

# PhD Day 2020

## Dan doktorata 2020



# Contents

- Preliminary research results..... 3
  - Basic medical sciences - preliminary research results..... 4
  - Clinical medical sciences - preliminary research results ..... 26
  - Public health and healthcare - preliminary research results ..... 90
- Thesis proposals ..... 95
  - Basic medical sciences - thesis proposals..... 96
  - Clinical medical sciences - thesis proposals ..... 114
  - Public health and healthcare - thesis proposals..... 168

**Preliminary research results**

Basic medical sciences - preliminary research results

**Poster Title:** Dynamics of ectopic bone formation using autologous bone graft substitute containing recombinant human bone morphogenetic protein 6 in an autologous blood coagulum and recombinant human bone morphogenetic protein 2 on an absorbable collagen sponge

**PhD candidate:** Natalia Ivanjko

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

**Mentor(s):** Academician Slobodan Vukičević

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** BMPs require a suitable delivery system or carrier to prevent the dispersion from the site of application while allowing gradual release resulting in osteoblastic differentiation with subsequent new bone formation. Carrier has to be biocompatible, easily and costly manufactured for large scale production, non-immunogenic, and enable vascular and cellular invasion. Bovine collagen was the first used as a BMP carrier although it has major disadvantages including compressibility, increased growth factor release, heterotopic bone formation and need for large BMP doses. Autologous blood coagulum (ABC) is a novel biocompatible carrier for BMPs. It suppresses foreign body response, promotes tight rhBMP6 binding with plasma proteins within the fibrin meshwork and allows a sustained in vitro release of rhBMP6. The aim of this study was to compare dynamics of ectopic bone tissue formation between a novel biocompatible device for bone healing consisting of recombinant human bone morphogenetic protein 6 (rhBMP6) in an autologous blood coagulum (ABC) and the only commercially available BMP based osteoinductive device consisting of rhBMP2 on absorbable collagen sponge (ACS).

**Materials and methods:** In vitro activity of rhBMP2 and rhBMP6 was tested on C2C12 mouse myoblast cell line, stably transfected with the BRE-Luc construct. In order to examine in vivo dynamics of ectopic bone formation, osteoinductive implants were implanted subcutaneously in axillary region in rats. The rhBMP6 in ABC and rhBMP2 on ACS were tested at doses of 5 and 20 µg per implant on day 7, 14 and 35 following implantation. New ectopic bone was analysed on histology sections stained by hemotoxylin & eosin stain. To follow, visualize and quantify new ectopic bone formation, implants were scanned after explantation using 1076 SkyScan µCT device.

**Results:** In vitro activity of rhBMP2 and rhBMP6 tested on C2C12 cell line stably transfected with the BRE-Luc construct showed that both recombinant proteins are active in comparison to their standards. Histology sections revealed that on 7th day after implantation newly formed bone was not present in rhBMP2 + ACS implants. On the other hand, in rhBMP6 + ABC implants endochondrally formed bone was present. On the 14th day, rhBMP6 + ABC implants were completely ossified while rhBMP2 + ACS implants were only partially ossified. On 35th day after implantation, bone was present in all histological sections of implants containing lower (5 µg) or higher (20 µg) dose of rhBMP2 + ACS implants and rhBMP6 + ABC implants. MicroCT analyses of implants showed that rhBMP6 in ABC was superior to rhBMP2 on ACS at higher doses (20 µg) on day 7, 14 and 35 after implantation. At lower doses (5 µg), rhBMP6 in ABC was superior to rhBMP2 on ACS on 7th and 14th day, but not on the 35th day after implantation. Amount of newly formed bone was dose dependent for both rhBM2 on collagen and rhBMP6 in ABC.

**Discussion:** In this study we demonstrated that rhBMP6 in ABC was superior to rhBMP2 on ACS on days 7, 14 and 35 at lower and higher doses. Novel autologous carrier for rhBMP6 (ABC) is creating a permissive environment for rapid bone formation compared to commercially available ACS containing rhBMP2. Even though all tested formulations showed extensive amount of bone, bone formation was dose dependent and formulations containing higher dose (20 µg) of both rhBMP2 and rhBMP6 induced significantly larger amount of bone than formulations containing lower dose (5 µg).

