a range of 0-15. Statistical analysis of protein expression was performed by GraphPad Prism software (Kruskal-Wallis test). Results were considered statistically significant when $p < 0.05$.

Results: Analysis of protein expression of LGALS3 in biopsy samples showed that its expression in PCa epithelium is statistically significantly lower than in BPH epithelium and tumor surrounding "healthy" tissue. Stromal expression of LGALS3 did not show any differences between PCa, BPH, and tumor surrounding "healthy" tissue. Analysis of protein expression of APC in biopsy samples showed significantly higher expression in PCa epithelium compared to BPH epithelium, while stromal expression was significantly higher in BPH compared to PCa and tumor surrounding "healthy" tissue. Immunohistochemical analysis done on radical prostatectomy samples was in accordance with results obtained on FFPE biopsy samples for both genes.

Discussion: With LGALS3 protein expression being significantly lower in PCa compared to BPH and tumor surrounding "healthy" tissue, this gene represents an interesting target for PCa biomarker research. When assuming that protein expression is regulated by DNA methylation, it will be interesting to see whether higher DNA methylation is found in PCa compared to BPH. Regarding APC, the differences in epithelial and stromal expression between two pathologies point out the need to further research to examine its biomarker potential. The DNA methylation analysis would be an additional step to elucidate this matter, which will be done as a part of this thesis.

MeSH/Keywords: prostate cancer, benign prostate hyperplasia, immunohistochemistry, biomarkers

Poster code: R-01-05-065

Poster Title: RASSF1A as a Diagnostic Tool for Seminoma

PhD candidate: Dora Raos

Part of the thesis: Metilacija nestaničnoga DNA gena RASSF1A u krvi i ejakulatu kao epigenetski biomarker u pacijenata sa seminomom testisa

Mentor(s): Professor Davor Ježek, MD PhD, Associate Professor Nino Sinčić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Testicular germ cell tumors (TGCTs) are the most common malignancies among young men with an overall increasing incidence. TGCTs are divided into seminomas (SE) and nonseminomas (NS) with both arising from germ cell neoplasia in situ (GCNIS). DNA methylation is an extensively researched epigenetic modification due to its high chemical and biological stability. Nowadays, cell-free DNA (cfDNA) methylation pattern in liquid biopsies is investigated as a tool for noninvasive diagnostics. RASSF1A represents one of the eight isoforms of RASSF1 gene, a tumor suppressor whose inactivation influences tumor initiation and development. Epigenetic inactivation of RASSF1A was detected in various cancer types and it represents one of the strong emerging biomarkers for TGCT. Analysis of RASSF1A DNA methylation in TGCT are currently done mostly on genomic DNA (gDNA), and has shown to be able to discriminate between SE and healthy tissue but its methylation pattern in cfDNA was investigated in ejaculate samples. In this study, RASSF1A methylation pattern in cfDNA from blood and ejaculate samples and as well as in gDNA tissue samples of seminoma patients will be investigated.

Materials and methods: This study was conducted by the School of Medicine University of Zagreb with the collaboration of the University hospital Centre "Sestre milosrdnice" and the University Hospital Center Zagreb. Blood and ejaculate samples were collected from twenty-four seminoma patients before and after surgery. As a control group, thirty-five healthy volunteers were recruited and their samples of blood and ejaculate were collected. CfDNA from blood and ejaculate plasma and gDNA from seminoma tissue were isolated according to the optimized protocol. After isolation, cfDNA and gDNA concentration were measured. DNA methylation analysis was done by pyrosequencing. Protein expression of RASSF1A was analyzed by immunohistochemistry. Morphometric analysis of the immunohistochemical signal was performed according to the standard protocol on the light microscope in collaboration with pathologists. Surrounding tumor-free tissue with preserved normal spermatogenesis has been considered healthy tissue. The statistical analysis was conducted with statistical tests, Mann-Whitney U test, and Kruskal–Wallis test by ranks. All p values less than 0,05 will be considered significant. The analysis was performed using the statistical program GraphPad Prism 7.

Results: No difference in RASSF1A methylation pattern was detected in cfDNA from preoperative and postoperative blood samples and no statistically significant difference was detected between preoperative and postoperative ejaculate samples. There was no difference between RASSF1A methylation pattern in blood samples from healthy volunteers and preoperative or healthy volunteers and postoperative samples,as well as between control and patient ejaculate preoperative and postoperative samples were found. Statistically, a significant difference was found in protein expression between healthy tissue and seminoma tissue.

Discussion: Decreasing trend of RASSF1A protein expression from HT to TGCTs, confirms the inactivation of these tumor suppressor genes in SE. Decreased protein expression of RASSF1A in SE but no differences in RASSF1A methylation pattern in cfDNA between control blood and ejaculate samples could still indicate that RASSF1A is epigenetically inactivated in SE just methylation profile from gDNA is not reflected in methylation pattern in cfDNA.

MeSH/Keywords: seminoma, biomarker, RASSF1A

Poster code: R-01-05-106

Poster Title: Wnt5a, beta-catenin and SUFU protein expression in IUGR placentas

PhD candidate: Ida Marija Šola

Part of the thesis: Uloga proteina SUFU u povezivanju Wnt i Hedgehog signalnoga puta u posteljica s intrauterinim zastojem u rastu

Mentor(s): Professor Ljiljana Šerman, MD PhD, Associate Professor Krunoslav Kuna, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Wingless (Wnt) and Hedgehog (Hh) signaling pathways are evolutionary conserved cell- signaling systems decisive for cell differentiation, migration, invasion, and apoptosis. They are crucial for trophoblast epithelial-mesenchymal transition (EMT) and placentation. Intrauterine growth restriction (IUGR) denotes fetal incapability of achieving its genetically determined growth potential, due to the placental, fetal or maternal pathology and is diagnosed in about 10% of pregnancies. Identification of growth- restricted fetuses, appropriate pregnancy monitoring and delivery timing is crucial since there is increased risk of fetal and neonatal morbidity and mortality. Abnormal placentation causes utero- placental insufficiency and chronic hypoxia thus leading to IUGR. In the current study we explored the expression of positive regulators of Wnt pathway Wnt5a and beta-catenin, and negative regulator of Hh signaling pathway Suppressor of Fussed (SUFU) in placentas from pregnancies complicated with IUGR, compared to placentas obtained from uncomplicated physiologic pregnancies.

Materials and methods: Formalin- fixed paraffin- embedded samples of 14 term placentas from physiologic pregnancies and 14 placentas from pregnancies complicated with IUGR were used for the study. Expression levels of mRNA for Wnt5a, beta-catenin and SUFU were analyzed by quantitative real- time PCR (qRT-PCR). Wnt5a, beta-catenin and SUFU protein expression were semi-quantitatively analyzed using immunohistochemistry (IHC) in three different parts of placental tissue: trophoblasts, stroma, and endothelial cells of placental villi. DNA methylation in the SUFU gene promotor was analyzed by methylation- specific PCR (MSP).

Results: Wnt5a protein expression was significantly higher in endothelial cells of placental villi in placentas from IUGR compared with the control group. Beta-catenin protein expression was also significantly higher in endothelial cells of placental villi as well as trophoblasts from IUGR placentas compared with physiologic ones. SUFU protein expression was significantly higher in trophoblasts from IUGR placentas compared with control group. mRNA expression of Wnt5a, beta-catenin and SUFU was analyzed in whole placental tissue in both IUGR and control group. Expression levels of mRNA for Wnt5a, beta-catenin and SUFU were higher in IUGR, compared to control group, but the difference was not statistically significant. SUFU gene promotor was unmethylated in both IUGR and control group.

Discussion: Collectively, our data demonstrate the difference in Wnt5a, beta-catenin and SUFU protein expression in placentas from IUGR compared to placentas from uncomplicated pregnancies. Also, SUFU gene promotor was unmethylated in both IUGR and control group suggesting other epigenetic mechanism could be involved in placental SUFU gene expression. Taken together, this data demonstrates that Wnt and Hh signaling pathway as well as possibly other epigenetic mechanism of placental gene expression could be significant in pathology of utero- placental insufficiency which is important since it is one of the main causes of IUGR. Further studies, preferably bigger with more tissue samples, are needed to elucidate these mechanisms and their clinical significance.

MeSH/Keywords: Wnt5a, beta-catenin, SUFU, placenta, IUGR, Wnt signaling pathway, Hh signaling pathway

Poster code: R-01-05-124

Poster Title: What is the role of Notch1 in the development of hepatic fibrosis?

PhD candidate: Dino Šisl

Part of the thesis: Uloga signalnog puta Notch u jetrenim zvjezdolikim stanicama tijekom razvoja mišjeg modela jetrene fibroze

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

Affiliation: University of Zagreb School of Medicine

Introduction: Hepatic fibrosis is a common feature of various liver diseases, and is characterized by activation of hepatic stellate cells (HSC), the principal source of alpha smooth muscle actin (αSMA) myofibroblasts in the liver. The pathophysiological role of Notch activation has been well established, but the role of Notch1 in activated HSCs is still not sufficiently investigated.

Materials and methods: In the present research, we first used two common murine models of hepatic fibrosis, a six-week carbon tetrachloride (CCL4) treatment, and a four-week 0.1% DDC-supplemented diet, and subsequently analysed the expression of Notch-related gene sin the hepatic tissue. In the next set of experiments, we used double transgenic SMACreΔRbpjκΔ mice in which Notch1 signalling pathway was specifically inhibited in myofibroblasts by application of tamoxifen during the development of fibrosis.

Results: In the CCL4 model, qPCR analysis showed an upregulation of Notch2, Hey1, HeyL, and Jag2, while DDC-induced fibrosis was associated with increased expression of Notch2, Notch3, Hey1, Hes1, HeyL, Jag1 and Jag2. Notch1 inhibition did not change the degree of fibrosis significantly, as evidenced by similar histological Sirius red liver staining and similar tissue expression of COL1A1 and ACTA2 both in the control (SMACre-ΔRbpjκΔ) and Notch1 inhibited (SmaCre+ΔRbpjκΔ) mice group.

Discussion: So far, our data do not support the conclusion that Notch1 signalling in myofibroblasts contributes to the development of hepatic fibrosis in neither CCL4 nor DDC model.

MeSH/Keywords: Animal models, Biology of the immune system, Chronic inflammation and fibrosis

Poster code: R-01-06-042

Poster Title: Notch 1 inhibition increases osteoclast progenitor activity in the mouse model of rheumatoid arthritis

PhD candidate: Maša Filipović

Part of the thesis: Notch signalni put u osteoklastnim progenitorima potaknutim mišjim modelom reumatoidnoga artritisa

Mentor(s): Professor Danka Grčević, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Osteoclasts mediate periarticular and systemic bone loss in rheumatoid arthritis (RA). 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+CCR2+ cells, are specifically increased in arthritis. We previously identified an increased frequency of OCPs expressing Notch receptors in arthritic mice. Several studies suggested that Notch signaling modulation affects the course of experimental arthritis. We aimed to determine the effects of Notch receptor signaling inhibition on OCP activity and arthritis severity in murine collagen-induced arthritis (CIA).

Materials and methods: Male C57/Bl6 and DBA mice were immunized with chicken type II collagen and treated with i.p. injections of anti-Notch 1 neutralizing antibodies (1mg/kg). Notch receptor 1 through 4 expression on OCPs was analyzed by flow cytometry in periarticular bone marrow (PBM) and spleen (SPL). Gene expression of Notch receptors, ligands and transcription targets as well as osteoclast differentiation genes RANK, cFos and cFms was determined by qPCR from tissues and sorted OCPs. FACS sorted OCPs were stimulated by osteoclastogenic factors (M-CSF and RANKL), in control, IgG, Jagged (Jag)1 or Delta-like (DLL)1 coated wells, with or without anti-Notch 1 antibodies. 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 25% and 40% positive OCPs in PBM and 35% and 20% in SPL respectively), with a significant increase of Notch 2 expression in arthritis. Seeding OCPs on DLL1 coated wells significantly increased while seeding on Jag1 coated wells significantly decreased osteoclastogenesis as reflected on the number of TRAP+ osteoclasts and expression of osteoclast differentiation genes. The addition of anti-Notch 1 antibodies to ligand-stimulated OCPs resulted in an increased number of TRAP+ osteoclasts, partially reversing Jag1 inhibition. In vivo treatment with anti-Notch 1 antibodies did not affect total OCP frequency, but increased expression of Notch 4 both in PBM and SPL as seen by flow cytometry and qPCR. Additionally, anti-Notch 1 treatment stimulated Notch transcription factors HES and HEY. Both PBM and SPL cultured OCPs from anti-Notch 1 treated mice produced a higher number of large TRAP+ osteoclasts, doubling the area covered with osteoclasts compared to untreated mice. Increased osteoclastogenesis in vitro was further confirmed by an increased expression of osteoclast differentiation genes in the treated group.

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 anti-Notch 1 neutralizing antibodies enhanced osteoclastogenesis in CIA model, implying an inhibitory role of Notch 1 signaling in osteoclast differentiation. As Notch 2 expression is increased on OCPs of arthritic mice, we next plan to determine the effects of Notch 2 neutralization on osteoclast activity and arthritis severity.

MeSH/Keywords: collagen-induced arthritis; osteoclast progenitor; Notch; DLL1; Jag1

Poster code: R-01-06-047

Poster Title: Adult upper cortical layers marker CUX2 in neurons of the transient cellular compartments of the developing human brain

PhD candidate: Terezija Miškić

Part of the thesis: Transcription factor CUX2 and post-transcriptional factor CELF4 in neurons of synapse-enriched layers during human fetal corticogenesis

Mentor(s): Associate Professor Željka Krsnik, MD PhD, Associate Professor Mladen-Roko Rašin, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: CUX2 is a homeobox gene expressed in the neurons of differentiated upper cortical layers in the adult cerebral cortex. Until now, CUX2 expression pattern throughout human fetal cortical development was not profoundly studied, although it has been shown in the human SP during the late midgestation. Possible CUX2 role in rodents is affiliated with synaptic development and molecular specification, while its role in human cortical development is mostly unknown. This PhD thesis is focused on the molecular and laminar characterization of CUX2 neuronal populations within the human cortex and possibly exploring its role during neurodevelopment.

Materials and methods: CUX2 protein and RNA expression patterns are identified using immunohistochemistry, immunofluorescence, and RNA-scope on formalin fixed paraffin-embedded of postmortem human brains from 10 to 38 post-conceptional weeks (PCW) of the Zagreb Neuroembryological Collection. Coronal sections were cut through the cerebral hemisphere at the level of the striatum, either in its rostral part or alternatively, the closest midlateral cortex that was available. To characterize CUX2 cellular populations, different molecular markers were used, including Reelin and Calretinin to visualize marginal zone (MZ) cells, and MAP2 and Neuroserpin to visualize differentiated subplate (SP) cells.

Results: In the early fetal phase of cortical development (12-13 PCW), CUX2 positive cells were present in the pre-subplate, SP in formation, and the upper third of the marginal zone (MZ), evenly spaced in the tangential direction and partially co-localizing with Reelin (Cajal-Retzius cell marker). During the midfetal development (15-25 PCW) CUX2 positive cells were found in the MZ, SP, and likewise, strong protein expression was shown in the cortical plate (CP). In the newborn neocortex, CUX2 protein was expressed in the cells of gyral white matter (WM), along with the cells of layers I-III, and SP remnant. In addition, CUX2 RNA expression was shown in the adult PFC in the upper layers of the post-SP phase. In summary, besides being expressed in migratory neurons of prospective upper cortical layers, CUX2 was expressed in the diverse subpopulation of transient postmigratory SP and MZ neurons of the human cortex.

Discussion: We showed that CUX2 is expressed in the migratory projection neurons destined for the upper cortical layers during their migration within the transient fetal compartments. Besides, we have found CUX2 expressed in the postmigratory, the earliest differentiated neuronal population of the two transient cortical compartments: SP and MZ. Dynamic changes in the population of neurons that do not belong to the characteristic CUX2-expressing upper cortical layers suggest the developmental, multifunctional role of CUX2 during prenatal human corticogenesis. Given the Cux2 functions reported in rodents, we suggest that the expression of CUX2 in postmigratory SP and MZ neurons in human is involved in the processes of molecular specification, axonal projection, dendritic development, and synaptogenesis.

MeSH/Keywords: human cortical development, layer markers, molecular specification

Poster code: R-01-08-011

Poster Title: Moderate perinatal hypoxia in the neonatal rat causes lifelong changes at the molecular, structural, and behavioral levels

PhD candidate: Sara Trnski

Part of the thesis: Promjene perineuronskih mreža u mozgu štakora nakon kontrolirane kratkotrajne perinatalne hipoksije

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

Affiliation: University of Zagreb School of Medicine

Introduction: This research aims to investigate the effect of moderate non-invasive perinatal hypoxic injury in young and adult rats at the level of brain histoarchitectonics, the occurrence of inflammatory processes and cell degradation, the behavioral outcome, and changes in the composition and structure of perineuronal nets (PNN's) and parvalbumin interneurons (PV), which to our knowledge have not yet been investigated.

Materials and methods: In the randomized study, 86 Wistar Han rats were subjected to general hypoxia (pO2 73mmHg/2h) or normoxia, 24h after birth. A modification of the hypoxia-inducing protocol according to Kaur et al was made. Blood samples from the jugular veins taken immediately after hypoxic treatment were used for acid-base status analysis. For Western blot and IHC method rat brains at 2.5h, 8h, and 24h after hypoxic treatment were used. At young (P30) and adult (P70) age each animal was submitted to a battery of behavioral tests in the following order: open field, hole board, T-maze, social choice, with one day of a break between each experiment. After behavioral testing animals were sacrificed for analysis of the number of PNN's and PV neurons in coronal sections of the cingulate cortex at the levels bregma -1.56 mm -1.92 mm for the midcingulate area, and -2.04 mm -2.92 mm for the retrosplenial area. All statistical tests were conducted using Prism8 (GraphPad Software, Inc., La Jolla, CA, USA) and JMP 11.2 (SAS Institute Inc., Cary, NC, USA).

Results: Analysis of acid-base status shows a decrease in bicarbonate (HCO3-) and partial pressure of carbon dioxide in the blood (PCO2) and an increase in lactate, which indicates that general tissue hypoxia has occurred. The Western blot method showed a gradual increase of the hypoxia-inducible factor 1α, and a decrease of the cytochrome-C-oxygenase (subunit 4, isoform 1) expression, revealing moderate brain lesion in the rats subjected to hypoxia. Our results show that perinatal hypoxia causes early changes in morphology and distribution of microglial activity, especially in the cingulate cortex and subventricular zone, prominently 24h after hypoxic injury. The behavioral testing displayed significant hyperactivity and a slower pace of learning in the rats that were exposed to perinatal hypoxia. The closer examination of the cingulate cortex at the age of 4 months, by WFA- and anti-parvalbumin immunohistochemistry, disclosed a significantly increased number of PNN's and PV in the medial and retrosplenial areas.

Discussion: This research provides a better understanding of the relationship of a histological substrate (extracellular and cellular markers) of the mechanism of perinatal brain development damage after a short hypoxic episode in the perinatal period, and their connection with motor and cognitive-behavioral outcome. Further research on this new animal model of controlled short-term perinatal hypoxia brain damage in rats is expected to provide new insights into fetal and perinatal brain damage in the rodent brain and for comparative and translational studies of fetal and perinatal brain damage mechanisms in humans.

MeSH/Keywords: rain development, neuronal connectivity, cingulate cortex, hyperactivity

Poster code: R-01-08-030

Poster Title: Diversity of somatostatin neurons in the human prefrontal cortex

PhD candidate: Ivan Banovac

Part of the thesis: Molecular characteristics of supragranular layer interneurons in the human frontal cortex

Mentor(s): Dora Sedmak, PhD, research associate, Professor Zdravko Petanjek, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: GABAergic neurons in the cerebral cortex can be classified into three mostly non-overlapping populations – parvalbumin, 5-HT3A-receptor and somatostatin expressing cells. Other classifications of GABAergic neurons also exist, with a classification based on expression of calcium binding proteins (parvalbumin, calretinin and calbindin) being one of the most prevalent. A significant portion of the 5-HT3A-receptor expressing cell population also expresses calretinin, while the somatostatin cell population is thought to largely overlap with the calbindin cell population. Somatostatin expressing cells are found in all cortical layers, except layer I, and display a variety of phenotypes.

Materials and methods: Using double labelling immunofluorescence and RNAscope in situ hybridization we characterized the somatostatin cell population in the human prefrontal cortex (PFC). We used the following combinations of markers for immunofluorescence: anti-somatostatin with anti-NeuN or anti-calbindin antibodies. Using the RNAscope Multiplex Fluorescent assay we simultaneously visualized somatostatin mRNA and somatostatin peptide or calbindin protein. We analyzed histological slides from five adult specimens and evaluated the results in two regions of the PFC – Brodmann areas 9 and 14. There were no observable qualitative differences between the two areas and, therefore, the results presented are applicable to both regions of the PFC.

Results: After thorough qualitative analysis, we found that somatostatin cells in the supragranular layers were small (soma diameter of approximately 10 µm), had a high expression of somatostatin mRNA and a relatively low expression of somatostatin peptide. Furthermore, almost all of the supragranular somatostatin cells colocalized with calbindin, indicating that somatostatin and calbindin positive cells represent a single cell population in the supragranular layers. In the infragranular layers, somatostatin cells were large (soma diameter of approximately 20 µm) with complex dendritic morphology, typically had a lower expression of somatostatin mRNA and a high expression of somatostatin peptide, and did not express calbindin protein. All somatostatin cells were also NeuN positive, confirming that this is indeed a neuronal cell population.

Discussion: In conclusion, in the human PFC, supragranular and infragranular somatostatin cells represent two distinct subpopulations of somatostatin neurons that likely have significant functional differences and could be involved in different neural circuits.

MeSH/Keywords: GABAergic neurons, somatostatin, calbindin, prefrontal cortex, in situ hybridization, immunohistochemistry

Poster code: R-01-08-033

Poster Title: Automated Detection of Intraretinal Fluid, Subretinal Fluid, and Pigment Epithelial Detachment in Optical Coherence Tomography Images

PhD candidate: Marin Radmilović

Part of the thesis: Uloga bradikinina u razvoju dijabetičkog makularnog edema

Mentor(s): Professor Aleksandra Dugandžić, MD PhD, Professor Zoran Vatavuk, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Optical coherence tomography (OCT) is a critical diagnostic tool in retinal disorders, especially those affecting the macular area. In various exudative maculopathies, the presence of intraretinal fluid (IRF), subretinal fluid and hyperreflective material (SRF/SRHM), or pigment epithelial detachment (PED) indicates distinct disorders and directly reflects their exudative activity. The evaluation of these OCT changes in routine clinical practice is expert-dependent and only indirectly quantitative, as commercial OCT algorithms measure gross changes in overall retinal thickness, without precise differentiation and quantification of the underlying lesions. An automated process offering detection and quantification of these distinct changes would provide more precision in both clinical and preclinical (animal) research, and ultimately in clinical practice. The objective of this study is to investigate the applicability of a machine learning method for automatic detection of IRF, SRF/SRHM, and PED from a database of OCT images with exudative maculopathies.

Materials and methods: Spectral-domain OCT macular volumes of 23 eyes with exudative maculopathy were collected on a Cirrus HD-OCT 500 platform (Carl Zeiss Meditec AG, Jena). Raw images were extracted and 1136 selected B-scans were manually annotated in an open-source image manipulation program (GIMP) by an expert grader. The annotation involved: 1) tracing inner limiting membrane (ILM), retinal pigment epithelium (RPE) and Bruch membrane (BM) as layers relevant for precise localization of exudates and for retinal thickness measurement, and 2) tracing IRF, SRF/SRHM, and PED. A subset of 75 B-scans was reannotated by the same expert grader and annotated by a second expert grader to calculate the intraobserver and interobserver errors. The annotated scans served as a template for a machine learning algorithm. The algorithm was evaluated using a leave-one-out volume validation and Dice score calculation, and was compared against the inter-observer and intra-observer Dice score.

Results: The Dice scores of the algorithm, the inter-observer, and the intra-observer segmentation were: 0.692, 0.735, and 0.844 for IRF segmentation; 0.891, 0.876, and 0.924 for SRF/SRHM segmentation; and 0.866, 0.860, and 0.912 for PED segmentation.

Discussion: The IRF segmentation proved to be the least reliable in both manual and automated segmentation, but the algorithm came close to the inter-observer error. In SRF/SRHM and PED segmentation, the algorithm even outperformed the inter-observer Dice score. These results indicate that the proposed algorithm can be used for effective automatic detection and calculation of IRF, SRF/SRHM, and PED in OCT scans of eyes with exudative macular disorders.

MeSH/Keywords: Computer-Assisted Image Processing, Optical Coherence Tomography, Diagnostic Imaging, Retina

Poster code: R-01-08-038

Poster Title: The effect of ganglioside composition on the submembrane localization of sodium/potassium ATPase, calcium ATPase and neuroplastin in mouse brain

PhD candidate: Borna Puljko

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

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

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

Introduction: Gangliosides are sialic acid-containing glycosphingolipids that reside in the outer leaflet of the plasma membrane, with their localization being more robust in lipid rafts (LR). Gangliosides are known to modulate the structure, function and localization of membrane proteins. Being particularly abundant in nervous tissue, they play an important role in signaling events affecting neural development and the pathogenesis of certain neurodegenerative diseases. Aim of this preliminary study was to ascertain the effect of ganglioside composition on the sumbmembrane localization of electrogenic pumps, Na+/K+ ATPase (NKA) and plasma membrane Ca2+ ATPase (PMCA), and of synaptic glycoprotein neuroplastin (Np) across different membrane subdomains in brain tissue of mouse models with impaired synthesis of gangliosides. Np has been shown to co-localize with PMCA, thus having an impact on intracellular Ca2+ homeostasis.

Materials and methods: We used cortical tissue of infant St8sia1 null and adult B4galnt1 null mice and their corresponding wild-type (WT) littermates. Animals were sacrificed, cortices neuroanatomically dissected, and tissue homogenates were prepared. Membrane proteins were isolated from the homogenates of both null mouse models and their corresponding WTs, and Np65, NKA and PMCA expression was analyzed by Western blot followed by ImageLab quantification. LR and non-raft (nLR) membrane fractions were obtained by ultracentrifugation in discontinuous sucrose gradients, and submembrane localization of Np, NKA and PMCA was analyzed by Western blotting, followed by ImageJ quantification.

Results: Data gathered from the experiments revealed a different expression and localization pattern of Np, NKA and PMCA across LR and nLR membrane subdomains in cortical tissue of mice with altered ganglioside composition. The overall expression of all studied proteins was higher in St8sia1 null mice compared to WT mice with their localization being more robust in the nLR membrane subdomains. In B4galnt1 null animals the overall expression of all three proteins was lower than in WT mice, with the localization being more prominent in LR subdomains. Regarding the localization within the membrane, there is an observed difference even between WT animals, which we can attribute to the different age of the animals.

Discussion: Results of this preliminary study indicate a prominent shift for Np, NKA and PMCA between different membrane subdomains, depending on specific ganglioside composition and the age of the animals. We hypothesize that these proteins, especially Np, change submembrane localization during development and aging. This is especially interesting since ganglioside composition and concentration also change with aging. Our ongoing experiments will elucidate the expression and submembrane localization of Np, NKA and PMCA in animals of different age and in different stages of development. We propose that gangliosides may modulate the function of Np, NKA and PMCA, and thus contribute to maintenance of the ion homeostasis in nervous tissue.

MeSH/Keywords: glycosphingolipids; Na+/K+ ATPase; plasma membrane Ca2+ ATPase; neuroplastin; lipid rafts

Poster code: R-01-08-048

Poster Title: Novel transgenic mouse line for in vivo imaging of inflammation related Tlr2 promoter activity in Tlr2-deficient mice after ischemic brain lesion

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: University of Zagreb School of Medicine

Introduction: Tlr2 receptor on the surface of microglial cells activates microglia-mediated inflammation after ischemic lesion of the mouse brain. We developed a new transgenic mouse line with bioluminescent reporter for in vivo imaging of inflammation related Tlr2 promoter activity: C57BL/6-Tyrc-Brd-Tg(Tlr2-luc/gfp)Kri-Tlr2tm1Kir/Gaj. The aim of this study was to characterize neuroinflammation in the novel mouse line and to compare it with previously established mouse model of Tlr2-mediated inflammation after ischemic lesion of the brain.

Materials and methods: The study was conducted on 3 months old transgenic male mice strains with bioluminescent reporter: C57BL/6-Tyrc-Brd-Tg(Tlr2-luc/gfp)Kri/Gaj and C57BL/6-Tyrc-Brd-Tg(Tlr2-luc/gfp)Kri-Tlr2tm1Kir/Gaj (each group n=4). Mice underwent 30 min transient middle cerebral artery occlusion (tMCAO) followed by reperfusion. Before the surgery and 3 days after tMCAO, mice were examined for the neurological deficit and their brains were imaged by high-resolution magnetic resonance imaging (MRI; Bruker 7T BioSpec). In vivo bioluminescent imaging (BLI; Perkin Elmer IVIS Spectrum) was performed before the surgery and at day 2 after tMCAO using luciferin. At day 3 mice brains were isolated and immunohistochemistry was preformed using microglial markers Iba1, Tlr2, and bioluminescent marker luciferase (Luc).

Results: Bioluminescent signal for luciferin at the day 2 indicated increase of Tlr2 transcription after ischemia in both mouse strains. Novel mouse line C57BL/6-Tyrc-Brd-Tg(Tlr2-luc/gfp)Kir-Tlr2tm1Kir/Gaj had reduced intensity of BLI signal 2 days after brain ischemia compared to control strain C57BL/6-Tyrc-Brd-Tg(Tlr2-luc/gfp)Kir/Gaj. Also, novel mouse line had smaller size of ischemic lesion 3 days after MCAO in comparison to control strain. Immunohistochemistry showed differences in shape of microglia in both mouse strains between ipsilateral and contralateral brain hemisphere using microglial marker Iba1, but Tlr2 was detected only in control strain C57BL/6-Tyrc-Brd-Tg(Tlr2-luc/gfp)Kri/Gaj. Presence of luciferase was confirmed by immunohistochemistry in both mouse strains.

Discussion: Expression of Tlr2 promoter is highest 2 days after ischemic lesion. Winters et al. showed decreased expression of Tlr2 promoter in Tlr2-deficient mice after brain ischemia using qPCR method. We observed the similar effect in out novel mouse line using in vivo bioluminescence. Novel transgenic mouse line C57BL/6-Tyrc-Brd-Tg(Tlr2-luc/gfp)Kir-Tlr2tm1Kir/Gaj is a suitable model for in vivo monitoring of Tlr2 promoter activity regardless of presence of the Tlr2 receptor.

MeSH/Keywords: Ischemic Stroke, Mouse, Inflammation, Microglia, Toll-Like Receptor 2

Poster code: R-01-08-061

Poster Title: The role of uroguanylin's signaling pathways in ischemic stroke development

PhD candidate: Martina Ratko

Part of the thesis: Uloga urogvanilina u razvoju ishemijskog moždanog udara

Mentor(s): Professor Aleksandra Dugandžić, MD PhD

Affiliation: Laboratory of cellular neurophysiology, Croatian Institute for Brain Research, Centre of Excellence for Basic, Clinical and Translational Neuroscience, School of Medicine, University of Zagreb

Introduction: Stroke is one of the leading causes of mortality and disability in industrialized countries. The vast majority of these patients experience ischemic stroke and, consequently, many studies focus on characterizing key pathological changes that underlie this disease. Recent research has shown that the activation of guanylate cyclase (GC)-A leads to a decrease of brain lesion size following middle cerebral artery occlusion (MCAO), while the same was not confirmed for GC-B. Since uroguanylin (UGN) activates guanylate cyclase C (GC-C) in neurons and a Ca^{2+}-dependent but GC-C-independent signaling pathway in astrocytes, the aim of this study was to investigate the potential role of UGN and its two signaling pathways in the development of ischemic stroke.

Materials and methods: In this study, MCAO was performed on mice lacking either UGN (UGN KO) or GC-C (GC-C KO) and their wildtype (WT) littermates. Volumetric analyses of lesion and oedema sizes were calculated from MR images taken before and 24 h after stroke induction. 48 h after stroke, increase in intracellular Ca^{2+} concentration upon UGN stimulation was recorded on astrocytes in brain slices of ipsilateral and contralateral hemispheres. Neurological impairment due to stroke was assessed 24 h and 48 h after MCAO. Immunohistochemical staining of brain slices was performed with GC-C, NeuN, and GFAP antibodies.

Results: GC-C KO, but not UGN KO, mice developed smaller lesions compared to their WT littermates. WT animals exhibited a stronger Ca^{2+} response to UGN stimulation in the ischemic penumbra than in similar regions of the cortex of the healthy hemisphere. This stronger activation was gone in GC-C KO animals because, when compared to their WT littermates, there was a statistically significant reduction in Ca^{2+} signaling in the ischemic penumbra. UGN KO animals showed the same intensity of Ca^{2+} response as WT animals in the area affected by stroke, but the signal lasted longer. Immunohistochemical staining showed GC-C expression solely on neurons in the healthy hemisphere, but GC-C became expressed in astrocytes in ischemic penumbra of WT and UGN KO animals.

Discussion: GC-C KO animals develop smaller ischemic lesions compared to their WT littermates, suggesting that activation of GC-C is not neuroprotective as shown for GC-A. The possible reason is that WT and UGN KO animals exhibit a stronger Ca^{2+} response to UGN stimulation in the ischemic penumbra while this stronger activation is not present in GC-C KO animals. Since GC-C becomes expressed on penumbral astrocytes where both signaling pathways for UGN exist, one possible explanation is that UGN activates cGMP-dependent Ca^{2+} channels. This hypothesis is supported by similar changes in Ca^{2+} response in UGN KO and their WT littermates accompanied with no difference in stroke volume. GC-C-dependent increase in intracellular Ca^{2+} concentration in astrocytes leads to the development of larger ischemic lesions. However, the effects of the GC-C-independent Ca^{2+} signaling pathway seem to be irrelevant for stroke development.

MeSH/Keywords: guanylate cyclase C, middle cerebral artery occlusion, calcium signalling, MR imaging, astrocytes

Poster code: R-01-08-063

Poster Title: Mouse stroke model by occlusion of the middle cerebral artery – collection of samples and first observations

PhD candidate: Damir Lisjak

Part of the thesis: Uloga živčanih matičnih stanica u kontroli regulirane stanične smrti nakon ishemijskog oštećenja mozga miša

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

Affiliation: University of Zagreb School of Medicine

Introduction: One of the most common cause of death and disability in the world is ischemic brain stroke. This condition is caused by decrease or complete interruption of blood flow due to blockage of the arteries. Hypoperfusion leads to hypoxia and hypoglycaemia, followed by oxidative stress, excitotoxicity, inflammation, and cell death. The main goal of our research is to compare brains of mice affected by stroke which received transplants of neural stem cells to their controls.

Materials and methods: Standardised method of middle cerebral artery occlusion (MCAO) for 30 min was performed to cause ischemic stroke in C57/BL6 mice. Neural stem cells (NSC) were isolated from telencephalic wall of 14 days old embryos, cryopreserved in liquid nitrogen, and after thawing, passaged two times before transplantation. Transplantation was performed by stereotaxic device (Kopf 9000LS) into the striatum. Lesion and edema volume were measured in vivo by MRI imaging system (Bruker BioSpec 70/20 USR). Before every MRI session, neurological status assessment was performed on every animal. Eighty (80) animals were divided into four groups: A-Sham, B-MCAO, C-MCAO + NSC complete cultivation medium, D-MCAO + NSC. Half of each group was sampled at day two after MCAO, and other half at day five after MCAO. For the purpose of q-PCR and Western blot fresh tissue was obtained from animals, divided into ipsilateral and contralateral hemispheres, snap frozen and stored at -80°C. Hemispheres were pulverised in liquid nitrogen using cell crusher, lysed in convenient buffer and stored at -20°C prior to analysis.

Results: Fifty- one (51) animals were divided in four groups: A – 20, B – 15, C – 6 and D -10. Performed MRI scanning revealed that one day after surgery lesion and edema were present in cortex and striatum. From all animals brain hemispheres were isolated, homogenised in liquid nitrogen and stored at -80°C in form of powder. Protein lysates were prepared from ipsilateral hemispheres of B-MCAO group. Western blot analysis performed on days 2 and 5 revealed that GFAP was upregulated, while MAP2 was downregulated. Interestingly, we observed that animals which underwent stereotaxic surgery after stroke, both in groups C and D, recovered faster than animals affected by stroke.

Discussion: Here we present our first observations after performing operations and collection of tissue from 51 mice. One day after MCAO, MRI revealed that stroke regularly affects region supplied by the middle cerebral artery. We have not seen unexpected lesion distribution. First results with markers Map2 and Gfap revealed that reactive gliosis and neuronal degradation is occurring from day two towards day five in brain affected by ischemic stroke. Most interestingly, it seems that skull drilling for stereotaxic procedure helps to reduce intracranial pressure caused by stroke edema and helps animals to recover faster.

MeSH/Keywords: ischemic brain stroke, regulated cell death, neural stem cells, regenerative therapy

Poster code: R-01-08-064

Poster Title: Optimization of neural stem cell differentiation to neurons and astrocytes

PhD candidate: Valentina Hribljan

Part of the thesis: Utjecaj matičnih stanica na nekroptozu stanica živčanog sustava in vitro uzrokovanu hipoksijom

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

Affiliation: University of Zagreb School of Medicine

Introduction: Diseases affecting the nervous system, which include neurodegenerative diseases and stroke, affect millions of people worldwide and represent a big burden to every nation's health system. In order to make needed scientific progress, it is critical to have a reliable disease model. Here we are presenting a model based on precise optimization of the protocol for differentiation of neural stem cells to neurons and astrocytes. This approach allows performing experiments on the effects of hypoxia on the cells of the nervous system.

Materials and methods: Neural stem cells (NSC) were isolated from the telencephalon of 14-day old mouse embryos and cultured in suspension in a growth medium containing DMEM/F12, 1 % antibiotic penicillin/streptomycin (P/S), 1 % N2 and 2 % B27 supplements, growth factors 20 ng EGF (epidermal growth factor) and 10 ng FGFb (basic fibroblast growth factor). As NSC divide, they stay together forming neurospheres that are dissociated into single cells using enzyme mix accutase when they reach 150-200 um in diameter. Single cells generated by dissociation can be seeded for further passage, or differentiation in plates coated with poly-D-lysine and laminin – components of extracellular matrix. Two media for differentiation were used – medium 1 (M1) composed out of DMEM/F12, 1 % P/S, 1 % N2, and 2 % B27 plus supplements; and medium 2 (M2) containing everything as M1 with the addition of 1 % fetal bovine serum (FBS). Cells were fixed with 4 % paraformaldehyde (PFA) at days 1, 4, and 8 of differentiation. After 3-4 days in culture, half of the medium was exchanged. These media were of the same composition as written for M1 and M2, except Neurobasal was used instead of DMEM/F12. Immunocytochemistry (ICC) was performed using primary antibodies against Nestin (a marker of neural stem cells), MAP2 (microtubule-associated protein 2, component of cytoskeleton found in dendrites of neurons), panaxonal neurofilaments (neurofilament light, medium, and heavy chain found in axons of neurons), and GFAP (glial fibrillary acidic protein found in astrocytes). DAPI was used to stain nuclei. Images were taken using a confocal microscope.

Results: After 24 h of differentiation (day 1), cells were >95 % Nestin positive in both media (M1 and M2) and they exhibited similar morphology. After 2-3 days in culture, we observed increased cell death in cells grown in M1, but not in M2. We repeated the experiment three times, and each time we made the same observation. The difference was visible in ICC images on days 4 and 8: cells cultivated in M1 contained a lot of fragmented nuclei compared to cells grown in M2. In M2 cell death was a much more rare event. As a consequence, the density of both astrocytes and neurons was greater in M2. Neurites of neurons in M2 were longer than those ones observed in M1 – that was the case both with dendrites (MAP2) and axons (pan axonal neurofilament).

Discussion: By comparing two different media compositions, we have found the medium which allowed us to differentiate NSC to neurons and astrocytes with a satisfactory level of survival and morphology. This step was critical since cell cultures cultivated using "a routine" approach described in the literature exhibit cell death which then covers the effects of hypoxia. This model enables us to generate a sufficient number of neurons and astrocytes in vitro, in comparison to primary neuronal cultures, which then allows us to perform a larger amount of experiments with a smaller number of animals sacrificed.

MeSH/Keywords: neural stem cells, differentiation, neurons, astrocytes

Poster code: R-01-08-068

Poster Title: Analyses of mitophagy caused by hypoxia during differentiation of the neural stem cells

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: Cells damaged by external factors, such as hypoxia, can improve their survival by help of the repair mechanisms. Autophagy is one of the most effective cellular repair mechanism. Mitophagy is a specific form of autophagy that participates in processes of mitochondrial fusion and fission. These actions are described during cell differentiation and during cellular damage repair. One of the most important pathways by which mitophagy is activated includes proteins Parkin and FUNDC1. Parkin acts as E3 ubiqutin ligase and plays an important role in ubiquitination of outer mitochondrial membrane proteins. FUNDC1 is outer mitochondrial membrane protein that initiate mitophagy of hypoxia damaged cells. Here we report investigation of influence of a short term hypoxic damage on levels of mitophagy in differentiating cells of the nervous system.

Materials and methods: To investigate influence of hypoxia and mitophagy on developing cells of the nervous system we used neural stem cells obtained from the telenchephalic wall of 14.5 days old mouse embryos. Neural stem cells were cultivated in differentiation medium for 7 days. On day 7 they were exposed to 1% of oxygen for 6 hours followed by 4 hours of reoxygenation and then analyzed using immunocytochemistry. Mitochondria were stained with Tomm20, marker specific for mitochondrial outer membrane, while levels of mitophagy were estimated by detecting levels and localization of Parkin. To evaluate amount of FUNDC1, we performed Western blot analysis of days 1, 5 and 7 of differentiation under normoxic and hypoxic conditions. Quantification and statistical analysis were obtained using CellProfiler, Imaris, ImageJ, and GraphPad Prism software.

Results: In cells of the nervous system affected by hypoxia on day 7 of their differentiation significantly increased levels of both Parkin and Tomm20 were detected. Parkin in cells affected by hypoxia showed tendency of forming large protein complexes, which were typically present at the outer membrane of mitochondria. Redistribution of Parkin to outer membrane of mitochondria was accompanied by changes in mitochondrial morphology. Results of Western blots quantification revealed that FUNDC1 increases during differentiation of the neural stem cells. Various stages of differentiation react in various ways in regard to activation of FUNDC1 after hypoxic damage.

Discussion: Here we are shedding light on the involvement of Parkin and FUNDC1 in mitophagy. Our results suggest that Parkin, one of the central elements of ubiquitin ligase complex reacts very early to hypoxic damage and starts marking mitochondria, most probably destined for degradation. Also, it has been shown that the expression of FUNDC1 is increased in all time points under hypoxic condition as well as after hypoxic reperfusion injury. Since FUNDC1 directly interacts with LC3 fragment of autophagosome, we suggest that it also plays an important role in activation of mitophagy during differentiation of the nervous system.

MeSH/Keywords: stem cells, autophagy, mitophagy, hypoxia, Parkin, FUNDC1

Poster code: R-01-08-070

Poster Title: Association of receptive music therapy with plasma levels of epidermal growth factor, interleukin-8 and neurofilament light chain in women with breast cancer

PhD candidate: Brigita Vilč

Part of the thesis: Association of receptive music therapy with plasma levels of epidermal growth factor, interleukin-8 and neurofilament light chain in women with breast cancer

Mentor(s): Associate Professor Marina Šagud, MD PhD, Professor Goran Šimić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Applying complementary interventions such as receptive music therapy (MT) can serve as a powerful multidisciplinary approach in cancer care. Music listening creates an avalanche of cognitive and emotional reactions, which intensifies neural activity involving specific regions in both hemispheres and enhances functional connectivity. The modulatory effect of music is evident in limbic and paralimbic structures, particularly the amygdala, hippocampus, ventral striatum and nucleus accumbens but also in midbrain and orbitofrontal cortex. The functional connectivity of these areas is disrupted in anxiety and depression. In the diagnosis of depressive disorder, elevated levels of the cytokine IL-8 in the blood and CSF may serve as diagnostic markers. Also, high level of IL-8 has been associated with the invasiveness of the malignant process. EGF plays a role in cancer risk and disease prognosis. The changes in EGF levels are also seen in anxiety and depression disorders. High levels of neurofilaments represent a general index of axonal damage. Their plasma levels thus can be used to monitor or predict the state of disease or the effectiveness of a particular therapeutic procedure. Altogether, plasma EGF, IL-8, or NfL levels may reflect an underlying mechanism affecting molecular pathways involved in generation of anxiety and depression symptoms in women with breast cancer.

Materials and methods: This study will be a randomized clinical trial for a hundred women with breast cancer, 18-65 years of age, during the perioperative period and on adjuvant chemotherapy. In the intervention group the receptive MT will be applied through listening to music using a music player and headphones; perioperatively twice a day for 30 minutes (during 3 days), and while receiving chemotherapy for 1 hour (12-24 weeks, depending on the type of cytostatics used - anthracyclines or taxanes). In the control group, the standard treatment and care will be provided, but without MT. At three time points, anxiety and depression symptoms and quality of life will be measured using the Hospital Anxiety and Depression Scale, Beck Depression Inventory-II, EORTC QLQ-C30, and Adaptive Functions of Music Listening Scale, simultaneously with measurements of the plasma levels of EFG, IL-8, and NfL. Instrumental classical music will be used, with a track database set by researcher and selection depending on the preferences of the participants (volume 65-80 dB), respecting the following features: tempo 60-80 bpm, continuous rhythm, gentle melodic lines, and consonant harmonic progressions.

Results: Due to COVID-19 pandemic and sensitive health of participants, it was unfortunately not possible to start the research in the previous period.

Discussion: Due to COVID-19 pandemic and sensitive health of participants, it was unfortunately not possible to start the research in the previous period, but we expect the following scientific contribution. Receptive music therapy affects the levels of EGF, IL-8, and NfL in plasma during the perioperative period and chemotherapy in women with breast cancer, and on the symptoms of anxiety and depression associated with the malignant process, as a basis for the future implementation of receptive music therapy in the complementary treatment of breast cancer.