**MeSH/Keywords:** bone morphogenetic protein 2, bone morphogenetic protein 6, bone regeneration

**Poster code:** R-01-01-128

**Poster Title:** Novel autologous bone graft substitute containing rhBMP6, autologous blood coagulum and bioceramics induces ectopic bone formation in rats and posterolateral lumbar spine fusion in rabbits

**PhD candidate:** Nikola Štoković

**Part of the thesis:** Ectopic bone induction by osteoinductive 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:** In order to define optimal properties of a novel autologous bone graft substitute (ABGS) containing rhBMP6 within the autologous blood coagulum (ABC) and bioceramic particles as a compression resistant matrix (CRM) we explored the influence of their chemical composition, size and ratio with ABC on the osteoconductive and osteoinductive properties of ABGS in rat subcutaneous implant assay. Based on obtained results, we tested new ABGS with CRM at a functional ectopic site in clinically relevant posterolateral spinal fusion (PLF) model in New Zealand white rabbits.

**Materials and methods:** A series of rat subcutaneous implant assays were conducted with various ABGS compositions. Tested bioceramic particles included tricalcium phosphate (TCP), hydroxyapatite (HA) and biphasic bioceramic (BCP), containing TCP and HA in 80/20 ratio of different particle sizes (small 74-420  $\mu\text{m}$ , medium 500-1700  $\mu\text{m}$  and large 1000-4000  $\mu\text{m}$ ). RhBMP6 was either mixed with ABC or lyophilized on CRM prior to use with ABC. In addition, we compared bone induction capacity of ABGS containing large particles and five different CRM/ABC ratios. The experiments were terminated on day 21 and implants were analysed by  $\mu\text{CT}$  and histology. In the PLF rabbit model, the ABGS implants (n=6 per group) containing rhBMP6, ABC and medium bioceramic particles (BCP or TCP 500-1700  $\mu\text{m}$ ) were implanted bilaterally between transverse processes of the lumbar vertebrae L5-L6. All animals were euthanized on day 50 after surgery. To visualize new ectopic bone formation, lumbar spine was scanned by  $\mu\text{CT}$ . The success of spinal fusion was analysed on  $\mu\text{CT}$  sections through the anterior and posterior transverse process. Furthermore, lumbar spine was palpated and the mobility of fused transverse processes was tested.

**Results:** All tested compositions with CRM induced in vivo formation of a bone-ceramic structure (BCS) consisting of newly formed bone guided by bioceramic particle scaffold.  $\mu\text{CT}$  analyses revealed that tested ABGS formulations induced an extensive amount of new ectopic bone. TCP and HA (used individually or combined forming BCP) have shown appropriate osteoconductive properties and based on new formed bone as well as the trabecular parameters it was not possible to determine the superiority of one material to others. Also, there were no obvious differences between the two methods of rhBMP6 application in the bone volume appearance. Importantly, ABGS compositions containing higher CRM/ABC ratios induced more bone than formulations containing lower CRM/ABC ratios. The most optimal ratio which we further translated in PLF model seemed to be in the range from 0,1 to 0,125 g CRM per 500  $\mu\text{L}$  of ABC. Due to superior handling properties and uniform distribution of medium particles in implant as well as low resorbability of HA in time, we have chosen medium, 500-1700  $\mu\text{m}$  BCP and TCP particles to be tested in PLF rabbit model. In this experiment the total fusion success rate was above 90% and the same result was obtained by both analysis of spinal fusion on  $\mu\text{CT}$  sections and by palpatory mobility testing.  $\mu\text{CT}$  analyses revealed that an extensive amount of newly formed bone was present in all experimental groups and that there was no significant difference among experimental groups regardless the type of bioceramics used (BCP vs TCP) and the method of rhBMP6 application (mixed with blood vs lyophilized on bioceramics). Bone formation and osseointegration were confirmed on histological sections through newly formed bone between transverse processes. Successful spinal fusion between adjacent transverse processes was confirmed radiologically ( $\mu\text{CT}$  sections and  $\mu\text{CT}$  analyses), by palpatory segmental mobility test and on histological sections.

**Discussion:** All tested ABGS formulation induced extensive bone formation which along with bioceramic particles formed complex, bone-ceramic structure. However, chemical compositions and particle size affect short term and long term properties of newly bone-ceramic structure. Therefore, particle properties might be adjusted to fit specific need of various complex clinical indications. Tested ABGS formulations containing rhBMP6, ABC and bioceramic granulate successfully induced spinal fusion in rabbit PLF model and might be an innovative and original biological approach for achieving a successful lumbar spine fusion in clinical practice.

**MeSH/Keywords:** Bone morphogenetic proteins (BMPs), BMP6, autologous bone graft substitute (ABGS), bioceramics, posterolateral spinal fusion (PLF)

**Poster code:** R-01-01-129

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

**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 a member of the transforming growth factor-beta superfamily of proteins and is the most abundant protein of all BMPs in bone. Proteins from this group display osteogenic activity, however, the role of BMP3 in bone tissue is considered antagonistic to other BMPs due to its negative regulation of osteogenesis. Mouse models with overexpressed BMP3 show delayed ossification with spontaneous rib fractures and thinner cortical bone. On the other hand, BMP3 deletion results in increased bone mass and trabecular bone in mice. BMP3-related research up to this day is insufficient and there are no reports of BMP3 and BMP6 interaction. The aim of this research is to explore the differences between Bmp3 knock-out (KO) and wild type (WT) mice regarding osteoblast precursor differentiation and new ectopic bone formation.

**Materials and methods:** Von Kossa staining was performed on osteoblasts derived from BMSCs isolated from WT and KO mice. Stained area fraction percentage was assessed to determine the effect of BMP3 and BMP6 on cell differentiation. Blood coagulum containing BMP6 was implanted in mice axillary region and after two week-incubation, implants were removed and new bone formation was analyzed using micro-CT and histology.

**Results:** In most cases, BMSCs from KO animals exhibited a better response concerning osteoblast differentiation. A striking difference was observed with the combination of differentiation agents and BMP6, which display a synergistic effect. BMP3 KO mice showed more newly formed ectopic bone compared to WT animals. This result was seen in H&E staining and confirmed with micro-CT quantification.

**Discussion:** BMSCs from KO animals differentiate into osteoblasts in a larger number than those from control animals in almost all cases. In vivo, it seems that BMP3 present in WT animals diminished the osteoinductive property of BMP6 in blood coagulum. Larger sample size is needed for conclusive results and reduced deviation, but these preliminary results show that BMP3 is an important part of osteoblast precursor differentiation, as well as bone formation. Its role is antagonistic compared to BMP6, which is in accordance with published research.

**MeSH/Keywords:** Bone marrow, Mesenchymal Stem Cells, Bone Morphogenetic Protein 3, Osteogenesis

**Poster code:** R-01-01-130

**Poster Title:** The effect of astaxanthin on testicular torsion-detorsion injury in rats

**PhD candidate:** Marko Bašković

**Part of the thesis:** The effect of astaxanthin on testicular torsion-detorsion injury in rats

**Mentor(s):** Professor Davor Ježek, MD PhD

**Affiliation:** University of Zagreb, School of Medicine

**Introduction:** Astaxanthin (C<sub>40</sub>H<sub>52</sub>O<sub>4</sub>) is a pigment that belongs to the family of the xanthophylls, the oxygenated derivatives of carotenoids whose synthesis in plants derives from lycopene. Common sources of natural astaxanthin are the green algae *Haematococcus pluvialis*, the red yeast *Phaffia rhodozyma*, as well as crustacean byproducts. One of the most important properties of astaxanthin is its antioxidant properties. Astaxanthin shows a specific antioxidant activity 4 times stronger than lutein, 10 times stronger than beta-carotene and even 500 times stronger than vitamin E. Testicular torsion is the most dramatic and potentially serious of the acute processes affecting the scrotal contents because it may result in the loss of the testicle. Testicular torsion involves the rotation of the testicles around the longitudinal axis of 180 or more degrees, followed by an interruption of circulation in the testis. If not recognized in time, this condition may result in ischemic injury and loss of the testis. The aim of this study is to investigate the potential protective effect of astaxanthin on testicular torsion-detorsion injury in rats.