MeSH/Keywords: music therapy, breast cancer, epidermal growth factor, interleukin-8, neurofilament light chain, anxiety, depression

Poster code: R-01-08-072

Poster Title: The effects of bradykinin type 2 receptor deficiency on ischemia-reperfusion injury of the brain and retina in diabetes mellitus

PhD candidate: Anja Barić

Part of the thesis: The effects of diabetes mellitus on the development of edema and microglial response in a mouse model of cerebral and retinal ischemia by changing the expression of bradykinin receptors

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

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

Introduction: Diabetes mellitus (DM) increases the risk of ischemia development in the brain and retina, and exacerbates the ischemic damage resulting in poor recovery and increased mortality. Ischemia triggers the release of bradykinin (BK), an inflammatory mediator considered to have a detrimental effect in the acute phase of both brain and retinal ischemia. The activation of the BK signaling pathway is modified in the presence of DM. The aim of this study was to determine the effects of bradykinin type 2 receptor (B2R) deficiency in the acute phase of brain and retinal ischemia in diabetic mice.

Materials and methods: In order to induce simultaneous brain and retinal ischemia, male diabetic C57Bl/6-Ins2Akita/J (Akita) mice, diabetic B2R knockout B6.Cg-Ins2Akita/Bdkrb2tm1Jfh/SmiJ (Akita/B2R-KO) mice and their non-diabetic controls C57Bl/6J (WT) and B6.129S7-Bdkrb2tm1Jfh/J (B2R-KO) were subjected to a 30-minute intraluminal middle cerebral artery occlusion (MCAO). Blood glucose concentration and HbA1C levels were measured to confirm DM. Additionally, blood and intraocular pressure were measured before surgery. Seven days prior, and on the 1st and 3rd day after MCAO the animals were scored for neurological deficit followed by fundus photography, fluorescein angiography and magnetic resonance imaging (MRI) using the 7T BioSpec 70/20 US system. The obtained images were analyzed in ImageJ.

Results: B2R deficiency had no effect on blood glucose concertation, HbA1C levels, blood or intraocular pressure compared to controls. However, B2R deficiency resulted in significantly higher body weight in diabetic Akita/B2R-KO compared to Akita mice. In the first three days after MCAO, there were no differences in survival or ischemic lesion size between groups. The MRI volumetric analysis of the 1st and the 3rd day after ischemia induction showed prominent swelling of both ipsilateral hemisphere and the measured chorioretinal layer of the ipsilateral eye caused by vasogenic edema formation. In the case of brain ischemia, non-diabetic B2R-KO mice showed less ipsilateral hemisphere tissue swelling on the 1st day, which was reflected in ameliorated neurological status compared to their controls. Neither diabetic nor non-diabetic B2R deficient mice showed edema resolution on the 3rd day, which was evident in both Akita and WT group. Similarly, by the 3rd day the swelling of the chorioretinal layer of the ipsilateral eye persisted only for the non-diabetic B2R deficient mice, while the chorioretinal thickness in other groups partially reverted towards baseline levels. B2R deficient mice showed severe necrosis and partial loss of the capillary network caused by retinal ischemia.

Discussion: Our preliminary results show that during the first three days after MCAO the changes caused by ischemia with subsequent reperfusion correlate between the retina and the brain. There was no significant difference in ischemic lesion size between groups. In non-diabetic animals, B2R deficiency lead to better neurological status and less ipsilateral brain tissue swelling while at the same time it exacerbated edema formation on the 1st day after ischemic induction in the retina. These opposing effects of B2R deficiency were no longer obvious when diabetes was present alongside ischemia. However, in the brain of both non-diabetic and diabetic B2R deficient animals we observed an extended period of edema retention compared to controls. In conclusion, the effects of B2R deficiency in the acute phase of brain and retinal ischemia are partially concealed in the presence of DM.

MeSH/Keywords: diabetes mellitus, bradykinin receptor type 2, brain and retinal ischemia

Poster code: R-01-08-082

Poster Title: Influence of Toll-like receptor 2 deficiency on neuroplastin and ATPases expression in mouse brain

PhD candidate: Mario Stojanović

Part of the thesis: Utjecaj nedostatka receptora sličnog Tollu 2 na izražaj neuroplastina i ATPaza u mozgu miša

Mentor(s): Professor Svjetlana Kalanj-Bognar, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Transmembrane proteins, neuroplastin (Np), and Toll-like receptor 2 (TLR2) have distinct roles during different stages of brain development and maturation. Np isoforms (Np55 and Np65) are related to synaptic plasticity and neuronal ion homeostasis by interactions with plasma membrane Ca2+ ATPases (PMCA). TLR2 is mostly expressed on microglia, but a much broader cellular localization of TLR2 during brain development has been established. As both Np and TLR2 are implicated in neuro-architecture, we hypothesize that the lack of TLR2 affects membrane lipid-protein environment and aim to clarify a potential interplay of selected membrane proteins by analyzing neuronal membrane phenotype in TLR2 knock-out (KO) mice.

Materials and methods: Tissue samples of cortex, cerebellum, and hippocampus derived from male TLR2-KO and age-matched control (C) mice (N=36+36) were used for systematic biochemical profiling encompassing protein and glycolipidomic analyses. The Western blot (WB) method investigated the expression of Np55, Np65, PMCA, Na+/K+-ATPase (NKA). Synaptosome enriched fractions were analyzed by liquid chromatography with tandem mass spectrometry (LC-MS/MS) to address the synaptic proteome modifications. Isolation of membrane fractions enabled detecting the shifts in protein distribution across lipid rafts (LRs) and bulk membrane (nonLRs) in TLR2-KO vs C brain tissue. The ion exchange capacity of P-type ATPases, NKA, and PMCA, was determined by spectrophotometric activity assay. Transcriptional variations of selected genes were evaluated by qPCR. Tissue sections were analyzed by immunofluorescence and confocal microscopy. Finally, gangliosides were extracted, purified, and separated with high-performance thin-layer chromatography.

Results: MS analysis of synaptic proteome revealed astonishing differences affecting numerous sets of protein systems, particularly those implicated in neurotransmission, pre-/post-synaptic arrangements, energy metabolism, axon/dendrite cytoskeleton, and myelin sheath modifications. WB revealed increased Np55, Np65, and NKA signal in cortex, cerebellum, and hippocampus in TLR2 vs C. These findings were confirmed at a transcriptional level using qPCR. WB analysis of total PMCA and 4 PMCA isoforms showed that lack of TLR2 is associated with increased expression of PMCA in the cortex, PMCA3 in the cerebellum, and PMCA2 in the hippocampus. Lipid raft isolation revealed significant shifts in protein distribution between LRs and bulk membranes. In TLR2-KO cortex as compared with controls, PMCA 1 and 2, and NKA, have been observed to localize more abundantly in LRs; synaptophysin was more abundant in both LRs and nonLRs while GluR2 was dramatically dislocated from LRs. NKA activity was found to be lower in the cortex and unchanged in hippocampal tissue, while PMCA activity was decreased in the hippocampus of TLR2 KO vs C. Preliminary analysis of tissue sections by immunofluorescence and confocal microscopy indicates changes of Np expression corresponding to WB and LC-MS/MS data.

Discussion: We found that TLR2 deficiency leads to alterations of expression, localization, and function of proteins associated with synaptic plasticity and ion homeostasis. Data obtained by multiple level analysis approaches suggest that changes are related to Np and P-type ATPases abundance but also encompass synaptic milieu, particularly glutamatergic transmission system, axon, and dendrite cytoskeleton organization and mitochondrion energy metabolism. Further investigation may clarify previously unknown roles of TLR2 in neuron-microglia interactions, synaptic connections arrangement, and neurotransmission.

MeSH/Keywords: Neuroplastin, Toll-like receptor 2, synaptic transmission, synaptic plasticity, ion homeostasis

Poster code: R-01-08-104

Poster Title: The effects of bradykinin receptor type 2 deficiency in different stages of brain and retinal ischemia development

PhD candidate: Helena Justić

Part of the thesis: Uloga bradikininskog receptora tipa 2 u razvoju ishemijske ozljede mozga i mrežnice

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

Affiliation: University of Zagreb School of Medicine

Introduction: The exact role of bradykinin as one of the key inflammatory mediators released during brain and retinal ischemia is still unknown. Previous studies showed both detrimental and protective effects of bradykinin receptor type 2 (B2R) activation over the course of the ischemic lesion progression. The aim of this study was to clarify the role of B2R in brain and retinal ischemia by longitudinal in vivo multimodal assessment of cerebral and retinal ischemic injury.

Materials and methods: In order to induce simultaneous ischemic injury of the brain and retina, 4 month old male C57Bl/6J (WT) and C57Bl/6J/Bdkrb2tm1Jfh/SmiJ (B2R-KO) mice underwent a 30-minute middle cerebral artery occlusion (MCAO) by filament insertion through the common carotid artery. Seven days before and 2, 9, and 35 days after MCAO the animals were scored for neurological deficit, subjected to fundus photography and fluorescein angiography, and imaged using a 7T BioSpec 70/20 USR magnetic resonance (MR) system. The scans included a high-resolution T2-weighted anatomical scan and a T2-map scan of the brain and the ipsilateral eye. After the last imaging session, the brains and eyes were isolated and processed for histological analysis.

Results: MCAO produced big ischemic lesions encompassing the cortex and striatum in both groups, which were characterized by vasogenic edema formation in the acute phase, followed by resolution of edema by day 7 and severe tissue loss in the chronic phase, significantly more pronounced for B2R-KO. The changes in measured chorioretinal thickness correlated with the progression of brain ischemia, showing thickening 2 days after MCAO, followed by thinning in the chronic phase. There was no difference in the MR measured chorioretinal thickness between the two animal groups. Fundus photography and fluorescein angiograms visualized necrotic regions, loss of capillary network and thinning of the vessels after ischemia. Hematoxylin/eozin stained retinal slices demonstrated morphological disruptions of the ipsilateral retinas with pronounced loss of cells in the ganglion cell layer and thinning of the plexiform layers 35 days after MCAO.

Discussion: Although previous studies showed diverse effects of B2R activation after ischemic injury, the exact long-term effects of B2R activation were not elucidated. Using longitudinal multimodal in vivo MR assessment, this study showed that B2R deficiency leads to poorer recovery and significant brain tissue loss compared to control animals, supporting the evidence of protective B2R actions in the chronic phases of ischemic brain injury. The retinal responses to ischemia corresponded to those in the brain and demonstrated necrotic lesions, edema formation and vascular changes, supporting the evidence of simultaneous ischemia of the brain and retina.

MeSH/Keywords: Bradykinin, ischemia, brain, retina

Poster code: R-01-08-108

Poster Title: The effect of Tlr2-mediated inflammation on apoptotic cell death after ischemic lesion of the mouse brain

PhD candidate: Paula Josić

Part of the thesis: Utjecaj upale na odumiranje stanica procesom apoptoze nakon ishemijske lezije mišjeg mozga

Mentor(s): Professor Srećko Gajović, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Ischemic stroke is a leading global health problem with a need for improvement of therapeutic strategies. Previous research shows that substantial and long-term induction of Toll-like receptor 2 characterizes the innate immune response after ischemic stroke. However, it is still unclear whether the induction of immune response after ischemia is protective or detrimental. Our hypothesis is that the reduction in immune response after ischemia increases apoptosis. Therefore, the aim of this research is measure the scope of apoptosis after ischemic stroke in vivo and to determine the effect of Tlr2-mediated inflammation on apoptosis.

Materials and methods: In this study, 12 wild type and 11 Tlr2-/- mice with ubiquitous luciferase expression were used. Neurological deficiency scoring, bioluminescence and magnetic resonance imaging were conducted for all mice at baseline, 2, 7, 14 and 28 days post ischemic stroke. Ischemic brain lesion was induced with transient middle cerebral artery occlusion. T2-weighted and T2 map sequence scans were performed on 7 T MRI machine. Apoptosis was measured using bioluminescence with caged Z-DEVD-aminoluciferin substrate. Immunohistochemistry was performed on brain cryostat sections with NeuN and cleaved caspase-3 antibodies. For statistical analysis the Shapiro-Wilk normality test, two-way ANOVA, and Tukey or Sidak post-hoc analysis were used. Statistical significance was defined as $p < 0.05$.

Results: To determine the extent of apoptosis, bioluminescence with caged Z-DEVD-aminoluciferin in Tlr2-deficient mice was compared to that of wild type mice. To measure stroke volumetry post-surgery, magnetic resonance imaging was performed. On day 2 post stroke, Tlr2-/- mice had significantly less tissue swelling than wild type controls. On days 14 and 28 post stroke, Tlr2-/- suffered a statistically significant larger loss of ipsilateral tissue compared to wild type controls. There were no statistically significant differences in neurological deficits between the two groups. Furthermore, Tlr2-/- animals had better survival, with four living to day 28, in contrast to only one survivor among the control group. In both groups, bioluminescent signal decreased after ischemic lesion compared to baseline values, increasing again on day 4 for the Tlr2-/- group and on day 7 for wild type group. Differences in total bioluminescence flux were not significant between groups.

Discussion: Tlr2-/- mice have a reduced Tlr2-mediated immune response, while the wild type controls have a normal Tlr2-mediated immune response. Therefore, the significantly reduced swelling on day 2 post-stroke in Tlr2-/- mice compared to wild type controls can be explained by a weaker immune response to stroke induction in Tlr2-/- mice. However, the significantly larger ipsilateral tissue loss in Tlr2-/- mice on days 14 and 28 post-stroke suggests that there is a mechanism by which normal inflammation preserves tissue. Better survival of Tlr2-/- animals possibly suggests that either smaller initial lesion volumes or reduced inflammation and its following effects, or a combination of all, contribute to survival through the chronic phase post-stroke.

MeSH/Keywords: bioluminescence, caged Z-DEVD-aminoluciferin, apoptosis, stroke, inflammation

Poster code: R-01-08-110

Poster Title: Split luciferase reporter system for the detection of human tau protein oligomerization in living yeast cells

PhD candidate: Klara Zubčić

Part of the thesis: Utjecaj proteotoksičnog stresa na agregaciju i toksičnost tau proteina čovjeka izraženog u kvascu Saccharomyces cerevisiae

Mentor(s): Mirta Boban, PhD, research associate, Professor Goran Šimić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the accumulation of tau protein aggregates in cells of the affected brain regions and the consequent neuronal death. The formation of large aggregates is preceded by early-stage tau oligomers, however the molecular pathways that initiate tau aggregation are unclear. To better understand early steps in tau pathology, we aimed to use a tool for studying tau oligomerization in living cells, based on the luminescent reporter NanoBiT, in which protein-protein interaction results in the complementation of the luciferase NanoLuc, and consequently in generation of luminescence. Since molecular pathways of protein aggregation are largely evolutionarily conserved, we selected a simple cell model, yeast Saccharomyces cerevisiae.

Materials and methods: This research was conducted on yeast strains of Saccharomyces cerevisiae isogenic to wild type strain S288C. Gene constructs were obtained by molecular cloning using standard recombinant DNA techniques (enzymatic restriction and ligation) and by homologous recombination in yeast. Expression level of fusion proteins was examined in total cell lysates by the Western blot, using antibodies against epitope tags (HA and V5) or tau protein (Tau 5). Luminescence intensity was measured in living yeast cells using a microplate reader and NanoGlo luciferase assay (Promega) and the signal was normalized to cell density, determined by OD600. Tau toxicity was examined by comparing the reproductive capacity of Tau-expressing and control cells using growth assays. Serial dilutions of exponentially growing cells were spotted on SCD agar plates and incubated for at least 48 h.

Results: To construct Tau-NanoBit reporter for the detection of tau oligomers in living yeast cells we separately fused two luciferase subunits to the C-terminus of the human tau protein and verified their protein expression. We also constructed negative control constructs, including a construct that express untagged tau and a construct in which large luciferase subunit is fused to Tdh3, a yeast protein with no reported tau interaction. Fusion constructs were introduced into in wild-type strain and two mutants: (1) pho85Δ mutant with increased tau phosphorylation and increased levels of sarkosyl-insoluble tau, and (2) rpn4Δ mutant with downregulated proteasome levels. We tested whether the expression of Tau-NanoBit is toxic to yeast cells by growth assays. Growth analysis demonstrated that expression of the Tau-NanoBit fusions did not affect the growth of the wild type yeast cells, or pho85Δ and rpn4Δ mutant cells. Wild type cells expressing Tau-NanoBit constructs exhibited the luminescent signal that was similar with the levels in cells expressing the negative control constructs, suggesting the absence of tau oligomerization. Tau-NanoBiT luminescence was elevated in the pho85Δ and rpn4Δ mutants, indicating that tau phosphorylation and proteasome downregulation affects tau oligomerization. Furthermore, the finding that Tau-NanoBiT expression did not affect cell growth suggests that the tau oligomerization does not affect viability and reproductive capacity of yeast cells.

Discussion: Our results showed increased activation of Tau-NanoBiT reporter in pho85Δ mutant, suggesting that tau phosphorylation promotes tau oligomerization in yeast cells. Rpn4 is a proteasome biogenesis regulator that cooperates with the unfolded protein response. The observed increased luminescence in rpn4Δ mutant may point to tau protein degradation pathway and indicate proteotoxic stress as a factor in Tau oligomerization.

MeSH/Keywords: Alzheimer's disease, Protein aggregation, Tau, Protein-protein interaction, Luciferase, Yeast Saccharomyces cerevisiae

Poster code: R-01-08-116

Poster Title: Association of psychological factors with digital activity and physical isolation during the COVID-19 disease pandemic

PhD candidate: Vanja Kopilaš

Part of the thesis: Povezanost psiholoških čimbenika s digitalnom aktivnošću i osobnom izolacijom tijekom pandemije bolesti COVID-19

Mentor(s): Professor Srećko Gajović, MD PhD, Assistant Professor Lovorka Brajković, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Coronavirus disease (COVID-19) pandemic has negatively affected entire world. We wanted to analyze the psychological and emotional states of participants in different pandemic-related contexts, with a focus on their digital and physical distancing behaviors.

Materials and methods: The study compared psychological and behavioral features of participants being in a direct contact with a COVID-19 infected person or in a country with rapidly ascending cases (Italy) to otherwise unaffected individuals in Croatia (those knowing not to be in the contact with the same infected person, or unrelated participants without such information). To address the psychological and emotional consequences of pandemic a survey was designed in which nine validated psychological and emotional scales were combined with two custom-made questionnaires on digital (online) and physical (off-line) pandemic-related behavior as well as an open-ended question on individual comments on pandemic.

Results: Affected Italy and CRO-contact groups showed higher self-perceived post-traumatic avoidance scores than the control CRO-no contact and CRO-unrelated groups. Avoidance was correlated with higher newly introduced Digital Activity and Physical Distancing Scores. Italy group in lockdown had higher self-perceived scores for depression, stress, post-traumatic intrusion and avoidance, as well as highest digital activity and physical distancing than the not-in-lockdown Croatian groups. Unlike Italian participants, the CRO-contact group had no other alterations than unexpectedly lower post-traumatic hyperarousal when compared to other groups.

Discussion: Our results suggest that lockdown and general emergency measures have more impact on people than direct contact with an infected person. Since we live in the digital society, our study provides some practical application showing how digital environment should be taken into account when discussing mental health.

MeSH/Keywords: depression, anxiety, loneliness, digital activity, social isolation

Poster code: R-01-08-120

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. The great importance of perinatal brain damage, such as intraventricular and periventricular haemorrhage, with its developmental and social repercussions, more detailed studies of prenatal development of telencephalic vascularization points to the necessity of better evaluation of these processes. The aim of this study was to investigate developmental changes of vascular network, in correlation with transient cerebral cytoarchitectonic zones, using a Rapid Golgi staining method on specimens from the "Zagreb Neuroembryological collection". We focus on the transient foetal "subplate zone", because of its positive role in structural plasticity after the perinatal brain lesion. The results of this study, which clearly defines the concept of transient vascular zones, will contribute to a better understanding of the pathophysiological mechanisms of intraventricular and periventricular bleeding in premature babies.

Materials and methods: Blood vessels were analyzed on serial rapid Golgi-impregnated sections, while adjacent Nissl stained sections were used for the appropriate delineation 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.

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: Previous studies of vascularization of the human fetal telencephalic wall did not take into account the existence of transient and very prominent fetal zone, the subplate zone. This zone is located on the border of white matter and cortical plate, and disappears in the perinatal period. It is possible that the fate of its vascular network is similar to the fate of its transient neural elements. Some elements degenerate, and some reorganize in the cortical plate and the white matter. With respect to that, it is reasonable to conclude that the perinatal period is characterized by reorganization of vascular network of the subventricular-ventricular zone and the subplate zone. The results of this study show the following: from the end of embryonic period, and throughout the entire fetal period, the vascular network of the human telencephalic wall transforms from a bilaminar pattern (radially oriented vessels in the upper part of wall, and irregular and profusely branching vessels in deeper part of fetal telencephalic wall) to the trilaminar pattern (with an intermediate vascular layer that develops within the subplate zone). The vascular networks in the telencephalic wall are also 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.

MeSH/Keywords: development, vascularization, telencefalon, subplate zone

Poster code: R-01-08-148

Clinical medical sciences – preliminary research results

Poster Title: COGNITION AND ANESTHESIA

PhD candidate: Tea Fabijanić

Part of the thesis: The comparison of cognitive function, symptoms of depression and anxiety, and quality of life in patients after hip fracture surgery under general or regional anesthesia

Mentor(s): Associate Professor Daniela Bandić Pavlović, MD PhD, Associate Professor Marina Šagud, MD PhD

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

Introduction: Hip fractures are among the most common injuries in the general population, particularly in the elderly. As such, they represent significant public health care problems regarding short-term and long-term medical treatment. Postoperative cognitive dysfunction is relatively common in elderly patients after hip surgery, but the exact mechanism of its onset is still unclear as well as contributing factors. There is also increased incidence of depression and anxiety. Both affect the recovery after surgery, slow it down and reduce the quality of life. The type of anesthesiological procedure may also influence postoperative cognitive changes, but there is no data if general and local anesthesia differently affect cognition. The aims of the present trial were to investigate the influence of general and regional anesthesia on cognition, anxiety and depression, and quality of life in patients after hip fracture surgery under regional or general anesthesia.

Materials and methods: Patients will be divided into two groups, and the hip surgery would be carried out under regional anesthesia or general anesthesia, and monitored after surgery. Patients will be tested before and after surgery with Montreal Cognitive Assessment Scale, F-A-S verbal fluency test, Hospital Anxiety and Depression Scale and EQ-5D-5L quality of life questionnaire to evaluate postoperative cognitive deficits, depression and anxiety and health-related quality of life. The data will be extrapolated and compared.

Results: We plan to recruit 140 patients dividende into 2 groups, 70 patients in one group who received regional anesthesia and 70 patients in the other who were under general anesthesia. We have enrolled so far 22 patients (19 female, 3 male), of which 3 patients didn't pass initial cognitive testing and were excluded from research. We have prospectively collected data for 19 patients (11 patients in the regional anesthesia group and 9 patients in the general anesthesia group). Patients were tested preoperatively and once after 24-48h postoperatively.

Discussion: Research is still ongoing.

MeSH/Keywords: hip fracture, surgery, regional anaesthesia, general anaesthesia, postoperative cognitive dysfunction, depression, anxiety, quality of life

Poster code: R-02-01-100

Poster Title: Skin microbiome in patients with periocular dermatitis

PhD candidate: Iva Ferček

Part of the thesis: Investigation of the skin microbiome changes in patients with periocular dermatitis

Mentor(s): Assistant Professor Rok Čivljak, MD PhD, Professor Liborija Lugović Mihić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: The human microbiome consists of all microorganisms residing on or in our bodies. According to studies performed by molecular methods, the skin microbiome is even more diverse than previous cultivation-based studies have shown. Periocular dermatitis (PD) includes skin changes in periocular region which are frequent in clinical practice and present challenges in treatment. Specifically, a vast number of various diseases (infectious diseases; atopic dermatitis, contact allergic or irritant dermatitis, photoallergic or phototoxic dermatitis, rosacea, drug allergic reactions; all connective tissue diseases and autoimmune diseases) can manifest in that region, and diagnostics are sometimes limited and rarely take into consideration the microbiologic composition of the skin, i.e. the microbiome. Despite numerous scientific findings, there are still no clinical studies conducted on patients with PD that would examine composition of microbiome using molecular methods.

Materials and methods: An observational case-controle study was designed to investigate periocular skin microbiome and identify its changes in periocular dermatitis. The study was approved by the ethics comitee of the University of Zagreb and conducted according to the principles of the Declaration of Helsinki. All participants were enrolled between December 2020 and May 2021, after providing written informed content. Inclusion criteria for all subjects included age 18 or older, for PD patiens diagnose of PD made by an dermatovenereologist and for controls no history of skin or allergic disorders. Exclusion criteria for all subjects included use of systemic drugs 30 days/application of local antibiotics or corticosteroids 7 days/bathing and showering 12 hours - prior to skin swabbing. The composition of microbiome was analyzed from visible skin changes in the periocular region in PD patiens. Samples of the skin microbiome were collected using individually packed, sterile swab tipped with syntetic material (Zymo Research, Irvine, CA, USA) that had been soaked in sterile 0.15 M NaCl. Periocular region skin was swabbed for 30 seconds while rotating the swab. Swab tips were broken into DNA/RNA shield collection tube (Zymo Research, Irvine, CA, USA) and immediately stored at -80°C while they awaited further processing. DNA from the specimen was extracted manually using commercial kit specific for microbial DNA purification ZymoBIOMICS DNA mini kit (Zymo Research, Irvine, CA, USA). Prior to DNA isolation, specimen had been exposed to shaking on MagNA lyser (Roche Applied Science,UT, USA) in order to disturb cell wall. DNA quality and yield were evaluated by agarose gel and Qubit fluorometer (Life Technologies Corporation, Carlsbad, CA, USA). After purification of the PCR products, both T-RFLP and NGS will be performed. The results will be bioinformatically and statistically analyzed. Smears for Malassezia genus and scraping for Demodex mite were also performed. The surface of the skin was gently craped or briskly swabed in the affected areas. The material collected was transferred from the blade or swab to the slide and examined under scanning and 10x objectives.

Results: The 11 participants with PD included 8 women and 3 men, all Caucasian, aged 22–73 years. Symptom onset in patients with PD varied from 2 days to > 10 years. Test for Malassezia genus was positive in 2 patients and for Demodex mite in other 2 patients. After DNA isolation and purification DNA concentration in collected samples was: 6,8 ng/µl, 17,6 ng/µl, 11,1 ng/µl, 6,18 ng/µl, 5,31 ng/µl, 20,12 ng/µl, 16,1ng/µl, 6,7 ng/µl, 10,2 ng/µl, 17,8 ng/µl, 20,8 ng/µl,

Discussion: The occurrence of periocular skin changes is linked to the epidermal barrier disorder, activation of the innate immune system, and fluctuations of the skin microbiome. Recent studies have shown that, among healthy individuals, the skin of the periocular region is inhabited by bacteria from the Actinobacteria, Proteobacteria, Firmicutes, and Bacteroidetes genera. Given the fact that DNA yield on skin is much lower than for example in gut, few isolation kits were used before finding the most appropriate. The minimum DNA concentration for performing NGS analysis is 5 ng/µl and this amount was reached in patients samples. Possible changes in the composition of microbiome will potentially contribute to the understanding of the etiopathogeneses of periocular dermatitis and more efficient treatment of skin lesions.

MeSH/Keywords: periocular, dermatitis, NGS, microbiome

Poster code: R-02-02-056

Poster Title: Influence of BRAF V600 mutant allele percentage on treatment response in metastatic melanoma

PhD candidate: Nika Franceschi

Part of the thesis: Influence of BRAF V600 mutant allele percentage on treatment response in metastatic melanoma

Mentor(s): academic Mirna Šitum, Assistant Professor Ivan Šamija, MD PhD

Affiliation: University of Zagreb School of Medicine, Department of dermatovenereology and Department of oncology and nuclear medicine, University hospital center Sestre milosrdnice

Introduction: The incidence of malignant melanoma, the most aggressive skin cancer, has been increasing for the last 40 years. Approximately 50-60% of melanomas carry a BRAF mutation and its determination is necessary for selection of metastatic disease treatment, as these patients can be treated with targeted BRAF and MEK inhibitor therapy. Combined treatment with BRAF and MEK inhibitors is a newer therapeutic option which significantly improves progression-free survival and overall survival compared to BRAF inhibitor monotherapy. However, initial response is often followed by an acquired resistance in many patients. Studies have shown that melanoma lesions exhibit heterogeneity of BRAF mutations. As a result of intratumor heterogeneity, differences in response to BRAF and MEK inhibitor treatment between melanomas with different percentages of BRAF mutant alleles could be expected. The mechanisms of acquired resistance to BRAF and MEK inhibitors have been extensively studied. However, data regarding biomarkers that predict response to therapy are limited.

Materials and methods: The study will be conducted on 80 BRAF V600 positive subjects with metastatic melanoma treated with BRAF and MEK inhibitors. Tissue samples of melanoma metastases will be analyzed when available, otherwise samples from the primary melanoma will be used. All samples will be reviewed by a pathologist who will determine the tumor cell content and subsequently DNA extraction will be performed using commercial kits. Real-time PCR will be used to determine the frequency of BRAF V600 mutant alleles. Patients will be monitored for 2 years during treatment and progression free survival will be used as clinical outcome measure which will be determined by clinical, imaging and laboratory methods. Overall survival will be determined over the same period, as well.

Results: During the first year, 25 newly diagnosed stage IV melanoma patients that are candidates for BRAF and MEK inhibitor therapy were included in the study, as well as 55 BRAF V600 positive patients already being monitored and treated with BRAF and MEK inhibitors at the Department of dermatovenereology and Department of oncology and nuclear medicine, University hospital center Sestre milosrdnice. A slightly higher number of male (55%) than female patients (45%) is included in the study. Patient's age varies from 30 years to 82 years, with most patients aged between 60 and 70. Of the 80 tumor samples, 12 (15%) are primary tumor tissue samples and 68 (85%) are metastasis. The BRAF V600E mutation was identified in 76 (95%) patients, while BRAF V600K mutation in 4 (5%) patients. A somewhat higher number of patients (68.75%) is receiving the treatment combination dabrafenib and trametinib. After a positive BRAF V600 mutation was identified, histopathological samples of the 25 newly diagnosed stage IV melanoma patients were sent to a pathologist, as well as 55 samples of the BRAF V600 positive patients already being treated with BRAF and MEK inhibitors. The pathologist is currently examining the tissue samples and will assess the tumor cell content, after which BRAF V600 mutant allele frequency will be determined. Patients are currently being monitored during treatment and all clinical, imaging and laboratory findings are being documented.

Discussion: Melanoma is most commonly diagnosed at an older age, therefore the average age of patients (67) included in this study was expected. In Croatia, a somewhat higher number of male patients is diagnosed with melanoma each year, explaining the slightly higher number of male patients in the study. Since more than 80% of BRAF mutations are V600E, a higher proportion of these mutations in contrast to V600K was expected as well. The treatment combination dabrafenib and trametinib was the first BRAF and MEK inhibitor combination approved in Croatia, which may be the reason it was prescribed in more patients.

MeSH/Keywords: Melanoma, Proto-Oncogene Proteins B-raf, Mutation, Molecular Targeted Therapy, Treatment Outcome, Tumor Biomarkers

Poster code: R-02-02-102

Poster Title: Biomechanical gait analysis in patients with ankylosing spondylitis

PhD candidate: Vedran Brnić

Part of the thesis: Biomechanical gait analysis in patients with ankylosing spondylitis

Mentor(s): Frane Grubišić, PhD, research associate, Igor Gruić, PhD, research associate

Affiliation: University of Zagreb School of Medicine; Department of Rheumatology, Physical Medicine and Rehabilitation, Sestre milosrdnice University Hospital Centre; University of Zagreb Faculty of Kinesiology

Introduction: Ankylosing spondylitis (AS) is a chronic inflammatory rheumatic disease affecting predominantly sacroiliac joints and axial skeleton. It leads to a decrease in spinal mobility, postural alteration and gait impairment. Considering the importance of gait in functional independence, there is a need for its objective assessment that can be utilized in the timely inclusion of targeted gait kinesitherapy program as part of the rehabilitation process. Such objective assessment can be accomplished through instrumental biomechanical analysis of kinetic and kinematic gait parameters. A pedobarographic kinetic analysis can be used as an indicator of postural deviation, while kinematic analysis complements this method and provides further insight into compensatory gait mechanisms. Therefore, the main objective of this study is to assess alteration of gait pattern through kinetic pedobarographic and kinematic parameters in AS patients compared to controls. Moreover, we want to establish correlations of these parameters with disease duration, clinical measures - Bath Ankylosing Spondylitis Functional Index (BASFI), Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and Bath Ankylosing Spondylitis Metrological Index (BASMI) as well as radiographic spinal structural changes measured by modified Stoke Ankylosing Spondylitis Spine Score (mSASSS).

Materials and methods: This is a cross-sectional study. 32 consecutive patients with the diagnosis of AS and 32 age- and sex-matched controls without disease or condition that could affect gait will be included. All participants are patients aged 18-70, treated at the Sestre milosrdnice University Hospital Centre, Department of Rheumatology, Physical Medicine and Rehabilitation. Pedobarographic and kinematic gait parameters of all participants will be recorded, as well as demographic and anthropometric characteristics. RS Scan Footscan Plate will be used for pedobarographic analysis and MS Kinect v2 Motion Sensor cameras will be used for kinematic analysis. Clinical characteristics, disease duration, BASFI, BASDAI, BASMI and mSASSS scores of AS patients will also be assessed.

Results: The study is currently in the phase of data collection. Until now, 3 male participants were included (1 AS patient and 2 controls) and their pedobarographic and kinematic gait parameters were recorded.

Discussion: The results of this study will contribute to a better understanding of gait alteration in AS patients and will help in the design of targeted gait kinesitherapy program. This study is also the first to correlate radiographic spinal structural changes measured by mSASSS with kinematic gait parameters in AS patients.

MeSH/Keywords: ankylosing spondylitis, gait analysis, walking, kinematics, kinetics

Poster code: R-02-03-090

Poster Title: Cigarette smoking during pregnancy: effects on metallothionein and trace elements in mother-newborn pairs

PhD candidate: Iva Miškulin

Part of the thesis: The association of cigarette smoking with the concentration of metallothionein and MT2A gene methylation in the mother-infant pairs

Mentor(s): Assistant Professor Lana Škrgatić, MD PhD, senior research associate, Jasna Jurasović, PhD, research advisor

Affiliation: Clinical Hospital Centre Zagreb and University of Zagreb School of Medicine, Zagreb; Institute for Medical Research and Occupational Health, Zagreb

Introduction: Cigarette smoking is one of the most important sources of exposure to toxic metals cadmium (Cd) and lead (Pb) in the general population. Pregnant women who smoke have higher concentrations of Cd in the blood and placental tissue, which may interfere with the transfer of essential elements to the foetus during pregnancy. Metallothioneins (MTs) are cysteine-rich low molecular weight metalloproteins that bind and transfer elements in the body. They are primarily involved in homeostatic regulation of the essential elements, zinc, copper and iron (Zn, Cu and Fe). Under condition of exposure to toxic metals, such as Cd and Pb, synthesis of MT is increased, which may play a protective and detoxifying role under acute and cause increased internal toxic metal accumulation under chronic toxic metal exposure.

Materials and methods: The cross-sectional study includes 74 mother-newborn pairs after obtaining all required ethics approvals. Participants' inclusion criteria were: healthy woman with a full term (≥37 weeks of pregnancy) vaginal birth without serious health problem during pregnancy or chronic illness. After obtaining informed consent, a questionnaire was used to collect data on smoking habit (number of smoked cigarettes per day before and during pregnancy) and other possible sources of environmental or occupational metal exposure, parity, weight gain during pregnancy, and newborn data (sex, birth weight and length, and APGAR vitality assessment after birth). Maternal urine samples were taken before and the entire placenta and blood samples from the umbilical cord and maternal peripheral vain within one hour after delivery. Parameters determined were concentrations of MT2A in serum and placental tissue, metals (Cd, Pb, Zn, Cu and Fe) in serum, blood and placenta, and cotinine in urine. The effect of smoking as a source of metal exposure was assessed by comparing two study groups: a) smokers (n = 37), women who smoked during pregnancy and had urinary cotinine ≥100 ng/mL, and b) non-smokers (n = 37), women who have never smoked and had urinary cotinine <LOQ.

Results: There were no differences between smokers and non-smokers in parity, body mass index before pregnancy and before delivery, and weight gain during pregnancy. All newborns were of excellent health with the highest median value of APGAR score 10, both at minutes 1 and 5 after birth. Smoking vs. non-mothers had significantly higher median Cd concentration in their blood (0.683 vs. 0.307 µg/L) and placenta (8.07 vs. 6.33 µg/kg) and no difference in cord blood Cd (0.029 vs. 0.028 µg/L). In all of the measured samples, Pb levels were higher in smokers than in non-smokers, which was significant only in the placenta (maternal blood: 9.32 vs. 8.30 µg/L, cord blood: 6.55 vs. 6.07 µg/L, placenta: 2.86 vs. 2.07 µg/kg). Smoking mothers had significantly lower Fe in serum (0.760 vs. 1.06 mg/L) and placenta (94.4 vs. 109 mg/kg) and higher Zn in placenta (11.1 vs. 10.7 mg/kg) than non-smokers. All mothers and newborns had similar MT2A levels in the serum and placenta.

Discussion: Smoking vs. non-smoking postpartum women have higher Cd levels in the blood and placenta, whereas Cd levels in newborns are similar and low due to known limited Cd transport through the placenta. We confirm transplacental Pb transfer proposed to occur by the passive diffusion. Our results are not in line with other authors' data on smoking-related increased placental Cd levels accompanied by increased placental MT expression attributed to the enhancement of free radical production caused by metals and other chemicals from cigarette smoke. Complex interactions between maternal exposure to toxic metals, oxidative stress, epigenetic and genetic markers (methylation of MT2A gene and its polymorphisms) related to cigarette smoking are yet to be evaluated.

MeSH/Keywords: cadmium; cigarette smoking; metallothionein; placenta; pregnant women

Poster code: R-02-05-037

Poster Title: Preoperative assessmentof myometrial invasion and infiltration of cervical stroma with three dimensional ultrasound (VCI method) in women with endometrial cancer type 1

PhD candidate: Velena Radošević

Part of the thesis: Preoperative assessment of myometrial invasion and infiltration of cervical stroma with three dimensional ultrasound in women with endomet

Mentor(s): Assistant Professor Goran Vujić, MD PhD, Assistant Professor Marija Milković Periša, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Myometrial invasion of endometrial cancer has been identified as a significant prognostic factor and when expressed as a percentage (< or > 50%) is used in FIGO classification. It is calculated by determining the depth of myometrial invasion and the total thickness of myometrium. Infiltration of cervical stroma is also important prognostic factor. Diagnostic methods in preoperative selection of low and high risk patients are of great importance in aim to decrease procedure radicality and following complications. Transvaginal 2D ultrasound is standard and the most simple visual method in preoperative evaluation of endometrial carcinoma.

Materials and methods: 19 patients with diagnosed endometrial cancer were hospitalized, treated and analyzed at the Department of Gynecological Oncology and the Department of Gynecological Surgery of University Hospital Centre Zagreb, from November 1st 2020. until May 1st 2021. The analysis included patients with diagnosed endometrioid cancer of the uterine body, grade I and II (Type 1) and by whom minimally hysterectomy with bilateral salpingooforectomy was performed. Prior to the surgery patients were examined by 2-D and 3-D transvaginal ultrasound (VCI method). The operative material analyzed by pathologist for final histopathological diagnose and depth of invasion. Findings of 2-D and 3-D transvaginal ultrasound were compared with a histopathological finding regarding the thickness of myometrium, the depth of the largest invasion in the myometrium and infiltration of cervical stroma were recorded. Data were analyzed using IBM SPSS Statistics version25. The concordance of 2-d and 3-D findings were calculated with kappa test and McNemar with $\alpha < 0,05$.

Results: The accuracy of 2-D and 3-D ultrasound in assesing deep myometrial invasion was 68,4% vs. 89,5 %; McNamar p=0,125, κ =0,406. The accuracy of VCI in assesing cervical involvment was was 89,5 %, same as the accuracy of 2D ultrasound 89,5% with apsolute concordance κ =1.

Discussion: Our results (on a small number of patients) show that preoperative 3D sonography (VCI method) predicts with reasonable accuracy deep myometrial invasion and cervical involvement in women with endometrial cancer type 1. Our initial 2-D US accuracy for miometral invasion (68,4 %) is lower than literary reported accuracy range (76 %-84 %,) but our 3-D accuracy 89,5 % is above the reported range. The benefit of additional 3D sonography is evident. Regarding the 2-D accuracy for stromal invasion our result are in concordance with literature range (78%-89%), and there were no additional benefits from 3-D sonography. Recent practice guidelines for assessment of endometrial cancer have included MRI as a method of preoperative investigation. The diagnostic accuracy of VCI in the our study is comparable to that of MRI. However, MRI is less accessible, more expensive and more time consuming to perform than is 3D ultrasound. Therefore 3D tranvaginal ultrasound (VCI method) method should have a role in preoperative imaging.

MeSH/Keywords: endometrial cancer, myometral invasion depth, cervical infiltration, 3D ultrasound, VCI

Poster code: R-02-05-099

Poster Title: Influence of continuous positive airway pressure in patients with obstructive sleep apnea on the cardiovascular system

PhD candidate: Tea Friščić

Part of the thesis: Influence of continuous positive airway pressure in patients with obstructive sleep apnea on plasminogen activator inhibitor-1 value

Mentor(s): Assistant Professor Edvard Galić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Obstructive sleep apnea (OSA) is the most common sleep disorder characterized by intermittent upper-airway obstructions leading to recurrent hypoxemia and hypercapnia. OSA is still an underdiagnosed condition although evidence is suggesting an independent association with higher rates of cardiovascular morbidity and mortality. The pathophysiology behind the increased cardiovascular risk is still unclear, as well as if the use of mainstream therapy (continuous positive air pressure (CPAP)) can reverse it. It is known that the OSA impact on the cardiovascular system is multifactorial including sympathetic activation, oxidative stress, inflammation and metabolic dysfunction. Cardiac biomarkers, such as troponin and NT-proB-type Natriuretic Peptide (NT-proBNP), have been investigated in OSA patients showing variable results regarding the response to the CPAP treatment. Plasminogen activator inhibitor-1 (PAI-1) which is the main physiological inhibitor of the fibrinolytic system showed a functional role in atherosclerosis, metabolic disturbances and chronic stress. In patients with OSA, elevated PAI-1 values were found correlating with the severity of OSA. The aim of this study is to determine the influence of therapy with CPAP in patients with diagnosed severe OSA on PAI-1 and NT-proBNP values as well as on other cardiovascular parameters that can be measured noninvasively.

Materials and methods: In this prospective cohort study the participants were selected after performing an overnight polysomnography, with confirmed severe OSA (AHI≥30). Data was collected before CPAP therapy was introduced and after a minimum of 6 months of CPAP use for at least 4 hours per night. At each visit patients had resting blood pressure and heart rate measured, and details of current medication use and health behaviors were documented through a structured interview. Blood samples were taken for the analysis of laboratory parameters including NT-proBNP. PAI-1 values will be measured with ELISA after all the samples are collected. Participants were provided with a 24-hour Holter electrocardiogram, 24-hour blood pressure Holter and echocardiography. The study exclusion criteria were: patients under the age of 18, pregnant women, those with AHI <30, severe chronic obstructive pulmonary disease, severe chronic renal insufficiency, atrial fibrillation, those with acute heart failure, cerebrovascular insult transitory ischemic attack or acute coronary syndrome in the last 6 months, those taking anticoagulant therapy, and known psychiatric disorders that could affect compliance.

Results: The study is still in progress with all the patients recruited but some of them waiting for the follow-up appointments. We enrolled 58 patients, from whom 40 of them had a follow-up. The median age was 52±9,9 years and 45 (77%) of them were men. The average BMI was 34 kg/m2. We found that 27.5% of patients were smokers, 53.4% had hypertension, 13.8 % had diabetes, 3.4% had COPD and 1.7% had a history of previous cardiovascular disease. The mean duration of adherence to CPAP therapy was 5.2 hours per night, and the mean AHI decreased from 56.2 events per hour at baseline to 4.5 events per hour during follow-up. The average total score of the Epworth Sleepiness Scale, intended to measure daytime sleepiness, decreased from 9,5 at baseline to 4.9 at follow-up. The remaining data collected from the study is still not sufficient for proper statistical analysis.

Discussion: The majority of our patients were middle-aged, obese and with a severe OSA. Average adherence to CPAP therapy in patients who had their follow-up is 5.2 hours per night which should be, according to previous studies, sufficient for changes in biomarker levels and other measurements regarding the cardiovascular system. Our study group is almost free of the cardiovascular disease burden because of the exclusion criteria we made, with a purpose to compare subclinical changes of the cardiovascular system and without the bias that would be present because of the therapy that is usually given to those patients, such as anticoagulants. Previous studies didn't yield a definite and uniform answer about OSA's influence on the cardiovascular system and the effect of therapy with CPAP on cardiovascular risk reduction. Our study may show a positive effect of CPAP therapy on the cardiovascular system in patients with OSA.

MeSH/Keywords: obstructive sleep apnea, continuous positive airway pressure, plasminogen activator inhibitor-1, cardiovascular risk

Poster code: R-02-09-004

Poster Title: The association of PNPLA3, EGF and Notch3 gene polymorphisms with alcoholic liver disease and hepatocellular carcinoma

PhD candidate: Ana Bainrauch

Part of the thesis: Association between the single nucleotide polymorphisms for PNPLA3, NOTCH3 and EGF with the risk of hepatocellular carcinoma occurrence in patients with alcoholic liver cirrhosis

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

Affiliation: University of Zagreb School of Medicine

Introduction: Alcohol abuse may lead to alcoholic cirrhosis and hepatocellular carcinoma (HCC). Once liver cirrhosis has developed, there is 1-8% annual risk of HCC. We investigated single nucleotide polymorphisms of PNPLA3, EGF and Notch3 as genetic risk factors for alcoholic liver disease (ALD) and its progression to HCC.

Materials and methods: DNA was isolated from the blood of 245 patients transplanted in the University Hospital Merkur due to ALD (124 without HCC and 121 with HCC) and 70 control patients (without liver disease). Single nucleotide polymorphisms (SNPs) for PNPLA3 (rs738409), EGF (rs4444903) and Notch3 (rs1043996) were determined by PCR using commercially available TaqMan assays. DNA isolation and PCR analysis were performed at the Laboratory for Molecular Immunology at Croatian Institute for Brain Research. Associations between SNPs and ALD or HCC were examined by SNPStats software.

Results: All SNPs were in Hardy-Weinberg equilibrium (p>0.05). Minor allele frequencies were 41%, 42% and 27% for PNPLA3, EGF and Notch3, respectively. PNPLA3 (rs738409) was associated with higher risk for ALD in codominant (OR95%CI = 6.15 (2.28-16.60) for GG vs CC genotype and 2.78 (1.55-4.97) for GC vs CC genotype), dominant (OR95%CI = 3.36 (1.94-5.82) for GG/GC vs CC), recessive (OR95%CI = 3.71 (1.42-9.69) for GG vs GC/CC) and log-additive model (OR95%CI = 2.60 (1.69-3.99) for G allele). This SNP was also associated with a higher risk for HCC in codominant (OR95%CI = 2.88 (1.37-6.03) for GG vs CC), recessive (OR 95%CI = 2.72 (1.43-5.17) for GG vs GC/CC) and log-additive model (OR95%CI = 1.63 (1.14-2.34) for G allele). EGF (rs4444903) was not associated with a risk for fibrosis development. However, it was associated with a mildly increased risk for fibrosis progression to HCC in a dominant model (OR95%CI = 1.92 (1.11-3.32), for GA/AA vs. GG). Notch3 (rs1043996) was associated with lower risk for ALD in codominant (OR95%CI = 0.30 (0.13-0.70) for GG vs AA), recessive (OR95%CI = 0.32 (0.14-0.72) for GG vs GA/AA) and log additive model (OR95%CI = 0.63 (0.43-0.94) for G allele). There was no association between the Notch3 genotype and risk for HCC (p>0.05).

Discussion: SNP of PNPLA3 (rs738409) is a considerable risk factor for the ALD and its progression towards HCC, while EGF (rs4444903) might be an additional risk factor for HCC development. Notch3 (rs1043996) genotype is associated with a lower risk for the development of ALD.

MeSH/Keywords: Alcoholic liver disease, liver transplantation, hepatocellular carcinoma, PNPLA3, EGF, Notch3

Poster code: R-02-09-054

Poster Title: Nutritional status and pulmonary rehabilitation outcome of patients with chronic obstructive pulmonary disease

PhD candidate: Davorka Muršić

Part of the thesis: Pulmonary rehabilitation outcome in relation to the nutritional status of patients with chronic obstructive pulmonary disease

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

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

Introduction: Chronic obstructive pulmonary disease (COPD) is one of the leading causes of death in developed countries of the world and one of the leading public health problems. The treatment of those patients is complex and, in addition to pharmacological treatment, non-pharmacological measures, such as pulmonary rehabilitation are very important. In recent years, a lot of attention was attributed to evaluation of nutritional status of COPD patients, with bad prognosis in patients with poor nutritional status. There is still no standardised method for assessing patients' nutritional status, including COPD patients. Subjective Global Assessment (SGA) questionnaire allows the integration of medical history and clinical examination data, allowing the physician to perform a rapid assessment of nutritional status. Additionally, bioelectrical impedance analysis (BIA) is a simple and non-invasive method which indirectly estimates body composition. Biochemical methods for measuring nutritional status are serum albumin and prealbumin.

Materials and methods: The study included COPD patients who were referred for pulmonary rehabilitation to the Clinic for Lung Diseases Jordanovac, UHC Zagreb in the period of September 2020 until April 2021. Patients were grouped according to SGA questionnaire (A – well-nourished, B - mildly/moderately nourished or C - severely malnourished). All patients met the following inclusion criteria: age > 40 years, both sexes, minimum 10 pack years, FEV1 values <80%, FEV1/FVC < 0.7, signed informed consent. Exclusion criteria were malignant or cardiovascular disease, unregulated arterial hypertension, acute inflammatory conditions, lung diseases other than COPD, inability to perform lung function tests, patients with pacemakers, kidney or liver insufficiency and diseases of the musculoskeletal system. All patients had the same pulmonary rehabilitation program for 4 weeks. Patients were measured by BIA (FFM, FFMI and phase angle values) and they filled in questionnaire of the degree and severity of symptoms (CAT). Lung function testing, 6-minute walk test (6MWT) and blood sampling for required laboratory tests were performed (leukocytes, fibrinogen, CRP, lipid profile, prealbumin, albumin). At the end of the program all measurements performed before the start of the pulmonary rehabilitation program were repeated.

Results: There were 4 (57,1%) male and 3 (42,9%) female patients with average age of 66,3±8,8 years. According to SGA questionnaire 4 (57,1%) patients were in group A, 2 (28,6%) patients were in group B and only 1 (14,3%) patient was in group C. FVC was 82,4±23%, FEV1 was 37,3±11,1%, 6MWT was 342,9±113,1 m, values of leukocytes were 8,4±2,3x109/L, fibrinogen 4,54±1,6 g/L, CRP 3,6±3,1 mg/L, cholesterol 4,9±1,5 mmo/L, HDL 1,47±0,5 mmo/L, LDL 2,9±1,3 mmo/L, triglyceride 1,2±0,5 mmo/L, prealbumin 0,25±0,04 g/L and albumin 46,7±2,5 g/L. FFM was 55,19±12,3kg, FFMI 18,4±2,7kg/m2, phase angle 5,9±0,7°, CAT result was 17,4±8,3. There were no statistical differences between fibrinogen, CRP, lipid profile values, FVC and FEV1 percentage, 6MWT distance prior and after pulmonary rehabilitation. There where statistical differences between leukocytes values and CAT questionnaire results which were lower after pulmonary rehabilitation.

Discussion: COPD patients referred to pulmonary rehabilitation program in our study had lower functional exercise performance (<350 m), as expected, and after pulmonary rehabilitation, a clinically significant increase in 6MWT was observed (>35m). Patients were well-nourished according to SGA questionnaire, BIA, serum albumin and prealbumin values. Because the number of subjects is too small, we cannot compare whether there is a difference in the outcome of pulmonary rehabilitation depending on nutritional status.

MeSH/Keywords: COPD, pulmonary rehabilitation, nutritional status

Poster code: R-02-09-088

Poster Title: Analysis of selected laboratory and echocardiographic parameters of cardiac microtrauma before and after the game in rugby players

PhD candidate: Petra Radić

Part of the thesis: Analysis of selected laboratory and echocardiographic parameters of cardiac microtrauma before and after the game in rugby players

Mentor(s): Associate Professor Zdravko Babić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: It has been known for a long time and confirmed by a number of epidemiological studies that daily physical activity is beneficial for the prevention of many diseases, especially cardiovascular. Nevertheless, in the case of professional athletes who are exposed to a high level of endurance or repetitive physical impatcs, intense physical training can have a detrimental effect on myocardial function. Rugby is a high-intensity sport, with moderate static (10-20%) and moderate dynamic (50-75%) components and a large number of bodily injuries within a single game. Transient heart injuries, but also possible long-term adverse effects in rugby players, are visible from serum elevations of NT-proBNP and cardioselective enzymes. It is important to bare in mind that the athlete's myocardium physiologically adapts to chronic exposure to training and competition which is also echocardiographically visable. Research to date has not yet fully elucidated the clinical significance of frequent thoracic injuries in contact sports, such as rugby, on myocardial function.

Materials and methods: This prospective reasearch will include 34 examinees, players of the First Croatian Rugby League. The study protocol will be approved by the Ethics committees and written informed consent will be obtained from each subject. At the initial examination, which will be done two days before the regular game in the competition season, we will be performing our protocol as following: taking medical history, doing clinical examination, measuring standard vital and anthropometric parameters, 12-channel ECG, transthoracic echocardiography and standard laboratory findings (complete blood count; creatinine and urea; lactates; AST, ALT and LDH; sodium, potassium, calcium and magnesium; CK; MbCK) and NT-proBNP and hs-troponin I values. The same examination pattern will be repeated 2 to 4 hours after the match in whitch the subject played at least 60 minutes. Echocardiographic examination will include analysis of the following parameters: left ventricular ejection fraction; left vetricle dimension; left ventricle diastolic function, E/A and E/E', 2D and 3D left ventricle volume, global ventricular strain and left ventricle tissue Doppler imaging deformation study. All measurements will be at the same time of day to avoid circadian variation in values. Data collected in this study will be displayed grafically and in tables. We will be using descriptive statistics with appropriate measures of central tendency and variability. The normality of the distribution of the values of individual variables will be determined by the Shapiro-Wilk test. To determine the significance of the difference between pre- and post-match findings, we will use a two-tailed Wilcoxon Signed Rank Test for Matched Pairs.

Results: Preliminary results in the assessed group od 10 rugby players suggest that there is a decrease in total global longitudinal left ventricular deformity due to high static-dynamic loading during rugby match. The observed LV GLS range varied from -15.9% to -22.1% (mean, -19.7%; 95% CI, -20.4% to -18.9%). The estimated odds of having decrease in LV GLS was 1.24 times more likely (98.3% CI: 0.997, 1.55) after a high static-dynamic loading endured during a rugby match. However, there was no difference in levels of hs Troponin I (p= 0.53, 95% CI -3.79 to 1.81) and levels of NTproBNP (p=0.010, 95% CI -0.2 to 1.08) before and after the rugby match.

Discussion: Our investigation provides evidence that decrease of longitudinal measures of myocardial deformation, specifically LV GLS, is associated with high static-dynamic loading during rugby match. Additionally the results of this research may be expressed as a recommendation for preventive screening of athletes and protective measures in contact sports for early detection of risk factors and prevention of sudden cardiac death.

MeSH/Keywords: rugby, microtrauma, myocardium, left ventricle

Poster code: R-02-09-094

Poster Title: Myocardial Remodeling in Ischemic Heart Disease – Imaging from Cell to Organ Level by Synchrotron Propagation Based X-Ray Phase-Contrast Imaging

PhD candidate: Ivo Planinc

Part of the thesis: Myocardial structural analysis with synchrotron X-ray tomographic imaging in heart failure

Mentor(s): Associate Professor Maja Čikeš, MD PhD, Patricia Garcia Canadilla, PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Cardiovascular research is in an ongoing quest for a superior imaging method to integrate gross-anatomical information with microanatomy, combined with quantifiable parameters of cardiac structure. In recent years, synchrotron radiation-based X-ray Phase Contrast Imaging (X-PCI) has been extensively used to characterize soft tissue in detail. The objective was to use X-PCI to comprehensively quantify ischemic remodeling of different myocardial structures, from cell to organ level, in a rat model of myocardial infarction.

Materials and methods: Myocardial infarction-induced remodeling was recreated in a well-established rodent model. A transmural MI was induced by left coronary artery ligation through a left thoracotomy in 8 to 11 week-old Wistar male rats, 4 rats with induced MI, and 1 control healthy rat were used. Ex vivo rodent hearts were imaged by propagation based X-PCI using two configurations resulting in 5.8μm and 0.65μm effective pixel size images.

Results: The acquired datasets were used for a comprehensive assessment of macrostructural changes including the whole heart and vascular tree morphology, and quantification of left ventricular myocardial thickness, mass, volume, and organization. On the meso-scale, tissue characteristics were explored and compared with histopathological methods, while microstructural changes were quantified by segmentation of cardiomyocytes and calculation of cross-sectional areas.

Discussion: PB X-PCI offers a powerful research tool to study tissue remodeling following an MI using a whole heart, 3D, non-destructive, non-staining or contrast-based technique, feasible for the quantification of myocardial remodeling at whole-organ, tissue, vascular as well as cellular level in ex vivo model of ischemic heart disease.

MeSH/Keywords: infarct remodeling, ischemic heart disease, imaging, X-ray, myocardial structure.

Poster code: R-02-09-098

Poster Title: Circadian patterns of large artery stiffness in patients with different types of sleep apnea

PhD candidate: Antun Koprivanac

Part of the thesis: Circadian patterns of large artery stiffness in patients with different types of sleep apnea

Mentor(s): Vedran Premužić, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: It is known that sleep breathing disorders (OSA, CSA, mixted type od apnea) are complexly associated with increased cardiovascular risk in these patients. Epidemiological studies reported increased incidence of arterial hypertension, heart faliure, coronary and cardiovascular disease. Main part of disorders in based on hypoxemia and hypercapnia induced sympathetic hyperactivity (joined with parasympatetic disfunction) as a causal factor in the development of hypertension. Arterial stiffness is valuable predictor of cardiovascular morbidity and mortality. Hypothesis of the study is that patients with sleep breathing disorders lacking night decrease of arterial stiffness will have increased cardiovascular risk, accelerated vascular aging and consequently increased cardiovascular mortality compared to patients with normal physiological decreasment of arterial stiffness during the night. Main aim od study is to determine the values of office and 24-hour measured large artery stiffness in patients with OSA, CSA and mixed type sleep apnea and analyze the relationship of pulse wave velocity with other cardiovascular risk factors (smoking, hyperlipidemia, age, obesity, positive family history of previous CV disease).

Materials and methods: For each patient, the following data will be collected - demographic data about the patient, morphological characteristics, smoking and sleep habits, disease history and accompanying conditions, data on chronic drug therapy, Epworth sleepiness scale questionnaire, complete laboratory acid-base status (initial examination), 24-hour urine excretion of albumin, sodium and potassium (day after initial examination), arterial pressure, office and 24-hour measured large artery stiffness (initial examination and day after), CNAP, spirometry. Analysis of damage to target organs will be done - left ventricular hypertrophy (ECG),microalbuminuria, increase in serum creatinine, sexual dysfunction, depending on the presence or absence nocturnal fall in the stiffness of the large arteries. The clinical course of patients and the stiffness of the large arteries will be monitored during hospitalization and after hospitalization through agreed outpatient controls in the periods from 3, 6 and 12 months.

Results: Current pandemic situation compromised study plan dynamics. For reasonable circumstances out of 120 patients that had been planned for study enrollment only 5 patients were initiated. All 5 patient are form subgroup of patients with obstructive sleep apnea. Median age was 56,2 years. Male to female ratio was 4:1. The mean body mass index was 34.1± 2,4 kg/m2. For all 5 patients initial data parameters were collected including Epworth sleepiness scale questionnaire, complete laboratory acid-base status, 24-hour urine excretion of albumin, sodium and potassium, arterial pressure, office and 24-hour measured large artery stiffness , CNAP, spirometry. Further statistical analysis was not performed because of small patient number

Discussion: In future if conditions will allow this study to continue it will provide insight into the etiology of central sleep apnea and its association with possible sympathetic hyperactivation. Also will help to determine the percentage of patients with pre-existing but unrecognized vascular aging and increased CV risk

Acknowledgments: Vedran Premužić, PhD, University of Zagreb School of Medicine

MeSH/Keywords: sleep apnea, arterial stiffnes, cardiovascular risk

Poster code: R-02-09-105

Poster Title: BAFF, CXCL9 AND CCL15 IN DIFFERENT CLINICAL CHRONIC GRAFT VERSUS HOST DISEASE PHENOTYPES

PhD candidate: Ana Zelić Kerep

Part of the thesis: Proupalni citokini u različitim kliničkim manifestacijama kronične bolesti presatka protiv primatelja

Mentor(s): Assistant Professor Dražen Pulanić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Chronic graft versus host disease (cGVHD) is a serious late post-allogeneic hematopoietic stem-cell transplantation (alloHSCT) complication. The proposed immunological pathogenesis model (Cooke and colleagues, The Biology of Chronic Graft-versus-Host Disease, BBMT, 2017) consists of three distinct phases: inflammation, immune dysregulation, and sclerosis. BAFF (B-cell activating factor) is a cytokine vital to B-cell survival and is a promising cGVHD biomarker. CXCL9 (C-X-C motif ligand 9) is an interferon-gamma inducible chemokine stimulating T-cell migration to target tissues, and CCL15 (C-C motif ligand 15 or MIP5 – macrophage inflammatory protein 5) is a monocyte/macrophage chemoattractant. This study hypothesizes that clinical cGVHD phenotypes are associated with different blood cytokine profiles.

Materials and methods: Patients (78 samples from 57 patients) were evaluated in a multidisciplinary protocol (4/2017 – 12/2019; Croatian Science Foundation project IP-2016-06-8046) and grouped into 3 phenotypes: inflammatory (n=16), sclerotic (n=15), mixed (n=15). Patients who were treated with alloHSCT without cGVHD were controls (n=11). Also, patients were evaluated by a multidisciplinary team and clinical manifestations were documented in detail. Patients with active infection and overlap cGVHD were excluded. Cytokines were analyzed by commercial essays (BAFF and CXCL9 – both Quantikine, CCL15 -DuoSet ELISA kit, R&D Systems, Inc., Minneapolis, MN, USA).

Results: The cGVHD patient group had a median age of 47 years, 59% were female, 71% received alloHSCT due to AML/ALL/MDS, and 47% of patients did not receive immunosuppressive therapy at assessment. There were no significant differences between groups, except in time from cGVHD diagnosis to enrollment (ANOVA, p=0.001) and from alloHSCT to enrollment (ANOVA p<0.001), which was longest in the sclerotic group in both cases. Except for sclerotic, other groups were not significantly different on the post hoc analysis (age, sex, level of immunosuppression, underlying disease), except in time from cGVHD diagnosis to enrollment and from alloHSCT to enrollment, which was longest in the sclerotic group. Overall, median time from cGVHD diagnosis to enrollment was 5 months. Median time from alloHSCT to enrollment for all patients was 27 months. For the sclerotic group the median was 63 months. Time to diagnosis/enrollment and cytokine level data was log transformed for statistical analysis as geometric means. Both BAFF (Figure 1, ANOVA p=0.025) and CXCL9 (Figure 2, ANOVA p<0.0001) levels were significantly lower only in patients with a sclerotic phenotype. However, there was no statistically significant difference in levels of CCL15 between the groups (Figure 3, ANOVA p=0.281).

Discussion: These preliminary results suggest the biological relevance of applying Cooke´s cGVHD phenotype model to human studies. It is a step toward developing studies based on biological subgrouping, rather than using heterogeneous cohorts and organ functional impairment staging, which has the potential to yield biologically and clinically relevant biomarkers able to guide clinical decisions.

MeSH/Keywords: chronic graft-versus-host disease, clinical phenotypes, biomarkers, cytokines, immunological model

Poster code: R-02-09-115

Poster Title: Von Willebrand factor and Factor VIII in chronic Graft-versus-Host Disease

PhD candidate: Antonela Lelas

Part of the thesis: Von Willebrand factor and Factor VIII in chronic Graft-versus-Host Disease

Mentor(s): Assistant Professor Dražen Pulanić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Chronic Graft-versus-Host Disease is a serious late complication after allogeneic hematopoietic stem cell transplantation (alloHSCT) with heterogeneous presentation and poorly understood pathophysiology that includes inflammation and endothelial dysfunction. Factor VIII (FVIII) and von Willebrand Factor (VWF) are coagulation factors known as biomarkers of endothelial dysfunction and inflammation in different settings, and therefore could serve as interesting candidate biomarkers of cGvHD.

Materials and methods: Research included patients who underwent alloHSCT in University Hospital Centre Zagreb. An extensive history and physical examination using established NIH cGvHD-related measurements were performed by the Multidisciplinary cGvHD team together with detailed laboratory evaluation including FVIII, VWF:Ag and VWF:Ac analysis. Descriptive statistic and non-parametric analyses were performed. Variables that showed significant univariate correlations were used in multivariate logistic regression (MLR) to identify the most predictive for FVIII, VWF:Ag and VWF:Ac in cGvHD patients.

Results: 70 cGvHD patients and 41 controls were analysed. Median age of cGvHD patients was 42, 50% females, 91.5% underwent alloHSCT for hematologic malignancies, 55.7% had myeloablative conditioning and 52.9% matched related donor. Median time from HSCT to study was 450.5 days and from cGvHD diagnosis to study 82 days. There were no demographic neither transplant related significant differences between cGvHD patients and controls beside stem cell source (peripheral blood 71.4% vs 51.2%, p=0.041) and history of acute GvHD (70.0% vs 22.0%, p< 0.001). Majority had moderate (52.9%) or severe (42.6%) NIH global cGvHD score, 57.2% active cGvHD by clinician´s impression. Median number of organs involved by cGvHD was 3 (1-6), and the most frequently involved organs were mouth, skin and eyes (52.0% each). cGvHD patients compared to controls had higher FVIII levels (median 206 (52-453)% vs 182 (51-406)%, p=0.044, reference range 50-149%) and higher VWF:Ag (median 261.6 (76.6-601)% vs 203.2 (51.9-600)%, p=0.030, reference range 50-160%), while VWF:Ac showed a trend toward higher levels (median 253.4 (54-601)% vs 178 (48.6-601)%, p=0.084, reference range 50-150%). Patients had higher GGT (p=0.002), lower anticardiolipin IgG (p=0.001) and IgM (p=0.003), and lower albumin (p=0.018) than controls, without differences between other laboratory parameters. Univariate analysis showed that among cGvHD patients higher FVIII was associated with worse Karnofsky score (KS) (p=0.031) and performance score (PS) (p=0.030), higher leukocytes (p=0.031), cholesterol (p=0.003), triglycerides, AST, ALT, GGT, LDH, and lower albumin. Higher VWF:Ag and VWF:Ac in cGvHD patients were associated with worse KS and PS (p< 0.001), with more active cGvHD (p<0.001), worse NIH cGvHD liver (p=0.042 ; p=0.039) and NIH cGvHD mouth (p=0.012 ; p=0.009), higher total NIH score (p=0.044 ; p=0.005), higher number organs involved (p=0.013 ; p=0.003), higher ESR, monocytes, D-dimers, AST, ALT, GGT, LDH, triglycerides, β-2-microglobulin, ferritin, total proteins, IgA and lower albumin. MLR analysis showed leukocytes (p=0.018) and cholesterol (p=0.010) as the strongest predictor of FVIII (r2=49.8%; p<0.001), while strongest predictor of VWF:Ac was number of organs involved by cGvHD (r2=71.7% ; p=0.031).

Discussion: It has been confirmed that for some cardiovascular and autoimmune diseases VWF discriminates acute phase from remission much earlier than clinical manifestations or other acute phase reactants enhancing thus earlier modification of therapy and improving outcome of disease. High levels of vWF have been described among alloHSCT survivors, but its biomarker potential has not been evaluated previously. Although high levels of FVIII has been historically attributed to its physiological connection to vWF who serves as its carrier, recent reports outlined prognostic importance of FVIII:vWF ratio suggesting different mechanism of their regulation. Results of this study detected high FVIII and VWF levels in cGvHD patients with possible reflections to cGvHD manifestations, what needs to be further confirmed in larger longitudinal studies.

MeSH/Keywords: chronic Graft-versus-Host Disease, allogeneic hematopoietic stem cell transplantation, Von Willebrand Factor, Factor VIII, biomarkers

Poster code: R-02-09-121

Poster Title: Comparing sensitivity of standard visual regional wall motion abnormality assessment and 2-d myocardial strain analysis in acute coronary syndrome without ST segment elevation

PhD candidate: Daniel Lovrić

Part of the thesis: Usporedba osjetljivosti procjene kontraktilnosti miokarda standardnom ehokardiografijom i studijom deformacije miokarda u akutnom koronarnom sindromu bez ST elevacije

Mentor(s): Professor Jadranka Šeparović-Hanževački, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Visual assessment of regional wall motion abnormalities (RWMA) on echocardiography represents the current standard in assessing the impact of coronary artery disease (CAD) induced changes in myocardial contractility. Although it has been proven to predict long-term outcomes it has been hard to rely on in acute situations due to the patient dependent variance in image acquisition quality and interoperator variability. We hypothesised that regional 2DS assessment due to the evaluation of longitudinal shortening that is barely visible to the naked eye could potentially be more sensitive than visual RWMA assessment in detecting ischemia induced loss of contractility in non-ST elevation acute coronary syndrome (NSTE-ACS).

Materials and methods: We performed a retrospective analysis of patients admitted through our Emergency Room to the Cardiology Department from January 2013 till December 2015 with the diagnosis of NSTE-ACS. Patients who did not undergo coronary angiography, patients with known prior coronary artery disease and patients who did not receive an echo in the 24 hours prior to angiography were excluded, as were the patients with images not adequate for 2D strain analysis. A total of 123 patients fulfilled the criteria and were included in the analysis and 4 different clinicians blinded to laboratory and ECG results performed 2DS analysis of global and regional 2D longitudinal peak systolic strain (LPSS) according to the 18-segment model prior to coronary angiography. Regional wall motion abnormalities (RWMA) as interpreted by the clinician performing the original echo exam were categorised according to the wall motion score guidelines.

Results: RWMA assessment shows good predictive power of the region of ischemia due to coronary artery stenosis location in LAD and LCx, but not in RCA. However, LPSS was significantly more precise overall (mean sensitivity 75.6% vs 39.5%, P<0.001) for all three vessels. Statistically significant difference was present even after accounting for potentially confounding factors like arterial hypertension, smoking, alcohol, atrial fibrillation, valvular disease, age or prior medical therapy.

Discussion: We have shown that a decrease in LPSS is significantly more accurate in detecting ischemia-induced loss of myocardial contractility than the visual assessment of RWMA in patients with NSTE-ACS. Our findings imply that 2DS should be employed as a supplementary tool during the echo assessment of patients with NSTE-ACS.

MeSH/Keywords: regional wall motion assessment, acute coronary syndrome, coronary stenosis localization, regional 2D strain, myocardial ishemia

Poster code: R-02-09-138

Poster Title: Prognostic significance of extranodal extension in oral cavity cancer patients with occult neck metastases

PhD candidate: Matija Mamić

Part of the thesis: Prognostic significance of the extent of extranodal extension in clinically node negative oral cavity squamous cell carcinoma patients

Mentor(s): Associate Professor Ivica Lukšić, MD PhD, Danko Muller, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Reports in the literature suggest that extranodal extension (ENE) in head and neck tumors is not a binary characteristic. Phenotype biomarkers such as extent of ENE can be helpful in risk stratifying patients with metastatic neck setting. The goal of this study was to determine a quantitative extent of ENE in a group of surgically treated oral cavity cancer (OCC) patients within a clinically node-negative (cN0) setting, in order to investigate its prognostic significance with respect to survival and recurrence.

Materials and methods: Pathological examination of 174 OCC patients who were primarily surgically treated with tumor resection and elective neck dissection in time period from January 2009 to March 2015 at the Department of Oral and Maxillofacial Surgery, University Hospital Dubrava, Zagreb was performed. Data of ENE presence, its extent (in millimeters), patients and tumors characteristics were statistically analyzed with respect to disease-free survival (DFS) and overall survival (OS).

Results: Ninety patients (51,7%) were identified with occult nodal disease, with 41 patients (23.6%) presenting with ENE. Receiver operating characteristics (ROC) curve analysis set threshold at 1.9 mm as an optimal ENE cutoff regarding both DFS and OS. Patients were divided by extent into minor ENE (≤1.9 mm) and major ENE (>1.9 mm) subgroups. Subgroup with minor ENE had significantly higher DFS and OS rates compared with major ENE.

Discussion: Extranodal extension (ENE) in patients with OCC is an important prognostic factor affecting DFS and OS. Various studies tried to determine clinically relevant ENE, by studying qualitative or quantitative characteristics of affected lymph node. Some authors reported macroscopic ENE as prognostically significant, while others found no differences between microscopic and macroscopic ENE in terms of final outcome. Some studies tried to define clinically relevant ENE by further grading of microscopic ENE. Results of our study determined prognostic extent of ENE with the cutoff value at 1.9 mm determined by ROC curve analysis. At this survival discriminatory cutoff microscopic extent, patients were divided into low-risk (minor ENE; ≤1.9 mm) and high-risk (major ENE; >1.9 mm) subgroups. Patients with minor ENE have significantly higher rates of 3-year and 5-year DFS and OS, which suggests the need for stratification of patients presenting ENE.

Acknowledgments: Special thanks to Marko Lucijanic, MD, PhD for assistance and advice regarding data processing.

MeSH/Keywords: oral cancer; squamous cell carcinoma; lymphatic metastasis; extranodal extension; prognosis

Poster code: R-02-10-001

Poster Title: Prognostic significance of tumor-infiltrating lymphocytes in oral cavity squamous cell carcinoma

PhD candidate: Mia Lorencin

Part of the thesis: Povezanost limfocitne infiltracije primarnog tumora s probojem čahure u metastatskom limfnom čvoru u bolesnika s planocelularnim karcinomom usne šupljine

Mentor(s): Assistant Professor Martin Jurlina, MD PhD, Danko Muller, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Squamous cell carcinoma of the oral cavity is one of the most common malignancy of the head and neck with a poor prognosis despite advances in treatment. The International Immuno-Oncology Biomarkers Working Group has published a detailed method for the semiquantitative analysis of lymphocyte infiltration of solid tumors on hematoxylin-eosin slides. In this paper we present the preliminary results of analysis of associations between the percentage of lymphocytic infiltration of the primary tumor and survival outcomes.

Materials and methods: The research was performed on tumor tissue of primary squamous cell carcinoma of the oral cavity of patients who were primarily surgically treated in the Department for Maxillofacial and Oral Surgery of the Medical Faculty of the University of Zagreb in the period from 1.1.2009. to 31.12.2014. and who were followed for a minimum of 5 years. Inclusion criteria: pathohistological diagnosis of squamous cell carcinoma of the oral cavity, including all anatomic subsites according to AJCC, 8th Edition; tumor material available for analysis, available data on patients' age, sex and outcome from the archive of medical history and outpatient controls, and the Cancer Registry of the Croatian Institute of Public Health. Patients who had previously been treated for a malignant tumor in the head and neck area were excluded from the study. pT and pN category were reevaluated. The degree of lymphocytic infiltration was analyzed according to the method for assessing lymphocytic infiltration of solid head and neck tumors described and recommended by the International Immuno-Oncology Biomarkers Working Group. Univariable and multivariable analyses were used to determine the associations between examined variables and overall survival (OS), disease-specific survival (DSS) and disease-free survival (DFS) using the Cox regression model. In multivariable analysis, only those variables that appeared statistically significant in the univariable analysis were included for the estimated models. Hazard ratios and 95% confidence intervals were reported for all analyses. P-value of <0.05 was considered statistically significant. The statistical analysis was performed using IBM SPSS Statistics and MedCalc.

Results: Preliminary results on 20 patients showed that the percentage of tumor-infiltrating lymphocytes (TILs) in the stromal part (sTILs) ranged from 5% to 90%, in the intratumoral part (iTILs) it ranged from 0% to 35%. Cut-off points were applied to stratify the tumors as having low or high TILs. The most clinically relevant cut-off point was 20% (low TILs ≤20%; high TILs >20%) at the invasive front. Four tumors (20%) had low TILs, while 16 tumors (80%) had high TILs. No association was found between sTILs and age, sex, TNM stage or WHO grade (P>0.05). In the univariable analysis low sTILs (≤20%) at the invasive front was shown to be a poor prognostic factor for OS (HR 2.45; 95% CI, 1.71-3.56; P< 0.001). sTILs showed a promising prognostic value for prediction of OS (HR 2.62; 95% CI, 1.76-3.82; P < 0.001), DSS (HR 1.98; 95% CI, 1.08-3.68; P =0.040), and DFS (HR 1.92; 1.17-3.29; P = 0.020).

Discussion: Recent research continues to stress the role of immune cells in modifying cancer invasion and metastasis. There is currently a lot of research effort to standardize the evaluation of TILs on HE slides. Many studies showed that a strong lymphocytic infiltration is associated with better outcome of different malignant tumors. These results could be due to the destruction of tumor cells and anti-tumor effect of the immunological system. The evaluation of TILs could have prognostic value in oral squamous cell carcinoma. The method introduced by the International Immuno-Oncology Biomarkers Working Group is simplistic and inexpensive and could easily be included in the routine pathology report. Findings on TILs in this study could be indicative of usefulness of routine measurement of immune response in oral squamous cell carcinoma, which in the future might enable us to classify patients into risk groups according to TILs and therefore provide a personalized treatment approach.

MeSH/Keywords: Oral cavity, squamous cell carcinoma, tumor-infiltrating lymphocytes, prognosis

Poster code: R-02-10-027

Poster Title: The relationship between tumor budding and tumor deposits and their relation to clinical-pathological parameters in patients with colorectal carcinoma

PhD candidate: Zdenko Bilić

Part of the thesis: Odnos tumorskoga pupanja i tumorskih depozita te njihova povezanost s kliničko-patološkim značajkama u bolesnika oboljelih od kolorektalnoga karcinoma

Mentor(s): Assistant Professor Mario Zovak, MD PhD, Alma Demirović, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Colorectal carcinoma (CRC) occupies the third place of incidence in men and the second place in women with about 1.8 million newly diagnosed cases and 860 000 deaths in the world annually. In addition to the earlier well-known parameters of the TNM classification, some other pathohistological and morphological tumor features have been detected lately as important prognostic factors, some of which are tumor budding (TB) and tumor deposits (TD) which we will study in our work. TB represents local dissemination of a small group of dedifferentiated tumor cells (less than 5 cells) at the invasive edge of the carcinoma into the surrounding tissue. It is considered to represent the histological manifestation of epithelial-mesenchymal transition (EMT). These events are probably related to the increased tumor aggressiveness and consequently worse prognosis. TD represents local extramural clusters of tumor cells in discontinuity with the main tumor mass and are not associated with lymph nodes or vascular and neural structures and are also related to a worse prognosis. A literature review did not reveal any published papers on the relationship between tumor budding and tumor deposits.

Materials and methods: A cross-sectional study with retrospectively collected data will be conducted at the Department of Pathology and Cytology and the Department of Surgery, Sestre milosrdnice University Hospital Center (SMUHC). We will use the archival materials of the Department of Pathology and Cytology will be used together with all relevant patients' clinical data available in the medical records and archives of the Department of Surgery in at least 70 patients operated due to CRC between January 1st, 2009 and December 31st, 2013. For each patient, all the tissue blocks stained with H&E will be analyzed to review the pathohistological characteristics and to estimate the presence of TD. Tumor budding will be determined using the International Tumor Budding Consensus Conference (ITBCC) recommendations, published in 2016. In addition, routine H&E stained tissue sections in which the largest number of tumor buds will be determined by the above method will be further analyzed by PAN-CK staining to estimate the number of buds more accurately.

Results: 1227 patients underwent potentially curable surgical resection for suspected CRC, all with adequately medical data entered into the database. The average age was 67 years. There were 61% male patients (N = 754) and 39% female patients (N = 473). The most common localizations of CRC were: rectum and rectosigmoid transition (43%), left-sided CRC (28%), right-sided CRC (21%), transverse colon (4%). Of the total number of patients, those who underwent emergency surgery procedures (N=158) and patients in whom the diagnosis of colorectal adenocarcinoma was not confirmed by pathological analysis (N=117) were excluded from further analysis. Also, the patients who had a recurrence of previously operated colorectal adenocarcinoma (N=38), metastatic disease present at the primary surgical procedure (N=134), and patients who had previously undergone neoadjuvant chemoradiotherapy (N=7) were excluded from the study. Further pathohistological analysis of the remaining 767 patients is underway. They will be divided into two equal groups, the group with present tumor deposits (at least 35 patients) and the control group (at least 35 patients) without present tumor deposits. The presence of TB will be further assessed separately in these groups.

Discussion: Preliminary research results show that the age-sex structure of the operated patients from CRC in SMUHC is similar to those in the literature data. Also, the other clinical and pathological data, such as localization, corresponds to previously conducted researches. Further pathological and statistical analysis of the results regarding the distribution of TB and TD in selected cases follows to check for a possible association of TB and TD in CRC. The possible association of TB and TD could indicate that in the early stage of tumor progression, it is more likely to be due to the direct spread of dedifferentiated tumor budding cells into the surrounding stroma and less likely due to the lymphogenic or perineural spread. New findings on tumor budding and tumor deposits could in the future influence a change in therapeutic modalities in the treatment of patients with colorectal cancer.

MeSH/Keywords: Colorectal carcinoma, CRC, epithelial-mesenchymal transition, EMT, tumor budding, TB, tumor deposits, TD

Poster code: R-02-10-128

Poster Title: Prediction of methicillin-resistant staphylococcus aureus in infections after vascular surgical reconstructions

PhD candidate: Inga Đaković Bacalja

Part of the thesis: Prediktivni model za pojavu infekcije meticilin rezistentnim Staphylococcus aureus nakon vaskularnih kirurških rekonstrukcija

Mentor(s): Assistant Professor Tomislav Meštrović, MD PhD, Professor Zdenko Sonicki, MD PhD

Affiliation: Medical University Zagreb

Introduction: Vascular surgical site infections (VSSI) are accompanied by a significant mortality and morbidity. Among these, methicillin-resistant Staphylococcus aureus (MRSA) infections are particularly frequent and severe, frequently accompanied by anastomotic disruptions, graft occlusions and bleeding.

Materials and methods: A retrospective analysis of risk factors for MRSA infections after vascular reconstructive surgery will be performed. The data will be collected retrospectively from medical histories and electronic database in University hospital centre Zagreb, Division for vascular surgery, in the period from 1996 to 2021. Preoperative, operative and postoperative variables will be included. A group of the variables of the observed patients will be explored by descriptive statistics, by data mining and by univariant statistical analysis.

Results: So far, partial data for time periods from 1996 to 2003 and from 2014 to 2016 have been analysed. During the earlier from the abovementioned periods, MRSA infections comprised around 40% of VSSI's, resulting in amputation in around 10% of patients and lethal outcome in 8,5%. The usage of the infrainguinal synthetic graft was the most important risk factor for development of MRSA infection. Additional risk factors associated with MRSA infection included patient's age, diabetes, hypertension, smoking history, duration of hospitalization and severity of infection according to Szilagyi. In the period from 2014 to 2016, data from 27 patients with VSSI's were collected. Outcomes of treatment of infection after vascular reconstruction were divided into survival, amputation and death. Of the 27 subjects, 14 survived without amputation, 7 were amputated, and 6 of them died. The average age of deceased patients was 70 years, 83% were smokers, 100% had hypertension, 50% were adipose and 50% of this group had a reoperation ("Re-Do procedure"). Observing the group with poor outcomes (death + amputation), we got a slightly younger average age of 67.46 years. Adipose patients appeared to have had better outcome (69%). The most common causative agent in the group with poor outcome was Pseudomonas (61.5%), while the male gender was highly predominant in all groups. Unlike in the previously investigated period (before 2013), when MRSA predominated as a causative agent, Pseudomonas was dominating in this period, while other causative agents occured less frequently. Resistant bacteria were isolated from 13 patients out of 27 (48%). Predominantly, these were suprainguinal bypasses in 46%, but an increase in infraingual synthetic bypasses was also noticed in relation to the total sample (38%). In the group of patients in whom resistant bacteria were isolated, we also observed the incidence of early infections in as many as 69% of cases. Random forest analysis revealed suprainguinal synthetic graft, renal insufficiency, severity of infection according to Szilagyi and body mass as significant variables related to outcome. Although there were not many MRSA infections in this period, preliminary random forest analysis revealed isolation of VRE (presumably as a result of vancomycin treatment), Szilagyi score, early infection, presence of G- bacteria or MSSE, as well as re-operation, as risk factors for MRSA infection. Decision tree analysis also revealed false aneurysm resection, suprainguinal graft, body mass lower than 77 kg, and isolation of Pseudomonas as variables relevant for classification into group with poorer outcomes.

Discussion: High mortality rate of 22% in the period from 2014 to 2016 confirms the severity of VSSI's and underscores the importance of unravelling contributing risk factors. Although these results are just preliminary, and much data remains to be harvested, there is still a hint of the changes in bacterial „landscape" over time, as well as changes in surgical technique, while sporadical data also imply the possibility of the existence of other factors contributing to poor outcome or development of infection. The research will, therefore, be expanded according to the influx of new data with potentially relevant variables, as well as with the analysis of risk factors for the emergence of VSSIs with other resistant bacteria apart from the MRSA. If possible, the trend analysis over the longer time period of one fourth of a century (years 1996-2021), will be performed.

MeSH/Keywords: methicillin-resistant Staphylococcus aureus, vascular graft infection, risk factors, clinical prediction rules

Poster code: R-02-10-131

Poster Title: Correlation of transection line and future liver remnant hypertrophy in associated liver partition and portal vein ligation for staged hepatectomy

PhD candidate: Ivan Romić

Part of the thesis: Correlation of transection line and future liver remnant hypertrophy in associated liver partition and portal vein ligation for staged hepatectomy

Mentor(s): Assistant Professor Goran Augustin, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) is an innovative surgical approach in two stages used in patients with advanced liver malignancies with inadequate postoperative future liver remnant (FLR). It includes liver transection and portal vein ligation in the first stage, which is followed by a 1-2 weeks of FLR hypertrophy. In the second stage, the diseased liver lobe is removed following the standard division of biliary structures. The exact underlying mechanisms of intensive FLR hypertrophy are still unknown and further clinical and fundamental studies are required to address these questions. The main goal of our multicentric study is to identify and analyze the impact of transection line and disease characteristics on the degree of remnant liver hypertrophy.

Materials and methods: Patients from 3 high-volume hepatobiliary surgical centers were included. Only patients with malignant disease limited to the liver and those who underwent both stages of ALPPS were analyzed. Relevant patient/disease related data were collected, and pre/post-procedural radiological images were evaluated using imaging-based CT volumetry software to obtain accurate measurements of the liver and tumor volumes. The correlation of transection lines and future liver remnant hypertrophy was studied as well as the impact of different disease characteristics on ALPPS success.

Results: Data from 85 patients were analyzed by now, and they were divided into 3 different groups according to liver transection lines (central n=39, left n=28, and right n=18). Overall, the median volumetric increase of FLR was 68% (range 23-94%) over the median interstage period of 9 days. FLR hypertrophy differed significantly among the studied groups: „Central line" group showed the most intensive FLR hypertrophy (72±31%) when compared to „Right line" group (59±27%) and „Left line" group (48±21%). Postoperative mortality and morbidity rate was comparable among the groups. However, patients with non-colorectal liver metastases had significantly lower 1-year survival when compared to patients with colorectal metastases (54.4% vs. 69.2%, p=0.02). In addition, diseased liver parenchyma and age greater than 65 years were significant factors for reduced FLR hypertrophy. Liver tumor volume was not predictive of reduced hypertrophy rate or 1-year survival.

Discussion: Preliminary results showed that 45% of patients underwent ALPPS with central transection line, which is expected due to a higher prevalence of liver metastases in the right liver lobe. Colorectal liver metastases were the most common indications for ALPPS (in 70% of cases), while in the non-colorectal group, the majority of patients had hepatocellular or cholangiocellular carcinoma. Furthermore, results demonstrate that FLR hypertrophy depends on the type of transection line, which suggests that a larger volume of deportalized liver may induce more intensive hypertrophy of the contralateral lobe. The exact causes for different hypertrophy rates are yet to be investigated, but hemodynamic and inflammatory changes may have an important role in hypertrophy induction. In addition, this analysis of the first 85 patients shows the feasibility and safety of ALPPS, especially when used in patients with colorectal liver metastases, since postoperative mortality and morbidity rate were comparable to standard liver resections. Significant prognostic factors of mortality were older age and diffuse liver diseases, and the final results of this dissertation will provide us a thorough analysis of other prognostic factors as well as factors that impact hypertrophy potential. Therefore, a larger number of patients will allow us to adequately test the hypothesis and confirm these preliminary results related to the correlation of transection line and FLR hypertrophy.

MeSH/Keywords: liver: hypertrophy; resection; staged

Poster code: R-02-10-142

Poster Title: Molecular epidemiology of neuroinvasive arboviruses in croatia

PhD candidate: Maja Bogdanić

Part of the thesis: Prevalence and molecular epidemiology of neuroinvasive arboviral infections in Croatia

Mentor(s): Assistant Professor Tatjana Vilibić Čavlek, MD PhD, Assistant Professor Vladimir Savić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: (Re-)emerging arboviruses represent a public health problem in many parts of the world. Among neuroinvasive arboviruses, tick-borne encephalitis virus (TBEV), West Nile virus (WNV) and Usutu virus (USUV) are most widely distributed. WNV strains are classified into several genetic lineages of which lineage 1 and 2 are the most widespread. Recent data from the European countries showed that WNV strains detected in humans mainly belong to lineage 2. USUV strains are grouped into 8 lineages: five European (1-5) and three African (1-3). Most human USUV strains belong to European USUV lineages (mainly Europe 2), however African lineages were also documented. The TBEV European subtype is most prevalent in western, northern and eastern Europe and European parts of Russia, with Far East and Siberian subtypes co-circulating in Eastern Europe.

Materials and methods: A total of 488 patients with neuroinvasive infection (meningitis, meningoencephalitis, myelitis) were tested for the TBEV, WNV, USUV, Toscana virus (TOSV), Tahyna virus (TAHV) and Bhanja virus (BHAV). Viral RNA was detected in cerebrospinal fluid and/or urine samples using a real-time reverse transcriptase-polymerase chain reaction (RT-PCR). Samples identified as positive using the real-time RT-PCR assays were subjected to conventional RT-PCR and Sanger sequenced.

Results: TBEV was confirmed in 43 patients (2017-2020), WNV in 61 (2017-2018), USUV in three patients (2018) and TOSV in three patients (2018-2019). One TBEV strain detected in the urine sample of a patient with severe meningoencephalitis (2017) belonged to TBEV European subtype. All 11 sequenced WNV strains (3 from 2017 and 8 from 2018) showed circulation of WNV lineage 2. One USUV strain from a fatal encephalitis case (2018) clustered within Europe 2 lineage.

Discussion: Molecular epidemiology of arboviruses detected in Croatian patients with neuroinvasive disease showed a similar pattern as in other European countries. However, there is certain diversity among TBEV, WNV and USUV strains detected in Croatia, respectively. This diversity is particularly evident among WNV detected in humans which group into three clusters indicating circulation of multiple WNV strains.

MeSH/Keywords: Neuroinvasive arboviruses, molecular epidemiology, Croatia

Poster code: R-02-16-023

Poster Title: Prevalence of human bocavirus in respiratory samples of children hospitalized for acute respiratory infection and insight in clinical presentation in cases of monodetection

PhD candidate: Maja Mijač

Part of the thesis: Epidemiološka i klinička obilježja infekcija dišnog sustava dječje dobi s dokazanim humanim bokavirusom

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

Affiliation: University of Zagreb School of Medicine

Introduction: Human bocavirus (HBoV) is relatively recently discovered parvovirus. In children HBoV 1 relates to respiratory infections, both upper and lower respiratory tract and most common clinical diagnosis are rhinitis, acute otitis media, pneumonia, bronchiolitis and exacerbation of asthma. Laboratory diagnosis of virus is molecular, mostly by PCR. Because of prolonged shedding, virus is possible to detect in asymptomatic person and often in combination with other respiratory viruses.

Materials and methods: From May 2017 to March 2021, a total of 957 patients younger than 18 years with presumed acute viral respiratory infection, were included from two Croatian hospitals: Children's Hospital Zagreb and General Hospital Karlovac, respectively. Nasopharyngeal and pharyngeal flocked swabs from each patient were collected. Multiplex RT-PCR for 15 respiratory viruses, including adenovirus, seasonal coronaviruses, parainfluenza virus types 1-4, rhinoviruses A/B/C, RSV type A and B, influenza type A and B, human bocavirus, human metapneumovirus and human enterovirus, was performed. Demographic and clinical data were collected by a retrospective review of patient medical records.