**Materials and methods:** Thirty-two Fisher rat were divided into 4 groups of 8 individuals. At the first (sham) group, a cut was made in the right inguinoscrotal region by which the ipsilateral testis took out and immediately returned to its natural position. In the second group, a 720° testicle torsion was performed, and detorsion after 90 minutes. In the third group, at the time of detorsion, astaxanthin was given intraperitoneally as a single dose of 75 mg / kg, while in the fourth group, astaxanthin was given intraperitoneally 45 minutes from the moment of detorsion at an equal dose. After 90 minutes of reperfusion the testis was removed and divided into two halves. On one half of the testicle biochemical research was conducted, while in the second half histological and immunohistochemical research were performed. Lipid peroxidation was determined by measuring malondialdehyde (MDA) in testis homogenates. The activity of superoxide dismutase (SOD) and glutathione peroxidase (GPx) were measured using ready-made kits and absorbance measurement. For the purpose of histological research, the preparations were dyed with haematoxylin and eosin (HE). Using a microscope, we measured the diameter of the tubule from which the mean diameter was obtained – MSTD for each group. Randomly, for each group, 20 tubules were selected. The diameter of the tubule was measured at a smaller and larger axis from which the mean diameter was obtained. Also, for each group, the Johnsen value was determined depending of the maturity of the germ cell. The number of apoptotic caspase-3 positive cells per 100 tubules (apoptotic index) was detected by the immunohistochemical method according to the protocol Id: 283 / Cell Signaling Technology®. For this purpose, the rabbit polyclonal antibody (Cleaved Caspase-3; Asp 175, Cell Signaling Technology®) was used. A nonparametric (distribution free) Kruskal-Wallis test was used to analyze the differences between the groups.

**Results:** The results of the histological part of the research are promising. There is a statistically significant difference in MSTD, at a significance level of 5% ( $\chi^2 = 55,733$ , DF = 3,  $p < 0,0001$ ). The results by groups are as follows; group 1 (mean = 224,874 / SD = 7,411), group 2 (mean = 174,371 / SD = 7,714), group 3 (mean = 183,036 / SD = 7,049), group 4 (mean = 191,678 / SD = 12,319). There is also a statistically significant difference for the Johnsen score, at a significance level of 5% ( $\chi^2 = 71,018$ , DF = 3,  $p < 0,0001$ ). The results by groups are as follows; group 1 (mean = 7,9 / SD = 0,308), group 2 (mean = 5 / SD = 0), group 3 (mean = 6,45 / SD = 0,510), group 4 (mean = 6,9 / SD = 0,308). Post-hoc analysis found that a statistically significant difference existed between all groups ( $p < 0,0001$ ). The biochemical and immunohistochemical results of the research are being processed and are expected in July 2020.

**Discussion:** Testicular torsion through several mechanisms leads to testicular dysfunction, spermatogenesis loss, permanent testicular damage and ultimately infertility of men. If multimodal (biochemical, histological, immunohistochemical) approach proves the protective effect of astaxanthin, research will significantly contribute to current knowledge and future research in this area for the purpose of future human research.

**MeSH/Keywords:** astaxanthin, testis, torsion-detorsion injury, ischemia-reperfusion injury, rat

**Poster code:** R-01-02-063



**Poster Title:** Sexual dimorphism of human lacrimal gland

**PhD candidate:** Koraljka Hat

**Part of the thesis:** Sexual dimorphism of human lacrimal gland

**Mentor(s):** Assist. Prof. Snježana Kaštelan, MD PhD, Danko Muller, PhD, research associate

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Sexual dimorphism represents differences in structural, physical, chemical and behavioral characteristics between two sexes of the same species. It has been shown in salivary gland, lacrimal gland, adrenal gland, pituitary gland, brain, liver, bone, metabolism of proteins, lipids and immune system. Previous studies of lacrimal gland have been mainly conducted on animal models. Published researches of human lacrimal gland have already suggested potential differences in structure between male and female lacrimal glands (tertiary sexual characteristics). Observed differences in structure of human lacrimal glands between sexes have not been studied adequately. Prevalence of dry eye syndrome rises with age and is much higher in females than in males. Considerably higher prevalence of dry eye in female population suggests sexual differences in human lacrimal gland function. Considering potential impact of structural differences on function, this study could contribute to better understanding of lacrimal gland dysfunction development. Also, this research is the first detailed stereological analysis of human lacrimal gland. Results of this study should broaden our knowledge of sexual dimorphism in histological features of human lacrimal gland.