Results: In four-year period, total of 957 patients were screened for respiratory viruses, 56% boys and 44% girls. Viral aetiology was proven in 739 (67%) patients. Most frequently detected virus was rhinovirus (38%), following RSV A/B (17%), adenovirus (15%) and parainfluenza 1-4 (11%). 73 patients (7,63%) were positive for human bocavirus, 41 male and 32 females, with median age 1,36 years. Among them, 39 had symptoms of upper respiratory tract infection (URTI), 29 of lower respiratory tract infection (LRTI) and in six of them both URTI and LRTI was diagnosed. To date, medical records were examined and clinical data collected for 53 patients with detected HBoV. Among them, 52% received antibiotic therapy and five of them received antiviral therapy, oseltamivir respectively. Five patients needed oxygen supplementation. Fortunately, none of the children needed mechanical ventilation. Chest radiography was performed for 23 children, 18 had pathologic findings of lower respiratory tract. Two patients had proven bacterial infection: one had streptococcal tonsillopharyngitis and other patient had S. pneumoniae in blood culture, while others had no bacterial isolates. In 60 cases HBoV was detected in combination with one or more respiratory viruses (82,19%) and in 13 cases it was discovered as monodetection (17,81%). For those children with sole HBoV detected, six of them had infection of upper respiratory tract (URTI), mostly presented as acute respiratory catarrh or unspecific febrile illness. Seven children had symptoms of lower respiratory tract infection (LRTI), with or without URTI, presenting as obstructive bronchitis or pneumonia, in three cases with pathologic findings on chest radiograph.

Discussion: During four-year period, HBoV was fifth most found virus in samples from children hospitalized for acute respiratory infection, presumed viral aetiology, with prevalence of 7,63%, mostly detected during winter months, with peak of detection in December and January, similar as main respiratory viruses. Consistent with other studies, this study also revealed high burden of HBoV co-detection with other respiratory viruses. However, our study found 13 cases of acute respiratory infection in which HBoV was the only pathogen detected. Half of those children had infection of lower respiratory tract, in some cases proven on X-ray, and in high percentage treated with antibiotics, which underlines need for continually human bocavirus detection. In conclusion, further efforts are needed for clarifying meaning of HBoV detection in respiratory samples. Presumed method is quantitative PCR, which is next step in our study.

MeSH/Keywords: human bocavirus, children, acute respiratory infection

Poster code: R-02-16-112

Poster Title: Frequency of BRAF V600E mutated alleles in patients with papillary thyroid cancer and its association with metastatic status

PhD candidate: Ivan Blažeković

Part of the thesis: Association of BRAF V600E mutant allele proportion with the dissemination stage of papillary thyroid cancer

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

Affiliation: University of Zagreb School of Medicine

Introduction: BRAF V600E mutations are the most common genetic alteration in patients with papillary thyroid cancer. Their prognostic value and association with aggressive features in patients with papillary thyroid cancer have been extensively studied but with conflicting results. Based on clonal evolution model and confirmed intralesional heterogeneity of thyroid papillary cancer we hypothesize that frequency of BRAF V600E mutated alleles might be associated with aggressiveness of thyroid papillary cancer. The aim of this study was to determine the frequency of BRAF V600E mutated alleles in patients with thyroid papillary cancer and to determine if it is associated with metastatic status.

Materials and methods: In this study we analysed thyroid papillary cancer samples from 72 patients (27 with localised disease, 35 with lymph node metastases, and 10 with distant metastases). DNA was isolated from formalin-fixed paraffin-embedded tissue and assessed for presence of BRAF V600E mutation using competitive allele-specific TaqMan polymerase chain reaction. Validated assay for BRAF V600E mutation and BRAF reference assay were used enabling us to calculate percentage of mutated alleles. To determine accurate frequency of mutated alleles in cancer cells, percentage of mutated alleles was normalized to the percentage of cancer cells in samples.

Results: BRAF V600E mutation was found in 47 (65,3%) out of 72 patients; 19 out of 27 (70,4%) patients with localised disease, 24 out of 35 (68,6%) patients with lymph node metastases, and 4 out of 10 (40%) patients with distant metastases. There was no significant difference between these groups of patients regarding presence of BRAF V600E mutation (p=0,15). Among patients with mutation, frequency of mutated alleles varied significantly, from 1% to 99%. Out of 47 patients with BRAF V600E mutation, 32 (68%) had frequency of mutated alleles < 30%, 11 (23%) had 30-60% mutated alleles which corresponds to heterozygous BRAF status, while 4 patients (8,5%) had frequency of mutated alleles >60%. There was no significant difference in frequency of BRAF mutated alleles between groups of patients without metastases, with lymph node metastases and with distant metastases (p=0,71).

Discussion: Our results indicate significant clonal heterogeneity in papillary thyroid cancer lesions regarding BRAF mutation status. No association was found between frequency of BRAF mutated alleles and metastatic status.

MeSH/Keywords: thyroid cancer, BRAF mutation, BRAF V600E mutation

Poster code: R-02-17-055

Poster Title: Personality traits, adherence and visual impairment in patients with primary open-angle glaucoma

PhD candidate: Dina Lešin Gaćina

Part of the thesis: Dimenzije ličnosti adherencija i oštećenje vida u bolesnika s primarnim glaukomom otvorenog kuta

Mentor(s): Associate Professor Darko Marčinko, MD PhD, Sonja Jandroković, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Glaucoma is a heterogeneous group of chronic ocular diseases characterized by retinal nerve fiber layer damage and corresponding visual field loss. Since high intraocular pressure is the only modifiable risk factor, regular application of topical hypotensive therapy in the form of drops is necessary for preventing progressive optic neuropathy. Nonadherence to glaucoma treatment is emphasized as an important clinical and economic burden. According to researches, psychological characteristics of patients are possible reason for such neglectful behavior. However, correlation among personality traits of glaucoma patients, adherence and consequent visual impairment is not still clear.

Materials and methods: This cross-sectional clinical study included 110 patients diagnosed with primary open-angle glaucoma (POAG). All participants underwent comprehensive ophthalmological examination. The main sociodemographic and clinical information were obtained from all patients. The Temperament and Character Inventory (Cloninger et al, 1993) was used in order to determine personality traits. Adherence pattern to the prescribed medication was assessed simultaneously by Čulig adherence scale (CAS). In addition, structural impairment was defined by retinal nerve fiber layer (RNFL) thickness measured by optical coherence tomography. Static automated perimetry (Octopus 900/G) was used for quantifying visual field loss, determined by parameter mean defect (MD). Using adequate statistic methods we will study correlation between personality traits, adherence to medication and visual impairment in patients with POAG.

Results: 110 POAG patients have been participated in the study so far. The study is currently in the phase of statistical analysis and preliminary results expected to be finalized in July 2021.

Discussion: The issue of non-adherence with prescribed therapy among glaucoma patients is major problem in clinical practice. Limited number of studies about personality traits and adherence among glaucoma patients have been conducted, taking into account the treatment outcomes. Hence, after receiving the results of this research, we will be able to evaluate impact of psychological characteristics of patients on adherence to treatment. Furthermore, regarding to results of the researches we could develop strategies for improving adherence and thereby reduce the rate of disease progression in glaucoma patients.

MeSH/Keywords: glaucoma; medication adherence; personality traits; structural visual impairment; functional visual impairment

Poster code: R-02-18-007

Poster Title: Macular perfusion analysed by optical coherence tomography angiography after uncomplicated phacoemulsification: benefits beyond restoring vision

PhD candidate: Ana Ćurić

Part of the thesis: Macular perfusion analysed by OCT angiography after cataract surgery by phacoemulsification

Mentor(s): Associate Professor Nenad Vukojević, MD PhD, Assistant Professor Mirjana Bjeloš, MD PhD

Affiliation: University of Zagreb School of Medicine, Faculty of Medicine, J. J. Strossmayer University of Osijek

Introduction: The purpose of the study is to investigate the changes of macular perfusion after uncomplicated phacoemulsification using OCT-angiography (OCT-A) OCT-A was performed before, 1 week, 1 month, and 3 months after surgery. Images with quality index (Q) ≥ 30, as computed by integrated software, were further quantitatively analysed with AngioTool 0.6 software (National Institute of Health, National Cancer Institute, Bethesda, USA).

Materials and methods: Superficial vascular complex (SVC), nerve fiber layer vascular plexus (NFLVP) superior vascular plexus (SVP), deep vascular complex (DVC), intermediate capillary plexus (ICP) and deep capillary plexus (DCP), as well as choroidal blood vessels and choriocapillaris (CC) were analysed for explant area (EA), vessels area (VA), vessels percentage area (VPA), total number of junctions (TNJ), junctions density (JD), total vessels length (TVL), average vessels length (AVL), total number of end points (TNEP), and mean lacunarity (ML). A comparison of pre- and post- operative values was made using non-parametric Friedman ANOVA test. The significance level was set to $P < 0.05$.

Results: Significant changes of vascular parameters in 55 eyes of 55 patients mostly reached plateau 1 week after surgery and remained stable up to 3 months after surgery, occurring in all retinal layers but not in choroid and CC. A significant increase in VA, VPA, TNJ, JD, TVL and AVL was found, followed by the decrease in TNEP and ML manifesting rise in blood supply of the central macula after phacoemulsification. The greatest increase in VPA (22.79%), TVL (16.71%), AVL (166.71%) and JD (29.49%) was in SVC. On the contrary, the greatest change of ML (− 53.41%) appeared in DVC.

Discussion: For the first time, this study revealed that uncomplicated phacoemulsification significantly improved macular haemodynamics. This perfusion alterations are most likely due to functional hyperaemia instead of inflammatory proces as the effect remained steady up to 3 months and average Q before and after phacoemulsification demonstrated no significant changes. We hypothesize that the effect is evoked by increased light intensity stimulation of retina after cataract removal. Thus, phacoemulsification in elderly population could have advantageous feature in addition to restoring visual acuity. This beneficial event could facilitate the decision-making process with regard to earlier timing for cataract removal in healthy aging patients.

MeSH/Keywords: Macula, Angiography, Retinal vessels, Blood supply, Phacoemulsification, Cataract

Poster code: R-02-18-024

Poster Title: Myocilin mutations in patients with glaucoma

PhD candidate: Tena Križ

Part of the thesis: Correlation of biomarkers BDNF, MYOC, IL-6 i PPARγ with optic nerve damage in glaucoma patients

Mentor(s): Professor Jadranka Sertić, MD PhD, Assistant Professor Mia Zorić Geber, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Primary open angle glaucoma (POAG) is chronic progressive optic neuropathy and one of the main causes of irreversible visual loss worldwide. It is most common type of glaucoma, typically asimptomatic until advanced visual field loss occurs. The exact mechanism of its occurance is still unclear, although some risk factors for POAG are identified among which intraocular pressure (IOP) is the most significant, and the only one we can affect. Also, genetic factors are increasingly researched and they are thought to play an important role in the development of glaucoma. Myocilin is protein encoded by the myocilin gene (MYOC) which plays an important role in the regulation of IOP. Mutations in MYOC accounts for 4% of adult POAG cases, and 10% of juvenile POAG. Two of the most common glaucoma causing variants of MYOC worldwide are Gln368* and Thr377Met. We have investigated the presence of mutations in MYOC gene in Croatian population with POAG.

Materials and methods: The study included 144 participants, 78 glaucoma patients and control group 66 patients with cataracts, 53 were male. Patients are from different parts of the country. Samples of genomic DNA were analyzed for two most common mutations in myocilin coding gene: p.Gln368* and p.Thr377Met. Exon 3 of the MYOC gene was amplified by PCR with specific primers. PCR fragments were sequenced bidirectionally using Big Dye® Terminator v3.1 Cycle Sequencing Kit and Applied Biosystems 3130xl Genetic analyzer.

Results: Mutation p.Thr377Met was detected in 3/78 (3.85%) patients with glaucoma. Mutation p.Gln368* was not found in any of the analyzed samples.

Discussion: Our study indicate that MYOC p.Thr377Met and p.Gln368* mutations are rare in Croatian POAG patients, but as common as in other populations. Also, this supports the use of DNA testing for relatives who share genetic background for early detection and treatment.

MeSH/Keywords: glaucoma, MYOC

Poster code: R-02-18-051

Poster Title: The relation between intracranial and intraocular pressure

PhD candidate: Maja Bakula

Part of the thesis: Odnos intrakranijskoga i intraokularnoga tlaka u bolesnika s akutnim povišenjem intrakranijskoga tlaka

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

Affiliation: University of Zagreb School of Medicine

Introduction: Since invasive methods of intracranial pressure measurement carry a number of possible complications, there is an interest for a surrogate non-invasive method of monitoring increased intracranial pressure. There are contradictions in literature about IOP and ICP correlation.

Materials and methods: We are conducting a prospective study and will include 30 participants. So far 12 patients were identified and included in the study and two of them were excluded since we could not finish measurements due to disease progression. Of 10 patients, 4 were female and 6 were men. The participants are patients who require continuous invasive intracranial measurement because of acute intracranial lesion, traumatic or nontraumatic etiology. Simultaneously we are measuring intraocular pressure during three days with handheld tonometer. The study will not include politraumatized patients, patients with injuries of the orbit, eye adnexa and eye, patients with heteroanamnestically obtained data on glaucoma disease and patients with corneal diseases. Patients suffering from hydrocephalus, brain tumors and other conditions that cause a chronic increase in intracranial pressure will also not be included in the study. The research is anonymous and in accordance with the basics of good clinical practice, the Helsinki Declaration, the Health Care Act of the Republic of Croatia (NN 121/03) and the Patients' Rights Act of the Republic of Croatia (NN 169/04). As the patients are not able to give their consent for the measurement of intraocular pressure due to the nature of the disease, the purpose and method of the research will be explained to the legal guardians. Intraocular pressure is measured at the same body position as intracranial pressure to avoid the possible impact of a change in body position on the values of both pressures. Intraocular pressure is measured by a non-invasive method using a portable hand-held tonometer after application of a local anesthetic (tetracaine 0.5%) to the eye. The portable tonometer (Tono-Pen XL Reichert Technologies) is useful because of the ability to measure pressure at different body positions, and the values obtained correlate well with the values of applanation Goldmann tonometry as the gold standard. Intraocular pressure is measured in both eyes in three consecutive measurements from which the mean IOP for each eye will be calculated. The cut-off value of the elevated IOP is > 21 mmHg. Intracranial pressure will be continuously monitored by EVD (external ventricular drain) with a cannula placed in the lateral ventricle of the brain, and connected to a pressure transducer. The cut-off value of elevated ICP is > 20 mmHg.

Results: Since we managed to collect only one third of planned measurements, we did not conduct any statistical analysis as the data would not be sufficient for adequate analysis. So far it seems that there is no evident rise in IOP values with ICP rise, but more data must be collected.

Discussion: Further measurements need to be done in order to achieve planned number of participants. Intraocular pressure measurement should be assesed if it could be an auxiliary tool for monitoring high intracranial pressure values.

MeSH/Keywords: ocular tonometry, intraocular pressure, intracranial pressure, intracranial hemorrhage, intracranial hypertension

Poster code: R-02-18-146

Poster Title: OUTCOMES FOR THE FIRST LINE TREATMENT FOR METASTATIC COLORECTAL CANCER IN THE UHC ZAGREB

PhD candidate: Nikša Librenjak

Part of the thesis: Prognostičko i prediktivno značenje indeksa sustavnog upalnog odgovora u liječenju bolesnika s metastatskim rakom debelog crijeva

Mentor(s): Professor Stjepko Pleština, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Metastatic colorectal cancer (mCRC) is an incurable disease for most of the patients with predominant treatment modality being palliative systemic chemotherapy to improve overall survival (OS). The addition of biological therapy to standard chemotherapy as well as the sequential use of all available treatments have improved prognosis in patients with mCRC with median OS currently reaching 2 to 3 years. Systemic inflammation plays a key role in pathophysiology of many conditions including cancer so it is important to identify prognostic biomarkers. Systemic inflammation response indices have been associated with worse prognosis in CRC stage II and III. However, there is still few data on exploring these indices in mCRC, especially in patients treated with modern protocols including monoclonal antibodies and beyond first line setting. Also, there is lack of information about relationship between the inflammatory indices and RAS and BRAF mutational status.

Materials and methods: The objective of this retrospective observational, single-centre study conducted in the University Hospital Centre Zagreb, was to investigate the outcomes of the first-line treatment for mCRC. Data were retrieved from medical records on 298 patients who began therapy between January 1st, 2016 and January 1st, 2019. All the eligible patients were aged at least 18 years, had histologically confirmed disease, and completed the first-line therapy by May 10th, 2021. Primary outcome was measured as progression-free survival (PFS). The patients continued the same treatment protocol until radiological and/or clinical disease progression, death, or unacceptable toxicity. Objective evaluation of response to therapy with computed tomography, carcinoembryonic antigen (CEA), complete blood count and biochemistry, was routinely performed every 6 cycles. Apart from age and sex, other variables were also obtained, including ECOG performance status, primary tumor sidedness, RAS status, and biological agent received. Cox regression analysis was used to examine the association between variables. Survival was estimated using the Kaplan-Meier method and curves of the specific subgroups were compared using the log-rank test. Results were expressed as hazard ratios (HR) with 95% confidence interval (CI) with statistical significance set at a confidence level of $P<0.05$.

Results: Regarding general characteristics, median age was 64.5 years, 44% more men than women were included in the study, and the majority of patiens were in good general condition (ECOG 0). Of the patients with determined RAS status, a similar number carried the mutation as well as being wild-type, while 16.8% had unknown mutational status. The majority of patients received bevacizumab in the first-line treatment, followed by an EGFR inhibitor, and 50 patients received no biological therapy. Overall PFS was 7.6 months (95% CI 6.44-8.76) and was positively associated with better performance status (ECOG 0), left sided primary cancer, and addition of biological agent to chemotherapy. Patients with left-sided primary cancer had median PFS of 8.7 months compared to 5.9 months for right-sided. Similarly, ECOG 0 was associated with better prognosis compared to ECOG ≥1, 8.6 vs 5.00 months, respectively. There was no statistical difference regarding different biological agents, although patients who received no biological therapy had significantly worse survival. No association was found for age, sex, or RAS mutational status.

Discussion: Our results are comparable with previously reported studies and support the clinical benefit of adding monoclonal antibodies to standard chemotherapy for mCRC. Further investigation is needed to evaluate the relationship between the inflammatory indices in assessing the effect of treatment according to modern guidelines and prognosis of patients.

MeSH/Keywords: metastatic colorectal cancer, prognosis, survival, cancer associated inflammation, systemic inflammation response index

Poster code: R-02-19-081

Poster Title: Neoadjuvant treatment outcomes of patients with locally advanced HER-2 positive breast cancer treated with anthracycline-taxane based chemotherapy with the addition of dual HER-2 targeted therapy

PhD candidate: Petra Vuković

Part of the thesis: Kliničko značenje tumorske hipoksije mjerenje izraženošću tumorskih biljega karbonične anhidraze IX I Bcl-2 u bolesnica s HER-2 pozitivnim rakom dojke liječenih neoadjuvantnom terapijom

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

Affiliation: 1 Department of Medical Oncology, University Hospital for Tumors, Sestre milosrdnice University Hospital Center, Zagreb, Croatia; 2 Department of Clinical Oncology, School of Medicine, University of Zagreb and Division of Oncology and Radiotherapy, Univer

Introduction: HER-2 positive breast cancer (BC) is an aggressive subtype that accounts for up to 20% of all BC. The neoadjuvant treatment approach is the preferred approach for the treatment of locally advanced HER-2 positive BC, allowing evaluation of tumor response in vivo. Neoadjuvant treatment outcome (pathological complete response (pCR) and residual disease) has been shown to be prognostic for the long-term outcome of BC patients.

Materials and methods: The analysis of patient characteristics and treatment outcome after neoadjuvant therapy of patients with locally advanced HER-2-positive BC was conducted at University Hospital for Tumors in Zagreb. Patients were treated with anthracycline-taxane based chemotherapy with the addition of dual anti-HER2 therapy (pertuzumab+trastuzumab) in a period from 2016-2019. Treatment outcome was classified using MD Anderson Cancer Center Residual Cancer Burden (RCB) calculator using measurements of the primary tumor (size and cellularity) and nodal metastases (number and size) to evaluate the response into four classes; RCB 0 (pCR), RCB-I, RCB-II, and RCB-III. pCR was defined as the absence of residual invasive cancer on the hematoxylin-eosin evaluation of the complete resected breast specimen and all sampled regional lymph nodes (ypT0/is ypN0).

Results: We identified 97 patients undergoing neoadjuvant therapy with dual HER-2 therapy for locally advanced HER-2 positive BC. Mean age was 53 years, 26.8% had hormone receptor (HR)-negative BC, and 73.2% had HR-positive BC. HER-2 positive status was determined by immunohistochemistry (3+) in 75% of patients, and in 25% by SISH. Overall, pCR rate was 45.36%. Among patients with residual disease majority had RCB-II class (RCB-I 24.5%, RCB-II 56.6%, RCB-III 18.9%). In respect to HR status, pCR rate was higher in HR-negative subgroup (pCR in HR-positive BC 40.8%; HR-negative BC 57.7%).

Discussion: Our results showed a lower pCR rate compared to cohort A in BERENICE study using the same treatment regimen (overall pCR 61.8%; pCR in HR-positive subgroup 51.6%, HR-negative subgroup 81.5%). Similarly to the previous reports, we observed a higher pCR rate in HR-negative compared to HR-positive subgroup of HER-2 positive BC.

MeSH/Keywords: breast cancer, HER-2, neoadjuvant therapy, trastuzumab, pertuzumab

Poster code: R-02-19-111

Poster Title: Risk factors for subsidence of modular revision hip endoprosthesis implanted using the transfemoral approach by Wagner

PhD candidate: Dino Bobovec

Part of the thesis: Rizični čimbenici za potonuće revizijske modularne bescementne endoproteze kuka ugrađene transfemoralnim pristupom prema Wagneru

Mentor(s): Professor Domagoj Delimar, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Total hip arthroplasty is a successful treatment for end-stage osteoarthritis, but the failure of its components still does occur and represents a devastating complication for the patient. Component failure most commonly develops as a result of mechanical loosening, dislocation, periprosthetic fracture, osteolysis, and infection. Modular fluted tapered stems are shown to be a reliable option for femoral component revision with a high rate of osseointegration and successful implant fixation. Specific revision situations like previously implanted unstable cemented femoral stem and/or Vancouver B type periprosthetic fractures represent a complex surgical challenge in which the transfemoral Wagner approach has a great value. Revision stem subsidence, however, may lead to the leg-length discrepancy, prosthesis instability, pain and ultimately require additional femoral stem revision surgery. The majority of published studies are limited due to the small number of cases in which the transfemoral Wagner approach was used, and by the short-term follow-up.

Materials and methods: A retrospective review of a single-centre surgical registry was performed. Patients who underwent a revision total hip arthroplasty, in which the uncemented modular fluted tapered stem (REVISION Hip/Anca-Ti6Al4V, LimaCorporate, Udine, Italy) was implanted using the transfemoral Wagner approach were identified. The patient's radiographic outcomes were assessed and analyzed. In this study, the significant subsidence was defined as more than 5 millimetres (mm), as suggested by many authors. Therefore, two groups were formed and analyzed, one with a subsidence of 5 mm and less, and the other with a subsidence of more than 5 mm.

Results: We identified 278 revision hip arthroplasties with a mean follow-up of 35 months. The median of subsidence in the group of 5 mm and less was 2 mm, and 17 mm in the group of subsidence of 5 mm and more. A negative correlation was found between the stem subsidence and the length of good contact between the medial and lateral cortical bone and the stem (medial, - 0.248; P < 0.001, lateral, 0.284; P < 0.001). For 200 mm stems, the percentage of good contact between femoral parts of stem and bone on the medial side was 40.5% (81.0 mm) for patients with subsidence of five or less mm, and 30% (60.0 mm) for the lateral side. For 140 mm stems, the percentage was 52.86% (74.0 mm) for the medial side and 40.36% (56.5 mm) for the lateral side.

Discussion: Modular fluted tapered stem became popular for femoral revisions due to its excellent mid-term to long-term survivorship and advantages, like modularity, prompt axial and rotational stability and decreased stress shielding. As risk factors for femoral subsidence have not been clearly defined in the literature yet, the purpose of this study was to identify radiographic risk factors for subsidence of modular fluted tapered stems implanted using the transfemoral Wagner approach. Our radiographic analysis revealed some modifiable factors that correlated with subsidence, such as bilateral bone-stem good contact zones which are shown to negatively correlate with the stem subsidence. We calculated lengths of good bone-stem contact zones, which should be achieved intraoperatively in order to prevent stem subsidence more than 5 mm. Values are calculated for both, 200 mm stems (on medial side contact should be at least 81.0 mm, and on the lateral 60.0 mm), and for 140 mm stems (medial-sided contact should be at least 74.0 mm and on lateral 56.5 mm). In conclusion, it is crucial to achieve good contact between the bilateral cortical bone and stem intra-operatively in order to reduce subsidence and to ensure longer implant survivorship.

Acknowledgments: None.

MeSH/Keywords: Hip revision arthroplasty, subsidence, risk factors, bone contact, transfemoral approach

Poster code: R-02-20-080

Poster Title: The effect of cancer stem cells and PD-L1 on extranodal extension in metastatic oral cancer

PhD candidate: Luka Manojlović

Part of the thesis: Correlation of expression of CD44, ALDH-1 and PD-L1 in oral cavity squamous cell carcinoma with regional lymph node extracapsular extension

Mentor(s): Professor Sven Seiwerth, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Oral cavity squamous cell carcinoma (OCSCC) is the sixth most common malignant tumor in the world. One third of OCSCC patients already develop regional lymph node metastases at the time of diagnosis. Combined therapy – therapy directed against cancer stem cells (CSC) and immunotherapy, is currently being intensively explored. Correlation between tumor agressiveness and cancer stem cell expression has been proven in OCSCC. It is known that they affect antitumoral immunity, in which programmed death receptor 1 (PD-1) plays an important role. Antibodies which prevent binding of PD-1 and its ligand, PD-L1, and enhance the immune response to the disease, are used in treatment of patients with immunohistochemically proven PD-L1 expression. This research will analyse the correlation between expression of CSC and PD-L1 in the primary tumors of the patients and extranodal extension (ENE) in positive lymph nodes, which is considered to be the worst prognostic factor in OCSCC patients.

Materials and methods: Tumor tissue specimens from 20 patients who were surgically treated for OCSCC with regional metastases at University Hospital Dubrava were obtained. The patients were divided into two groups, each comprised of 10 patients: those with regional lymph node tumor ENE and those without ENE. Tumor tissue specimens were routinely histologically processed and tumor tissue sections were immunohistochemically stained with antibodies for PD-L1 and cancer stem cell markers CD44 and ALDH1. Immunohistochemical staining was analysed and quantified based on the percentage of stained tumor cells and the intensity of staining. Association of immunohistochemical staining with standard clinicopathological parameters was analysed.

Results: Tumor tissue specimens of 19 male and 1 female patients aged between 31 and 80 years (median 55 years) were immunohistochemically stained for CD44, ALDH1 and PD-L1. The most frequent tumor localization was the tongue (n=10), followed by the floor of the mouth (n=5), gingiva of the mandible (n=3) and gingiva of the maxilla (n=2). According to the most recent TNM tumor staging, there were 5 T1, 2 T2, 7 T3 and 6 T4 stage tumors, and 5 N1, 5 N2 and 10 N3 (ENE) stage tumors. CD44 expression was positive more often in patients without tumor ENE (n=9) than in patients with ENE (n=5), and it also showed positive correlation with advanced tumor stage (T3, T4) (n=12). PD-L1 expression was more frequently positive in patients with tumor ENE (n=7) than in those without ENE (n=4). ALDH1 was negative in most patients. Positivity was observed in only 1 patient with ENE and in 3 patients without ENE. No significant association between expression of individual markers was detected.

Discussion: Numerous studies suggest that cancer stem cells have an important role in tumor local aggressiveness, invasion and destruction of surrounding tissue and vascular invasion. In our preliminary research results, CD44 was positive more frequently in advanced tumor stages, which indicates that cancer stem cell marker expression is positively correlated with tumor size and local invasiveness. However, no significant correlation with ENE was found. No association was found between ALDH1 and any of the investigated clinicopathological parameters. PD-1/PD-L1 interaction enables the tumor to evade antitumoral immunity and PD-L1 expression is an independent negative prognostic marker for patient outcome and survival. In this research, PD-L1 expression was associated with ENE, suggesting that those patients might benefit from anti-PD-L1 immunotherapy. The complete research will provide more conclusive results.

MeSH/Keywords: oral cavity squamous cell carcinoma; cancer stem cells; immunohistochemistry; immunotherapy

Poster code: R-02-23-022

Poster Title: GMNN, PCNA and EZH2 gene and protein expression in diffuse large B-cell lymphoma.

PhD candidate: Katarina Horvat Pavlov

Part of the thesis: Ekspresija gena i proteina GMNN, PCNA i EZH2 u difuznom B-velikostaničnom limfomu

Mentor(s): Professor Slavko Gašparov, MD PhD, Associate Professor Petra Korać, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Diffuse large B-cell lymphoma (DLBCL) is the most frequent type of adult non-Hodgkin lymphoma. Classification of DLBCL has been subjected to many changes due to constant research and new clinical, biological and molecular findings. However, majority of cases remains unclassifiable to any of specific categories, and are classified as not otherwise specified subtype (DLBCL-NOS). Gene expression profiling enabled determination of two subtypes of DLBCL-NOS; prognostically favourable subtype with gene expression similar to germinal centre B cells (GCB) and prognostically adverse subtype with gene expression similar to activated B cells (non-GCB). Due to high technological and financial demands of gene expression profiling, expression of proteins determined by immunohistochemical staining is widely used as adequate surrogate.   Genes GMNN, PCNA and EZH2 are some of decisive in process of replication and chromatin reassembly. Mutations of genes included in the process of replication and chromatin reassembly allow accumulation of errors and represent one of the main steps in tumorigenesis.

Materials and methods: Preliminary analysis has been conducted on 10 formalin fixed, paraffin embedded (FFPE) tonsile tissues (control group) and 10 FFPE lymph nodes from patients with the diagnosis of DLBCL NOS (10 DLBCL GCB and 10 DLBCL non-GCB). All tissue samples were revised by three experienced hematopathologists. Non-contact laser capture microdissection was than performed on FFPE. Microdissected germinative centres and DLBCL tumour parts were thawed and subjected to RNA isolation. Relative quantification of GMNN, PCNA and EZH2 was analysed using TBP1 gene as endogenous control. Immunohistochemical staining was performed on FFPE sections with primary antibodies.

Results: No significant difference was found comparing gene expression between two subtypes of DLBCL NOS. Comparing gene expression of both subtypes of DLBCL NOS as one group, lymphoma cells showed significant downregulation of GMNN ($p<0.01$) in comparison to germinative centre B-lymphocytes. Comparison of protein expression between DLBCL NOS and germinative centre of tonsils revealed statistically significant lower expression of PCNA ($p<0.01$) in DLBCL NOS.

Discussion: Pilot project has been conducted in order to determine if all analysis planned to be performed are functional on paraffin imbedded tissue samples that have been archived for over a decade. Staining with hemalaun eosin, microdissection, RNA isolation, Q-RT PCR as well as immunohistochemical staining all gave results that were reproducible, and initial analysis were adequate. Results are insufficient for major conclusions, but pilot project proved the designe of the study to be feasible.

MeSH/Keywords: DLBCL, GMNN, PCNA, EZH2, lymphoma, lymphoma genesis

Poster code: R-02-23-097

Poster Title: Partial Dedifferentiation of Human Mesothelioma

PhD candidate: Sunčana Sikirić

Part of the thesis: Utjecaj mitohondrijskog energijskog metabolizma i visoke koncentracije glukoze na ekspresiju gena pluripotentnosti SOX2 i NANOG i vijabilnost mezoteliomskih stanica in vitro

Mentor(s): Professor Sven Seiwerth, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Pluripotency genes, such as OCT4, NANOG and SOX2, are a key regulator of tumor differentiation and therefore may be critical for the clinical outcome of malignant tumors. The purpose of this study was to examine whether human malignant mesothelioma cell line Mero-14 expresses pluripotency genes (OCT4, NANOG and SOX2) and whether their expression is affected by reactive oxygen species (ROS) produced by the mitochondria.

Materials and methods: The Mero-14 cell line was used as an in vitro mesothelioma model. To stimulate mitochondrial ROS production, cells were treated with the mitochondrial electron transport chain complex III inhibitor - antimycin. mitoTEMPO was used to scavenge mitochondrial ROS. Immunocytochemistry was used to determine the expression levels of proteins OCT4, NANOG and SOX2, while vimentin and cytokeratin 7 were used as controls. ROS generation was identified using CM-H2DCFDA fluorescence indicator and quantified using the EVOS imaging system.

Results: Mero-14 cells exhibited NANOG and SOX2 expression and no expression of OCT4. Antimycin A dose-dependently increased mitochondrial ROS generation. At lower, but not higher concentrations antimycin A enhanced NANOG expression. Although SOX2, vimentin and cytokeratin 7 were expressed in Mero-14 cells the use of antimycin A did not affect their expression levels. mitoTEMPO abrogated antimycin A induced increase in NANOG expression.

Discussion: Immunocytochemical quantification of protein expression levels in cell culture is highly sensitive and accurate, due to the ability to identify individual cells and normalize the signal to background. It is shown that Mero-14 cells express pluripotency genes NANOG and SOX2. ROS generated by mitochondria can induce NANOG expression and as such may trigger reprogramming of mesothelioma cells toward more malignant phenotypes. Pluripotency genes OCT4, SOX2 and NANOG are markers of cancer stem cells. This experiment showed that antimycin A treatment enticed high expression of NANOG in cells, which can correspond to cancer stem cells features. This indicates that mesothelioma reprograming with mitochondrial ROS likely upregulates cancer stem cells.

MeSH/Keywords: Differentiation, mesothelioma cell line, mitochondrial ROS, pluripotency genes

Poster code: R-02-23-107

Poster Title: Expression and prognostic significance of PD-L1 in HER2-positive breast cancer

PhD candidate: Melita Perić Balja

Part of the thesis: Expression and prognostic significance of PD-L1 in HER2-positive breast cancer

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

Affiliation: University of Zagreb School of Medicine, Department of Pathology, Sestre milosrdnice University Hospital Center

Introduction: HER2-positive breast cancers (BC) occur in about 20% of all BC, with two intrinsic subtypes: a luminal subtype with positive hormone receptors, and a true HER2 enriched with negative hormone receptors. The response to therapy and disease prognosis is not identical for both groups. Patients with locally advanced HER2-positive BC receive dual anti-HER2 therapy in a neoadjuvant setting (NAC). After NAC, patients undergo surgery when pathologists assess tumor response to treatment based on the pathological TNM and the residual cancer burden (RCB). Approximately half of the patients achieve a complete pathological response (pCR), and the patient's prognosis correlates with residual disease. During tumor progression, the immune system creates strong lymphocyte infiltrations into the tumor area (TILs). In the process of recognizing antigens on tumor cells, the key protein is the programmed cell death receptor (PD-1) on lymphocytes. The binding of PD-1 on lymphocytes to the PD-L1 ligand on tumor cells results in inhibition of the cytotoxic action of T cells. By blocking that interaction, the antitumor immune response is reactivated. Monoclonal immunotherapy with PD-L1 inhibitors is used in the treatment of metastatic triple-negative BC that express PD-L1. We hypothesized that patients with HER2-positive and PD-L1-positive BC have a poorer response to NAC and a shorter time to disease progression compared to those with PD-L1-negative. Therefore, we determined the immunohistochemical expression of PD-L1 in residual tumor and TILs after NAC of HER2-positive BC and compared it with pathohistological parameters of BC and the disease progression.

Materials and methods: The study is performed on paraffin-embedded archival material of 63 patients with HER2-positive BC who did not achieve a complete response to NAC in the period from 01.01.2015. to 31.12.2018. Tumors were grouped in Luminal HER2-positive (Lum/HER2), and HER2-positive (HER2E). All clinical and pathohistological data are taken from medical records from Sestre milosrdnice University Hospital Center. The local recurrence and locoregional or distant metastases are considered disease progression. The median follow-up time was 26 months. Histological materials with the highest proportion of tumor and TILs were selected for PD-L1 analysis. Immunohistochemical staining was performed in automated staining device Ventana BenchMark® using rabbit monoclonal antibody to PD-L1 (Ventana, clone SP142, RTU). The result is recorded as a percentage of stained cells, and the cut-off value for a positive result is 1%, according to the guidelines for triple-negative BC. Differences in pathohistological characteristics according to PD-L1 status (positive/negative) were analyzed by χ^2 test and Fisher's exact test.

Results: The mean age of patients with HER2-positive BC who did not have a pathological complete response (pCR) to NAC was 56±11.87 years. The mean tumor size was 16±1.49 mm, and 51% of patients had positive lymph nodes. Immunohistochemical staining was performed on 23 HER2E and 40 Lum/HER2 BC. Our preliminary results show that TILs are positive for PD-L1 in 27/63 cases (42.9%), while tumors were PD-L1-positive in only 6.3%. Although TILs were more often positive for PD-L1 in the HER2E (52.2%) than in the Lum/HER2 (37.5%), there was no statistically significant difference (χ^2=1.28; P=0.257). There was no statistically significant difference in the average age at diagnosis, the percentage of positive lymph nodes, and the mean size of the tumor between groups. Overall, 23.8% of patients had disease progression, with a statistically significant difference between groups. Patients with HER2E had disease progression in 41.7% of cases compared to 12.8% in Lum/HER2 (χ^2=6.82; P=0.009). Lum/HER2 tumors had a significantly higher RCB score than HER2E (χ^2=6.17, P=0.045), but the average time to disease recurrence was longer (27.46±6.84 vs. 23.8±17.58). The median time to disease recurrence was 17 months.

Discussion: According to our unpublished data, about 60% of HER2E and 35% of Lum/HER2 BC archive complete pathological response to anti-HER2 neoadjuvant therapy. Despite targeted therapy, one-third of patients have disease progression within 2 years. Our results show that almost 50% of HER2-positive patients with BC have PD-L1 positive TILs, with the possibility of administering therapy with PD-L1 inhibitors and prolonging the time to disease recurrence.

MeSH/Keywords: breast carcinoma, HER2, PD-L1, neoadjuvant therapy

Poster code: R-02-23-114

Poster Title: Characteristics of patients with thin basement membrane nephropathy combined with focal segmental glomerulosclerosis

PhD candidate: Matija Horaček

Part of the thesis: Obilježja bolesnika s nefropatijom tankih glomerularnih bazalnih membrana udruženom s fokalnom segmentalnom glomerulosklerozom

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

Affiliation: University of Zagreb School of Medicine

Introduction: Alport syndrome (AS) and thin basement membrane nephropathy (TBMN) are part of a spectrum of disorders caused by mutations in COL4A3, COL4A4 and COL4A5 genes, which encode chains of collagen IV (COL4), the main component of glomerular basement membranes (GBM) in humans. The main manifestation of these disorders is haematuria but they differ in progression and severity. Recent studies show that TBMN is not a benign disorder with favorable long-term prognosis because some patients present with proteinuria and develop focal segmental glomerulosclerosis (FSGS) with progression to end-stage renal disease. Also, COL4 mutations have been detected in some cases of genetic FSGS and therefore the spectrum of disorders caused by these mutations has been broadened.

Materials and methods: We plan to conduct a combined retrospective and prospective research. Patients will be identified from the register of renal biopsies of the Department of Nephropathology and Electron Microscopy of the University Hospital Dubrava and will be divided into two groups. The inclusion criteria will be diffuse thinning of GBM found on electron microscopy (first group) and the same finding associated with the presence of FSGS on light microscopy (second group). After identification, all available clinical and pathohistological data will be collected. Next generation sequencing of COL4A3, COL4A4 and COL4A5 genes will be performed on the iSeq100 System platform (Illumina, San Diego, CA, USA) at the Department of Pathology, Faculty of Medicine, University of Zagreb. We will use Illumina VariantStudio software for variant analysis. Potential new-found mutations will then be confirmed by standard dye-terminator Sanger sequencing on ABI310 (Applied Biosystems) with BigDye v1.1 chemichals (Thermo Fisher Scientific, Waltham, MA, USA). We will review the results using Vector NTI software (Thermo Fisher Scientific, Waltham, MA, USA). All clinical, histological and genetic data will be statistically analyzed using SPSS 19.0 software for Windows (SPSS Inc., Chicago, IL, USA) and MedCalc 11.4.2.0. (MedCalc Software bvba) and genotype-phenotype correlation will be performed.

Results: As part of our research project we performed NGS for 403 patients and their family members. Among them, 191 had a pathogenic or likely pathogenic variant in either COL4A3, COL4A4 or COL4A5 genes, 34 had a variant of uncertain significance and 178 didn't have pathogenic or likely pathogenic variants. According to the listed inclusion criteria, around 90 patients have been selected for this research but an overall data analysis and genotype-phenotype correlation is yet to be performed. As for now, we collected clinical and pathohistological data and analyzed phenotype of novel and frequent variants in tested population for the purpose of a better insight into the clinical course of each variant.

Discussion: For the past 20 years, AS and TBMN research have focused on the correlation of genotype and phenotype. Several studies show that COL4 mutations cause a wide range of renal disorders from TBMN to X-linked and autosomal forms of AS. Recently, COL4 mutations were identified in patients with familial FSGS, broadening the spectrum of structural disorders of GBM. Although TBMN, caused by autosomal COL4 mutations, was considered a benign disorder with an excellent prognosis, several publications convincingly show that a number of patients developed proteinuria and FSGS and progressed to ESRD. Our aim is to determine clinical, pathohistological and genetic characteristics of patients with TBMN associated with FSGS and to compare them to patients with isolated TBMN. It is also important to determine which COL4 variants are present in our population and how their type and location affect the clinical course of the disease. These findings are essential for an adequate therapeutic and prognostic approach to patients as well as the basis for further research of therapeutic interventions.

MeSH/Keywords: Alport syndrome, thin basement membrane nephropathy, focal segmental glomerulosclerosis, collagen IV

Poster code: R-02-23-132

Poster Title: A high-impact bodyweight exercise program significantly improved bone mineral density and body composition in children with inflammatory bowel disease in remission

PhD candidate: Ivana Trivić

Part of the thesis: Uloga tjelesne aktivnosti u nastanku i liječenju poremećaja mišićno-koštane mase u djece oboljele od kroničnih upalnih bolesti crijeva

Mentor(s): Professor Sanja Kolaček, MD PhD

Affiliation: University of Zagreb School of Medicine; Children's Hospital Zagreb, Croatia

Introduction: Inflammatory bowel disease (IBD) in children is associated with unfavourable alterations in body composition such as decreased bone mineral density (BMD) and reduced lean body mass (LBM), even when stable remission has been achieved. Regular exercise is associated with muscle mass accrual and improved bone health. The aim of our study was to investigate the effect of an exercise program on BMD and body composition in children with IBD in remission.

Materials and methods: A total of 42 paediatric IBD patients in remission (25 boys; aged 15.3 ±2.08 years; disease type: Crohn's disease (CD) n=22, ulcerative colitis (UC) n=18, inflammatory bowel disease-unclassified (IBD-U) n=2) were recruited to participate in a 6-month home-based exercise program involving high-impact bodyweight exercises. Total body less head (TLBH) dual energy X-ray absorptiometry (DXA) was used to measure BMD, expressed in g/cm2 and as age-and sex-based Z-scores, and to assess fat mass (FM) and LBM, expressed in grams and as age-, sex- and height-based Z-scores at baseline and at the completion of the program. Prior to starting the exercise program, and after its completion, a medical examination and evaluation of physical fitness was done, and patients' daily caloric intake was assessed using a three day food intake record. Adherence was assessed using daily activity logs.

Results: Based on baseline and endpoint TBLH DXA measurements, study participants experienced an increase in total body mass from 53.32±11.02 kg to 56.24±12.27 kg (p<0.001), with an increase in BMD (from 0.947±0.135 g/cm2 to 0.983±0.144 g/cm2, p<0.001), FM (from 16.59±7.28 kg to 17.41±7.23, p<0.001) and LBM (from 36.90±8.53 kg to 38.41±10.16 kg, p=0.012). BMD Z-score increased significantly (from -0.336±1.075 g/cm2 to -0.261±0.979 g/cm2, p=0.020), whilst LBM Z-score did not significantly change (from -1.69±1.25 to -1.71±1.44, p=0.908). When analysed separately, CD patients experienced a significant increase in BMD (p<0.001), BMD Z-score (p=0.017) and LBM (p<0.001), while in UC and IBD-u patients only BMD improved significantly (p=0.001). Similarly, male patients experienced an improvement in BMD (p<0.001), BMD Z-score (p=0.043) and LBM (p<0.001) without a significant change in LBM Z-score (p=0.126), while female patients improved only in the BMD (p=0.046). Dietary intake did not change significantly during the intervention period. Regression model found that there was no association between improvement of BMD and LBM and variables such as age at diagnosis, duration of the disease and the use of biological therapy. No adverse events were noted, and only one participant withdrew from the study.

Discussion: Participants experienced significant improvement in BMD and BMD z-score, as well as in FM and LBM. Subgroup analysis showed that only CD patients and male study participants experienced improvement in all aforementioned parameters, whilst UC, IBD-U and female patients experienced solely improvement in BMD.

MeSH/Keywords: inflammatory bowel disease, children, body composition, physical exercise

Poster code: R-02-24-008

Poster Title: Contribution of the whole exome sequencing in the identification of genetic variants associated with childhood-onset systemic lupus erythematosus and IgA vasculitis - preliminary research results

PhD candidate: Mario Šestan

Part of the thesis: Contribution of the whole exome sequencing in the identification of genetic variants associated with childhood-onset systemic lupus and IgA vasculitis

Mentor(s): Professor Marija Jelušić, MD PhD, Professor Carola G. Vinuesa, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: The research is focused on identification of novel and rare gene variants in patients with possible monogenic forms of two complex autoimmune diseases: childhood-onset systemic lupus erythematosus (cSLE) and IgA vasculitis (IgAV).

Materials and methods: In this multiple case study "trios" containing proband case with cSLE or IgAV and parents (including other informative family members) with severe, atypical clinical features, syndromic characteristics, early onset of the disease, resistance to conventional therapy and/or family pattern of occurrence underwent genomic testing using WES. For WES DNA samples were enriched and sequenced by Illumina HiSeq 2000 (Illumina, inc). The exomes of these sequences were analysed through bioinformatics pipeline: single nucleotide variants (SNV) and small insertions and deletions (indels) were called from the aligned data using SAMtools. Two in silico panels were used for performing targeted, outcome-driven analyses of WES data. The first in silico panel of genes included 75 genes known to cause monogenic SLE, interferonopathies or found within GWAS SLE loci. The second in silico panel of genes included Vasculitis and Inflammation Panel of 166 genes associated with vasculitis and autoinflammatory diseases. Using both bioinformatics pipeline and predictive tools including Polyphen 2, SIFT and CADD narrowed the list of potential candidate genes that could be causing the disease. Applying WES analysis the mode of inheritance of the variants will be analyzed. The variants will be classified according to the American College of Medical Genetics classification guidelines.