**Materials and methods:** Samples are human lacrimal glands collected during 60 autopsies. Donors are deceased patients from University Hospital Dubrava, University Hospital Center Sestre Milosrdnice and University Hospital Center Zagreb also eligible for eyeball or cornea donation and with signed informed consent. 30 donors will be male and 30 female. Tissue samples will be fixated in 10% buffered formalin solution and processed for further histological analysis. Qualitative histological analysis will be performed using binocular light microscope Nikon Alphaphot (Nikon, Japan) and transmission electron microscope JEOL JEM-1400 (JEOL, Japan). Results of qualitative analysis will be descriptive. Quantitative (stereological) analysis will be performed using Weibels 32 point multipurpose test system with light microscope Nikon YS 100. Immunohistochemical analysis will be performed using primary antibody for androgen and estrogen receptors. Numeric density of estrogen (Nva) and androgen receptors (Nve) expression will be calculated using stereological analysis methods. Statistical analysis will be performed, P values <0,05 will be considered significant. Expected duration of research is 24 months.

**Results:** Collection of samples has been extremely prolonged. Samples from 30 donors have been collected so far. To increase the number of potential donors, we have obtained ethics committee approvals from University Hospital Center Sestre Milosrdnice and University Hospital Center Zagreb! Unfortunately, analysis has not started yet due to considerable technical problems after the earthquake in Zagreb and COVID-19 epidemiological situation. 16 samples have been collected from female donors and 14 from male donors. Average age of donors was 69 years. Further results are expected in the next 6 months.

**Discussion:** Previous studies of lacrimal gland have already suggested differences in structure and function between sexes. Most of the studies have been conducted on animal models. Only few studies published so far have been conducted on human models. Their results have confirmed differences in structure between male and female lacrimal glands using light microscope and descriptive methods. Roen et al. have found that 75% of human lacrimal glands collected during autopsies show microscopical abnormalities with periductal fibrosis and chronic inflammation as the most common finding. 52% of glands after the age of 50 shows signs of periductal fibrosis and 74% shows some degree of ductal changes. Obat et al. have studied acinus atrophy, periductal fibrosis, interlobular ductal dilatation, periductal lymphocyte infiltration and fat infiltration in human lacrimal gland specimens using light microscopy. Their findings have been consistent with findings of Roens group confirming sexual differences in human lacrimal gland microstructure. Obviously, observed differences in structure of human lacrimal glands between sexes have not been studied adequately! To test our hypothesis, we have to finish this research!

**MeSH/Keywords:** sexual dimorphism, lacrimal gland, human, histology, stereological analysis, immunohistochemistry, androgen receptors, estrogen receptors

**Poster code:** R-01-02-069

**Poster Title:** Pentadecapeptide BPC 157 improved ketamine-induced recognition memory deficit and social deficit in rats

**PhD candidate:** Andrea Zemba Čilić

**Part of the thesis:** Effects of pentadecapeptide BPC 157 and interactions with the NO system in pharmacological models of psychosis and antipsychotic side effects in rats

**Mentor(s):** Assoc. Prof. Alenka Boban Blagaić, MD PhD, Professor Predrag Sikirić, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** L-NAME, NOS-inhibitor, and L-arginine, NOS-substrate, relation, the effect on schizophrenia-like symptoms. Rats received (mg/kg i.p.) medication (BPC 157 (0.01), L-NAME (5.0), L-arginine (100.0) given alone and/or together) at 5 min before the challenge for the acutely disturbed motor activity (dopamine indirect / direct agonists (amphetamine (3.0), apomorphine (2.5)), NMDA receptor non-competitive antagonist (MK-801 (0.2))), or catalepsy, (dopamine receptor antagonist haloperidol (2.0)). Alternatively, BPC 157 10 µg/kg was given immediately after L-NAME 40.0 mg/kg i.p. To induce or prevent sensitization, we used chronic methamphetamine administration, alternating 3 days during the first 3 weeks, and challenge after next 4 weeks, and described medication (BPC 157, L-NAME, L-arginine) at 5 min before the methamphetamine at the second and third week. Given alone, BPC 157 or L-arginine counteracted the amphetamine-, apomorphine-, and MK-801-induced effect, haloperidol-induced catalepsy and chronic methamphetamine-induced sensitization. L-NAME did not affect the apomorphine-, and MK-801-induced effects, haloperidol-induced catalepsy and chronic methamphetamine-induced sensitization, but counteracted the acute amphetamine-induced effect. In combinations (L-NAME+L-arginine), L-NAME counteracts all counteractions L-arginine-induced, except that in the acute amphetamine-rats. Unlike L-arginine, BPC 157 maintains its counteracting effect in the presence of the NOS-blockade (BPC 157+L-NAME) or NO-system-over-stimulation (BPC 157+L-arginine). Illustrating the BPC 157-L-arginine relationships, BPC 157 restored the antagonization (BPC 157+L-NAME+L-arginine) when it had been abolished by the co-administration of L-NAME with L-arginine (L-NAME+L-arginine). Finally, BPC 157 directly inhibits the L-NAME high dose-induced catalepsy. Further studies would determine precise BPC 157/dopamine/glutamate/NO-system relationships and clinical application. Now, we explored the effects of pentadecapeptide BPC 157, L-NAME and L-arginine on cognitive and negative symptoms of schizophrenia.

**Materials and methods:** We evaluated ketamine-induced performance deficits in the novel object recognition task (NORT) [1-3, 5], and ketamine-induced social deficit in the social interaction test (SIT) [4, 5]. Acute treatment (mg/kg i.p.) with ketamine (3.0) [1] lead to impaired cognitive flexibility; subchronic treatment with ketamine (8.0) [4] to social deficit in rats. In NORT, rats received (mg/kg i.p.) medication (BPC 157 (0.01), L-NAME (5.0), L-arginine (100.0) given alone and/or together at 5 min before the ketamine. In subchronic SIT, ketamine was administered for 3 days, while saline, BPC 157, L-NAME and/or L-arginine were given 30 minutes prior to testing after the third dose of ketamine.

**Results:** Concerning D data, a significant main effect of ketamine (K), and a significant interaction between ketamine, BPC 157 (B) and L-arginine (A) was evidenced (\* $p < 0.001$ ). BPC 157 significantly improved ketamine-induced recognition memory deficit even in presence of L-NAME (\* $p < 0.001$ ). L-NAME (N) counteracted effect of L-arginine. (Figure 1) As shown in figure 2, BPC 157 and L-NAME alleviated the subchronic ketamine-induced social interaction deficit (\* $p < 0.001$ ), whereas the L-arginine showed no effect. When the test agents were combined, social interaction was blocked (\* $p < 0.001$ ).

**Discussion:** Pentadecapeptide BPC 157 was efficacious in counteracting the behavioural effects related to the blockade of the NMDAR. In principle, based on the effect of administration of L-NAME and/or L-arginine, characteristic presentations of NO pathways were identified (ketamine-induced effects (L-arginine-L-NAME opposite effects; antagonization), whereby the effect of BPC 157 was always overwhelming. It can be concluded that BPC 157 counteracted the effects of ketamine, and that there exists an interaction with the NO-system.

**Acknowledgments:** Prof. Predrag Sikirić, MD PhD

**MeSH/Keywords:** pentadecapeptide BPC 157, ketamine, NORT, SIT

**Poster code:** R-01-03-132

**Poster Title:** The effect of pentadecapeptide BPC 157 on contrast-induced nephropathy in rats

**PhD candidate:** Ivica Kocman

**Part of the thesis:** The effect of pentadecapeptide BPC 157 on contrast-induced nephropathy in rats

**Mentor(s):** Professor Predrag Sikirić, MD PhD, Assoc. Prof. Ingrid Prkačin, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** The intravascular use of iodinated contrast media (CM) for diagnostic or therapeutic purposes can cause contrast-induced nephropathy (CIN). Established CIN is associated with increased morbidity and mortality particularly in patients with chronic kidney disease. Proposed mechanisms of CIN is direct toxic effect of CM to the tubular epithelial cell and medullary hypoxia due to intrarenal vasoconstriction. Cell death (apoptosis) ensues. There is no clinically effective pharmacological therapy for CIN. Stable gastric pentadecapeptide BPC 157 (BPC 157) is cytoprotective agent. BPC 157 has pleiotropic beneficial effects in different organ, in different animal models with the same dose regimen. Lethal dose was not reported and there are no adverse effects in clinical trials. We hypothesize that BPC 157 would reduce CIN in rats.