Results: Overall, 17 patients met the inclusion criteria and, among them, in 7 patients with cSLE and 1 patient with IgAV the total number of 15 novel and/or rare variants that may possible contribute to disease were discovered. One variant was classified as pathogenic (class V) according to the American College of Medical Genetics classification guidelines. That was structural, frame-shift variant in exon 34 of KMT2D gene (NM_003482.3:c.8626delC; 55 reads C, 56 reads delC), predicted to truncate the protein (p.Gln2876Serfs*34), occurred "de novo" in patient with cSLE and Kabuki syndrome resulting in KMT2D loss of function. In the same patient additional missense mutations in C1S and C1R gene were found, both predicted to be pathogenic with high CADD scores, however, probably with no direct impact on the disease manifestations, since only homozygous C1r and C1s deficiency are associated with monogenic SLE, while the patient was heterozygous for these mutations. Other 12 variants were classified as variants of uncertain significance (VUS). These are gene variants that encode proteins involved in various cellular signaling pathways, especially related to tyrosine phosphorylation, post-transcriptional modification of mRNA, signal transduction shared by many cytokines, regulation of apoptosis and RNA binding.

Discussion: In the new epoch of personalized medicine, implementation of next generation sequencing has improved the diagnostics and treatment of patients with autoimmune diseases. Nevertheless, such quantity of data raised the problem of interpretation of genetic variants and their use for therapeutic purposes. Undeniably, pathogenic variants often represent only a small percentage of all the variants reported, while there is growing number of variants that we are still not able to clearly define and use in the clinical context, such as VUS, that limit the clinical utility of genetic information. This has to prompt the scientific community to develop methods to properly categorize VUS and escalate the amount of practicable information from next generation sequencing.

MeSH/Keywords: Systemic Lupus Erythematosus, Henoch-Schönlein Purpura, Whole Exome Sequencing, Mutations, Monogenic

Poster code: R-02-24-085

Poster Title: Differential diagnosis of β-thalassemia and sideropenic anemia in children based on decision trees

PhD candidate: Daniel Turudić

Part of the thesis: Differential diagnosis of β-thalassemia and sideropenic anemia in children based on decision trees

Mentor(s): Associate Professor Ernest Bilić, MD PhD, Paško Konjevoda, PhD, senior research associate

Affiliation: University of Zagreb School of Medicine

Introduction: We present the preliminary results of our study with the aim to discriminate β-thalassemia from iron deficiency anemia (IDA) using common mathematical indices.

Materials and methods: A CBC sample of 100 children (aged 6 months - 18 years) treated for β-thalassemia and IDA in the Department for Pediatric Hematology and Oncology UHC Zagreb was randomly selected. Only children diagnosed with microcytic hypochromic anemia (MCV < 80 fl; MCH < 26 pg; hemoglobin < 109 g/l) were enrolled in the study. Diagnosis of β-thalassemia was confirmed with a hemoglobin A2 level > 3.5% by liquid chromatography while serum iron levels <4 umol/L and serum ferritin levels <15 ng/dl indicated iron deficiency anemia (IDA). Children below < 6 months of age or with other diseases that could cause microcytic hypochromic anemia were excluded. Sensitivity, specificity, and receiver operating characteristic (ROC) analysis of most common discriminating indices (Matos & Carvalho, Mentzer Index, RDW Index, Green and King, Ehsani Index) used in the differential diagnosis of these two diseases was calculated using MedCalc.v15.2 statistical software. The nonparametric nature of the CBC sample was assessed using the Kolmogorov–Smirnov test. Mann–Whitney test was used to investigate differences between the two groups. The area under the ROC curve was calculated for each index and their differences were assessed. A p-value < 0.05 was considered significant.

Results: Among the 5 tested indices, the Ehsani index correctly diagnosed the highest number of children with β-thalassemia but failed to properly recognize children with IDA (sensitivity 92%, specificity 46%). The most commonly used Mentzer index showed similar results (sensitivity 88%, specificity 48%). The best ratio between sensitivity and specificity was observed for the new Matos & Cavalho index (sensitivity 74%, specificity 88%) with the highest area under the ROC curve. Pairwise comparison of ROC curves observed a significant difference between Matos & Cavalho index and the remaining four tested indices (RDWI p<0,0008; Ehsani p<0,0001; Green and King p<0,0001; Mentzer p<0,0001). Kolmogorov–Smirnov test for normal distribution of CBC values showed a p>0,05 while Mann–Whitney U test for independent samples showed a p<0.05 difference between IDA and β- thalassemia.

Discussion: Our results show that the most optimal index for discriminating between β- thalassemia and IDA in analyzed children is Matos & Cavalho Index. Therefore, it is more appropriate for discernment than the other analyzed indexes. All indexes with low specificity (Mentzer, Ehsani, Green and King) were of low validity as they have a low proportion of IDA correctly identified as such. We aim to complete our research using the new decision tree-based machine learning algorithms (J48 and rpart) to construct an optimal discriminating decision tree for distinguishing these two diseases.

MeSH/Keywords: sideropenic anemia, beta thalassemia, decision tree, children

Poster code: R-02-24-109

Poster Title: Predictive factors of mental health in children and adolescents with juvenile idiopathic arthritis

PhD candidate: Lana Žigić Antić

Part of the thesis: Prediktivni čimbenici mentalnog zdravlja djece i adolescenata s juvenilnim idiopatskim artritisom

Mentor(s): Lovro Lamot, PhD, research associate, Professor Nataša Jokić-Begić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Mental health is integral part of overall health and is defined as a condition in which a person realizes their abilities, can cope with everyday stress, work productively and fruitfully, and contribute to his own community. Early study shows that there are some risk factors (peer isolation, school pressure, low self-esteem, poor self-image, parental conflicts, unemployment, etc) and protective factors (personality traits, family support, authorative parenting style, parent and social support etc.) of mental health. Juvenile idiopathic arthritis (JIA) is one of the most common chronic diseases in children and adolescents and an important cause of short-term and long-term disability. Children and adolescents with JIA face many difficulties including family dependency, peer isolation, and a range of physical disabilities which can compromise developmental tasks and thus impair the mental health of the child.

Materials and methods: We used The Stait-Trait Anxiety Inventory (train anxiety), The Stait-Trait Anxiety Inventory for Children (trait anxiety), Anxiety Sensitivity Index, Childhood Anxiety Sensitivity Index, Family Support Scale, Social Support Perception Questionnaire, Clinical Outcomes in Routine Evaluation – Outcome Measure, Clinical Outcomes in Routine Evaluation – Young Person, Mental Health Checklist – Short Form, The Juvenile Arthritis Multidimensional Assessment Report, The Juvenile Arthritis Disease Activity Score, Visual – Analog Scale and General Data Survey made specifically for this study. Using adecuate statistica methods we will study predictive and risk factors of mental health in JIA children and adolescents.

Results: Battery of measuring instruments was administered in 29 JIA patients aged 9-18 years, monitored at the Pediatric Clinic of the Sestre milosrdnice University Hospital Center in Zagreb, Croatia. Part of the questionnaire was completed by a parent, as well as by the attending pediatric rheumatologist. According to epidemic reasons of COVID-19 we were limited in conducting all required respondents due to much rarer and more necessary first check-ups and controls. Finally, after conducting all planned respondents, a statistical analysis will be made.

Discussion: Mental health of JIA children and adolescents is neglected problem in the domain of health. To the best of our knowledge, this is the first study to examine the risk and protective factors of mental health in children and adolescents with JIA, as well as to examine anxiety sensitivity in this population. This research will allow us to examine and define predictive mental health factors as well as the importance of anxiety sensitivity in children and adolescents with JIA. A significant contribution of this research is the use of measuring instruments with excellent psychometric characteristics. It will provide a better understanding of factors influencing the assessment of the general condition and activity of the disease, as well as enhanced apprecitaion of the difficulties faced by patients, along with the planning of preventive strategies.

MeSH/Keywords: mental health, juvenile idiopathic arthritis, JIA, children, adolescents, predictive factors, anxiety, anxiety sensitivity, family support, social support, biopsychosocial model.

Poster code: R-02-24-122

Poster Title: The role of arterial spin labeling magnetic resonance imaging technique in differentiation Alzheimer's disease from vascular dementia

PhD candidate: Tena Sučić Radovanović

Part of the thesis: The role of arterial spin labeling magnetic resonance imaging technique in differentiation Alzheimer's disease from vascular dementia

Mentor(s): Assistant Professor David Ozretić, MD PhD, Assistant Professor Hrvoje Budinčević, MD PhD

Affiliation: Clinical Hospital "Sveti Duh", University of Zagreb School of Medicine

Introduction: ASL (arterial spin labeling) is a non-invasive MR imaging technique for measurement cerebral blood flow (CBF). Dynamic susceptibility contrast (DSC)-magnetic resonance imaging (MRI), computed tomography (CT) perfusion imaging, single-photon emission tomography (SPECT), and H2[15O] positronemission tomography (PET) are well-established methods for investigating blood flow in neurological diseases and measure perfusion by dynamic imaging of the passage of a contrast agent. By contrast, ASL generates an image by magnetically "labeling" water molecules as an endogenous tracer as they travel to an organ of interest. CBF values expressed as the volume of blood per volume of tissue per minute (ml/100 g/min) obtained with ASL correlate with golden standard PET. Previous research showed interesting comparison of CBF in different types of dementia comparing to healthy subjects. In patients with Alzheimer's disease global hypoperfusion was observed and also regional hypoperfusion in medial occipital, medial temporal and particularly parietal lobes, while similar hypoperfusion was observed in posterior cingulate cortex, precuneus and some parts of frontal lobes in comparison with healthy population, while patients with vascular dementia have diffuse cerebral hypoperfusion. For Alzheimer's disease diagnosis two regions that should be thoroughly observed are posterior cingulate cortex and precuneus.

Materials and methods: In this cross-sectional study 60 patients with Alzheimer's disease and 60 patients with vascular dementia will take part during two year period. All subjects will undergo MRI at Clinical Hospital "Sveti Duh" using 3.0-T Siemens Magnetom Vida scanner equippped with 64-channel head coil. After standard MRI brain protocol, pulse ASL images and 3D MPRAGE T1-weighted sequence will be obtained. The PASL data will be processed using the SPM8 software and ASL toolbox. The inclusion criteria are the following: confirmed diagnosis of vascular dementia (according to NINDS-AIREN criteria) and Alzheimer's disease (based on NIA-AA clinical criteria and Hachinski ischemic score) and age of subjects 50 and above. The degree of cognitive impairment will be assessed with MMSE (<23) and MoCA (<26). The non-inclusion criteria are the following: internal carotid artery stenosis > 70%, cardiac decompensation (NYHA IV), ischemic stroke in posterior cingulate cortex and precuneus, aphasia, contraindications for MRI scan, reversible causes of dementia, mixed dementia and other types of dementia. The exclusion criteria are the following: phobia, inability of subjects to lay down calmly and other brain diseases that affect cognitive function. Due to the pandemic we managed so far to include 5 patients with Alzheimer's disease. All patients meet the inclusion criteria with no non-inclusion and exclusion criteria.

Results: Preliminary results show that in obtained perfusion maps all 5 patients with Alzheimer's disease have decreased perfusion in posterior cingulate cortex and 4 out of 5 patients with Alzheimer's disease have decreased perfusion in precuneus.

Discussion: In forthcoming 15 months all other patients will undergo MRI following described scanning protocol and PASL data will be processed for precise quantification of CBF in order to determine if there are differences in CBF in frontal, parietal, occipital and temporal lobes, and of course in posterior cingulate cortex and precuneus in Alzheimer's disease and vascular dementia. Correlation of CBF values with degree of cognitive impairment and duration of disease will be determined. Preliminary results show that ASL imaging technique could differentiate Alzheimer's disease from vascular dementia based on specific pattern of perfusion in Alzheimer's disease.

MeSH/Keywords: Keywords: magnetic resonance imaging, ASL imaging technique, perfusion, Alzheimer's disease, vascular dementia, posterior cingulate cortex, precuneus

Poster code: R-02-25-059

Poster Title: Ten-year prognostic value of computed tomography specific scores for coronary atherosclerotic burden in patients with suspected coronary artery disease

PhD candidate: Petar Medaković

Part of the thesis: The estimation of total coronary atherosclerotic burden by coronary computed tomography angiography and its association with ten-year survival

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

Affiliation: University of Zagreb School of Medicine, Special Hospital Agram, Zagreb

Introduction: Current cardiovascular risk assessment focuses mainly on the stenosis severity of the coronary vessel lumen. Integration of the additional indices such as plaque extent, location and composition of the plaque may improve risk stratification for future cardiac events. Coronary computed tomography angiography (CCTA) with its high negative predictive value allows accurate exclusion of the coronary artery disease (CAD) and additionally comprehensive assessment of the aforementioned plaque indices. Several computed tomography (CT) based scores for coronary atherosclerotic burden assessment have been developed and suggested for the prediction of cardiovascular events among patients with CAD. However, none of them is recommended as the sole prognostic test, hence their ability for accurate long-term prognosis remains to be defined. Therefore, the objective of this study is to evaluate long-term (ten-year) prognostic value of computed tomography-adapted Leaman score (CT-LeSc) in comparison with previously suggested CT based scores in patients with suspected coronary artery disease.

Materials and methods: Cohort of patients selected from the clinical database of Special Hospital Agram Zagreb. For 261 patients referred to coronary computed tomography angiography (CCTA) from January the 1st to June 30th, CAD risk factors, demographic and clinical data, CAD pre-test probabilities by the Diamond-Forrester model and Morise score were obtained. Causes and dates of death were crosschecked from registry of Croatian Institute of Public Health ending with 30th June 2018. For each patient Agatston calcium score, CT-LeSc, SIS and SSS were calculated. We conducted Kaplan-Meier survival curves and proportional Cox regression models for outcome-all cause death and cardiovascular death.

Results: Patients baseline demographic and clinical characteristics are presented in Table1. CAD was diagnosed in 72% of patients and 57.4% of them had non-obstructive CAD. The median CT-LeSc in the entire cohort was 3.22 and in patients with CAD 5.22. The cut-off value for high coronary atherosclerotic burden was estimated using tercile distribution within the total patient sample and CT-LeSc≥5.52 was considered as high burden. Among patients with CAD 46.8% had a high coronary atherosclerotic burden and 19.5% with non-obstructive CAD had a high CT-LeSc≥5.52.The mean SIS and SSS values were 3.72 and 4.4, respectively. The mean follow-up was 120.6±16.1.months, and 26 deaths by all cause (10 cardiac deaths) were recorded. High CT-Leaman score was significant predictor for all-cause (logrank test= 19.7742, p<0.0001) and CV death (logrank test=14.917, p=0.0001) compared with lower CT-Leaman score. (Figure 1 and 2) SIS and SSS ≥ 5 were also significant predictors for CV death, logrank test=9.3048, p=0.0023 and logrank test=7.9181, p=0.0049, respectively. In proportional Cox regression model adjusted for previously mentioned covariates, significant predictors for CV death were; age (p=0.0481) hyperlipidaemia (p=0.0105) and high CT-Leaman score (p=0.0023), area under the ROC curve (AUC) was 0.893. (Table 2)

Discussion: Results confirm that high coronary atherosclerotic burden assessed by CT-LeSc, SIS and SSS is associated with significantly higher all-cause and cardiovascular mortality. In proportional Cox regression model statistical significance remained for high CT-LeSc even when adjusted for other traditional cardiovascular risk factors. Several previous studies demonstrated that considerable proportion of cardiac events arise from non-obstructive coronary lesions with soft composition and larger plaque volumes and that proximal location and supply dominance are also important prognostic indices. All selected CT based scores significantly predicted CV mortality. The estimates of extent, plaque composition and stenosis severity of CAD should be considered during diagnostic and clinical treatment of coronary patients especially in patients with non-obstructive disease. Conclusion: Coronary atherosclerotic burden assessed by specific CT based scores has significant prognostic value for long-term cardiovascular and all-cause mortality.

MeSH/Keywords: coronary artery disease, coronary CT angiography, CT-adapted Leaman score, mortality

Poster code: R-02-25-084

Poster Title: Comparison of magnetic resonance biopsy with a systemic biopsy in a patient with a negative prostate biopsy

PhD candidate: Sven Nikles

Part of the thesis: Comparison of magnetic resonance biopsy with a systemic biopsy in a patient with a negative prostate biopsy

Mentor(s): Professor Boris Ružić, MD PhD

Affiliation: University of Zagreb School of Medicine, Department of Urology Sestre milosrdnice University Hospital Centre

Introduction: Prostate cancer is the most common cancer of the male population. In the selection of prostate biopsy candidates, the history of PSA (prostate specific antigen), clinical examination and more recently MRI (Magnetic Resonance Imaging) of the prostate are used. With the introduction of mpMRI and fusion biopsy role of conventional TRUS (transrectal ultrasound) biopsy slowly faded. Pathohistological evidence of the disease is of particular importance for the decision of treatment. Prostate biopsy is an invasive but mandatory procedure with rare but not negligible potential complications. There are several methods of prostate biopsy, of which a systemic or planar prostate biopsy and cognitive or targeted MRI-guided prostate biopsy are available. The lack of systemic biopsies is a poor detection of atypical lesions. The multiparametric MRI (mp-MRI) has demonstrated its accuracy and reproducibility in detecting, locating and evaluating the extent and aggressiveness of prostate cancer.

Materials and methods: This prospective study will be conducted in the period of 36 months and will include 300 male patients with elevated PSA and/or positive DRE (digital rectal exam), and a previous negative TRUS biopsy. Before the second, repeated prostate biopsy all patients will be randomly divided into two groups. In the first group, 150 patients will undergo multiparametric magnetic resonance imaging (mpMRI), followed by cognitive fusion biopsy of the prostate. In the PIRADS 3-5 group 10 systematic biopsy cores and 3 targeted biopsy cores per suspected lesion will be sampled. If the mpMRI finding is PIRADS ≤ 2 then only systematic biopsy will be performed. In the second group, 150 patients will undergo only a classic 10 core template repeated TRUS biopsy without prior image processing. The detection of suspected lesions will be labeled and graded according to Prostate Imaging Reporting and Data System, version 2 (PI-RADS v2). The study is approved by the Ethics Committee of Sestre milosrdnice University Hospital Center, and each patient signed an informed consent.

Results: Preliminary results: In the assessed population of 145 patients there was no difference between the groups in PSA levels (p = 0.44, 95% CI -3,89 to 1,72) or in DRE finding (p = 0.69, 95% CI -0.12 to 0.08) but PC detection rate differed significantly (p=0,007, 95% CI -0,05 to 0,32). When we compared detection rate of clinically significant PC, according to Epstein criteria, the detection rate of PC was statistically significant in the mpMRI group, 18 (27,7%) vs 6 (7,5%) (p=0,001). We also compared these two cohorts by analyzing each PC core. Detection rate of targeted cores was significantly higher when compared with any other group. Every fourth core on targeted biopsy was positive.

Discussion: A diagnostic dilemma in patients who have had a prior negative biopsy and persistent suspicion of PCa can, in part, be solved with more precision and accuracy by using mpMRI. When a suspicious lesion is found on mpMRI, there is demonstrated improvement in clinically significant cancer detection compared to TRUS biopsy. It is of particular importance that the visualization of the tumor allows better sampling of difficult to reach tumors in the anterior and apex of the prostate. Additionally, mpMRI prior to repeated biopsy could be used to reduce unnecessary prostate biopsies in some patients. The performance of mpMRI relies on the ability of mpMRI to identify clinically significant cancer, but also on the operator dependent reading and interpretation, as well as diagnostic accuracy of biopsy itself.

MeSH/Keywords: prostate cancer, mpMRI, systemic prostate biopsy, cognitive prostate biopsy

Poster code: R-02-28-021

Poster Title: Functional urethral length and maximal urethral closure pressure before radical prostatectomy as an early postoperative continence recovery predictors – pilot study

PhD candidate: Mirko Bakula

Part of the thesis: Functional urethral length and maximal urethral closure pressure before radical prostatectomy as an early postoperative continence recovery predictors

Mentor(s): Assistant Professor Tvrtko Hudolin, MD PhD, research advisor

Affiliation: University of Zagreb School of Medicine

Introduction: Urinary incontinence (UI) is one of the most common complications of radical prostatectomy (RP). Impaired urethral sphincter function is generally considered to be the most important contributing factor of UI. In this research, the urodynamic method of Urethral Pressure Profile (UPP) was used to evaluate Functional Urethral Length (FUL) and Maximal Urethral Closure Pressure (MUCP) in the patients before open retropubic RP and correlate with the postprostatectomy continence recovery. Objective of this research is to evaluate preoperative FUL and MUCP as an early continence recovery predictors.

Materials and methods: 38 patients aged 49 to 78 (65.7) undergoing RP were characterized and included in this prospective cohort study. UPP was performed prior to the surgery. The severity of UI and bothersome were assessed using fully validated International Consultation on Incontinence Questionnaire – Urinary Incontinence short form (ICIQ-UI SF) and number of pads used in 24h. Patients were interviewed about the use of urinary pads and asked to fill out the ICIQ-UI SF before and 2, 8, 16 and 24 weeks after RP. Endpoint of the study is definied as no UI. Ethical Committee approval and Informed consent from all the participants have been obtained. Statistical analysis of correlation was performed using Pearson's correlation coefficient and regression.

Results: The mean value of FUL (mm) and MUCP (cm H2O) was 67.8 (31 – 94) and 82.8 (37 – 150), respectively. Performed statistical analysis indicate a negative linear relationship between the independent variable of FUL and MUCP and the dependent variables of ICIQ-UI SF symptom score and number of pads used per day (p=0,002) in the observed time frame. Preoperative shorter FUL and lower MUCP are associated with a higher risk of remaining incontinent in the same period.

Discussion: Preoperatively evaluated FUL and MUCP seem to be valuable prognostic factors for the early continence recovery after open retropubic RP. Further investigation on a larger patient cohort is needed to asses the role of UPP in the preoperative management of patients waiting for RP.

Acknowledgments: None to declare.

MeSH/Keywords: Urinary incontinence; radical prostatectomy, functional urethral length; maximal urethral closure pressure; urethral pressure profile

Poster code: R-02-28-126

Poster Title: The interplay between psychological distress and autonomic nervous system symptom burden

PhD candidate: Anamari Junaković

Part of the thesis: The impact of the symptoms of anxiety, depression and stress on the result of the COMPASS-31 questionnaire

Mentor(s): Associate Professor Mario Habek, MD PhD, Assistant Professor Milena Skočić Hanžek, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Psychological distress in the form of anxiety or depression is a common comorbidity in patients with disorders of the autonomic nervous system (ANS). Therefore, we aimed to evaluate the influence of depression, anxiety and stress on ANS symptom burden.

Materials and methods: Consecutive patients referred to the Laboratory for testing of the ANS, Zagreb, Croatia for the evaluation of dysautonomia (N=524, mean age 43.98, 371 females) and healthy controls (N=88, mean age 41.15, 57 females) completed validated Croatian versions of the Depression Anxiety Stress Scales 21 (DASS-21) and Composite Autonomic Symptom Score 31 (COMPASS-31). There was no difference in age and sex between groups (p⏷0.05).

Results: Significantly more patients had severe or extremely severe depression, anxiety and stress compared to healthy controls (50 vs 2, p=0.036; 143 vs 4, p⏷0.001 and 63 vs 2, p=0.008; respectively). All three subscales of DASS-21 and COMPASS-31 were significantly higher in patients compared to healthy controls (all p⏷0.001). There was a significant correlation between depression, anxiety and stress subscales of DASS-21 and COMPASS-31 in both patients (rs=0.444, p⏷0.001, rs=0.501, p⏷0.001 and rs=0.413, p⏷0.001, respectively) and healthy controls (rs=0.382, p⏷0.001, rs=0.423, p⏷0.001 and rs=0.461, p⏷0.001, respectively). COMPASS-31 values were significantly higher in patients with DASS depression score ⏷ 9, anxiety score ⏷ 7 and stress score ⏷ 14 (all p⏷0.001).

Discussion: Reported psychological distress is common in patients referred to the autonomic laboratory, and our study demonstrates that they are interwoven in the complex pathophysiological and clinical picture of ANS disorders.

MeSH/Keywords: autonomic nervous system, COMPASS-31, depression, anxiety, stress

Poster code: R-02-30-009

Poster Title: Assessment of cognitive functions in psoriatic arthritis patients

PhD candidate: Kristina Kovač Durmiš

Part of the thesis: Assessment of cognitive functions in psoriatic arthritis patients

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

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

Introduction: Systemic inflammation, increased cardiovascular risk, chronic pain, associated depressive symptomatology and medications like glucocorticoids and methotrexate may have certain impact on cognitive decline in inflammatory rheumatic diseases. Data suggest cognitive impairment in patients with various inflammatory rheumatic diseases as well as psoriasis (PsO), but data on cognitive status in PsA patients are lacking. A recent cognitive status study in PsA patients showed high incidence of mild cognitive impairment (MCI) associated with worse functional status of joint disease and greater fatigue. No control group was assessed and MCI diagnosis was based on Montreal Cognitive Assessment (MoCA) test results. The aim of this study was to assess cognitive functions in PsA patients in relation to control group of healthy individuals.

Materials and methods: In the cross-sectional study three patients diagnosed with PsA according to the Classification criteria of Psoriatic Arthritis (CASPAR) were consecutively included in the test group. None of the healthy controls were assessed. The research was conducted in the Department of Rheumatology and Rehabilitation at the University Hospital Centre (UHC) Zagreb. Sociodemographic and clinical data was collected through medical interview and clinical examination. Standard parameters of PsA and psoriasis severity, including disease activity, and functional status were assessed by clinical examination, patient reported outcome measures and calculation of composite outcome measures. Routine laboratory testing was performed after overnight fasting period. Depressive and anxiety symptoms were tested by Beck Depression Inventory-II (BDI-II) and State-Trait Anxiety Inventory (STAI). MoCA test and Trail Making Test (TMT) A and B were used for cognitive status assessment. Each participant fulfilled all inclusion and none of the exclusion criteria and signed informed consent prior to the study entry. The study protocol was approved by UHC Zagreb and School of Medicine Ethics Committee. The research was done according to the principles of Declaration of Helsinki and good clinical practice.

Results: Study included 2 male and one female PsA patient aged 56, 49 and 72 years, respectively. Educational status was similar, with completed primary and high school. All participants were current non- smokers and had dyslipidemia. Two patients were diagnosed with arterial hypertension. The body mass index (BMI) was in the range from 25.0 kg/m2 to 29.9 kg/ m2. All participants had longstanding skin disease and active peripheral arthritis with 8,10, and 15 tender and 6, 3, and 10 swollen joints in the 68/66 joint count, respectively. Health Assessment Questionnaire (HAQ) scores among participants were equal or lower than 1.0 and Dermatology Life Quality Index (DLQI) scores were equal or lower than 5.0. MoCA test results among participants were 22, 28, 29 out of 30, respectively.

Discussion: This study revealed foreseen comorbidities in PsA patients such as dyslipidemia and arterial hypertension. All participants were classified as overweight according to the BMI values. Functional status evaluation showed no significant impairment in physical function measured by HAQ as well as no significant impact of psoriasis on health-related quality of life measured by DLQI. MoCA scores in two of three patients were within the normal range, and one had mild impairment in testing results. The study has several major limitations, primarily due to extremely low number of participants enrolled. Additionally, none of the healthy controls was assessed. Given the above-mentioned limitation, no meaningful conclusions are possible.

MeSH/Keywords: psoriatic arthritis, cognition, cognitive impairment, Montreal Cognitive Assessment, Trail Making Test

Poster code: R-02-30-137

Public health and healthcare – preliminary research results

Poster Title: Is there a simple relationship between school environments and unhealthy Body Mass Index in second and third-grade children in Croatia?

PhD candidate: Maja Lang Morović

Part of the thesis: Školsko okruženje kao prediktor prekomjerne tjelesne mase i debljine u djece drugih i trećih razreda osnovne škole u Hrvatskoj

Mentor(s): Associate Professor Sanja Musić Milanović, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Childhood overweight and obesity present a growing worldwide public health problem due to its negative effects on all aspects of lifelong health. In order to tackle this problem, interdisciplinary public health interventions are the only effective approach. This joint, intersectoral work of professionals from both public health and educational backgrounds, should ideally be implemented in the settings where children from all socioeconomic backgrounds spend the majority of their time, schools. To implement successful interventions, it is vital to understand the relationship between different aspects of school environments and an unhealthy Body Mass Index in children. The purpose of this paper is to analyze simple relationships between Croatian school physical activity and nutrition environments and unhealthy Body Mass Index in children attending second and third grade.

Materials and methods: This paper is a part of the Childhood Obesity Surveillance Initiative conducted by the World Health Organization Regional Office for Europe. The goal of this surveillance is to gain comparable, standardized data on childhood weight status. For the data collection purposes, three questionnaires were used: School Form, Parental Form, and Examiner's Form. The surveillance was administered in randomly selected 182 second and 182 third grades from 164 main elementary schools in Croatia. Index variables were calculated for the school physical activity environment and for the school nutrition environment. The relationship between these index variables as well as each school environment variable and school overweight prevalence was calculated using Pearson correlation. Two-tailed tests were and P-values <.05 were considered significant.

Results: The prevalence of unhealthy Body Mass Index ranged from 0 to 70%. School physical activity environment index variable with a scale from 0 to 7 ranged from 3 to 7. School nutrition index variable, with a scale from 0 to 17 ranged from 7 to 16. No significant relationships were found between school nutrition variables and an unhealthy Body Mass Index in children. Also, the tests failed to show significant relationships between school physical activity environments and the prevalence of school overweight and obesity in children.

Discussion: Results show that schools vary in a great deal in ways they support children's physical activity and nutrition. However, these variations are not related to unhealthy Body Mass Index in children in a simple manner. From this starting point, more complex analyses ought to be made to implement directed public health interventions aimed at halting the rise in childhood overweight and obesity.

MeSH/Keywords: School environments, child, student, obesity, health promotion

Poster code: R-03-02-067

Poster Title: Impact of Pre-Pregnancy BMI on Blood Glucose Levels in Pregnancy and on the Anthropometry of Newborns - Preliminary Insights from The Croatian Islands' Birth Cohort Study (CRIBS)

PhD candidate: Nives Fuchs

Part of the thesis: Relationship of stress and depression in pregnant woman with pregnancy outcomes and anthropometry of newborn

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

Affiliation: University of Zagreb School of Medicine

Introduction: It is well known that the biological, physical and social environment, to which a child is exposed early in life may lead to disease or disability in childhood and adulthood. Various risk factors acting during critical periods of development in pre- and early postnatal life play an important role in the etiology of these non-communicable diseases. Early identification of all biological, environmental and behavioral risk factors for poor pregnancy outcomes and metabolic syndrome related disorders is important for the development of preventive and early intervention strategies. The main goal of this study was to investigate the influence of body mass index (BMI) before pregnancy and glucose levels in the second trimester on birth weight, birth length, and head circumference of newborns and to determine the trend of measured anthropometric variables of pregnant women and derived indices during pregnancy. The sample consisted of 171 healthy pregnant women and their newborns from the Croatian Islands' Birth Cohort Study.

Materials and methods: Pre-pregnancy BMI was taken from pregnancy booklet. Extensive medical and biochemical were collected during pregnancy and after birth. Comprehensive questionnaires were filled in during pregnancy and postnatal at particular child developmental milestones. Peripheral blood of pregnant women was taken between the 22nd and 26th week of gestation, fasting glucose in pregnant women was measured at the licensed biochemical laboratory at the Dubrava University Hospital. Fasting glucose values >5.1 mmol/L were considered elevated according to the reference values of Croatian Society of Medical Biochemistry and Laboratory Medicine. Anthropometric variables (bodyweight, height and waist circumference) were measured according to the International Biological Program. Anthropometry of newborns (body length, weight, head circumference) was measured and converted to z-scores.

Results: There was a significant positive correlation between pre-pregnancy BMI and fasting glucose levels between the 22nd and 26th week of gestation . Likewise, there was a significant positive correlation between pre-pregnancy BMI and birth weight, birth length and birth head circumference of the newborns .We also tested the correlation between newborn anthropometry between mothers with different levels of glucose in the second trimester (22nd and 26th week of gestation). The sample of mothers was defined in two groups: normal glycaemic mothers and hyperglycaemic mothers. We detected that hyperglycaemic women gave birth to significantly heavier newborn girls than normal glycaemic women.

Discussion: A limited number of studies has previously focused on variables of pre-pregnancy BMI, glucose level and newborn length, weight and head circumference. Only several previous studies have focused on the investigation of fasting blood glucose as a predictor of birth weight among neonates of non-diabetic mothers and have detected a positive correlation between elevated glucose levels of mothers in pre-pregnancy and increased birth weight of the newborn. In this study only the difference in birth weight of newborn girls was statistically significant. The results were expected due to a small sample, although the trend of increased birth weight, birth length and head circumference of newborns of hyperglycaemic mothers is observed. In conclusion, a growth trend has been observed between pre-pregnancy BMI, fasting glucose during pregnancy and the anthropometry (body length, weight and head circumference) of newborns in the CRIBS study. We can conclude based on our results that there is a minor influence of pre-pregnancy BMI on the anthropometry of newborns.

MeSH/Keywords: Croatian Islands' Birth Cohort Study, pre-pregnancy BMI, fasting glucose in pregnancy, anthropometry, newborns

Poster code: R-03-02-077

Poster Title: Pharmacoeconomic aspect of moderate and severe psoriasis with biological therapy versus conventional therapy

PhD candidate: Ante Orbanić

Part of the thesis: Pharmacoeconomic aspect of moderate and severe psoriasis with biological therapy versus conventional therapy

Mentor(s): academic Mirna Šitum, Professor Stjepan Orešković, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Psoriasis is a chronic, recurrent, autoimmune skin disease. It has several clinical variants that differ in severity, location, longevity, and shape and pattern of the scaling. It affect 1-3% world population, with men and women being equally affected, which is around 125 million people. Treatment for patients with moderate and severe psoriasis include phototherapy or systematic therapy. When a patient do not respond and/or has contraindications on systematic therapy of phototherapy (at least two treatments), it is eligible to receive biological treatment. Total direct and indirect health care costs of psoriasis for patients in world are calculated at $11.25 billion annually, with work loss accounting for 40 percent of the cost. Since there is no cure for psoriasis, only treatment for reducing symptoms, there is always challenge to find the best treatment to improve patients quality of life as soon as possible.

Materials and methods: We conducted a retrospective study including data from all patients having moderate and severe psoriasis that were treated with ustekinumab and acitretine in Sestre milosrdnice University Hospital Center in Zagreb in the years 2016.-2020. We are going to calculate and evaluate direct cost of using ustekinumab and acitretine. Data about outpatient direct costs included: number and type of visits to specialists, number of laboratory blood tests and the data about pharmacotherapy. Direct medical cost will be compared between acitretin and ustekinumab by determining a decremental cost-effectiveness ratio (DCER) by dividing the difference in costs by the difference in Quality Adjusted Life Years (QALYs) between the groups. Furthermore, the Net Monetary Benefit (NMB) per patient will be calculated using the formula: Willingness to Pay (WTP)* effect (difference in QALY) - costs. This results in the net amount of money saved, when the possible loss of QALY is corrected for, using different WTP levels per QALY.

Results: We included in our research 25 patients using ustekinumab and 25 patients using acitretin. Those patients had diagnose of moderate or severe psoriasis and severity of psoriasis was determined by dermatologist. We also collected data about direct cost which are essential to perform pharmacoeconomic analysis so we could test non-inferiority of an inexpensive generic (acitretin) in comparison with a novel, more costly intervention.

Discussion: Research revealed that comparing ustekinumab and acitretin by analyzing direct cost (including non-effective treatment that was given before biologic treatment and to analyze cost-effectiveness using method which takes into consideration cost of each treatment) could show that ustekinumab in treatment of severe and moderate psoriasis shows non inferiority compared to acitretin using comparative effectiveness research method to show that a less costly medication is not worse than the current standard with regard to safety and/or efficacy.

MeSH/Keywords: psoriasis, pharmacoeconomic, ustekinumab, acitretin

Poster code: R-03-02-086

Poster Title: Evaluation of health resort thermal and mineral waters capacity, using balneological potential

PhD candidate: Damir Andabaka

Part of the thesis: Procjena lječilišnoga kapaciteta termomineralnih voda korištenjem balneološkoga potencijala

Mentor(s): Professor Jagoda Doko Jelinić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Balneology is the study of therapeutic use of thermal and mineral waters. In many countries is an independent specialty (Turkey, Spain, and Italy) or competencies (Germany, Austria, Serbia, Hungary, Portugal, and France). The usage of thermal and mineral water in Croatia dates back in Roman times but is not recognized as independent specialty. Aim of this paper is development of method for evaluation of health resort capacity based on balneological potential. Based on the literature search, check the connection between the balneological composition and the diagnoses and diseases treated with thermal and mineral water. Based on literature data, physicochemical and microbiological analysis, and well capacity, a method will be developed to estimate the number of patients with specific diagnoses and the size and number of pools or tubs optimal for a particular well.

Materials and methods: Literature search is made with keywords thermal and mineral water, spas, balneotherapy and balneology. Also, data from Croatian Institute for Public Health about patients who were sent to special hospitals in period from 2016.- 2019. were collected. In 2019. we analyzed thermal water from Tuheljske Toplice on chemical and microbiological parameters.

Results: Systematic review about efficacy of balneotherapy on fibromyalgia confirms that balneotherapy could improve the symptoms of fibromyalgia including pain depression and minor symptoms. Also, balneotherapy and spa therapy may be considered as useful interventions for the management of stress conditions. Balneotherapy in sulphur waters has potential in treatment of upper respiratory tract diseases according to meta-analysis. Microbiological analysis of swimming pool in Tuheljske Toplice which use thermal water without chlorination showed contamination with total coliforms, Escherichia coli, Pseudomonas aeruginosa.

Discussion: Many systematic reviews show that balneotherapy with thermal and mineral water has beneficial effect on many chronical diseases. Main deficiency of studies is limited data and high heterogeneity. Individual clinical studies of health resorts show results in favor for balneotherapy but systematic reviews mostly can't find enough evidence for that claims. According to literature search balneotherapy with Sulphur water has beneficial effect on upper respiratory disease. There is two springs of Sulphur water in Croatia, Istarske toplice i Varaždinske Toplice, that could be used for treatment of upper respiratory disease. Microbiological analysis of thermal water showed that swimming pools should be build with special care and that number of bathers should be limited.

MeSH/Keywords: thermal water, mineral water, spa, balneotherapy, balneology

Poster code: R-03-02-123

Poster Title: Integration processes within palliative care model

PhD candidate: Dorja Vočanec

Part of the thesis: Determinants of the long term care integration process in the Republic of Croatia based on a palliative care model

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

Affiliation: University of Zagreb School of Medicine

Introduction: In Croatia, palliative care has been developing as an integral part of the health care system since 2014. The development is in accordance with the integrated care concept, emerging in many countries. Integrated care is a result of coherent set of organizational and clinical methods and models designed to create continuity, coherence and cooperation within and between different sectors of care. However, there is a number of implementation problems following integration processes in health care. The aim of this paper is to identify positive and negative determinants for the integration of care.

Materials and methods: A desk research including National legislative and strategic framework, national reports on the implementation of these frameworks, and international reports on the state of health and health care in Croatia, has been performed to: 1. Identify changes in three prominent domains according to strategic documents on the development of palliative care: development of new organizational structures, empowering stakeholders, and removing barriers to the provision of integrated palliative care. 2. Obtain a force field analysis of integration processes within the palliative care model.

Results: Integration policy processes or organizational changes have been identified and described within three key domains: the development of new organizational structures, stakeholders' empowerment, and removing barriers to the provision of new integrated palliative care, for the period 2014-2020. Minimum standard of palliative care resources per county have been achieved in more than half counties, many of which have more resources than the required minimum. Changes have been introduced to legal acts, reporting systems and payment methods. Palliative care competencies for few professions have been defined, and educational courses as part of continuous professional education introduced. Volunteer activities have been emerging. The resources that have been developed, such as mobility aid rentals, hospital palliative care teams, IT software, have provided the basis for both horizontal and vertical integration of care. Force field analysis of integration processes within the same three domains shows forces driving the integration of care, including (inter)national initiatives and demand for the integration of care, and need-based approach to solutions. Forces hindering integration of care include lack of change management, and silo mentality.

Discussion: The progress visible in the domains of new organizational structures, stakeholders' empowerment, and removing barriers to the provision of new integrated palliative care show the sustainability of the model. Although there is a national plan and an overall legislative framework for the development of palliative care in Croatia, there are large differences between individual regions. The nuclei from which palliative care develops and spreads differ significantly. Furthermore, the force-filed analysis of integration processes has shown there are directions in the health care system of opposite logic, therefore hindering the integration of care. The results obtained indicate that numerous national and local determinants determine the implementation of integrated care, so bottom-up approach and processes mapping analysis are necessary for the integration of care. The challenge of further research is to formulate specific questions for the focus groups, using these as starting points.

MeSH/Keywords: Integration, Health care organization, Palliative care

Poster code: R-03-02-127

Poster Title: MicroRNAs expression and biochemical parameters in maternal and neonatal pairs in association with cigarette smoke exposure - preliminary results

PhD candidate: Adrijana Dorotić

Part of the thesis: Assessment of microRNAs 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: Exposure to maternal smoking can adversely affect fetal development in utero with mechanisms involving changes in microRNAs (miRNAs) that regulates gene expression. The aim of the study was to determine the expression profile of candidate miRNAs (miRNA-1537, miRNA-190b, miRNA-16, miRNA-21, miRNA-146a) and compare them with selected biochemical parameters (glucose, uric acid, triglycerides, total cholesterol and LDL-cholesterol), in samples of maternal and cord blood plasma depending on the maternal smoking habit.

Materials and methods: This retrospective cross-sectional study was carried out in 66 mother-infant pairs (31 non-smokers and 35 smokers) recruited during 2018. The expression of miRNAs were determined in the Department of Medical Chemistry, Biochemistry and Clinical Chemistry, University of Zagreb, School of Medicine (Zagreb, Croatia) using Qiagen (Hilden, Germany) methodology in maternal plasma (MP) and umbilical cord plasma (UCP), while biochemical parameters were determined in Department of Medical Laboratory Diagnostics University Hospital 'Sveti Duh' (Zagreb, Croatia) by Atellica Solution analyser (Siemens, Erlangen, Germany) using methods that are proposed by Croatian Chamber of Medical Biochemists. Normality of data was tested using D'Agostino-Pearson test with MedCalc software (Ostend, Belgium). Since all parameters did not follow normal distribution, non-parametrical statistical tests were used and results were presented as median and interquartile range. Expression of miRNAs is presented as ΔCt value. Differences and correlation between groups were tested using Mann-Whitney U-test and Spearman correlation with Statistica programme (TIBCO Software, Palo Alto, USA). Statistical significance was set at $P<0.05$.

Results: Statistically significant difference for median (IQR) between smokers and non-smokers was found in MP for ΔCt miRNA-16 (0.43 (1.5) vs. 1.01 (1.9), respectively, P=0.028) and for uric acid (255 (75) umol/L vs. 293 (60) umol/L, respectively, P=0.036)⍰, and in UCP for ΔCt miRNA-146a (2.95 (1.2) vs. 3.45 (0.9), respectively, P=0.036). Moderate correlation was found between various miRNA in MP - miRNA-1537 vs. miRNA-190 (r=0.65, P<0.001), miRNA-190 vs. miRNA-21 (r=0.65, P<0.001), and miRNA-21 vs. miRNA-146 (r=0.69, P<0.001).

Discussion: Our results showed that there is a statistically significant difference in the expression of miRNA-16 and uric acid depending on the smoking habit in MP and miRNA-146a in UCP. The results of selected epigenetic and biological indicators and their associations can provide a new insight into the risks of smoking for maternal and infant health.

MeSH/Keywords: microRNAs, cigarette smoking, prenatal exposure

Poster code: R-03-02-133

Poster Title: Selection of the workers who are exposed to excessive noise at the workplace

PhD candidate: Roko Žaja

Part of the thesis: Uloga koncentracije kortizona u slini i značajki slušnih evociranih potencijala moždanog debla u procjeni rizika od ranog profesionalnog oštećenja sluha bukom

Mentor(s): Assistant Professor Mihael Ries, MD PhD, Associate Professor Milan Milošević, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Occupational safety engineers regularly monitor the level of noise on the workplace in order to prevent excessive exposure and hearing impairment. Workers who work in noisy environment are usually exposed to vibrations and volatile chemicals that can additionally damage their hearing. They usually suffer from mild or moderate hearing impairment or do not have any hearing problem if they are younger or work in noise briefly. Occupational and sports medicine physicians have detailed information regarding workers' hearing status, but in the risk assessment of the early occupational noise-induced hearing loss risk, all aforementioned agents should be equally considered. Therefore, the aim of the first step of exploration of the role of salivary cortisone concentration and auditory brainstem response characteristics in assessment of the early occupational noise-induced hearing loss risk was to consult an engineer about noise exposure and determine the maximum number of workers who do not have hearing impairment and are willing to participate in the study.

Materials and methods: The principal investigator compiled a list of inclusion criteria of the study and shared it with occupational safety engineer in January 2020. The engineer explored the list of 225 workers who were exposed to any kind of noise at the workplace and singled out those who were exposed to noise greater than or equal to 85 decibels for eight hours, who were not exposed to carbon disulfide, toluene or xylene at the workplace, who were aged 19-30 years and worked in the noisy environment from one to two years. All workers worked in the processing of aircraft parts. Differences between continuous variables (age) were analysed with Student's t-test. All P values below 0.05 were considered significant. All statistics was run on IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, NY, USA).

Results: One hundred and eight workers (48%) were exposed to noise greater than or equal to 85 decibels for eight hours at the workplace and did not work with ototoxic agents (carbon disulfide, toluene, xylene) at the same workplace. There were 86 men and 22 women who fitted the inclusion criteria. Men were slightly younger than women, 21.0±2.7 vs. 24.1±1.9 years, P=0.024.

Discussion: Almost a half of workers exposed to noise fitted the inclusion criteria and could be considered for the future research. Occupational safety engineer met them and all agreed to participate. As expected, the majority of workers were men, which will not supposed to have influence on the measurement of auditory brainstem response nor salivary cortisone concentration. Workers who worked with ototoxic chemicals were spending their working week in premises other from places where potential participants worked. The sample of potential participants (N=108) seemed to be large enough but the number will be final after checking the list of exclusion criteria. The features of the criteria are earlier sudden hearing loss, current use of oral and intranasal corticosteroids, surgical treatments of ears, chronic otitis media and dizziness associated with hearing loss and tinnitus. All conditions will have to be absent among study participants. According to the power analysis, there should be at least 50 workers exposed to noise and included in the original study. One hundred and eight workers will be examined individually at the workplace by the principal investigator and will be included in the study eventually. Since COVID-19 pandemic influenced the number of employees from January 2020 until now, we are planning to repeat the selection of participants according to the inclusion criteria, and to continue with the rest of the research methods.