**Materials and methods:** Male Wistar rats were randomly divided to treated (bolus BPC 157 10 µg/kg bm ip) and control (equivalent volume of 0,9% sodium chloride) group of 6 rats each. Single dose of iohexol (1600 mg/kg bw iodine) was administered into the tail vein. After 24, 48 and 72 hours caudal vena cava blood samples were collected to determine serum creatinine levels by Jaffe colorimetric method. Following sacrifice the right kidneys were morphological semiquantitative evaluated for acute tubular necrosis (ATN), apoptosis, cytoplasmic vacuoles, proteinaceous cast, medullary congestion and interstitial edema on HE staining slides. Immunohistochemically expressions characteristics (positivity, intensity) of B-cell lymphoma 2 (Bcl2) and Bcl2 associated X (BAX) protein and histochemical terminal deoxynucleotidyl transferase dUTP Nick-End Labeling (TUNEL) assay were performed concerning cell apoptosis. Oxidative stress markers were measured in left kidneys. Lipid peroxidation in term of malondialdehyde (MDA) was measured using thiobarbituric acid reactive substances (TBARS) assay. Kidney superoxide dismutase (SOD) activity was determined using the SOD kit following the manufacturing protocol.

**Results:** Morphological control group showed strong and very strong kidney injury according to apoptosis both proximal and distal tubules in all study period. In treated rats at 24 hours there was mild grade of apoptosis but at 48 and 72 hours AKI features were reduced. MDA concentration in kidney is reduced in BPC 157 treated rats already at the beginning of the treatment.

**Discussion:** BPC 157 reduced AKI morphological features after iv administration iohexol in rats. BPC 157 outweigh increased oxidative stress in rat kidneys. BPC 157 could be agent for pharmacological therapy of CIN.

**MeSH/Keywords:** contrast-induced nephropathy, pentadecapeptide BPC 157

**Poster code:** R-01-03-137

**Poster Title:** Identification of HIV-1 transmission clusters in Croatia, 2014 - 2017: evidence for the forward spread of HIV-1 resistant variants

**PhD candidate:** Maja Oroz

**Part of the thesis:** Molecular diversity of human immunodeficiency virus type 1 and the role of transmission chains in the local spread of infection in Croatia

**Mentor(s):** Professor Josip Begovac, MD PhD, Snježana Židovec Lepej, PhD, research advisor

**Affiliation:** University of Zagreb School of Medicine, University Hospital for Infectious Diseases

**Introduction:** Phylogenetic analysis is a useful tool for identification of HIV-1 transmission clusters and analysis of biological characteristics of individual clusters. The aim of the study was to determine and characterize transmission networks that are responsible for the forward spread of HIV-1 infection and resistant variants in Croatia.

**Materials and methods:** We analyzed 403 (95.9%) of 428 newly HIV-diagnosed persons who entered clinical care at the University Hospital for Infectious Diseases, Zagreb. The entire protease HIV-1 gene (PR, codons 1-99) and a part of the reverse transcriptase HIV-1 gene (RT, codons 1-240) were sequenced by using a validated in-house method. Mutations were determined by using Surveillance Drug Resistance Mutation list. HIV subtype was determined with Rega HIV-1 subtyping tool, version 3.0. Sequences subtyped as B were selected for phylogenetic inference. For each sequence in the dataset 10 closest sequences were determined with BLAST search. Phylogenetic trees were constructed with PhyML 3.0, while the Figtree version 1.4.3 was used for tree visualization. Transmission cluster were defined as sequences  $\geq 3$  patients from Croatian cohort with the approximate likelihood ratio test value  $> 0.90$ .

**Results:** Subtype B was found in 368 (91%) of patients. The overall prevalence of transmitted drug resistance (TDR) was estimated at 16.4% (n=66/403). The most prevalent TDR patterns were T215S, 4.9% (n=20/403); K101E, 3.5% (n=14/403); T215S + L210W, 2.2% (n=9/403); V32I+I47V+T215E/D+L100I+K103N, 1.9% (n=8/403); M41L+T215L, 0.7% (n=3/403). Phylogenetic analysis identified 19 local transmission clusters, of which 5 clusters (26%) were responsible for the forward spread of resistant HIV-1 viral strains (Figure 1). Characteristics of these clusters are presented in Table 1. Patients in clusters (n=347) were more frequently MSM (319/358 vs. heterosexuals 24/39,  $p < 0.001$ ), of younger age (median: 35 vs 39 years,  $p = 0.02$ ), infected in Croatia (305/348 vs. infected abroad 39/52) and had a recent infection (77/81 vs. chronic 270/322  $p = 0.009$ ) (Table 2).

**Discussion:** In this study we found a high prevalence of TDR and identified 5 local transmission clusters responsible for the spread of HIV-1 resistant strains in Croatia.