MeSH/Keywords: noise; criteria; workplace

Poster code: R-03-03-089

Research proposals

Basic medical sciences – research proposals

Poster Title: In vitro and in vivo determination of potential interaction between bone morphogenetic protein 6 (BMP6) and serotonin and their mutual influence on glucose and bone metabolism

PhD candidate: Marina Milešević

Part of the thesis: The impact of bone morphogenetic protein (BMP6) and serotonin on glucose and bone metabolism

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

Affiliation: University of Zagreb School of Medicine

Introduction: The role of bone morphogenetic protein 6 (BMP6) was demonstrated in the bone as the potent osteoinductive protein and glucose metabolism, and the pathogenesis of type II diabetes. Serotonin (5HT) is a well-known monoamine neurotransmitter and outside the central nervous system, it is involved in the regulation of the endocrine function of the pancreas in high glucose concentration, as well as a signaling molecule that influences bone metabolism. In clinical practice, proper control of diabetes is essential for the prevention of adverse effects on the organism, such as a negative impact on the bone quality that causes increase fracture risk in patients with type I and type II diabetes. Since the bone and glucose metabolism are strongly interrelated, and the BMP6 and the 5HT demonstrate their ability and influence in glucose and bone metabolism as individual molecules, it is important to find a link between their effects on glucose and bone metabolism as well as their involvement in the pathogenesis of type II diabetes.

Hypothesis: BMP6 and 5HT have a synergistic effect on glucose and bone metabolism and loss of functional BMP6 in mice will negatively affect the 5HT system and will cause changes in glucose metabolism and occurrence of type II diabetes.

Aims: Investigate the potential interaction between BMP6 and 5HT in bone and glucose metabolism, and their role on the pathogenesis of type II diabetes and alterations in bone turnover in vitro on the pancreatic endocrine cell line (INS-1) and in vivo on BMP6 knockout mice.

Materials and methods: In vitro, the effects of BMP6 and 5HT on cellular processes related to glucose metabolism, insulin secretion, and the 5HT on the pancreatic endocrine cell line (INS-1) will be examined by measuring insulin and 5HT secretion using ELISA kits and conducting a gene expression analysis. In vivo, changes in glucose metabolism in BMP6 knock-out mice and wild-type mice will be investigated with standard metabolic assays, and changes in the 5HT system in multiple organ systems will be investigated with immunohistochemistry and gene expression analysis. Potential alterations in bone metabolism will be investigated by determining the concentration of specific bone turnover markers and micro CT analysis of bone tissue.

Expected scientific contribution: The results of the research will determine the functional association between BMP6 and 5HT in bone and glucose metabolism contributing to a better understanding of their complex biological role that may lead to a new therapeutic strategy in the treatment of type II diabetes.

Acknowledgments: /

MeSH/Keywords: BMP6, serotonin, bone metabolism, glucose metabolism

Poster code: T-01-01-041

Poster Title: Epigenetic status and expression of SALL4 in normal and impaired testicular development

PhD candidate: Dajana Krsnik

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

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

Affiliation: University of Zagreb School of Medicine

Introduction: Nonobstructive azoospermia (NOA), a severe form of male infertility, as well as precursor lesions of testicular tumors (GCNIS), seem to be results of molecular mechanisms disruption controling the germline development during fetal/early postnatal life. Epigenetic changes, like DNA methylation are responsible for gene expression modifications and can affect testicular cell development. SALL4 (Spalt-like transcription factor 4; Sal-like protein 4), gene that controls spermatogonial development, differentiation and proliferation, is overexpressed in some testicular tumors. Information of its expression and distribution in human infertile testis is limited, although it could be related to impaired spermatogonial development.

Hypothesis: CpG island of Exon1/Intron1 region of SALL4 is hypomethylated in early rat testis development as well as in human infertile testis with a diagnosis of NOA, which is associated with its overexpression, while decreased expression of SALL4 negatively affects in vitro testicular development.

Aims: Our aim is to analyse and compare DNA methylation status with RNA and protein expression profile of SALL4 in the human testis with NOA as well as in late fetal and early postnatal rat testis development in vivo and its silencing in a 3D culture by siRNA. Specific aims are: 1. Analyse SALL4 expression in late fetal and early postnatal rat testis development on the protein and mRNA level 2. Analyse and compare DNA methylation status with RNA and protein expression profile of SALL4 in late fetal and early postnatal rat testis development 3. Establish a 3D in vitro model of prenatal testicular culture 4. Determine the significance of the SALL4 in rat testis development by in vitro silencing its expression using siRNA 5. Analyse SALL4 expression in the FFPE (Formalin Fixed-Paraffin-Embedded) samples of testicular biopsies of azoospermic men on the protein and mRNA level 6. Analyse and compare DNA methylation status with RNA and protein expression profile of SALL4 in the FFPE samples of testicular biopsies of azoospermic men

Materials and methods: Fresh samples of fetal (GD20.5), neonatal (PND0.5; PND3.5; PND5.5) and adult (>3mo) rat testis as well as archival FFPE samples of testicular biopsies of azoospermic men will be included in this study. Human testis samples with 3 different diagnoses of NOA will be used: hypospermatogenesis (H), maturation arrest (MA), sertoli cell-only sindrom (SCOS). An in vitro testis organoid culture will be established to investigate siRNA-SALL4 effect on the germ cell fate analysing the proliferation and apoptosis. Fixed and paraffin embedded samples will be accessed by histological, immunohistochemical/immunofluorescence and sterological methods for expression and localization analysis of Sall4 proteins. Frozen rat samples will be stored on -80 °C and together with human FFPE samples, used for DNA and RNA isolation. DNA methylation of SALL4 will be determined by pyrosequencing, while gene expression analysis by qPCR method. Statistical analysis will be conducted in statistical program GraphPad Prism 7.0.

Expected scientific contribution: This research will contribute to knowledge on biological significance of epigenetic and expression changes of SALL4 gene and clarify its role in spermatogenesis. New insights about SALL4 could contribute to the potential use of these gene as a biomarker to assess the risk of infertility or offer a new molecular target for NOA therapy.

MeSH/Keywords: testis, male infertility, spermatogenesis, SALL4

Poster code: T-01-02-083

Poster Title: Predictive Model for Sperm Presence in Testes of Azoospermic Men Based on Magnetic Resonance Imaging

PhD candidate: Ana Planinić

Part of the thesis: Predictive Model for Sperm Presence in Testes of Azoospermic Men 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, the absence of spermatozoa in the ejaculate, is a condition that affects 10-15% of infertile men. Two-thirds of those men have nonobstructive azoospermia (NOA), the most severe form of male infertility caused by failure of spermatogenesis. Those men are then referred to testicular sperm extraction (TESE) which in these patients has a 50% success rate. 50% of patients are, thus, needlessly exposed to risks of the procedure. The only method currently used for predicting sperm retrieval is histological analysis. Half of the tissue sample obtained via TESE is histologically analyzed while the other half is cryopreserved. The shortcomings of this method are that sample analysis doesn't necessarily reflect the heterogeneous histology of the whole testis and that the method is invasive. Magnetic resonance imaging (MRI), on the other hand, allows imaging of the whole testis as well as parameter mapping and is also non-invasive. MRI parameters and metabolite concentrations obtained with magnetic resonance spectroscopy (MRS) have been correlated with sperm presence in the testis. Preliminary results of our group show that parameters obtained via ex vivo MRI are comparable to parameters obtained via in vivo MRI. ADC was significantly higher in tissue without sperm compared to tissue with sperm and T2 was elevated in cryopreserved compared to fresh tissue which could indicate changes in tissue consistency and diffusion of the medium into the tissue. These results indicate that parameters obtained via ex vivo MRI could improve the predictive value of a model predicting sperm presence in tissue as well as analyze the impact of cryopreservation on tissue.

Hypothesis: Testicular tissue parameters obtained with MRI and MRS allow the prediction of sperm presence in the sample and indicate changes in tissue metabolism and morphology due to cryopreservation.

Aims: The general aim of this study is to construct a predictive model for sperm presence in testicular tissue using MRI and MRS parameters and patient history information as well as determine the impact of cryopreservation on testicular tissue. The specific aims are: 1. To determine and compare the parameters obtained by MRI in fresh and cryopreserved testicular biopsies, in biopsies with and without sperm, and between different histological groups. 2. To determine and compare the levels of metabolites obtained by MRS in fresh and cryopreserved testicular biopsies, in biopsies with and without sperm, and between different histological groups. 3. Histologically analyze and evaluate testicular biopsies according to the Johnsen scale and compare fresh and cryopreserved samples. 4. Develop a predictive model for the presence of sperm in testicular biopsies based on tissue parameters obtained by magnetic resonance imaging and data from the patient's history.

Materials and methods: 30 patients with azoospermia will undergo testicular sperm extraction (TESE). Half of each tissue sample will undergo MRI and the other half will be cryopreserved and then imaged. MRI parameters (T1, T2, ADC, MTR, and FA) will be calculated as well as concentrations of metabolites from spectra obtained via MRS. After imaging, the samples will be histologically processed and evaluated according to Johnsen's score. A predictive model will be constructed using MRI parameters and patient history data that improve its predictive value.

Expected scientific contribution: A predictive model for sperm presence in testicular tissue would contribute to the development of this type of non-invasive diagnostics that, in clinical application, could eliminate the need for TESE in up to 40% of patients thus sparing them the risk of the procedure, recovery from surgery and the financial costs of fertility treatments. This research will also expand the knowledge about the impact of cryopreservation on testicular tissue.

MeSH/Keywords: Azoospermia; Infertility, Male; Sperm Retrieval; Magnetic Resonance Imaging; Magentic Resonance Spectroscopy; Cryopreservation

Poster code: T-01-02-125

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 - a procedure that carries a risk of acute bleeding, and cSDH recurrence is a common long(er)-term complication. On the other hand, disruption of antithrombotic treatment in patients in whom it is indicated increases the risk of occlusive incidents. Thus, 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 surgery for cSDH in relation to re-installment of their antithrombotic treatment.

Hypothesis: When indicated, it is possible to re-install antithrombotic treatment following chronic subdural hematoma evacuation without an increased risk of hemorrhagic complications, while still preventing occlusive incidents.

Aims: General objective is to evaluate benefit and risk of various modalities of antithrombotic treatment re-initiation in patients following chronic subdural hematoma evacuation. Specific objectives pertain to assessing benefits (prevention of occlusive events) and risks (death, intracranial bleeding or extracranial major or clinically relevant bleeding) regarding: a) re-installment of antiplatelet/anticoagulant treatment, "early" or "late" (vs. no re-installment); b) "early" vs. "late" re-installment of antiplatelet/anticoagulatn treatment; d) "early" vs. "late" antiplatelet vs. respective anticoagulant re-installment.

Materials and methods: Systematic review and (network) meta-analysis of observational and randomized controlled studies. Literature search. 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, Scopus, Elsevier Science Direct, Cochrane Database, Web of Science) and reference lists (manual search) will be included. Inclusion criteria. Included will be randomized control trials, non-randomized controlled trials, stratified cohort studies and case-control studies that comply with the PICO criteria: 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. Outcomes. 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 clinically relevant bleedings. Risk of bias awill be evaluated by two independent researchers using the Cochrane risk-of-bias tool for RCTs (RoB2) and for non-randomized studies of interventions (ROBINS-1) and the Newcastle-Ottawa scale (NOS). Data synthesis. 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.

Expected scientific contribution: The proposed research could, using vast available observational data, significantly increase the reliability of benefit/risk evaluation of antithrombotic treatment re-initiation following cSDH evacuation.

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

Poster code: T-01-08-066

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 lack of oxygen - hypoxia 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 development 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 (nestin, Map2, Dcx, Gfap) are common to damage caused by hypoxia and neurodegeneration.

Hypothesis: Change in Mbd1 gene expression is a common mechanism present during hypoxic neuronal damage in differentiation and in neurodegenerative changes investigated in an in vitro model.

Aims: The main goal is to investigate the role of MBD1 protein during normal and hypoxia - exposed development of nervous system cells and in an in vitro model of hyperhomocysteinemia - mediated neurodegeneration. The specific goals are: 1. To compare Mbd1 expression during neural stem cell differentiation under conditions of normoxia and hypoxia. 2. To investigate MBD1 expression in an in vitro model of neurodegeneration via hyperhomocysteinemia. 3. To investigate the assumed correlation in MBD1 activity and methylation status of DNA markers of nervous system cell differentiation - nestin, Dcx, Map2 and Gfap.

Materials and methods: Neural stem cells (NSC) will be isolated from telencephalon of 14-day old mouse embryo. NSC will be differentiated in vitro using a protocol by which most of the cells will differentiate to neurons and astrocytes. For hypoxia model differentiation will be performed in 21% and 1% of oxygen, respectively. For neurodegeneration model cells will be exposed to L-Homocystein during differentiation. We will analyse cells on Day 1, Day 3 and Day 7. During differentiation we will analyse cells markers for NSC (SOX2, DCX), neurons (MAP2, NEUN), astrocytes (GFAP), epigenetic marker (MBD1) and marker of DNA damage (yH2A.X) both on RNA (real-time PCR) and protein (immunocytochemistry and Western Blot) level. The analysis of epigenetic changes of markers of differentiation of cells of the nervous system will be performed by bisulfite conversion and pyrosequencing.

Expected scientific contribution: Since we do not know the role of Mbd1 in normal and impaired nervous system development, this study will answer the question of whether there is an association between Mbd1 expression, epigenetic changes, hypoxic impairment and neurodegeneration.

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

Poster code: T-01-08-069

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): Assistant Professor Goran Sedmak, MD PhD, Nenad Šestan, PhD

Affiliation: University of Zagreb School of Medicine; Yale University

Introduction: The neural circuits of the hippocampal formation (HIP) and entorhinal cortex (EC) are critical components of a widespread neural network for memory and representation of space and time. Based on cytoarchitectonic, cellular, and circuitry variations, the hippocampal-entorhinal system can be subdivided into functionally distinct subregions that gradually transition from the simple three-layered dentate gyrus (DG) and hippocampus (Cornu Ammonis, CA), and through more complex lamination of the subiculum (collectively referred to as the allocortex) to the six-layered EC (mesocortex). The molecular basis of the diversity of cell types in these subregions and their homology with bordering neocortical cell types and lamination remains poorly understood. Primate- or human-specific evolutionary innovations may underlie some region-selective aspects of hippocampal cell types, necessitating the study specifically of the human hippocampus. Within the human hippocampal-entorhinal system, some cell types and circuits are selectively vulnerable in normal aging and certain pathological processes, such as in Alzheimer's disease. A more detailed molecular profiling of this system will aid our understanding of human brain development and neuropsychiatric diseases.

Hypothesis: There is a significant diversity in the transcriptomic profile of cells in different subregions of the hippocampal-entorhinal system.

Aims: To survey the transcriptomic diversity and functional specification of the mesial temporal cortex and gain insights into neuronal and non-neuronal populations within this system. To reveal organizational principles underlying the specialization and function of the mammalian cerebral cortex and refine our understanding of the evolution of allo-, meso-, and neo-cortex. To integrate known regional and cell-specific differences in disease susceptibility with patterns of gene expression and identify candidate genes that may contribute to vulnerability/resilience in diseases of the human hippocampal-entorhinal system.

Materials and methods: Five regions (dentate gyrus, CA2-4, CA1, subiculum, entorhinal cortex) will dissected from 3 neurotypical "control" and 3 AD frozen brain specimens. Pulverized tissue will be lysed, homogenized, and nuclei isolated through density gradient ultracentrifugation. Single nucleus capture will be performed on microfluidic system (10x Genomics), followed by cDNA synthesis, RNA sequencing on HiSeq 4000 platform (Illumina), and data analysis. Following biostatistical analysis, identified genes of interest will be tested using immunohistochemistry and in-situ hybridization in tissue specimens.

Expected scientific contribution: To our knowledge, this will be the first detailed single-cell transcriptomic analysis of the adult human hippocampal-entorhinal system. It will allow us to uncover novel biology related to the molecular diversity of these cells and better understand evolution of allo-, meso-, and neo-cortex. Furthermore, it will potentially identify cell types and molecular mechanisms that contribute to disease susceptibility.

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

Poster code: T-01-08-073

Poster Title: Selective degradation of misfolded proteins in quiescent yeast Saccharomyces cerevisiae

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: Accumulation of misfolded proteins and their aggregates in cells is a hallmark of many neurodegenerative diseases, such as Alzheimer's, Parkinson's, and other, however the underlying causes of protein aggregation are largely unclear. To better understand the mechanism of disease onset, it is necessary to investigate how a healthy cell prevents the accumulation of abnormal proteins. To maintain protein homeostasis, cells have developed protein quality control system, a set of highly conserved molecular pathways for the refolding and degradation of terminally damaged proteins. The main pathway for degradation of misfolded proteins in the cell is the ubiquitin-proteasome system. Through this pathway, ubiquitin ligases attach ubiquitin molecules on proteins determined for degradation whereupon proteins are sent for proteolysis to the proteasome, a multiprotein complex localized in the nucleus and cytoplasm. Many cells in the human body do not divide, such as neurons or quiescent stem cells. Previous studies of the ubiquitin-proteasome system have been predominantly done on proliferating cells. In the proposed work, the aim is to investigate pathways that mediate selective protein degradation in quiescent cells, using yeast Saccharomyces cerevisiae. Based on proteasome reorganization in quiescent yeast, we assume that quiescent cells use specific pathways of selective protein degradation.

Hypothesis: Degradation pathways of misfolded proteins in quiescent yeast Saccharomyces cerevisiae do not involve ubiquitin proteasome system as in proliferating cells.

Aims: Main aim: To investigate molecular pathways of misfolded protein degradation in quiescent yeast S. cerevisiae. Specific aims: 1. Construct DNA plasmids for expression of model misfolded proteins in quiescent yeast S. cerevisiae. 2. To examine the stability of model misfolded proteins in quiescent yeast S. cerevisiae. 3. Determine the degradation pathways of misfolded proteins in quiescent yeast S. cerevisiae. 4. To examine whether misfolded proteins form inclusions in quiescent yeast S. cerevisiae.

Materials and methods: The research will be conducted in the Laboratory for Developmental Neuropathology of the Croatian Institute for Brain Research in collaboration with the Fred Hutchinson Cancer Research Center in Seattle, USA. The study will be performed on quiescent yeast cells S. cerevisiae that express specific misfolded proteins. To examine which degradation pathways target misfolded proteins in quiescent cells, we will use mutant strains lacking components of the specific degradation pathways, such as ubiquitin ligases. Degradation of misfolded proteins will be analyzed by monitoring protein levels via Western blot or by measuring the activity of reporter enzymes luciferase and β-galactosidase.

Expected scientific contribution: Based on the evolutionary conservation of selective protein degradation pathways from yeast to human, we expect that identification of pathways that target misfolded proteins in quiescent yeast cells will contribute to better understanding of the processes involved in protein aggregation in nondividing cells such as neurons.

MeSH/Keywords: misfolded proteins, aggregates, ubiquitin, proteasome, quiescence, yeast Saccharomyces cerevisiae

Poster code: T-01-08-113

Poster Title: Association of Alzheimer's disease biomarkers with inflammatory mediators and activation of microglia and inflammasome

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 microglial activation has been implicated in the pathogenesis of Alzheimer's disease (AD), but it is not known how it leads to neuronal dysfunction and cognitive decline. One of the possible mechanisms of the harmful microglia effect would be excessive inflammasome activation. In the context of AD, the most studied inflammasomes are NLRP1 and NLRP3. The uncontrolled activation of NLRP1 and NLRP3 is supposed to have an impact on both AD onset and progression but the exact mechanisms of the aberrant activation and time sequence of the pathological changes are yet not known. Many inflammatory mediators can be measured in bodily fluids such as plasma and cerebrospinal fluid (CSF), thus providing insight into the processes in the brain. However, previous measurements of cytokine and chemokine levels showed very inconsistent results. One approach to this problem would be determining more extensive cytokine and chemokine profiles, e.g. with a multiplex ELISA system. By comparing inflammasome and microglial activation and by determining the levels of about twenty pro- and anti-inflammatory mediators in the CSF and plasma from AD, mild cognitive impairment (MCI), and cognitively healthy control (HC) subjects, an attempt will be made to better clarify the role of inflammatory processes in the development and progression of AD.

Hypothesis: Comparison of AD, MCI, and HC subjects will show different profiles of the inflammatory mediators in the cerebrospinal fluid and plasma samples along with stronger microglia, NLRP3, and NLRP1 inflammasome activation in the hippocampal formation of the AD subjects relative to the HC group.

Aims: Main aim: To investigate inflammatory processes in AD by analysis of the plasma and CSF samples of HC, MCI, and AD subjects, and hippocampal brain tissue of the healthy and diseased subjects. Specific aims: 1. To determine the levels of about 20 pro- and anti-inflammatory cytokines and chemokines in the CSF and plasma samples and levels of AD biomarkers in the CSF (Aβ, p-tau, and total tau) of the AD, MCI, and HC patients and to examine their correlation. 2. To determine the concentration of ASC and TREM2 proteins in the CSF and plasma samples from AD, MCI, and HC patients, to correlate them with the levels of AD biomarkers in the CSF, and to determine their biomarker potential in the early diagnosis of the AD. 3. To investigate the microglia, NLRP3, and NLRP1 inflammasome activation in postmortal hippocampal formation tissue from AD patients and HC.

Materials and methods: This study will include at least 20 patients in each of the groups (AD, MCI, and HC). Patients will be recruited at Clinical Hospital Centre Zagreb and will be grouped based on clinical diagnosis and Mini-Mental State Examination (MMSE) status. Cytokine and chemokine levels (IL-1β, IL-2Ra, IL-10, IL-13, IL-17A, IL-18, IFN-γ, TNF-α, M-CSF, IP-10, RANTES, CTACK…) in CSF and plasma samples will be determined by multiplex ELISA (Enzyme-Linked Immunosorbent Assay) method. The standard ELISA method will be used for the determination of the other markers in the CSF (Aβ1-42, t-tau, p-tau181, TREM2, and ASC) and plasma (TREM2 and ASC). Microglial and inflammasome activation in the postmortem hippocampal formation tissue will be analyzed by immunohistochemical staining to visualize NLRP3, NLRP1, and microglial (Iba1, CD68, and HLA-DR) markers.

Expected scientific contribution: We expect to reveal new knowledge in regard to specific cytokine and chemokine profiles in plasma and CSF samples from HC, MCI, and AD subjects. For the first time, the measurement of such a large panel of pro- and anti-inflammatory molecules will be compared with the core CSF biomarkers of AD, microglial markers, and NLRP inflammasomes. Therefore, we hope to better clarify the role of immune changes and neuroinflammation in the onset and progression of AD.

MeSH/Keywords: Alzheimer's disease, microglia, inflammation, inflammasome, ELISA method

Poster code: T-01-08-117

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

PhD candidate: Janja Kopić

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

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

Affiliation: University of Zagreb School of Medicine

Introduction: The human neocortex is involved in sensory perception, motor functions, and complex cognitive functions. The human brain development is characterized by transient fetal zones of the future cerebral cortex where neural stem cells proliferate in proliferative zones (ventricular-VZ and subventricular zone-SVZ), progenitors migrate along radial glia through the intermediate zone-IZ, create the first synapses in subplate zone-SP, and reach their final destination in the cortical plate-CP. Cajal-Retzius cells in the marginal zone-MZ secrete protein Reelin into the extracellular space, involved in radial migration and laminar cortical organization. Neurodevelopment requires precise regulation of gene expression, including mRNA translation. RNA-binding proteins (RBP), such as CELF1, regulate which mRNAs undergo translation. Despite recent advances on the role of mRNA translation in cortical development, significant gaps in knowledge remain during human neurodevelopment. Here, we use immunohistochemistry on prenatal postmortem human brain tissue to analyze the laminar, sublaminar, and regional expression pattern of CELF1, determine subclasses of glutamatergic neurons that express CELF1, and the critical period when glutamatergic neurons obtain their identity.

Hypothesis: Since the RNA-binding protein CELF1 regulates mRNA translation in early progenitor cells involved in the production and regulation of the identity of glutamatergic neurons, we expect early laminar expression and different regional distribution suggesting CELF1 participation in the construction of architecture and regional differentiation of the human cerebral cortex during prenatal development.

Aims: To determine the spatio-temporal dynamics of the laminar expression pattern of the CELF1, which regulates the translation of mRNA during neurogenesis and determines the identity of glutamatergic neurons in different regions of pallium during prenatal development of the human cerebral cortex. Specific aims: 1. To determine the laminar expression pattern of CELF1 protein, and its regional distribution in the cerebral cortex of the frontal and occipital lobes during the early (8-12PCW) and middle fetal period (13-24PCW) 2. To investigate whether progenitor cells are located, except in proliferative zones, in other transient fetal zones of the future cerebral cortex, given the complexity of temporary fetal lamination in humans (Zunic Isasegi et al., 2018), such as recently described compartment MACC (multilaminar axonal-cellular compartment) 3. To determine subpopulations of cells expressing CELF1 in the cerebral cortex with markers specific for neurons of certain cortical layers: CTIP2, TLE4, FOXP2, SOX5, TBR1 (deeper layers), CDP, CUX1, CUX2 (superficial layers), and to determine the neurotransmitter profile (e.g. vGLUT) of CELF1-expressing cells.

Materials and methods: Experiments will be performed on the postmortem prenatal human brain specimens that are part of the Zagreb Neuroembryological Collection. All ethical standards according to Helsinki Declaration were respected and approved by the Ethics Committee of the Faculty of Medicine, University of Zagreb. Prenatal samples cover a time period of 8 to 24 postconceptional weeks (PCW). Paraffin-embedded samples were cut in the coronary plane into 8-20 µm thick sections using a microtome (Leica, SM2000R) and stained using immunohistochemistry, immunofluorescence, in situ hybridization, or RNA Scope. The medial, dorsal, and dorsolateral portions of the cerebral cortex of these samples will be visualized for comparative analysis with Olympus FV3000 confocal microscope.

Expected scientific contribution: The understanding of the role of the RNA-binding protein CELF1 in the process of neurogenesis, especially laminar and regional development will be improved, considering that it is still unknown in which developmental period the molecular identity of glutamatergic neurons in the human brain is established. In addition, new data on the regulation of translational mechanisms during the development of the cerebral cortex will contribute to a better understanding of the disfunction of the translational processes leading to neurodevelopmental and neurodegenerative diseases.

MeSH/Keywords: human cerebral cortex development, mRNA translation, RNA-binding proteins

Poster code: T-01-08-118

Clinical medical sciences – research proposals

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: Evaluation and correlation of ego strength, anxiety, depression and quality of life of parents with the severity of atopic dermatitis of their 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.

Hypothesis: Parents of children with atopic dermatitis have lower Ego strength and quality of life, and a higher level of anxiety and depression in relation to the parents of children who do not suffer from chronic skin diseases. The Ego strength of the parents of children with atopic dermatitis correlates to the intensity of the clinical picture in the affected child.

Aims: GENERAL OBJECTIVE: Investigate Ego strength, levels of anxiety and depression, as well as the quality of life of parents of children with atopic dermatitis and correlate to the intensity of the clinical picture in their children. SPECIFIC OBJECTIVES: 1. Investigate the strength of Ego strength of parents of children with atopic dermatitis with respect to age, gender, and the intensity of the clinical picture in their children. 2. Investigate the level of anxiety and depression in parents of children with atopic dermatitis with respect to age, gender, and the intensity of the clinical picture in their children. 3. To investigate the quality of life of parents of children with atopic dermatitis with respect to age, sex and intensity of the clinical picture in their children.

Materials and methods: The study will be conducted in the outpatient clinic and the Department of Pediatric Dermatology for 2 years on 72 subjects (36 in each analyzed group). The study will include parents of children with atopic dermatitis aged 6 months to 7 years. The control group would be the parents of healthy children who do not suffer from chronic skin diseases or other chronic diseases. The sample will exclude 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 will be 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 will be assessed with the Scoring Atopic Dermatitis index (SCORAD index). After signing the informed consent, the participants will 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 will be analyzed using descriptive and analytical methods.

Expected scientific contribution: The results of the proposed research could answer the question of how much the psychological characteristics of parents affect the treatment outcomes of atopic dermatitis in their children, while providing insight into the dynamics and relationship of psychological characteristics with the severity of the clinical picture. If the obtained results show a significant effect on the treatment outcomes, an attempt will be made to develop a clinical procedure with the aim of achieving a better treatment outcome in pediatric patients with atopic dermatitis.

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

Poster code: T-02-02-076

Poster Title: The value of cartilage biomarkers serum CS846 and urinary CTX-II in assessing the severity 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

Introduction: Hemophilia is rare sex-inherited disease of blood clotting affecting men. Bleeding into the joints cause hemophilic arthropathy (HA), most commonly of the elbows, knees, and ankles. Studies on the value of biomarkers in the assessment HA severity are based on correlation with radiological and clinical scores. To our knowledge there has been no study on the association of clinical and ultrasound (US) scores of joints with values of cartilage biomarkers serum CS846 and urinary CTX-II in patients with hemophilia. To date, these markers have no clinical application. There has been no study on proteomic blood analysis to identify markers of cartilage damage and to analyze the role of ADAMTS4 aggrecanase in HA.

Hypothesis: Patients with severe hemophilia will have higher values of CS846 and uCTX-II compared to patients with mild hemophilia and will positively correlate with US score of joints.

Aims: To determine association of the values of CS846 and uCTX-II with clinical and US scores of joints. To compare the values of CS846 and uCTX-II in patients with hemophilia and healthy men. To compare the values of CS846 and uCTX-II with the values of bone markers: osteocalcin, bone alkaline phosphatase and CTX-I in patients with hemophilia. Proteomic blood analysis to identify and quantify markers that may be associated with cartilage damage and to analyze the role of ADAMTS4.

Materials and methods: Patients with severe and mild hemophilia will be included in test and healthy men in control group, 46 subjects in each group. Blood and urine samples from all subjects will be taken for CS846 and uCTX-II analysis, and for bone marker analysis in patients with hemophilia. Mass spectrometry will be used for proteomic blood analysis of all subjects. HA will be assessed by clinical (classification recommended by the World Federation of Hemophilia) and US scoring of elbows, knees and ankles (HEAD-US protocol).

Expected scientific contribution: The results will show whether CS846 and uCTX-II can be used in clinical practice in assessing HA severity. To our knowledge, this is the first study on proteomic blood analysis to detect biomarkers associated with cartilage damage and the role of ADAMTS4 in HA.

MeSH/Keywords: hemophilic arthropathy, cartilage biomarkers, proteomics

Poster code: T-02-03-010

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

PhD candidate: Stjepan Čota

Part of the thesis: Učinkovitost terapijskog ultrazvuka energije 4500 Joula u liječenju kalcificirajućeg tendinitisa ramena

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

Affiliation: University of Zagreb School of Medicine; University Department for Rheumatology and Rehabilitation, Clinical Hospital Centre Zagreb

Introduction: Calcific tendinitis of the shoulder 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-ultrasound) and kinesiotherapy are commonly used in the treatment.

Hypothesis: T-ultrasound in combination with kinesiotherapy leads to a significantly better rehabilitation outcome than sham T-ultrasound in combination with kinesiotherapy in the symptomatic rotator cuff calcific tendinitis.

Aims: To determine whether T-ultrasound, in parameters that have been shown to affect, in combination with kinesiotherapy leads to a significantly better rehabilitation outcome, calcification size and pain reduction, increased shoulder mobility, improvement of the shoulder functional status, and overall rehabilitation outcome satisfaction, than sham T-ultrasound in combination with the same kinesiotherapy in the symptomatic rotator cuff calcific tendinitis.

Materials and methods: The study group will consist of 21 patients who will receive T-ultrasound (continuous mode, 1.5 W/cm2 for 10 minutes, total energy per treatment 4.500 J) in addition to kinesiotherapy (pendular exercises, range of motion, and shoulder stabilizer strengthening exercises for half an hour), while the control group will consist of 21 patients treated with sham T-ultrasound and the same kinesiotherapy for 4 weeks (5 times per week). The primary outcome of the study is a reduction in the calcification size before and after the treatment. Before and after the intervention, the following will be 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).

Expected scientific contribution: The results of the study will contribute to the overall knowledge about the effectiveness of T-ultrasound in the treatment and rehabilitation of symptomatic calcific tendinitis of the shoulder because for the first time the total applied energy of 4500 J per treatment, which is assumed to have a positive effect, will be evaluated.

MeSH/Keywords: rotator cuff; rehabilitation; exercise therapy

Poster code: T-02-03-012

Poster Title: Kegel exercises or extracorporeal magnetic innervation for stress urinary incontinence - which way is better?

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 Vladimir Banović, MD PhD

Affiliation: 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. It has been reported that SUI affects up to 35% of postmenopausal women, impacting unfavourably on the quality of life and overall mental health. Management of SUI is based mainly on available conservative treatment modalities. Although Kegel exercises and extracorporeal magnetic innervation (EMI) share the majority of their influences on the pathophysiology of SUI, it is unclear whether one of these routinely used treatment modalities is superior to another in terms of improvement of clinical outcomes or cost-effectiveness. To the best of our knowledge, no randomized controlled trials have so far directly compared these two treatment modalities.

Hypothesis: We hypothesized that EMI is more effective in reducing the number of episodes of urinary incontinence and improving quality of life compared to Kegel exercises in patients with SUI.

Aims: The primary outcome will be the effectiveness of the treatment as measured by the ICIQ-UI SF overall score, 8 weeks after commencement of treatment. Secondary outcomes will include: 1) the average increase in the vaginal pressure, as measured with the Peritron perineometer at 8 weeks; 2) women's satisfaction with treatment, measured by the PGI-I scale 3 months after the intervention has ended; 3) quality of life, measured by ICIQ-LUTSqol 8 weeks and 3 months after commencement of treatment.

Materials and methods: We will perform a parallel-group, randomised controlled trial compliant with the CONSORT reporting guidelines. The study will be conducted at the Department of Obstetrics and Gynecology, Clinical Hospital Center Zagreb, Croatia. Participants will be women aged 18 to 65 years who have previously given at least one birth (12 months or more before joining the study) who present with symptoms of SUI lasting at least 6 months, yet have not previously undergone treatment for it. In the first study arm, we include patients into 8-week high-intensity, home-based Kegel exercises regimen. The whole exercise programme is sent by nursing assistant on patients' e-mail address after initial enrollement and is tailored during 8-week period by the attending physician. In the second study arm, we include patients into 8-week EMI treatment scheme. All measurements will be done at enrollment, after the intervention is completed, and three months following the end of the intervention. We will use perineometer, bladder diary, ICIQ-UI SF and ICIQ-LUTSqol questionnaires and PGI-I scale. Descriptive statistics measures will be used to show the distributions of the participants. Categorical variables will be displayed as frequencies with their percentages. In order to calculate whether there are statistically significant differences initially, after the treatment and 3 months post-treatment in ICIQ-UI SF results between two groups (EMI vs. Kegel), we will conduct mixed-model analysis of variance. A p-value less than 0.05 will be considered statistically significant.

Expected scientific contribution: Our randomised trial will provide the first 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 exercises; extracorporeal magnetic innervation; quality of life

Poster code: T-02-05-014

Poster Title: Seroprevalence of IgG antibodies against pertussis in children aged 6 to 18 years

PhD candidate: Vedran Stevanović

Part of the thesis: Seroprevalencija IgG protutijela na hripavac u djece od 6 do 18 godina

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

Affiliation: University of Zagreb School of Medicine

Introduction: Pertussis is a vaccine preventable disease caused by Bordetella pertussis. Adolescents with pertussis continue to be a significant source of infection for incompletely vaccinated infants who are in harm for developing severe disease.

Hypothesis: Current immunization programs achieve different seroprevalence of IgG antibodies against pertussis in children aged 6 to 18 years.

Aims: The primary objective of our study is to determine the concentration of IgG-anti-PT in children older than 6 years.

Materials and methods: A single-center cross sectional seroprevalence study will be conducted in regularly vaccinated children and adolescents in age range 6 to 18 years at the University Hospital for Infectious Diseases in Croatia within 18 months. Serum samples will be collected and frozen at the study site until serological analysis.

Expected scientific contribution: Our proposed study will give an insight in seroprevalence of IgG-anti-PT in children and adolescents aged 6-18 years, estimate pertussis infection activity and compare it regarding inflicted immunization programs at the time.

MeSH/Keywords: seroprevalence, IgG antibody to pertussis toxin, pertussis, vaccination

Poster code: T-02-07-019

Poster Title: Impact assessment of increased intra-abdominal pressure on the width and collapsibility of the vena cava inferior

PhD candidate: Mia Rora

Part of the thesis: Procjena utjecaja povećanog intraabdominalnog tlaka na širinu i kolapsibilnost donje šuplje vene

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

Affiliation: University of Zagreb School of Medicine

Introduction: Evaluation of the diameter of the vena cava inferior (VCI) by ultrasound allows a rapid and non-invasive assessment of the volume status of patients in intensive care units. The change in the diameter of the vena cava inferior during respiration depends on the filling of the circulatory system and the type of respiration. Numerous papers compared central venous pressure and diameter of vena cava inferior. Based on these papers, tables were formed according to which ultrasound can be used to measure the central venous pressure in a patient by measuring the diameter of the VCI and the index of collapsibility of the VCI, which is valuable clinical data available noninvasively next to the patients bed. It is not known to what extent intra-abdominal pressure alters the diameter and collapse of VCI and affects volume status assessment.

Hypothesis: Increased intra-abdominal pressure leads to a decrease in the diameter of the inferior vena cava assessed by ultrasound.

Aims: General: To investigate the influence of elevated intrabdominal pressure on the diameter of the inferior vena cava. Specific: 1. Assess the impact of elevated intrabdominal pressure on inferior vena cava diameter and inferior vena cava collapse index. 2. Assess the impact of different levels of elevated intrabdominal pressure on the diameter and collapse index of the inferior vena cava and central venous pressure. 3. Calculate correction factors for the assessment of central venous pressure based on the diameter and index of collapse of the DSV in patients with elevated intra-abdominal pressure.

Materials and methods: Two models are envisaged that will allow us to test the effect of intra-abdominal pressure on the diameter and collapse of the vena cava inferior. One model includes patients with tension ascites, in whom there is a need to evacuate ascites. The second model includes patients without elevated intra-abdominal pressure. In the latter group, an increase in intra-abdominal pressure is planned to be achieved by placing the abdominal girdle. Both groups of subjects must have a urinary catheter inserted. By the method of connected vessels, through the measurement of intravesical pressure, we will measure the intra-abdominal pressure. In the first group of subjects, discharge of ascites will result in a decrease in intra-abdominal pressure, which is registered by measuring intravesical pressure. After reducing the intra-abdominal pressure by one unit of measurement, we will repeat the examination of the vena cava inferior with ultrasound, measure the anteroposterior diameter in inspiration and expiration, and calculate the index of collapse of the vena cava inferior. In case the subjects also have a central venous catheter placed, we will measure the central venous pressure before and after the ascites evacuation. In the second group of subjects, we will repeat the measurement of diameter of the vena cava inferior and calculate the collapse index for different units of increase in intra-abdominal pressure achieved by controlled filling of the belt and balloon with air.

Expected scientific contribution: The results of this study indicate how different levels of elevated intra-abdominal pressure affect the diameter and index of collapse of the vena cava inferior. The above would help us to correct the existing tables for the assessment of central venous pressure based on the assessment of the diameter and index of collapse of the vena cava inferior in patients with intra-abdominal hypertension. The results of this study could also offer the possibility of using ultrasound to assess intra-abdominal hypertension based on the measurement of the diameter of the vena cava inferior by ultrasound.

Acknowledgments: I would like to thank the Clinical Hospital Center Zagreb and the Clinic for Internal Medicine for enabling me to conduct this research.

MeSH/Keywords: diameter of the vena cava inferior, collapse index of the vena cava inferior, central venous pressure, intra-abdominal hypertension, intravesical pressure

Poster code: T-02-08-013

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 in the absence of fluid overload or heart failure, which lead to respiratory failure. The underlying pathophysiologic mechanism of ARDS is a diffuse inflammatory lung injury, causing non-cardiac lung edema. Although well known for decades, it is still 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, generating negative pleural pressure (Ppl). 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 then used as a surrogate for Ppl. To these days esophageal manometry remains the gold standard for the estimation of Ppl.

Hypothesis: In mechanically ventilated patients with ARDS who have preserved spontaneous inspiratory efforts it is possible to assess changes in pleural pressure (ΔPpl) by measuring changes in the inferior vena cava diameter (ΔVCI) with ultrasound (US).

Aims: The main aim of the study is to determine the relationship between the change in Ppl and the change in the inferior vena cava diameter during inspiration in mechanically ventilated ARDS patients with preserved spontaneous inspiratory efforts by simultaneous measurement of ΔPpl with esophageal manometry and ΔVCI with ultrasound. Secondary aim is to assess the impact of ventilatory modalities on this relationship.

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 (<20), 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 ΔPpl with esophageal manometry and three repeated measurements of ΔVCI 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 (ΔPpl) will be modeled in generalized linear models as a continuous, ordinal and binary variable. The main independent variable of interest (ΔVCI) will be treated as a continuous variable.

Expected scientific contribution: Finding the relationship between ΔPpl and ΔVCI with ultrasound as a non-invasive and inexpensive method would provide 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: T-02-08-025

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 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%. According to the Third International Consensus Definitions for Sepsis and Septic Shock from 2016 sepsis is defined as an increase of SOFA (Sequential Organ Failure Assessment) score ≥ 2 with suspected infection. Timely diagnosis and early interventions are crucial for disease outcome, especially in immunocompromised patients. We are witnessing an increase in the number of immunocompromised patients worldwide. Scoring systems like APACHE II (Acute Physiology And Chronic Health Evaluation II), SOFA and SAPS II (Simplified Acute Physiology Score II) were designed for prediction of in-hospital mortality. Various biochemical structures are being investigated in search for an ideal biomarker to ease risk stratification and predict hospital outcome. 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 regrading IL-6 and leukocyte CPD in these patients is scarce, mostly retrospective and does not offer a separate analysis of this patient group.

Hypothesis: We assume that higher initial values of IL-6 and leukocyte CPD in immunocompetent and immunocompromised patients with sepsis indicate a greater risk of lethal outcome.

Aims: We aim to investigate the value of PCT, IL-6 and leukocyte CPD for outcome prediction in patients with sepsis. Also, we will analyze the trend of PCT, IL-6 and leukocyte CPD during the investigation period in immunocompetent and immunocompromised patients with sepsis. The potential use of initial concentrations of PCT, IL-6 and leukocyte CPD for outcome prediction in each patient group will be assessed separately.

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. Gathered data will be analyzed using appropriate statistical tests. Sample size of 179 patients was calculated with the assumption of using binary logistic regression analysis as the adequate statistical model. This study is approved by the Ethics comity of the University Hospital Centre Zagreb.

Expected scientific contribution: The expected scientific contribution is 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: T-02-08-043

Poster Title: Ultrasonic assessment of diaphragm mobility in patients with systemic sclerosis with and without interstitial lung disase

PhD candidate: Anja Ljilja

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

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

Affiliation: University of Zagreb School of Medicine

Introduction: Systemic sclerosis (SSc) is a chronic autoimmune disease of unknown etiology. One of the most common cause of death in patients with SSc is interstitial lung disease (ILD). Results of the studies indicate that esophageal disease can independently contribute or be the cause of ILD. Diaphragm is the main muscle in physiology of respiration. Diaphragm mobility is known to be reduced in some chronic lung and autoimmune diseases.

Hypothesis: Patients with systemic sclerosis with ILD have decreased diaphragm mobility compared to the patients without ILD.

Aims: General objective: To compare diaphragm mobility in patients with SSc with ILD to the patients without ILD. Specific objectives: 1.To investigate the difference in diaphragm thickening fraction using ultrasound in patients with SSc with and without ILD; 2. To evaluate diaphragm thickness at total lung capacity (TLC) and functional residual capacity (FRC) in patients with SSc; 3. To examine whether esophageal disease is more common in patients with reduced diaphragmatic mobility.

Materials and methods: Participants in this study will be 50 adult patients with diagnosis of SSc who are in regular follow up in an outpatient clinic of the Department of clinical immunology, rheumatology and allergology, University Hospital 'Dubrava', aged 18-78 years. Duration of this cross-sectional observational research will be until december 2022.Criteria for exclusion will be diseases or conditions known to reduce diaphragmal function, chronic lung disease of etiology not specific to the patients with SSc, neuromuscular disease or autoimmune disease that could contribute to impaired diaphragmatic mobility or pathological values of lung function, BMI <18.5 and BMI> 40. In statistical analysis wil be included clinical and radiological parameters: age, sex, mMRC scale, significant comorbidities, therapy for SSc, smoking history, BMI, chest and waist circumference, spirometry, diffusion capacity for CO and modified Rodnan skin score. HRCT of the thorax will be used for evaluation of ILD. Warrick score will be calculated in order to evaluate extent and severity of ILD. Mobility and thickening of the diaphragm during the respiratory cycle will be evaluated by ultrasound. Amplitude of craniocaudal diaphragmatic mobility during normal and deep breathing will be measured. Diaphragmatic thickness will be measured at FRC and at TLC. Diaphragmal thickness fraction will be calculated with formula: (thickness at TLC- thickness at FRC) / thickness at FRC x 100.

Expected scientific contribution: This study will for the first time examine whether there is decreased mobility and diaphragm thickening fraction in patients with SSc and ILD compared to the patients without ILD. The expected contribution of this study is to identify diaphragmatic dysfunction in patients with SSc so that patients can be referred for pulmonary rehabilitation to strengthen inspiratory musculature, which also has a positive effect on esophageal reflux. Another expected contribution is to identify patients with decreased diaphragm function to which early screening for gastroesophageal disease to prevent development or progression of ILD should be done.

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

Poster code: T-02-09-002

Poster Title: Impact of previous chronic statin therapy on mortality in patients with AMI treated with pPCI

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 health system burden. Statins reduce the cholesterol level primary by inhibiting 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase. Despite clear evidence that statin treatment in both primary and secondary CV prevention will lead to a reduction in cardiovascular events and mortality, it is unclear whether previous chronic statin therapy has an impact on the mortality of patients with acute myocardial infarction (AMI).

Hypothesis: Early statin therapy reduces overall long-term mortality in patients with acute myocardial infarction treated with primary percutaneous coronary intervention (pPCI).

Aims: The aim of the study is to determine whether previous statin therapy affects the outcomes of patients treated with primary PCI for AMI. The primary objective of the study is to determine whether previous statin therapy affects the overall long-term mortality of patients treated with primary PCI due to AMI. The secondary objective of the study is to determine the impact of previous statin therapy on short-term CV mortality, long-term CV mortality, short-term overall mortality, SYNTAX score, left ventricular systolic function and NYHA status, angiographic findings (occlusion/stenosis of the culprit artery) and technical parameters of the procedure of patients with AMI treated with primary PCI.

Materials and methods: A prospective, clinical, non-interventional cohort study will be conducted, and patients treated for AMI in UHC Sestre milosrdnice Zagreb, between 01.06.2011. and 01.01.2019 with pPCI will be included. Patients will be divided in two groups: patients treated with statins before AMI and patients not treated with statins before AMI. Patients with no coronary angiography performed, patient with recurrent AMI and patients with terminal heart failure or other non-cardiac terminal disease that significantly affects outcomes will be excluded from the study. The registry of the Department for Invasive and Interventional Cardiology of UHC Sestre milosrdnice will be used as a data source. Mortality data will be collected from available medical histories, the mentioned registry, and from the National database of deceased. Additional data will be collected: general and demographic data, vital parameters, acute myocardial infarction characteristics, interventional data and a list of therapy used before and after the index event, echocardiographic parameters and laboratory findings. Their outcomes will be compared.

Expected scientific contribution: This is the first clinical study examining the impact of previous statin therapy on mortality in patients with AMI, including the impact on short-term and long-term mortality, and on cardiovascular and overall mortality. The effects of previous statin therapy on angiographic and echocardiographic findings after AIM will be further investigated.

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

Poster code: T-02-09-003

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. Homing and accumulation of lymphocytes in certain organs is manifested by tumor distribution which is important for classification and prognosis of the disease. Homing, proliferation, and apoptosis of lymphocytes are a consequence of interaction with the tumor microenvironment. Ibrutinib has significant interactions with the microenvironment in addition to inhibiting Bruton's kinase. Ibrutinib may induce significant redistribution of B lymphocytes from lymphoid organs to the peripheral blood prior to reducing tumor mass. 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

Hypothesis: In the first months of treatment of B-chronic lymphocytic leukemia ibrutinib causes changes in the expression of CD38, CD49d, CXCR4, CXCR5 molecules at the surface of clonal B-lymphocytes leading to tumor distribution between lymphoid organs and bone marrow in peripheral blood.

Aims: General aim: To determine the association of tumor cell immunophenotype with tumor distribution during ibrutinib treatment in patients with B-chronic lymphocytic leukemia. Specific aims: 1) Determine the effect of ibrutinib therapy on the change of expression patterns of molecules: CD38, CD49d, CXCR4, CXCR5. 2) Explore the level of expression of each molecule (CD38, CD49d, CXCR4, CXCR5) in samples sampled from different microenvironments (intraclonal heterogeneity).

Materials and methods: Patients with B-chronic lymphocytic leukemia who received ibrutinib will be included in this prospective, longitudinal, observational study. Untreated and previously treated patients will be included. Inclusion criteria are refractory/relapsed disease and indications for treatment according to the International Working Group for CLL and Croatian Cooperative Group for Hematologic Disease. Exclusion criterion is active chemotherapy treatment of other malignant disease. Patients will be monitored for six months after starting ibrutinib treatment. Time points are the beginning of the treatment, the third and the sixth month. Methods include complete blood count, computed tomography (CT) of the neck, the thorax, the abdomen and the pelvis and flow cytometry. Total tumor mass (TTM1) and tumor distribution (TD2) will be determined at specific time points in all patients. CT will be performed at the beginning of the treatment and in the sixth month in all patients. CT will be performed also in the third month of treatment in patients with splenomegaly and those with thoracic, abdominal and pelvic lymph nodes. Expression levels of the surface molecules CXCR4, CXCR5, CD38 and CD49d will be determined by flow cytometry on malignant CD5+ and CD19+ 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

Expected scientific contribution: This research will explain tumor distribution from lymphoid organs and bone marrow to the peripheral blood in patients with B-CLL treated with ibrutinib by changes of the expression of the CD38, CD49d, CXCR4 and CXCR5 molecules on the surface of malignant B lymphocytes.

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

Poster code: T-02-09-015

Poster Title: Prognostic role of C4d in primary IgA nephropathy and 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: IgA nephropathy (IgAN) is the most common primary glomerular disease, while focal segmental glomerulosclerosis (FSGS), a disease less common than IgAN, has the highest tendency of progression to end-stage renal disease (ESRD). Both diseases appear in primary and secondary forms. The pathogenesis of both diseases is different. IgAN is a typical immune complex disease with mesangial deposition of IgA and C3, while immune complexes are an uncommon finding in FSGS. According to recent research, complement system seems to be involved in pathogenesis of both diseases with potential prognostic implications. Up to 35 % of patients with IgAN have positive markers of complement activation via lectin pathway and they have worse disease outcome. 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 both diseases, we are still searching for novel prognostic factors. C4d is a potential candidate the role of which in primary IgAN and primary FSGS should be clarified.

Hypothesis: C4d is a significant predictor of ESRD development in primary IgAN and primary FSGS.

Aims: General aim of the study is to elucidate the role of complement system and C4d in primary IgAN and primary FSGS. Specific aims are: to determine prevalence and prognostic significance of C4d in both diseases, to determine prevalence of IgM, C3 and C1q in both diseases and their relation to disease outcome and to compare clinical, histological and laboratory features of patients regarding C4d positivity.

Materials and methods: This is a cohort study. Patients with biopsy proven primary FSGS and primary IgAN, 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, laboratory findings, including serum creatinine and 24-hours proteinuria, and histological data will be reviewed retrospectively. Kidney function will be estimated using CKD-EPI formula and expressed as eGFR in ml/min/1.73m2. 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. For the purposes of disease outcome analysis, follow up data and treatment regimens will be analyzed. Primary endpoint is a progression to ESRD (defined as the beginning of renal replacement therapy or the eGFR decrease up to < 15 ml/min/1.73m2) and secondary endpoint is a decline in eGFR > 50% from baseline value. C4d positive and negative group will be statistically compared regarding baseline data and disease outcome in both diseases. Prognostic value of C4d will be evaluated using Kaplan-Meier survival analysis and Cox regression model.

Expected scientific contribution: It is important to determine prevalence of C4d positivity in primary IgAN and primary FSGS due to different reports in literature. Establishing C4d as a new prognostic factor may have an influence on the diagnostic and therapeutic approach in both diseases.

MeSH/Keywords: IgA nephropathy, focal segmental glomerulosclerosis, C4d, prognostic factor, disease outcome

Poster code: T-02-09-016

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

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: Allogeneic haematopoietic stem cell transplantation (alloHSCT) is a standard treatment modality for haematological diseases that leads to a high infection rate. Sepsis is a life-threatening infection and its timely diagnosis is difficult due to the lack of accuracy of standard biomarkers. Early diagnosis of sepsis 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

Hypothesis: Diagnostic validity of presepsin (PSP) and glycoprotein YKL-40 in the recognition of sepsis is high and can distinguish septic from non-septic febrile episodes in patients in the early phase of allogeneic haematopoietic stem cell transplantation.

Aims: Primary: To determine the diagnostic validity of PSP and YKL-40 in distinguishing septic and non-septic febrile episodes during the early phase of alloHSCT. Secondary: 1. To compare the diagnostic validity of PSP and YKL-40 with the diagnostic validity of PCT and CRP 2. To determine the diagnostic validity of different combinations of PSP, YKL-40, CRP, and PCT 3. To determine the correlation of other clinical and laboratory parameters with serum concentrations of PSP and YKL-40. 4. Determine a model (combination of clinical parameters and laboratory markers) that ensures the best degree of differentiation of septic and non-septic febrile episodes 5. To determine whether there is a statistically significant difference in serum concentrations of PSP and YKL-40 between patients with positive and negative blood cultures.

Materials and methods: In this prospective, observational study we plan to include consecutively all febrile patients, 18 years of age and older with signed informed consent, during the pre-engraftment phase of alloHSCT treatment, and to analyze at least 62 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. Fever is defined as axillary body temperature ≥ 38 ° C measured twice in a 1 h interval or one measurement ≥ 38.5 ° C. Febrile episodes will be stratified into two groups, sepsis, and non-sepsis, according to the Sepsis-3 International Conference definition of sepsis as a suspected or documented infection with an acute increase in SOFA (Sequential Organ Failure Assessment) of a total score of ≥2. FE stratification will be 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 will be determined by the sandwich ELISA technique at the beginning of each febrile episode and every 48 hours during the first 7 days. The diagnostic performance of biomarkers will be assesed in terms of sensitivity, specificity, predictive values, likelihood ratios and area under the ROC (Receiver Operating Characteristics) curves.

Expected scientific contribution: We plan to study diagnostic validity in recognizing sepsis of scarcely (PSP) or never (YKL-40) investigated biomarkers in patients treated with alloHSCT. Stratification of patients will be performed according to Sepsis-3 criteria which have not been used so far. New biomarkers can contribute to a faster and more accurate diagnosis of sepsis, more rational use of antibiotics with better therapeutic success, all of which may ultimately result in a better outcome in patients treated with alloHSCT.

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

Poster code: T-02-09-020

Poster Title: Association of gene polymorphism MTHFR, KCNE1, and PITX2 with atrial fibrillation in overweight patients

PhD candidate: Rea Levicki

Part of the thesis: Association of gene polymorphism MTHFR, KCNE1, and PITX2 with atrial fibrillation in overweight patients

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

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

Introduction: Atrial fibrillation is the most common arrhythmia. Risk factors for atrial fibrillation development are advanced age, hypertension, diabetes, congestive heart disease, valvular heart disease, obesity and obstructive sleep apnea. Genetic influence on atrial fibrillation development is already well known, and probably caused by hyperhomocysteinemia and gene polymorphisms; MTHFR C677T, KCNE1 G38S, PITX2 rs2200733. Other studies show that KCNE1 and PITX2 gene are coding myocardial tissue around pulmonary veins, MTHFR C677T polymorphism has a known prothrombogenic and atherogenic effect.

Hypothesis: Gene polymorphisms MTHFR 677C>T (rs1801133), KCNE1 G38S (rs1805127), PITX2 (rs2200733) are risk factors for atrial fibrillation development in overweight patients.

Aims: Aim of the study is to prove that MTHFR, KCNE1 and PITX2 gene polymorphisms are expressed more in a group of overweight patients with atrial fibrillation compared with the control group of overweight patients without atrial fibrillation. Specific aims are: to prove that MTHFR, KCNE1 and PITX2 gene polymorphisms are associated with hypertension and diabetes development, to prove that MTHFR, KCNE1 and PITX2 gene polymorphisms are associated with left atrial enlargement shown on transthoracic echocardiography and to investigate if MTHFR, KCNE1 and PITX2 gene polymorphisms interractions are risk factors for atrial fibrillation development.

Materials and methods: We are comparing a group of minimal 170 overweight patients (BMI>25 kg/m2) with atrial fibrillation and 170 overweight patients without atrial fibrillation in the control group. Patients with: coronary heart disease, prostetic valve, mitral stenosis, transplanted heart, congenital heart disease and heart pumps will be excluded. Clinical examination with ambulatory blood pressure measurement, body weight and body height measurement, body mass index calculation, anamnestic data, laboratory analysis, electrocardiography, transthoracic echocardiography and genetic analysis will be performed to all of the patients and statistically analyzed. Polymorphism analysis MTHFR C677T (rs1801133), KCNE 1 G38S (rs1805127) and PITX2 rs2200733 will be performed using real time polymerise chain reaction (PCR) with prepared probes according to literature data. In statisticall analysis testing for normality of the distribution in numeric variables will be performed by Kolmogorov-Smirnov test. Differences in distribution of numeric variables between groups will be analyzed by T-test for independant samples or ANOVA for nonparametric equivalents. Differences in distribution between groups will be analyzed by Pearson χ2 test. Association between individual genotype and atrial fibrillation development will be analyzed by logistic regression models. Patients identity will be protected and specific identification code number will be used for each patient. Study will be conducted according to ethycal principals and clinical practice principals. Estimated time of study duration is two years.

Expected scientific contribution: Confiming association between polymorhisms MTHFR C677T (rs1801133), KCNE 1 G38S (rs1805127) and PITX2 rs2200733 and atrial fibrillation, hypertension and diabetes development in a group of overweight patients contributes to advanced undestanding in genetic predisposition for cardiovascular diseases development and possible prevention of cardiovascular comorbidities. Clinical contribution would be in reducing the number of procedures of pulmonary vein isolations in overweigth patients with polymorphisms MTHFR C677T (rs1801133), KCNE 1 G38S (rs1805127) and PITX2 rs2200733 because of expected high atrial fibrillation recurence.

MeSH/Keywords: atrial fibrillation, MTHFR, KCNE1, PITX2

Poster code: T-02-09-034

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

Introduction: Heart transplantation (HTx) is gold standard in treatment of terminal heart failure. 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 visible with intracoronary imaging techniques (e.g. intravascular ultrasound or optical coherence tomography). Extracorporeal photopheresis (ECP) is an immunomodulatory procedure that reduces graft rejection and possibly CAV.

Hypothesis: Addition of extracorporeal photopheresis (ECP) to modern immunosuppressive drug therapy reduces coronary artery intimal thickening (as an early sign of cardiac allograft vasculopathy) during first 12 months after heart transplantation.

Aims: Our aim 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 30 patients after heart transplant who will be randomized (by permuted block technique) 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.

Expected scientific contribution: 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. 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: T-02-09-045

Poster Title: Accurate renal function determination in the population of overweight patients

PhD candidate: Lana Gellineo

Part of the thesis: Točno određivanje bubrežne funkcije u populaciji s prekomjernom tjelesnom masom

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.

Hypothesis: 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.

Aims: 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.

Expected scientific contribution: 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.

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

Poster code: T-02-09-050

Poster Title: Diagnostic and prognostic role of presepsin in febrile neutropenia in hematological patients with lymphoproliferative diseases

PhD candidate: Karla Mišura Jakobac

Part of the thesis: Dijagnostička i prognostička uloga presepsina u febrilnoj neutropeniji kod hematoloških bolesnika s limfoproliferativnim bolestima

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

Affiliation: University of Zagreb School of Medicine

Introduction: Febrile neutropenia (FN) and sepsis are common complications of intensive chemotherapy and a major cause of non-relapsing mortality in patients with hematological malignancies. Patients with neutropenia often develop bacterial infections and in immunocompromised patients the infection is often difficult to diagnose due to the lack of a local inflammatory response and specific clinical sign. The most common used inflammation-rreleted biomarkers such as C-reactive protein (CRP) and procalcitonin (PCT) are not enough specific and sensitive in this group of patients and the early diagnostic marker is needed. Presepsin is immunologic biomarker, an early marker of sepsis, the circulating soluble form of CD14 subtype (sCD14-ST) but its efficacy in patients with lymphoproliferative diseases and febrile neutropenia is not well confirmed.

Hypothesis: Serum concentrations of presepsin in hematological patients with lymphoproliferative diseases and febrile neutropenia as a consequence of cytostatic therapy will be statistically significantly higher in the group with positive blood cultures compared to the group with negative blood cultures.

Aims: General aim is to determine the diagnostic and prognostic role of presepsin as an early indicator of bacteremia and sepsis in patients with lymphoproliferative diseases who developed FN during treatment with cytotoxic therapy. Specific aims are: to investigate value of plasma presepsin as compared with serum PCT and CRP, to show the dynamics of laboratory parameters and biomarkers of inflammation initially and 72 hours after the start of antibiotic therapy, to determine the correlation between the initial values of biomarkers of inflammation and the results of microbiological analysis and clinical / radiological signs of infection - assess whether biomarker dynamics can predict a response to initial empirical antimicrobial therapy and to assess whether there some factors that can predict the duration of neutropenia and the final outcome of treatment

Materials and methods: This prospective study will successively include all patients older than 18 years with lymphoproliferative diseases who develop FN during chemotherapy or immunochemotherapy. The examinees will be divided into two groups according to positive or negative blood culture. We will measure laboratory parameters, including CRP, PCT and plasma presepsin levels on the first day and after 72 hours. A serum sample for a presepsin analysis will be centrifuged and stored in a freezer at -80 ° C. The samples will be subsequently processed by ELISA. Immune status of each patient will also be determined by flow cytometry. We will record the duration of fever and the duration of neutropenia in days, the adequacy of initial empirical antimicrobial therapy, and the need for change. The result of microbiological processing and the isolated pathogen will be recorded in all patients.

Expected scientific contribution: The results of this study will contribute to a better understanding of the role of presepsin in the early diagnosis of bacteremia in hematological patients with lymphoproliferative diseases and FN. We expect that the results of this study will allow easier fever differentiation in hematological patients with neutropenia and thus allow a better and more targeted approach and outcome of treatment in certain cases in this specific group of immunocompromised patients.

MeSH/Keywords: febrile neutropenia, sepsis, lymphoproliferative diseases, presepsin

Poster code: T-02-09-057

Poster Title: Arterial stiffness and target organ damage in treated hypertensive patients

PhD candidate: Vladimir Prelević

Part of the thesis: Krutost krvnih žila i oštećenje ciljnih organa u liječenih hipertoničara

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.

Hypothesis: Measurements of pulse wave velocity (PWV) as a clinical indicator of major artery stiffness over 24 hours is a better predictor of target organ damage than ambulatory PWV measurements in treated patients with arterial hypertension.

Aims: The main goal of the study is to determine the association of 24h PVW measurements with target organ damages. Additional goals include analysis of 24h PVW characteristics in our group of subjects: variability, circadian pattern, night stiffness, increased morning stiffness, masked stiffness, and "white coat stiffness", and comparison of PVW values measured during 24 hours with values measured in the office. It is also planned to analyze the characteristics of 24 h pVW with the characteristics of 24 h arterial blood pressure measurement.

Materials and methods: It is plan to include 190 patients with essential arterial hypertension, older than 18 years with signed informed consent. Excluding criteria are: resistant hypertension, history of myocardial infarction and / or stroke, heart failure, chronic kidney disease (eGFR <60 ml / min / 1.73m2), malignant or inflammatory disease (eg rheumatoid arthritis, lupus, inflammatory bowel disease), patients with a life expectancy of less than 6 months, pregnant and lactating women, patients with amputation of one or more extremities, and patients with dementia. We will measure brachial arterial pressure with the Omrom M7 oscillometric device and the KMAT (MobiloGraph) device for continuous arterial pressure measurements. An ambulatory stiffness measurement and a 24-hour stiffness measurement using the Sphygmocor device, a mobilograph, an arteriograph, which are world-renowned "gold standards", will be performed. The thickness of the intima of the carotid medium and the mass of the left ventricle will be determined using appropriate ultrasound devices. We will determine the AB index using two devices - MicroLife and CAVI. Laboratory parameters which will be measured: glycaemia, Na, K, Ca, P, lipidogram, NtproBNP, 24h measured sodium, potassium and albuminuria. An ECG will be recorded to assess left ventricular hypertrophy. Appropriate statistical analysis will be used. The correlation of pWV measured over 24 h (mean or median) and PWV measured ambulatory will be tested by Student's t-test for dependent samples or ANOVA for three or more data groups. The circadian rhythm of PWV, ie statistically significant differences in PWV during 24 hours, will be tested with the ANOVA test. Relationship between patient characteristics, echocardiographic parameters, intima and carotid media thickness, 24h albuminuria, eGFR, fundus, AB index with pWV will be determined by multiple regression analysis. PWV reference values over 24 h will be determined by determining the percentile at a given time of day (2.5 - 97.5 or 5-95). To compare the characteristics of 24h PWV with 24h KMAT we will use contingency tables and the χ^2 test or Mc Nemar test.

Expected scientific contribution: We will obtain valuable data on the circadian rhythm of the pulse wave velocity, what is not only of scientific importance but also have significant clinical implementation.

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

Poster code: T-02-09-078

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

PhD candidate: Danilo Radunović

Part of the thesis: Uloga krutosti velikih krvnih žila i centralnog arterijskog tlaka u procjeni kardiovaskularnog rizika kod predhipertoničera i neliječenih hipertoničara

Mentor(s): Academician Bojan Jelaković

Affiliation: University of Zagreb School of Medicine

Introduction: Stiffness of large arteries, ie its measure used in clinical work, pulse wave velocity (PWV), is included in the guidelines as a sign of damage to target organs and a useful predictor of clinical course. Pulse wave velocity (PWV) is a clinical sign of stiffness of large arteries. 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.

Hypothesis: 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.

Aims: 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. To determine the values of ePWV in subjects classified into individual categories of arterial pressure; to determine predictive factors for elevated ePWV and compare them with predictive factors for measured major artery stiffness.

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 2018 (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. The estimated pulse wave velocity (ePWV) will be calculated using a validated equation. Statistical data processing will be done using SPSS statistical software.

Expected scientific contribution: 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. If the results are positive, then given the sample size and the length of follow-up, this study will significantly affect the guidelines and recommendations for the use of this method in everyday clinical work, especially when deciding to start drug treatment in patients with prehypertension, which has important medical benefits implications.

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

Poster code: T-02-09-095

Poster Title: Nonalcoholic fatty liver disease in acromegaly patients

PhD candidate: Maša Čavlina Ševo

Part of the thesis: Nealkoholna masna bolest jetre kod bolesnika s akromegalijom

Mentor(s): Professor Nadan Rustemović, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Nonalcoholic fatty liver disease (NAFLD) is one of the most important causes of liver disease in the world. It is closely linked to metabolic syndrome. Acromegaly is a rare condition that is a result of excessive production of growth hormone (GH) and insulin-like growth factor 1 (IGF-1). Its most common cause is pituitary adenoma producing GH. Among many metabolic effects of GH, induction of lipolysis and insulin resistance are the ones with proven role in etiology of NAFLD. Based on GH deficiency patients research in whom it is almost uniformly reported increased prevalence of NAFLD it could be hypothesized that GH and IGF-1 have protective effect on the liver. However, available research is scarce and inconsistent with few studies reporting increased prevalence of NAFLD in active acromegaly patients, explaining it with increased insulin resistance. Contrarily, a body composition study in acromegaly patients has found lower intrahepatic lipid content in those with active disease, as well as its increase after successful treatment, interpreting it with complexity of acromegaly being a state of significant insulin resistance with decreased total body fat.

Hypothesis: Non-invasive parameters of liver steatosis in acromegaly patients will be significantly reduced 6 months after successful treatment of acromegaly.

Aims: The aim of our study is to evaluate and compare non-invasive parameters of liver steatosis in acromegaly patients at diagnosis and 6 months after reaching remission of the disease. Our objectives are to evaluate relationship between non-invasive parameters of liver steatosis and fibrosis in acromegaly patients and anthropometric and biochemical parameters of patient's metabolic status, body composition, insulin resistance and IGF-1 and GH values.

Materials and methods: Study is designed as prospective observational study. All patients with newly diagnosed acromegaly who give the informed consent to participate in the study will be included. Acromegaly will be diagnosed according to current guidelines. Exclusion criteria are liver cirrhosis, hepatitis B or C infection and excessive alcohol consumption. All included patients will be assessed on two occasions, at diagnosis and 6 months after reaching remission of the disease (defined as normalization of IGF-1). On both occasions anthropometric data will be measured and serum samples will be taken to determine basic hematologic, biochemical and coagulation data, as well as GH, IGF-1 and insulin levels. Data will be used to calculate Body Mass Index, Fatty liver Index and NAFLD Fibrosis Score, as well as HOMA-IR score. Body composition will be measured using bioelectrical impedance method (on Tanita MC-780MA-N scale) and abdominal ultrasound and transient elastography with CAP will be performed in order to evaluate liver steatosis and fibrosis. Duration of this phase of the study is expected to be 24 months. Using sample size calculation methods we have calculated that minimal required population sample is 19 patients ($\alpha=0.05$; $\beta=0.20$). Sample size was calculated using data on prevalence of liver steatosis in acromegaly patients and reduction of Hepatic Steatosis Index after treatment of the disease. In statistical analysis we will use descriptive and analytic statistical methods. Depending on normality of distribution of numerical variables we will use parametric and nonparametric statistical tests. In all tests p value of <0.05 will be considered significant.

Expected scientific contribution: To our knowledge this is first prospective study using controlled attenuation parameter (CAP) in assessment of liver steatosis in acromegaly patients follow-up. We believe that our results will contribute to better understanding of relationship between GH and IGF-1 as well as other metabolic factors and liver steatosis in acromegaly patients.

MeSH/Keywords: NAFLD, liver steatosis, acromegaly

Poster code: T-02-09-147

Poster Title: Expression of nuclear epidermal growth factor receptor in squamous epithelial lesions of the oral cavity

PhD candidate: Marko Tarle

Part of the thesis: Klinički potencijal utvrđivanja ekspresije nuklearnog receptora epidermalnog faktora rasta kao biomarkera u epitelnim lezijama usne šupljine

Mentor(s): Associate Professor Ivica Lukšić, MD PhD, Danko Muller, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Oral squamous cell carcinoma (OSCC), as the most common malignant tumor of the head and neck is still a significant public health problem due to late diagnosis and poor overall survival (about 65%) despite numerous knowledge of risk factors, diagnostic, and therapeutic options. Identification of molecular biomarkers is a possible solution to this problem. Although the epidermal growth factor receptor (EGFR) is known to be expressed in more than 90% of squamous cell carcinoma of the head and neck, the role of nuclear epidermal growth factor (nEGFR) in the carcinogenesis of OSCC is unknown.

Hypothesis: The expression of nEGFR proportionally increases to number of pathohistological changes in the squamous epithelium of the oral cavity, ranging from precancerous lesions without dysplasia through precancerous lesions with dysplasia to OSCC. The amount of mEGFR and nEGFR expression correlates positively with an increase in the histological grade of OSCC and shorter overall survival.

Aims: Determination of the degree of nEGFR expression in healthy oral mucosa, precancerous lesions (leukoplakia, erythroplakia, oral lichen) and invasive OSCC with the use of immunohistochemical methods and determination of the relationship between nEGFR expression and histological and clinical characteristics of squamous epithelial lesions.

Materials and methods: In this retrospective study, tissue specimens were obtained from 100 patients with histologically confirmed precancerous lesions and OSCC, primary surgically treated at the Department of Maxillofacial Surgery, Dubrava University Hospital, between 1 January 2010 and 31 December 2014. Expression of tested molecular biomarkers (mEGFR, nEGFR, p53, cyclin D1, Ki67 and ABCG2) will be classified in 4 groups, depending on the percentage of stained cells or staining intensity, using immunohistochemistry. Control group consists of healthy oral mucosa tisssue of 50 patients surgically treated for benign tumors of the oral cavity.

Expected scientific contribution: Routine analysis of the expressions of mEGFR, nEGFR, and a range of other molecular biomarkers can help us analyze the risk of malignant alteration of precancerous lesions in invasive OSCC. The results of this research could contribute to the selection of high-risk patients and the best modality of treatment, better local and regional disease control, reduction of mortality and improvement of the quality of patients' life.

MeSH/Keywords: squamous cell carcinoma, oral cavity, biomarkers, nEGFR, immunohitochemistry

Poster code: T-02-10-026

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: Alopecia is defined as partial or total hair loss. The main division of alopecia is into scarring and non-scarring type. The most frequent type of non-scarring alopecia is androgenic alopecia which is hereditary and hormone-dependent. This type of baldness occurs in 95% of cases. It is estimated that androgenic alopecia affects between 50% and 80% of men. Researches 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 conservative treatment, but the only lasting solution is hair transplantation. Hair transplantation is the most frequent aesthetic surgical intervention in men. The FUE (follicular unit extraction) method is considered to be the most advanced approaches to hair transplantation. In this method, follicular units are extracted one by one from the donor area – usually the back of the head and lateral areas toward the earlobes – and transplanted into areas where hair is sparse or lacking. Every follicular unit contains 2,5 hairs on average.

Hypothesis: Hair transplantation improves life quality and psychosocial status among male adults with androgenic alopecia.

Aims: OVERALL AIM: To examine the quality of life and psychosocial functioning of mature men with androgenic alopecia before and after hair transplantation. SPECIFIC AIMS: To investigate the symptoms of depression, anxiety and stress in persons with alopecia and to investigate the correlation between degrees of alopecia and measures of psychosocial functioning and quality of life.

Materials and methods: Study will be carried out in the Dr Maletić Polyclinic in Zagreb and Daruvar. The expected duration of the study is two years. A minimum of 45 adult males will be included in the study. Further inclusion criteria are the presence of androgenic alopecia and informed consent for participation in the study. Patients with non-scarring, non-androgenic alopecia, scarring alopecia, and those with previous history of anxiety and mood disorders will be excluded from the study. All patients satisfying the inclusion criteria will fill out a questionnaire with personal data such as demographic data, clinical data, SF 36 self-reported measure of health questionnaire, DASS-21 (Depression, Anxiety and Stress Scale), Diener subjective well-being scale and MSPSS (Multidimensional Scale of Perceived Social Support). The questionnaires will be filled before hair transplantation and during control check-up one year after the surgery. Appropriate statistical software will be 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 surgery. If the distribution shows significant deviation from the normal one, the nonparametric Wilcoxon rank sum test will be used. The Pearson or Spearman correlation coefficient will be used to verify the connection between individual variables in the study. The 5% significance level will be used.

Expected scientific contribution: 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.

MeSH/Keywords: hair transplantation, alopecia, quality of life

Poster code: T-02-10-035

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 of 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.

Hypothesis: 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.

Aims: To examine the correlation of the degree of expression of the MAGEA3, the quantity of CD86 and CD163 macrophages, their mutual connection as well as the relation with other clinical-pathological features in patients operated due to pancreatic carcinoma.

Materials and methods: A prospective research will be conducted in which the archival materials of the "Ljudevit Jurak" Clinical department for pathology (UHC "Sestre milosrdnice") will be used together with all relevant patients' clinical data obtained from BIS (Hospital information system) in at least 60 patients operated due pancreatic carcinoma. All the tissue blocks will be 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.

Expected scientific contribution: The existence of a possible connection between the expression of MAGE-A3 and the type of intra-tumor macrophages could have a prognostic value and could lead to the development of new treatment modalities.

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

Poster code: T-02-10-087

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

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.

Hypothesis: By conducting early physical therapy (3rd postoperative day) in the form of thenar muscle electrostimulation combined with kinesitherapy in patients with surgically treated carpal tunnel syndrome (CTS) a faster recovery of sensory and motor function of the hand is achieved compared to delayed initiation of physical therapy and kinesitherapy conducted at home.

Aims: The general objective of this study was to determine the role of the use of early physical therapy on postoperative hand function recovery time in working-age patients with carpal tunnel syndrome. 1.To determine the optimal time to start rehabilitation of the hand, by comparing the application of early (day 3) and late (day 14) physical therapy in patients after CTS surgery. 2.To compare the results of the application of selected physical therapy modalities (electrostimulation (ES) of the thenar muscles and kinesitherapy, in relation to only kinesitherapy conducted at home) in patients after CTS surgery. 3.To compare the results of the self-assessed questionnaire (BCTQ; Boston Carpal Tunnel Questionnaire) with the clinical parameters of hand function, after surgical treatment of CTS. 4.To investigate the association between the results of the BCQT questionnaire and different modalities of physical therapy (ES with kinesitherapy, in relation to stand-alone kinesitherapy conducted at home), as well as the relation between the BCQT questionnaire results and the commencement of physical therapy (3rd and 14th postoperative day). 5.To compare the results of the functional status questionnaire DASH (Disabilities of the Arm, Shoulder and Hand) with the clinical parameters of hand function after surgical treatment of CTS, as well as to examine the relationship between the results of the DASH questionnaire in connection to the selected physical therapy modalities and the timing of physical therapy (3rd and 14th postoperative day).

Materials and methods: The study will include subjects with CTS divided into four groups; The first two groups consisting of patients who received physical therapy (electrostimulation (ES) of the thenar muscles with supplementary kinesitherapy) on the 3rd, and respectively, the 14th postoperative day. The third and fourth groups consisting of patients who, after proper education, will conduct only kinesitherapy at home — also on the 3rd and 14th postoperative day, respectively. 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).

Expected scientific contribution: Standardized protocol for the use of physical therapy after surgical treatment of carpal tunnel syndrome has not yet been established. After we conduct our research, we hope that the optimal time of starting physical therapy will be established, as well as the value of different modalities of physical therapy after surgical treatment of the carpal tunnel syndrome. Since most patients affected by CTS can be found among the working age population, standardization of treatment protocols will contribute to faster recovery of hand function, earlier return to work and thus reduce the socio-economic burden of CTS.

MeSH/Keywords: carpal tunnel syndrome, physical therapy

Poster code: T-02-10-136

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: Prognostička vrijednost serumske razine interleukina-6 i interleukina-8 kod dermalnih opeklinskih ozljeda kod djece

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

Hypothesis: We hypothesize that the relative decline of serum concentrations of IL-6 and IL-8 between the third and the eighth day after burn injury is negatively correlated with the duration of epithelialization of dermal burn injuries in children.

Aims: Primary aim: Determination of the relation of serum concentrations of IL-6 and IL-8 between the third and the eight day after injury and the duration of epithelialization of dermal burn wounds in children. Specific aims: 1. Measurement of serum concentrations of IL-6 and IL-8 in patients in whom an indication for skin transplantation has been made based on clinical appearance of the burn wound; 2. Determination of the relation of serum concentrations of IL-6 and IL-8 on the third day after injury with total body surface area affected with burn injury; 3. Determination of the relation of local infection with the dynamics of serum concentrations of IL-6 and IL-8 between the third and the eighth day after burn injury

Materials and methods: Measurement of serum concentrations of IL-6 and 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.

Expected scientific contribution: •If we will be able to prove that there is a significant correlation of serum concentrations of IL-6 and IL-8 with the duration of epithelialization of dermal burn wounds in children, this method will be useful for reaching a decision for indication for skin transplantation in dubious cases. This method could be especially useful for doctors in centres who are not in the position to use „Laser doppler imaging" an to those who rarely treat burn injuries.

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

Poster code: T-02-10-145

Poster Title: The value of PET/CT in patients with primary hyperparathyroidism and negative or inconclusive neck ultrasound and MIBI scintigraphy

PhD candidate: Eva Pasini Nemir

Part of the thesis: Fluor-18-choline positron emission tomography/computed tomography (PET/CT) value for localization of overactive parathyroid glands in patients with primary hyperparathyroidism and negative conventional diagnostic methods

Mentor(s): Professor Dražen Huić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Primary hyperparathyroidism (PHPT) is a common endocrine disorder and parathyroid surgery still represents the only curative approach. Preoperative localisation of hyperfunctioning parathyroid glands is very challenging, but it is crucial for minimally invasive parathyroid surgery. The aim of our study is to assess the value of F-18-fluorocholine positron emission tomography (PET/CT) in a very selected group of patients with negative or inconclusive neck ultrasound and MIBI scintigraphy and history of hyperparathyroidism for several years.

Hypothesis: F-18-fluorocholine PET/CT has a higher sensitivity and specificity as a diagnostic method for the localisation of hyperfunctional parathyroid glands compared to conventional methods of diagnosing primary hyperparathyroidism.

Aims: Main objective: to determine the specificity and sensitivity of fluoro-18-fluorocholine PET/CT in the diagnosis of hyperfunctional parathyroid glands in patients with primary hyperparathyroidism. Other goals: 1. To determine the localisation of overactive parathyroid glands. 2. To determine the intensity of choline metabolism within the parathyroid glands using the semiquantitative uptake value (standardized uptake value) 3. To compare the intensity of increased gland metabolism with serum PTH and Ca values, with gland size and with pathohistological findings. 4. To determine the minimum resolution of the PET/CT camera for the detection of hyperfunctional parathyroid glands. 5. To determine the most common pathohistological finding after surgery (adenoma or hyperplasia). 6. To compare serum PTH and Ca values before and after surgery.

Materials and methods: The study will include patients with clinically and laboratory-proven PHPT, after a negative ultrasound finding of the neck and a negative finding of Tc-99m sestaMIBI neck and thorax SPECT/CT. A minimum of 50 patients referred by endocrinologists with a clear indication for performing an imaging examination for the purpose of proving PHPT (elevated iPTH, elevated serum Ca) will be included in the study. Patients will get (both oral and written) explanation of the further procedure at the Clinical Department of Nuclear Medicine and Radiation Protection, University Hospital Center Zagreb. After fasting for at least 4 hours before the examination, patients will receive 100-150 MBq of F-18-fluorocholine intravenously. After 20 minutes, a neck and chest scan will be performed. A low-dose CT scan with PET examination will be performed (bed speed 3 minutes per position). Interpretation, processing and reconstruction of images will be performed using Siemens HD PET software with iterative TrueX and "time-of-flight" OSEM method. The pathological finding represents a focal accumulation of radiopharmaceuticals more intense than the surrounding activity, which is not in the locations of the known physiological accumulation of radiopharmaceuticals, without similar activity on the contralateral side. The metabolism of each lesion will be expressed semiquantitatively using the SUV (standardized uptake value). A clearly visible pathological accumulation of F-18-fluorocholine in the neck and mediastinum area will present a positive finding. The size of the lesions will be measured by CT. The PET/CT finding will be compared with the pathohistological finding, to determine the sensitivity and specificity of the method. In all cases, patients will be monitored for at least 3 months after surgery, with serum PTH and calcium values monitoring at least twice during that period.

Expected scientific contribution: The study will determine the value of F-18-choline PET/CT in the diagnosis of patients with primary hyperparathyroidism. We expect to further clarify the correlation between positive findings and pathohistological results. The anatomical localisation of the parathyroid glands and anatomical distinction from thyroid gland will be further clarified. The obtained results will be used to determine the clinical guidelines for better and easier management of patients with primary hyperparathyroidism.

MeSH/Keywords: F-18-fluorocholine; PET/CT; primary hyperparathyroidism

Poster code: T-02-17-036

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

PhD candidate: Roko Granić

Part of the thesis: The correlation between the presence of BRAF V600E and TERT promoter 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. Its high incidence puts a great strain on medical institutions the world over. During the last 60 years a five-fold incidence rise of differentiated thyroid cancer (DTC) has been detected but without the rise in mortality. Croatia is among top five European countries with the highest DTC incidence. Radioiodine (RAI) treatment of patients who underwent total thyroidectomy for DTC includes thyroid remnant ablation, treatment of locally invasive tumors as well as local and distant metastases. Patients with intermediate or high risk of the recurrence of the disease could benefit the most from this treatment. Despite recent scientific studies the presence of genetic mutations such as BRAF V600E i TERT promoter mutations and their influence on the disease course in DTC is not yet widely recognized or clinically accepted as a standard prognostic parameter.

Hypothesis: Patients with intermediate and high risk of the recurrence of the disease (according to American Thyroid Association`s - ATA 2015 guidelines), that underwent total thyroidectomy for DTC and that have promoter region mutation of TERT gene and V600E mutation of BRAF gene will have poorer response to RAI therapy as opposed to patients without those mutations, and therefore an unfavorable course of the disease.

Aims: The aim of this investigation is to determine whether there is a correlation between the presence of BRAF V600E mutation and TERT promoter mutation, and poorer response to RAI treatment.

Materials and methods: This retrospective cohort investigation will last for 3 years and will include 200 patients treated for thyroid cancer at the Dpt. of Nuclear medicine Sestre milosrdnice UHC. The presence of promoter region mutation of TERT gene and V600E mutation of BRAF gene will be determined in DTC samples stored postoperatively. Out of patients treated with RAI at our Dpt. during the last 10 years a group of patients with intermediate and high risk of the recurrence of the disease will be isolated. Out of that group a subgroup of patients with distant metastases (40-50 pts) will be analyzed separately as they usually show a different course of the disease so their response to RAI therapy must be evaluated in a different way. Both group`s (intermediate and high-risk pts. without distant metastases - M0 and high-risk pts. with distant metastases – M1) response to RAI therapy will be evaluated after a median of 40 months of follow up or after at least 2 courses of RAI therapy considering stimulated Thyroglobulin changes, Anti-thyroglobulin antibodies as well as RAI uptake status and other imaging findings (ultrasound, computed tomography etc.). The response is going to be classified as excellent response or non-excellent response (the latter includes incomplete biochemical, incomplete structural or indeterminate response). A laboratory phase of the investigation will include the isolation of DNA that will be tested for TERT promoter and BRAF V600E mutation. Data will then be statistically evaluated.

Expected scientific contribution: The presence of BRAF V600E and TERT promoter mutation in DTC could be considered a prognostic factor associated with poorer response to RAI treatment and thus a precursor of a more aggressive form of the disease. This should affect the treatment and follow up through better selection of high-risk patients. The financial benefit could also be achieved as the burden on medical system could be alleviated through diagnostics and treatment optimization.

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

Poster code: T-02-17-052

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

PhD candidate: Mihovil Plečko

Part of the thesis: Imunofenotipizacija koštanih i hrskavičnih prethodničkih stanica iz subhondralne kosti acetabuluma i glave bedrene kosti bolesnika s primarnim osteoartritisom kuka i sekundarnim osteoartritisom kuka uzrokovanim poremećajem kukova

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

Affiliation: University of Zagreb School of Medicine

Introduction: Osteoarthritis (OA) is a degenerative joint disease. The molecular 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 (sOA). One of important causes of secondary osteoarthritis 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 the use of mesenchymal stem cells (MSCs) may provide good treatment results. However, there is no study 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 samples of acetabular and femoral head subchondral bone samples from patients with hip pOA and DDH-OA.

Hypothesis: The proportion of osteochondroprogenitor populations is different in subchondral bone samples of patients with primary hip osteoarthritis and secondary hip osteoarthritis caused by developmental dysplasia of the hips.

Aims: To determine the proportion of cells expressing osteochondroprogenitor markers in samples of subchondral bone of Ac and Fh of patients with primary hip osteoarthritis and secondary hip osteoarthritis caused by developmental dysplasia of the hips.

Materials and methods: Samples will be taken from waste tissue of patients undergoing hip arthroplasty due to pOA or DDH-OA. Cells will be isolated from subchondral bone, stained with antibodies against osteochondroprogenitor markers (CD73, PDPN, CD146, CD164, GD2, CD140b, CD271, CD10) and will be analyzed by flow cytometry. In addition, immunohistochemical analysis will be performed if further clarification of the results is required.

Expected scientific contribution: Analysis of cell populations could reveal affected populations in a particular degenerative process. Population differences could indicate different response to damage and regional difference in regenerative capacity, which would expand the knowledge needed for biological treatment of pOA and DDH-OA.

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

Poster code: T-02-20-096

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

PhD candidate: Mislav Malić

Part of the thesis: Analiza funkcije osjeta okusa u kroničnoj upali srednjeg uha bez kolesteatoma

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

Affiliation: University of Zagreb School of Medicine

Introduction: It is well knownt that in chronic otitis media there is an imparement of gustatory function, however there is insuficient data on the effect of middle ear surgery on the impared 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 unafected side, before and after surgery.

Hypothesis: Surgical treatment of the unilateral chronic otitis media affects the gustatory function of the ipsilateral side of the tongue.

Aims: GENERAL AIM: To determine gustatory function in the patients with a unilateral chronic otitis media without cholesteatoma and compare the results with a healthy side, before and after surgical treatment. SPECIFIC AIMS: 1. To determine gustatory function with a "taste strips" test on a both sides of the tongue, before the surgery and one day and 3 months after the surgery. 2. To analyze subjective perception of the taste according to VAS scale. 3. To determine the differences in a specifics tastes (salty, sour, bitter and sweat) between healthy and diseased side, depending on the manipulation of the chorda tympani nerve.

Materials and methods: Patients will be divided in two groups, first group will have endoscopic surgery without elevation of the tympanic membrane and without the chorda tympani manipulation, and the second group of patients will have standard surgery that involves raising the tympanomeatal flap and manipulating the chorda tympani. Gustatory function testing will be administered using taste strips as described by Mueller. Total of 120 patients is planned to be included in the study, 60 in each group.

Expected scientific contribution: This research will show how the impaired gustatory function in chronic otitis media is affected by surgical treatment.

MeSH/Keywords: taste, gustatory function, chronic otitis media, myringoplasty

Poster code: T-02-21-143

Poster Title: Immunohistochemical expression of cytokeratin 5/6 and transcription factor GATA3 in muscleinvasive urothelial bladder cancer

PhD candidate: Robert Terlević

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

Mentor(s): Professor Božo Krušlin, MD PhD

Affiliation: Clinical hospital centre "Sestre milosrdnice", Zagreb, Croatia

Introduction: Bladder cancer is the fifth most common cancer in Croatia and seventh most common cause of cancer related death among males. Histologically 90% are urothelial carcinomas. Early forms are noninvasive, prone to recurrence and are treated with local therapy. A greater challenge is locally advanced (muscleinvasive) and metastatic disease, with 5-year overall survival of the latter being around 5%. By analyzing tumor transcriptomes advanced urothelial cancer can be subdivided into at least 2, and at most 6 molecular subtypes. Different subtypes are associated with differences in survival and response to chemo-radiotherapy. Using immunohistochemical analysis of protein expression in tumor cells cases can be assigned to a specific molecular subtype. The goal of this study is to classify tumors into molecular subtypes using immunohistochemistry and compare whether the molecular subtype influences survival depending on the specific treatment. This approach could lead to routine subtyping of advanced urothelial carcinoma cases and better therapy selection for each patient.

Hypothesis: The immunohistochemical expression of GATA3 is increased in the luminal type, and cytokeratin 5/6 in the basal type of bladder urothelial carcinoma which is correlated to better or worse survival, respectively, in patients with this tumor type.

Aims: OVERALL GOAL: classify cases of muscleinvasive bladder urothelial carcinoma into luminal and basal subtypes using the immunohistochemical expression of GATA3 and CK5/6 in tumor cells. SPECIFIC GOALS: 1. Collect and compare the relevant anamnestic, clinical and pathohistological dana of patients with muscleinvasive bladder cancer and collect the archived tissue samples. 2. Conduct the immunohistochemical analysis of patient tissue samples and determine the expression of specific proteins (GATA3, CK5/6) in tumor cells 3. Determine the tumor molecular subtype using the results of the immunohistochemical analysis 4. In each subgroup, determine the clinical outcomes in relation to primary therapy while considering the relevant covariates 5. Analyze the immunohistochemical expression of FGFR and p16 in order to subclassify the luminal group into subtypes (luminal papillary, luminal unstable)

Materials and methods: In this retrospective cross-sectional study tumor tissue samples obtained via transurethral bladder resection will be analyzed. Data of pathologic stage T2 cases in the period between 1.1.2011.-31.12.2020. will be collected from the archives of the Department of Pathology and Cytology Ljudevit Jurak at the Clinical Hospital Centre „Sestre milosrdnice", Zagreb, Croatia. Cases with metastatic disease or other primary malignant disease will be excluded. Institutional ethics board approval will be sought. Data collection and analysis Relevant patient information will be obtained from hospital electronic medical records. The collected tumor samples will be analyzed using routine H&E sections to confirm the initial diagnosis and select the most appropriate tissue block for immunohistochemical (IHC) analysis. The presence of tumor heterogeneity will be noted. IHC analysis will be performed on 5 microns thick, formalin fixed and paraffin embedded tissue sections from the most representative tumor block. IHC antibodies used in this study include GATA3 (klon L50-823), CK5/6 (klon D5/16B4), p16 (E6H4), FGFR3 (C51F2). Staining protocol and antigen-antibody visualization will be achieved according to manufacturer's instructions and using routine in-house methods. Signal localization will vary depending on the specific antibody. Positive staining will be considered when present in >10% of tumor cells. Statistical analysis Statistical analysis will include anamnestic and clinicopathological patient data. Survival will be compared using the monovariate Kaplan-Mayer method for specific molecular subtypes and using Cox multivariate analysis with age, sex, therapy and molecular subtype.

Expected scientific contribution: A scientific contribution in the field of muscleinvasive urothelial carcinoma classification into clinically relevant subgroups is expected. Recent dana suggest therapy response might be linked to tumor molecular subtype. With this work a contribution could be made toward predicting patient response to therapy, which could lead to better patient outcomes.

MeSH/Keywords: bladder cancer, urothelial carcinoma, molecular subtype, immunohistochemistry, survival

Poster code: T-02-23-046

Poster Title: Epigenetic changes and PTX3 protein expression and the presence of M1 and M2 macrophages in the placenta with intrauterine growth restriction

PhD candidate: Darija Mužinić

Part of the thesis: Epigenetske promjene i ekspresija proteina PTX3 te prisutnost M1 i M2 makrofaga u posteljici u trudnoći s intrauterinim zastojem u rastu ploda

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

Affiliation: University of Zagreb School of Medicine

Introduction: Fetal growth is the result of the interaction between its genetic potential and growth support received from its mother and the placenta. Intrauterine growth restriction (IUGR) is an important cause of neonatal morbidity and mortality. Disruption of the uterine microenvironment during the early stages of pregnancy (decidualization, implantation, and placentation) is one of important etiological factors for IUGR, showing endothelial dysfunction as well as M1/M2 macrophage polarization. Pentraxin 3 (PTX3) is an acute phase protein whose expression has been shown increased in placentas from pregnancies complicated by IUGR. Previous studies have confirmed that PTX3 expression is epigenetically regulated in certain tumors, but no studies have yet been performed suggesting epigenetic regulation of placental PTX3, nor a possible association with M1/M2 macrophage polarization in placentas from pregnancies complicated by IUGR.

Hypothesis: PTX3 protein expression will be increased, PTX3 gene will be hypomethylated and there will be more M1 macrophages in placentas from IUGR pregnancies compared to the placentas from normal pregnancies.

Aims: General aim is to investigate changes in PTX3 protein expression and DNA methylation in the PTX3 gene promoter and the number of M1 and M2 macrophages in the placentas in pregnancies with IUGR in relation to the placentas in normal pregnancies and to determine the relationship between these characteristics.

Materials and methods: The research will use archival samples, paraffin blocks of placental tissue collected during childbirth at the Clinic for Gynecology and Obstetrics, Clinical Hospital Merkur in the period from 2013 to 2021. Placental tissue samples have been analysed and archived at Clinical Department of Pathology and Cytology, Clinical Hospital Merkur. A minimum of 60 placental tissue samples from pregnancies with idiopathic intrauterine fetal growth restriction (IUGR) will be used in the study. The control group will be 40 samples of placental tissue from term pregnancies without complications. Primary antibodies used in the study will be PTX-3, CD68 PGM1, HLA-DR, CD163, Anti-MSR1, STAT1 and c-maf. DNA methylation in the promoter region of the PTX3 gene will be analyzed by methylation-specific PCR (MSP). Processing of all collected parameters will be done by descriptive statistics. The estimated duration of the research is 2 years, which will take place at the Clinical Department of Pathology and Cytology, Clinical Hospital Merkur and the Department of Biology, Faculty of Medicine, University of Zagreb, and ethical principles and legal regulations will be respected.

Expected scientific contribution: This research will contribute to knowledge about the pathogenesis of IUGR, investigate the role of PTX3 proteins and epigenetic modifications of the PTX3 gene, determine which macrophage phenotype dominates in the placentas with IUGR and try to determine whether there are cause-and-effect relationships of PTX3 and dominant macrophage phenotype (M1 or M2). The new insights that will be gained during the research could over time serve as an idea for possible biomarkers and therapeutic procedures that would help in the prevention and treatment of IUGR.

MeSH/Keywords: IUGR, placenta, PTX3, M1 and M2 macrophage polarisation

Poster code: T-02-23-058

Poster Title: The effect of activin A and P-selectin expression in liver tissue on the outcome of patients with biliary atresia

PhD candidate: Petra Džepina

Part of the thesis: Utjecaj pozitivnog imunohistokemijskog bojenja tkiva jetre na aktivin A i P-selektin na ishod pacijenata s bilijarnom atrezijom

Mentor(s): Associate Professor Ruža Grizelj, MD PhD, Professor Jurica Vuković, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Biliary atresia is a rare disease of unknown etiology which causes complete obliteration and disappearance of extrahepatic biliary ducts with cholestasis, liver inflammation, liver fibrosis, cirrhosis and fatal outcome if left untreated. The only relatively effective treatment is performing Kasai portoenterostomy which enables bile drainage into small intestine. If Kasai portoenterostomy proves to be uneffective, the only treatment that remains is the liver transplantation. Etiology of biliary atresia remains unknown, although there are many possible causes (viral infection, genetic predisposition, autoimmune response). Up until now, the research showed that immune system plays a large role in the development of biliary atresia which is proven by numerous inflammatory cell and adhesion molecule infiltration around the biliary ducts. In the past few years, there are many studies that claim specific proteins may be the new markers of liver inflammation and fibrosis. Activin A was found in the fibrotic liver in rats, and P-selectin may play a role in cholestatic liver injury.

Hypothesis: The outcome of patients with biliary atresia with activin A and P-selectin expression in liver tissue before surgery is worse than the outcome of patients with biliary atresia without activin A and P-selectin expression in liver tissue before surgery.

Aims: The aim of this study is to show connection between the activin A and P-selectin expression in liver tissue in patients with biliary atresia before surgery and the poor outcome of said patients. Investigate whether there are more complications after surgery in patients with activin A and P-selectin expression in the liver before surgery.

Materials and methods: The study will include patients with biliary atresia which were admitted and treated from 1986 to 2018 at the Department of Pediatrics, University Hospital Zagreb. We expect the study to last for 2 years and cover about 40 patients. The following variables will be extracted from medical records: demographic, prenatal, perinatal, treatment-related, biopsy findings, and post-surgical treatment-related. The liver paraffin-embedded samples from surgical biopsy will be gathered and activin A and P-selectin immunostaining will be performed. Immunostained sections will be evaluated for the qualitative expression of activin A and P-selectin.

Expected scientific contribution: To determine whether there is a connection between the activin A and P-selectin expression in the liver tissue and poor outcome of the patients with biliary atresia. To determine whether liver tissue activin A and P-selectin immunostaining has a predictive value in patients with biliary atresia.

MeSH/Keywords: biliary atresia, Kasai portoenterostomy, activin A, P-selectin

Poster code: T-02-24-005

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

PhD candidate: Iva Biondić Špoljar

Part of the thesis: Procjena učinka neoadjuvantne terapije kontrastnim ultrazvukomu u pacijenata s dijagnosticiranim karcinomom dojke

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 neoadjuvant therapy 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.

Hypothesis: CEUS is an efficient method for monitoring the effects of the neoadjuvant therapy in patients diagnosed with breast cancer.

Aims: General: Comparison of the results of CEUS and MRI on the effects of the neoadjuvant therapy on malignant lesions. Specific: Assessment of the response to NACT with conventional gray-scale ultrasound and CEUS parameters. Assessment of the response to NACT with MRI, based on morphologic, kinetic and diffusion-weighted MRI parameters. Analysis of the effectiveness of CEUS in relation to the MRI. Comparison of responses to the therapy in different molecular subtypes of breast cancer.

Materials and methods: The research will be conducted at the Department of Diagnostic and Interventional Radiology of the University Hospital "Dubrava". The observed group will consist of women of all ages who are diagnosed with breast cancer and are planned for having neoadjuvant therapy. Initial MRI and CEUS examinations will be done before the application of the therapy, as part of the diagnostic protocol. The applied therapy response will be monitored with both methods in the early stages of treatment and upon completion of the therapy. Follow-up examinations will be done on the same machines, using the same imaging protocols as the initial examinations.

Expected scientific contribution: Depending on the results of the research, contrast-enhanced ultrasound might have the basis for a wider clinical application in the analysis and monitoring of breast cancer patients. The used ultrasound contrast agent has been shown to be safe and acceptable for patients due to its composition, structure and elimination pathway from the body. In patients with contraindications for magnetic resonance imaging, it will be possible to monitor the effect of the therapy with this imaging method

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

Poster code: T-02-25-101

Poster Title: Dynamic contrast enhanced breast magnetic resonance imaging radiomics for prediction of response to neoadjuvant therapy in breast cancer patients

PhD candidate: Lucija Kovačević

Part of the thesis: Dynamic contrast enhanced breast magnetic resonance imaging radiomics for prediction of response to neoadjuvant therapy in breast cancer patients

Mentor(s): Professor Maja Prutki, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Neoadjuvant chemotherapy (NAC) is increasingly being used in the treatment of early and locally advanced breast cancer, as it can increase the rate of breast-conserving surgery while maintaining similar long-term outcomes as adjuvant therapy. After NAC, residual cancer burden (RCB), a reliable prognostic score, is used to quantify residual disease. Although the absence of residual invasive cancer in the breast and axillary lymph nodes (RCB 0) and minimal residual disease (RCB I) are the favourable outcomes of NAC, most hormone-dependent breast cancers do not achieve these outcomes following NAC. In other words, there is a need to develop biomarkers that will allow an optimal patient selection for NAC and save the non-optimal patients of toxicity and delays in treatment. Radiomics is a non-invasive technique that uses the standard of care images to extract large amounts of quantitative image features to develop predictive biomarkers. In this study, we use dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) radiomics to maximally exploit information from the existing images to develop biomarkers for NAC response prediction.

Hypothesis: It is possible to predict the final response to NAC using machine learning models based on the radiomic features of the intratumoral region of luminal B-like subtype breast cancer extracted from breast DCE-MRI performed before and after two cycles of NAC.

Aims: The aim is to determine the predictive value of a machine learning model developed based on radiomic features of breast cancer extracted from DCE-MRI done before and after the second cycle of NAC in the assessment of NAC response. Specific aims are 1. To determine the value of radiomic features of breast cancer extracted from the pretreatment breast DCE-MRI in the prediction of RCB 0 and RCB I; 2. To determine the value of radiomic features of breast cancer extracted from the breast DCE-MRI performed after two cycles of NAC in the prediction of RCB 0 and RCB I; 3. To explore the potential of breast cancer delta-radiomics features in the prediction of RCB 0 and RCB I.

Materials and methods: This retrospective study will include 150 patients with luminal B-like subtype breast cancer who underwent breast DCE-MRI before and after two cycles of NAC at the UHC Zagreb and have available RCB class. Based on the RCB class patients will be divided into two groups. Biopsy-proven invasive breast cancer masses will be manually segmented using ITK-SNAP-3.8.0. Radiomic feature extraction from segmented breast cancers will be performed using PyRadiomics. In this study, to validate our models we will use an independent holdout set of 20% of all prepared data obtained by stratified random sampling. The rest of the data is used for the development of NAC response classification machine learning models. Automatic models selection and hyperparameter tuning of the models will be conducted using Bayesian optimisation with 5-fold cross-validation (CV). The selected NAC response classification model with the best CV score will be evaluated on the holdout set. The area under the Receiver-operator characteristic (ROC) curve, the area under the Precision-Recall (PR) curve, and the F1 score will be used as evaluation measures.

Expected scientific contribution: We expect that the model based on radiomic features of luminal B-like subtype breast cancer and their changes that occur early during NAC will influence the type of treatment and its outcomes in patients.

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

Poster code: T-02-25-134

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, University of Ljubljana School of Medicine

Introduction: Assessment of the time since death or postmortem interval (PMI) 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, which are not applicable in the the late PMI. 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 metabolism is anaerobic and 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.

Hypothesis: The dynamics of the decrease in the proportion of viable chondrocytes during the time shows regularities and can be used to estimate the PMI.

Aims: 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 Žale City Cemetery in Ljubljana. Four osteochondral cylinders (macroscopically intact part, ICRS grade 0) will be removed with the osteochondral autograft transfer system from both lateral femoral condyles (Ø 6 mm, depth 20 mm). Every osteochondral cylinder will be immediately stored in a 2 mL tube filled with Dulbecco's 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 18–20 h at 37 °C. The degraded cartilage samples afterward will be washed through a cell strainer with 40 μm 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 suspension for FC will be treated with RedDot + 7AA-D (7-Aminoactinomycin D) to mark viable/non-viable chondrocytes.

Expected scientific contribution: The results of this research, as well as the used methods of sampling and processing will be the basis for further research and will contribute to better knowledge of chondrocyte biology.

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

Poster code: T-02-27-029

Poster Title: Determination of discriminant functional equations for the purpose of sex determination based on the osteometry of the humerus, radius and ulna of the victims of the Homeland War

PhD candidate: Anton Mažuranić

Part of the thesis: Određivanje diskriminantnih funkcijskih jednadžbi u svrhu utvrđivanja spola temeljem osteometrije nadlaktične, palčane i lakatne kosti žrtava Domovinskog rata

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

Affiliation: University of Zagreb School of Medicine

Introduction: Sex determination is an important, and often the first step in the process of identifying skeletal remains. It is based on differences in shape, size, and appearance of the bones between sexes called sexual dimorphism. Sex determination based on sexual dimorphism is conducted using morphological or osteometric methods. The osteometric method is based on discriminant equations. In order to be able to use discriminant equations, measures of anatomical characteristics of bones with known sexual dimorphism are needed. The advantage of the osteometric method is its objectivity and ease of use by inexperienced examiners. The disadvantage of the osteometric method is the fact that discriminant equations are population-specific. Humerus, radius and ulna are suitable for the osteometric determination of sex because they relatively strong and therefore are often well preserved, and due to a large number of anatomical features they can be used even when fragmented.

Hypothesis: Parameters previously determined on other populations as well as never before determined osteometric parameters on the humerus, ulna and radius will show statistically significant differences between the sexes in the sample of the contemporary Croatian population.

Aims: The primary aim of this study is to determine the discriminant equations for sex estimation based on measured parameters of the humerus, radius and ulna for the contemporary Croatian population. The primary aim will be accomplished through the following secondary aims. First, we aim to measure standard osteometric parameters of the humerus (7), radius (5) and ulna (4). Concomitantly, we aim to measure new osteometric parameters of the humerus (3), radius (2) and ulna (2). After the measurements are finished, we aim to test standard and new osteometric parameters for sex determination quality.

Materials and methods: The sample consists of skeletal remains of 60 males and 60 females victims of the Homeland War (1991-1995). Exclusion criteria will be pathological changes and antemortem, perimortem or postmortem damage to the bones. Sex of the skeletal remains was already determined morphologically, as well as by DNA analysis, and serves as the control measurement. To test the repeatability between the authors, measurements on randomly selected twenty male and twenty female skeletal remains will be performed by another colleague for comparison with the original results. To test the repeatability over time, the author of the study will repeat the measurements on randomly selected twenty male and twenty female bone remains for comparison with the original results.

Expected scientific contribution: This research will determine the importance of the osteometric method based on the humerus, radius and ulna in the sex determination of the skeletal remains. The research aims to obtain discriminant equations based on the parameters of humerus, radius and ulna for sex estimation for the contemporary Croatian population. Three new parameters on the humerus, two on the radius and two on the ulna will be tested for sexual dimorphism. The results of this research may have practical application in the future work of forensic medicine experts.

MeSH/Keywords: osteometric sex determination; long bones of the arm; discriminant functions; Homeland war victims

Poster code: T-02-27-103

Poster Title: Development of depression; anxiety and PTSD in patients hospitalized for COVID-19

PhD candidate: Dijana Lucijanić

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

Mentor(s): Professor Alma Mihaljević-Peleš, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Evidence from previous viral epidemics and the current SARS-CoV-2 epidemic indicates high rates of mental prevalence problems as short-term and long-term consequences. However, the broader picture of mental disorders among is still missing different populations. A study at a convalescent hospital in Hong Kong found that about 50% of patients recovered with SARS. One study compared patients with COVID-19 with people suffering from other forms of pneumonia (Yang et al., 2020). He found more severe symptoms of anxiety and depression in patients with SARS-CoV-2. Patients with severe symptoms could not get rid of the memory of struggle with SARS and those memories disrupted their daily activities.

Hypothesis: People who had a more severe clinical picture and a more pronounced systemic inflammatory response to SARS-CoV-2 virus corona infection compared to those who did not, we will find mental disorders, ie anxiety, depression, stress and PTSD symptoms.

Aims: GENERAL OBJECTIVE: To identify possible risk factors for anxiety, ptsp, and depression and to investigate the association between disease severity and the occurrence of psychopathology among COVID-19 survivors. SPECIFIC OBJECTIVES: 1.To examine the incidence of anxiety, depression, stress, and PTSD symptoms as measured by DASS-21 and IES-R during inpatient treatment for COVID-19 2. Examine the incidence of anxiety, depression and PTSD after 3 months in COVID-19 survivors 3. To examine whether the severity of the clinical picture of COVID-19 disease affects the occurrence of these mental disorders and their severity 4. To examine the relationship between the parameters of systemic inflammation and the occurrence of these mental disorders and their severity

Materials and methods: The subjects are patients from COVID-19, older than 18 years who are hospitalized in the respiratory center KB Dubrava. After an introductory interview and informed consent, demographic data will be taken and then two psychological questionnaires will be applied. In three months, patients will be contacted by phone and questionnaires will be filled out again. Criteria for inclusion: hospitalized patients, age above or equal to 18 years, positive PCR test for SARS-Cov-2, informed consent, after stabilization of the somatic condition Exclusion criteria: patients with disturbances of consciousness, poor somatic condition, patients on a respirator or in an intensive care unit, severe communication difficulties (dementia, deafness), previously diagnosed anxiety, depressive and whether ptsp disorders. Exclusion criteria: patients who do not survive, who will not answer the phone call at the control visit Variables: patient characteristics - demographic data, experience of vulnerability, information on whether they have been previously treated psychiatrically, symptoms of anxiety, depression, stress and ptsp, somatic comorbidities Intensity of systemic inflammation Severity of Covid-19 disease

Expected scientific contribution: A review of the literature did not find that there were studies investigating the association of the inflammatory response in SARS-CoV 2 infection, comorbidity and the onset of symptoms, anxiety, depression, and ptsp. A limited number of studies have examined stress, anxiety, and depression in patients with coronavirus infection during hospitalization because of the risk of spreading the infection to medical staff and the need to work in full protective equipment to have access to patients. We see a scientific contribution in the fact that if we find a connection between the severity of the disease and inflammation with mental health problems, we could more quickly diagnose and treat the resulting mental disorders. In such cases, professional support or treatment could be provided. What we expect in the future is the development of specific treatments for inflammation-related mental disorders

MeSH/Keywords: depression, anxiety, stress, PTSD, COVID-19

Poster code: T-02-29-053

Poster Title: Changes in the elements of medical professionalism in medical students

PhD candidate: Ivan Pavao Gradiški

Part of the thesis: Promjene sastavnica medicinskog profesionalizma studenata medicine tijekom studija

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

Affiliation: University of Zagreb School of Medicine

Introduction: Medical professionalism refers to the set of skills and values that characterise the essence of humanism in professional work. Since it's one of the core elements of ensuring high-quality and safe patient care the development of an instrument to measure and evaluate medical professionalism became an important task in formal medical education. In order to describe multidimensional nature of medical professionalism, a multi-scale profile describing empathy, teamwork and lifelong learning, as its core elements, has been created at the Thomas Jefferson University in Philadephia (USA). The usage of this instrument is widespread throughout the world and is translated on over 56 languages and used in over 80 countries. Empathy, as a characteristic, is cognitive by nature and is defined as the ability to understand the perspectives of another person as well as its inner experiences and feeling without intensive emotional engagement. The concept of empathy also includes the ability to communicate this understanding making the foundation for a good physician-patient relationship. Teamwork and inter-professional communication are key elements in maintaining and improving the quality of health care leading to better health outcomes and professional satisfaction. Physician's lifelong learning is a process enabling a student to master, improve and renew his own knowledge and skills while including a continuing motivation for learning.

Hypothesis: Elements of medical professionalism according to the Jefferson model in medical students differ on various study years.

Aims: Main aim: Investigation of associations between medical students burn-out syndrome, feeling of loneliness and sociodemographic factors with elements of medical professionalism according to the Jefferson model. Specific aims: Analysis of the elements of medical professionalism according to the Jefferson model in medical students of the University of Zagreb School of Medicine. Evaluation of the correlation between certain study-based and sociodemographic characteristics with the elements of medical professionalism. Evaluation of the correlation between burn-out syndromes in medical students with the elements of medical professionalism. Evaluation of the correlation between the medical students feeling of social and emotional loneliness with the elements of medical professionalism. Validation of the Croatian translation of the Jefferson scales of empathy, teamwork and lifelong learning.

Materials and methods: The study will be cross-sectional, and the subjects will be students of the first, third and sixth year of the University of Zagreb School of Medicine in Croatian language which will anonymously fill out a questionnaire consisting of The Jefferson Scale of Empathy, The Jefferson Scale of Attitudes toward Physician-Nurse Collaboration, The Jefferson Scale of Physician Lifelong Learning, The Social and Emotional Loneliness Scale for Adults, The Maslach Burnout Inventory-General Survey and a sociodemographic questionnaire. Validation of the Croatian translation of the Jefferson Scales will be completed using a panel of experts and a pilot group of students. Statistical analysis will be used to analyse the collected data and to fulfil set aims. This research will be performed as a part of international collaboration with the National Center of Documentation on Bioethics Institute from Spain and the University of Zagreb School medicine.

Expected scientific contribution: Contribution to the understanding of the elements of medical professionalism in medical students by analysis of the correlation between certain elements of medical professionalism with psychological and socio-demographical characteristics and factors pertaining to medical education.Validation of the Croatian translation of the Jefferson scales will enable further research of medical professionalism in Croatia, and it's comparison with medical professionalism in the world.

MeSH/Keywords: Medical professionalism, medical students, empathy, lifelong learning, teamwork

Poster code: T-02-29-060

Poster Title: The significance of correlation between brain neurotrophic factor and depression in patients after percutaneous coronary intervention

PhD candidate: Sara Medved

Part of the thesis: The significance of correlation between brain neurotrophic factor and depression in patients after percutaneous coronary intervention

Mentor(s): Professor Alma Mihaljević-Peleš, MD PhD, Associate Professor Joško Bulum, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Studies on the association between cardiovascular diseases and depression indicate changes in serum levels of brain-derived neurotrophic factor (BDNF) as a common biomarker. BDNF has the ability to regulate vascular growth and repair, the focus of survival, differentiation and preservation of neurons. Reduced blood level was observed in depression, heart failure, and acute coronary syndrome, and indicates a higher risk of a coronary incident in angina pectoris. However, the exact mechanism of BDNF action is not fully understood. A significant proportion of cardiovascular diseases are coronary artery diseases and the majority of patients undergo percutaneous coronary intervention (PCI) with stent placement. The presence of depression seems to delay recovery after PCI with stent replacement significantly.

Hypothesis: Lower serum concentrations of BDNF in patients undergoing PCI with stent placement are associated with a poorer outcome of depressive symptoms in those patients.

Aims: To determine the association of serum BDNF concentration with depressive symptoms in patients at the time and six months after PCI with stent placement.

Materials and methods: The study is designed as a prospective, cohort study with a 6-months follow-up. After performed PCI with stent placement, each patient that meets the criteria will be informed about the study, and upon singing the informed consent enrolled. For each patient medical data will be obtained and the investigator will conduct a structured interview and a clinical psychiatric examination alongside with completing psychiatic scales (Hamilton Rating Scale for Depression (HAM-D) and Montgomery-Asberg Depression Rating Scale (MADRS)). The participant will complete self-evaluating psychiatic questionnaires (Beck's Depression Inventory II (BDI-II) and Patient Health Questionnaire Physical Symptoms (PHQ-15)), cardiology questionnaires (Seattle Angina Questionnaires (SAQ-7) and Duke Activity Status Index (DASI)), and quality of life questionnaire (EQ-5D-3L). A sample of the participant's blood will be taken the morning after for BDNF analys using enzyme-linked immunosorbent assay. Subjects that meet the diagnostic criteria for a depressive episode will be given an appointment for an outpatient care at the Clinic of Psychiatry and Psychological Medicine. Six months after the enrollement, participants will be contacted and data from available medical records, a structured clinical interview, and a psychiatric examination will be taken. Subjects will complete BDI-II, DASI, PHQ-15, SAQ-7, and EQ-5D-3L, while the principal investigator will complete HAM-D and MADRS. Respondents who had been psychiatrically treated would have a Clinical Global Impression completed by a leading psychiatrist.

Expected scientific contribution: The role of BDNF as a possible biomarker in both cardiovascular and psychiatric disease.

MeSH/Keywords: brain derived neurotrophic factor, BDNF, depression, cardiovascular disease, percutaneous coronary intervention

Poster code: T-02-29-071

Poster Title: The impact of health care delivery through mobile team services on the process of recovery of people with severe mental illness

PhD candidate: Sarah Bjedov

Part of the thesis: The impact of health care delivery through mobile team services on the process of recovery of people with severe mental illness

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

Affiliation: Department of Psychiatry and Psychological Medicine, University Hospital Centre Zagreb

Introduction: Despite a sizeable evidence on benefits of community-based treatment for people with severe mental illness (SMI), its development is still at an early stage in Croatia, and it lacks specific and well-organized programs designed for this type of care. 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 is held at University Hospital Centre Zagreb and it provides an opportunity for people with SMI to receive care through newly implemented multidisciplinary community mental health teams (commonly named mobile teams), in addition to the current standard psychiatric treatment. Core members of these teams include at least one psychiatrist, nurse, psychologist, social worker and peer worker (person with lived experience of a severe mental illness), and the service delivery is based on Dutch model of Flexible Assertive Community Treatment. The treatment is focused on home visits to patients, and the interventions are patient-centered, with the purpose of achieving patients' 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, it is necessary to examine their impact on the process of recovery of people with SMI.

Hypothesis: People with SMI receiving additional care provided by mobile teams will show significantly better general functioning, higher health-related quality of life, less severe symptoms, fewer admissions to inpatient psychiatric care and fewer days of inpatient treatment than patients receiving only standard psychiatric treatment.

Aims: The overall aim of this study is to examine the impact of health care delivery through the services of newly implemented mobile teams on the process of recovery of people with SMI. The specific aims will include the comparison of 1) general functioning; 2) health-related quality of life; 3) severity of symptoms; 4) 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 will be 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 an open, prospective randomized clinical trial, in which participants are allocated either to the intervention group, which will receive care provided by mobile teams in addition to standard psychiatric treatment, or to the control group which will receive only standard psychiatric treatment. Participants will be patients with SMI aged 18 to 65 years. A structured psychiatric clinical interview will be used at enrollment for confirmation of the diagnosis based on ICD-10 criteria. Data collection and assessment will be performed at enrollment, and after 12 and 18 months. Global functioning, health-related quality of life and severity of symptoms will be measured using assessment and self-assessment scales, while data on the number of admissions to inpatient psychiatric care and the duration of inpatient stay will be collected from medical records.

Expected scientific contribution: The results of this study will contribute to the comprehensive evaluation of the newly implemented treatment model through mobile teams in Croatia. This 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: T-02-29-092

Poster Title: Value of cone beam computerized tomography angiography in treatment planning for Gamma Knife radiosurgery of intracranial arteriovenous malformations

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. Golden standard in diagnosing AVM is DSA which provides two-dimensional information, but volumetric data acquired with CT or MR are used in SRS treatment planning as well considering the importance of exact target volume definition. 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. Literature shows it can be used in SRS planning, combined with DSA it is safe and effective, it can be used for visualization of micro AVMs and also for ruptured AVMs. It provides detailed spatial resolution and changes AVM delineation during treatment planning when compared to MRA. Precise AVM nidus delineation is essential for the success of SRS – a volume that is too small can lead to under dosing and treatment failure while delineation of a volume that is too large results in healthy tissue radiation with increased adverse radiation effects

Hypothesis: Addition of Cone Beam CT angiography improves nidus delineation and localization during treatment planning for AVM gamma knife surgery

Aims: To explore impact of CBCT-A on nidus localization and delineation during gamma knife radiosurgery treatment planning of intracranial arteriovenous malformations through comparison with other imaging modalities To determine total intracranial radiation exposure from different methods and combination of methods of nidus delineation during gamma knife radiosurgery treatment planning To determine dosimetric implications of gamma knife treatment planning without the use of magnetic resonance

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 let us assess impact of CBCT-A. Sum of intracranial dose burden for each patient will be determined by adding doses from CBCT-A, DSA, CBCT during position verification immediately before radiosurgery and radiosurgery itself.

Expected scientific contribution: Comparison of CBCT-A with other imaging methods in exact delineation of AVM nidus during Leksell gamma knife radiosurgery treatment planning CBCT-A is potentially a useful method for detection of nidi less visible on MRI and DSA Research will assess value of CBCT-A in AVM treatment planning for patients with contraindication for MRI

MeSH/Keywords: arteriovenous malformations; gamma knife; stereotactic radiosurgery, cone beam CT angiography

Poster code: T-02-30-017

Poster Title: The role of ubiquitin C - terminal hydrolase L1 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 L1 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 hospitalization. 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 UCH - L1 and S100-B levels in patients with ES.

Hypothesis: Postictal serum values of UCH - L1 and protein S100 - B are elevated in patients with ES compared to their values in patients with PNES and healthy controls.

Aims: The main aim of this study is to compare the postictal serum values of UCH - L1 and protein S100 - B in patients with ES in relation to their values in patients with PNES. Specific aims: Investigate whether there is a difference in the serum values of UCH - L1 and protein S100 - B in patients with ES and in patients with PNES in relation to their values in healthy controls.

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

Expected scientific contribution: 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 . We believe that the identification of specific proteins in the central nervous system will provide important insight into the underlying mechanisms and impairments following ES and at the same time open opportunities for the identification of new biomarkers for the diagnosis of PNES.

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

Poster code: T-02-30-018

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

PhD candidate: Berislav Ruška

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

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

Affiliation: University of Zagreb School of Medicine

Introduction: Autonomic dysfunction (AD) is a possible manifestation of multiple sclerosis (MS). Exact pathophysiological connection between those two is unclear, however AD is suspected to be caused by neuroimmune interactions as well as demyelinating lesions that are affecting ANS. AD in people with multiple sclerosis (pwMS) is very important as it contributes to development of disability and can also be predictor of the future disease course. So far, studies have shown that subjective and objective AD is present in pwMS. However, studies investigating long-term development of AD during the disease course from the earliest stage of disease- clinically isolated syndrome (CIS) are lacking. Therefore, there is a need for studies on a long-term follow up of development of AD in pwMS from CIS, which is the proposed topic of this PhD thesis.

Hypothesis: In people with multiple sclerosis, during the disease course, there is a progression of autonomic dysfunction.

Aims: Primary aim is to study long-term development of autonomic dysfunction in people with multiple sclerosis from the stage of clinically isolated syndrome. Secondary aims are to: -study changes in specific domains of CASS after six years from baseline -study changes in subjective evaluation of AD after six years with COMPASS-31 questionnaire -compare results of ANS testing with relapses and disease progression -compare results of ANS testing with changes visible on MR of the brain and the spinal cord -find predictors of future AD in people with CIS

Materials and methods: This is a prospective cohort study, with 121 participants who have been diagnosed with CIS. Participants were enrolled from August 2014 until February 2016. All of the tests are performed in Laboratory for ANS testing at the Clinic of Neurology, University Hospital Centre Zagreb, Croatia. All of the participants were screened for drugs and diseases that could affect ANS function. At baseline, each participant had a thorough neurological exam with EDSS evaluation, completed COMPASS-31 questionnaire, completed brain MRI, and underwent a battery of neurophysiological tests for objective evaluation of ANS function. Battery of ANS tests included Quantitative Sudomotor Axon Reflex Test (QSART), heart rate and blood pressure responses to the Valsalva maneuver, heart rate response to deep breathing test and blood pressure response to passive tilt test. Results of these tests are expressed with ten-point Composite Autonomic Scoring Scale (CASS) divided into three domains- sudomotor (0-3 points), cardiovagal (0-3 points) and adrenergic (0-4 points), with each point higher meaning more severe impairment. All of the above tests will be repeated in four-cycles with two-year interval. In the meantime, all relapses, EDSS progression, new lesions on the MRI as well as new drugs will be noted.

Expected scientific contribution: Research of changes and impairment of ANS, during the MS disease course, will contribute to better understanding of autonomic dysfunction, which significantly contributes to disability and, therefore, quality of life in people with MS. Additionally, this research could also help elucidate complex and unclear mechanisms of interaction between immune and nervous system.

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

Poster code: T-02-30-049

Public health and healthcare – research proposals

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

PhD candidate: Nina Krajcar

Part of the thesis: Nasopharyngeal pneumococcal carriage and serotype distribution among children and adolescents after the introduction of the 10-valent pneumococcal conjugate vaccine

Mentor(s): Professor Goran Tešović, MD PhD

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Introduction: Streptococcus pneumoniae (Sp) remains a leading cause of both mucosal and invasive bacterial infections in children. The overall prevalence of nasopharyngeal (NP) carriage, as well as incidence of non-invasive and invasive (IPDs) pneumococcal diseases declined due to significant reduction in vaccine serotypes (VTs) carriage after implementation of pneumococcal conjugate vaccines (PCVs) in childhood immunization schedules 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. 10-valent PCV (PCV10) was introduced in the Croatian National Immunization Program (NIP) in 2019. Based on data collected before 2019, serotypes 14, 3 and 19A were the most prevalent serotypes in IPDs in Croatian population. Both serotypes 3 and 19A have high invasive potential which could lead to greater morbidity in children if their prevalence increases after the PCV10 introduction. However, a systematic study on NP carriage in Croatian children before and after implementation of PCV10 has not been conducted until now.

Hypothesis: Introduction of 10-valent PCV in the Croatian NIP increases prevalence of pneumococcal serotypes 3 and 19A in NP carriage among children and adolescents.

Aims: The aim of the study is to determine distribution and serotype replacement of NP Sp isolates before and after the introduction of 10-valent PCV in the Croatian NIP among healthy children (aged 6 to 48 months) and children (<18 years of age) with upper (acute otitis media) and lower (community acquired pneumonia) respiratory tract infections. The secondary aim is to analyse antimicrobial susceptibility among all NP Sp isolates.

Materials and methods: The study will be conducted in Zagreb metropolitan area in 4 paediatric consulting-rooms of primary health care centers and in emergency room of Paediatric Infectious Diseases Department at University Hospital for Infectious Diseases (UHID) within a 2-year period (November 2018 - November 2019; June 2021 - June 2022). A total of 1800 patients will be included (900 patients in each year of the study). The subjects will be divided in 2 groups: (a) healthy children (6 to 48 months of age) in which NP swab will be obtained during periodic systematic health examination and/or routine immunization and (b) children <18 years of age with isolated Sp from NP aspirate who will be treated in UHID for acute otitis media and/or pneumonia. Bacterial cultures of NP swabs/aspirates will be performed and Sp positive cultures will be identified by standard methods (optochin disc; bile solubility test). Susceptibility of Sp isolates to penicillin and erythromycin will be determined from minimal inhibitory concentrations by the gradient test method. Sp isolates will be serotyped by Quellung reactions with antisera. Prevalence ratios will be used to establish the impact of PCV10 implementation on pneumococcal NP carriage.

Expected scientific contribution: Change of serotype prevalence of NP Sp carriage after the introduction of PCV10 could significantly influence on the selection of vaccine when creating further Croatian NIP. Additional contribution is analysis of serotype replacement in country with high pre-vaccination prevalence of IPD caused by serotypes 3 and 19A which is a unique research opportunity.

MeSH/Keywords: Streptococcus pneumoniae, pneumococcal conjugate vaccine, children, serotype

Poster code: T-03-01-129

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 Ana-Maria Šimundić, MD PhD

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Introduction: Sport is extremely present in people's lives nowadays, either in recreational or professional form. The limits of physical fitness of professional athletes are broken, leading their body to overtraining and the possibility of various injuries, so it is important to know how to dose training and recognize the level of physical fitness. Medicine, as a science, in contact with sports comes not only in the form of treatment of sports injuries but also as a science that participates in the physical preparation of athletes themselves by monitoring and analyzing numerous biochemical markers during training.

Hypothesis: Progressive increase and decrease in training volume during the season causes a proportional degree of tissue damage induced and modulated by inflammatory factors which indirectly show the relationships and values of calprotectin and various biochemical markers of inflammation, markers of muscle injury, lipid markers and indicators of physical activity in professional athletes in different phases of physical fitness.

Aims: The aim is to examine the association of serum calprotectin and concentrations of different biochemical markers of inflammation, markers of muscle injury and lipid markers with indicators of fitness through four phases of dynamic monitoring of top athletes during one competitive season

Materials and methods: Respondents are professional athletes (water polo players) who have undergone multiple sampling at the following times: 1. at the beginning of the season (respondents refrained from strenuous training for at least 8 weeks before joining the survey), 2. in the training period of low intensity, 3. in the training period of high intensity, 4. at the end of the season. Analyzes will be performed on samples previously obtained by puncture from the antecubital vein, namely 3 mL of EDTA-whole blood, 10mL of serum and a 24-hour urine sample of all subjects. To assess the acute impact, whole blood and serum samples were collected 1-15 min after training from the antecubital vein of the dominant arm of each athlete, and to assess the chronic impact, 24-hour urine samples were collected 96 h after the last training period. Samples were frozen at -20 ° C (serum, plasma, hemolysate) and -80 ° C (urine) and will be stored until biochemical markers of inflammation, markers of muscle injury and lipid markers are analyzed. Creatine kinase (CK), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) will be analyzed by photometric UV methods; cholesterol, myoglobin and CRP will be analyzed by immunoturbidimetric method; HDL and LDL will be analyzed by a homogeneous enzymatic method; IL-6, troponin, cortisol and calprotectin will be analyzed by immunoanalytical methods. For each subject (athlete), anamnestic data were taken, including data on supplementation, and they were subjected to a performance tests. In the performance tests, tests of repetitive power, maximum power and static power were performed. These tests were performed in four phases (at the beginning of the season, in low-intensity training, in high-intensity training and at the end of the season) on the same day as blood and serum samples were taken and 96 h before 24-hour urine collection.

Expected scientific contribution: The following research should enlighten the possibilities in the application of certain biochemical markers, markers of muscle injury and lipid markers as markers of physical fitness in top athletes. If the hypothesis of this study is confirmed, direct, targeted measurement of certain markers could become the first method of choice for fitness monitoring in top athletes of various sports during the competitive season. The results of the research will be a valuable contribution to the further development of understanding and monitoring of physical fitness.

MeSH/Keywords: training, inflammation, biochemical markers

Poster code: T-03-03-093

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

PhD candidate: Maja Marković Zoya

Part of the thesis: Knowledge and Attitudes of Family Physicians and their Patients on Clinical Trials of Medicines for Regulatory Purposes: Cross –sectional Study in the Republic of Croatia, and in the countries of the Western and Southeastern Europe

Mentor(s): Professor Vladimir Trkulja, MD PhD, Associate Professor Hrvoje Tiljak, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Clinical drug trials are specific investigations in humans that are the basis for regulatory drug approvals and for decision making based on the principles of evidence-based medicine (EBM). Increase in general quality of patient care and beneficial effects on a broader economic system have been demonstrated in regions actively participating in clinical drug trials. Part of the public supports such investigations, but misconceptions and skepticism are common. General practitioners (GPs) are the first line of contact between the public and the healthcare system. The concept of primary care includes also an educational role. Hence, GPs can influence the attitudes in the population. We aim to assess the level of awareness and attitudes towards EBM and specifically towards regulatory clinical drug trials among GPs and their patients in Croatia and several other countries.

Hypothesis: Higher awareness about EBM in GPs is associated with their positive attitude towards clinical trials, which in turn is associated with a positive attitude of their patients.

Aims: Objective Our general objective is to explore the relationship between GPs' knowledge/awareness about EMB and their attitudes towards clinical drug trials, as well as the relationship between GPs' attitudes and attitudes of their patients – in Croatia and in several Eastern European countries (BiH, Macedonia, Serbia, Montenegro, Turkey) and several EU countries (Poland, Portugal, Spain). Specific objective 1. Assess the knowledge about EBM and attitudes towards regulatory clinical drug trials of GPs'. 2. Assess the relationship between GPs' knowledge and attitudes. 3. Assess attitudes towards regulatory clinical drug trials of their patients. 4. Assess the relationship between GPs' attitudes and attitudes of their patients. 5. Compare Croatia to participating Eastern European and EU countries.

Materials and methods: This is a cross-sectional, voluntary, non-profit survey. Subjects are GPs and their patients. In the first step, GPs will be offered to complete a questionnaire evaluating their knowledge and attitudes. In the second step, each participating GP will enroll at least 5 of her/his patients and will help them complete a questionnaire prepared specifically to assess patients' attitudes. We expect to enroll 50 GPs in Croatia and 250 of their patients. For each other participating countries, we expect to enroll 20 GPs and 100 of their patients. Following countries accepted participation: Croatia, BiH, Serbia, Montenegro, Macedonia, Turkey, Portugal, Poland and Spain. Each country has a coordinator who is leading the enrolment, and keeps the subject log. Each subject is tracked under specific code for the data protection purposes. Enrolment is currently in the process. Questionnaires for the GPs and their patients were validated in a pilot study. GP questionnaire has four sections: Demographic data, level of EBM knowledge, implementation of EBM knowledge, clinical trial attitudes. Questionnaire for patients contains following sections: Demographic data, clinical trial attitudes. Outcomes (knowledge, attitudes) as well as general data for physicians and patients will be summarized by country / region and will be analyzed in generalized hierarchical (mixed) models.

Expected scientific contribution: This research will create new insights into the knowledge and attitudes of GPs and the attitudes of their patients in respect to regulatory clinical drug trials and the impact that GPs attitudes might have on the general population. It will also help position Croatia relative to other included countries in this respect. Evaluating the relationship between the knowledge / attitudes of GPs with those of their patients can identify target groups towards which educational measures could be directed in order to improve the perception/understanding of the regulatory clinical drug trials in the general population.

MeSH/Keywords: Clinical drug trials, family medicine, awareness, attitudes

Poster code: T-03-04-062

Poster Title: Evaluation of the short-term intervention using the integrated, individual and interdisciplinary approach in the prevention of cardiovascular diseases in primary health care

PhD candidate: Ino Kermc

Part of the thesis: Evaluation of the short-term intervention using the integrated, individual and interdisciplinary approach in the prevention of cardiovascular diseases in primary health care

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, a growing public health problem and a significant economic burden for all health systems. 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.

Hypothesis: Developing an effective prevention model for patients at risk for CVD at the primary care level through the effective use of integrated care, individual and interdisciplinary approaches will reduce the incidence of cardiovascular disease.

Aims: GENERAL OBJECTIVE: to investigate the effectiveness of short-term prevention model for patients at risk for CV diseases using integrated care, individual and interdisciplinary approach at the level of primary health care SPECIFIC OBJECTIVES: 1. To determine the effects of the planned intervention on risk factors for the development of CVD in accordance with the guidelines for the prevention of CVD 2. Determine the importance of training family physicians to conduct a motivational interview 3. Determine the importance of patient enablement for activities related to CVD prevention 4. Determine the importance of organizing a system that allows the creation of multidisciplinary teams as part of preventive activities for CVD

Materials and methods: The study will be prospective cohort, subjects all over 18 years of age with the presence of one or more risk factors for CVD development. Sampling will be random, consecutive. One in five patients over the age of 18 with a risk factor for developing CVD who comes to a family medicine practice will be enrolled in the study, a total of 10 patients per office. Determination of risk factors in accordance with the guidelines for the prevention of CVD will be carried out at the beginning of the study and after 6 months. Intervention and control group will be formed in the area of the city of Zagreb. The research would include 20 family medicine practices that will participate in the intervention and 20 practices that will be the control group, which makes a total of 200 intervention participants and 200 control group participants. The intervention would include an individual approach in cardiovascular risk assessment, the creation of an individual treatment and monitoring plan that would include an individual approach in forming the interdisciplinary team needed in monitoring. In the control group, the family physician would work according to established clinical practice. After defining the intervention plan, and in accordance with the individual assessment of cardiovascular risk, risk factors would be monitored in accordance with the recommendations on the prevention of CV disease and would include a questionnaire on smoking, body weight, physical activity, blood pressure, blood lipids, diabetes, kidney function, medication adherence, medications, patient awareness of risk factors, lifestyle, alcohol consumption. In addition, measurements of height, weight, waist and hip circumference, blood pressure, blood glucose, total cholesterol, LDL, HDL will be performed. The intervention would also include an optional consultation with some of the members of the interdisciplinary team at the primary care level, depending on the assessment of the family physician and the type of risk factors.

Expected scientific contribution: A significant scientific contribution of this research is that short-term intervention will investigate the role of integrated care, individual and interdisciplinary approach at the level of primary health care in the prevention of cardiovascular disease

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

Poster code: T-03-04-119

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

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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.

Hypothesis: Decreased physical activity of the elderly during the COVID-19 pandemic is negatively related to their quality of life and self-assessment of their health and validation of the test method will not affect the validity of the questionnaire.

Aims: The main aim is to identify predictors of the quality of life of the elderly at the time of the COVID-19 pandemic. Specific aims:1. Investigate and analyze factors related to the quality of life of the elderly,2.Examine the association of self-assessed health of the elderly through eight dimensions (SF-36) with physical activity using the IPAQ questionnaire,3. Assess the quality of life of the elderly at the time of the COVID-19 pandemic, 4. Assess the level of physical activity during the COVID-19 pandemic, 5. Analyze the use of health care with respect to self-assessed health and physical activity,6. Comparison of whether physical activity is associated with disease outcome in patients with COVIDA-19

Materials and methods: Respondents will be citizens of the city of Zagreb aged 65 and over (500 will be patients of 10 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: 1. "Personal wellbeing index" questionnaire for measuring the quality of life 2. "Short form health survey" –SF-36- structured questionnaire with questions related to demographic characteristics and way of living, self-assessment of health 3. "International physical activity questionnaire" IPAQ-International Physical Activity Questionnaire And also we will ask respondents questions related to COVID-19 disease.

Expected scientific contribution: 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: T-03-04-130