**Acknowledgments:** This study was supported by the Croatian Science Foundation Grant IP-2014-09-4461

**MeSH/Keywords:** HIV-1 infection, transmission clusters, molecular epidemiology, phylogenetic analysis

**Poster code:** R-01-05-017

**Poster Title:** Spectropic analysis of tissue and DNA isolated from human placentas with intrauterine growth restriction

**PhD candidate:** Petra Kejla

**Part of the thesis:** Spectropic analysis of tissue and DNA isolated from human placentas with intrauterine growth restriction

**Mentor(s):** Assist. Prof. Sanja Dolanski Babić, MD PhD, Professor Ljiljana Šerman, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Intrauterine growth restriction (IUGR) is one of the leading problems in modern day obstetrics. Common cause of IUGR is placental malfunction. Biological basis of the placentation and many proteins that are differently expressed in the IUGR are the key to understanding this pathology. Protein expression is determined by the DNA structure changes commonly found in epigenetic modifications. We are isolating tissue and DNA from IUGR and control nonIUGR placentas to measure the vibration characteristics of the solutions using Infrared spectroscopy with Fourier transform infrared spectroscopy (FTIR). Elaborated FTIR spectra will be analyzed to determine the potential difference in the secondary DNA structure

**Materials and methods:** Frozen and paraffin tissue blocks are being used. We are using 30 placentas from physiological term pregnancies and 30 placentas from term pregnancies complicated with IUGR. Thin slices from control and IUGR placentas are being made. DNA is isolated following specific protocols and concentration and quality measured. Specimens are prepared for spectroscopy and FTIR spectroscopy is done. FTIR spectra will be normalized and statistical analysis done using principal component analysis.

**Results:** Paraffin blocks of IUGR and normal placentas were selected. We deparaffinized the blocks. Thin tissue cuts were prepared. DNA was isolated from the samples. DNA concentration and quality were checked. We have made preliminary measurements to determine protocols before the corona crisis and the earthquake and had to stop in the middle of the project. Due to the earthquake in March 2020 we needed to evacuate the whole laboratory and some of the equipment was damaged. Since we were not able to finish the measurements until the deadline for this publication, no results are yet available.

**Discussion:** Deoxyribonucleic acid (DNA) is a biological macromolecule responsible for the development and function of cells of all living organisms. The structure, conformation and interaction of DNA with the surroundings plays a key role in determining its biological functions, and so many efforts have been made to improve the understanding of DNA behavior in different environment. Experimental methods like FTIR and Raman measurements, recognized as powerful and non-destructive techniques that probe primary and secondary structure of DNA, do not have any restrictions regarding DNA length and in this regard describe perhaps more physiological regimes. Several bands of major importance in the IR spectrum of DNA are highlighted in the Figure 1. In the region between 1300-1800 cm<sup>-1</sup>, where base sites of DNA absorb strongly, we focus on 4 vibrational bands: carbonyl C6=O6 (guanine) and C2=O2 (thymine) at 1704 cm<sup>-1</sup>, C4=O4 (thymine) at 1661 cm<sup>-1</sup>, C8=N7 (adenine) at 1608 cm<sup>-1</sup> and C4=N3 (cytosine) and C8=N7 (guanine) at 1487 cm<sup>-1</sup> (Figure 1). In the sugar-phosphate DNA backbone region, 800-1300 cm<sup>-1</sup>, six more bands come into focus: asymmetric and symmetric PO<sub>2</sub> stretching mode (sensitive to even the slightest changes in the environment around phosphate groups) at 1234 and 1089 cm<sup>-1</sup>, respectively, sugar-phosphate stretching band at 1071 cm<sup>-1</sup>, deoxyribose stretching mode at 966 cm<sup>-1</sup>, an A and B form markers situated at about 860 cm<sup>-1</sup> and 835 cm<sup>-1</sup>, respectively. Our measurements that are planned will show exact changes absorbance for different wavenumber and the preliminary measurements strongly support this.

**Acknowledgments:** This publication was co-financed by the European Union through the Europe Regional Development Fund, Operational Programme Competitiveness and Cohesion, under grant agreement No. KK.01.1.1.01.0008, Reproductive and Regenerative Medicine - Exploring New PI

**MeSH/Keywords:** placenta, intrauterine growth restriction, FTIR, spectroscopy

**Poster code:** R-01-05-141

**Poster Title:** Knowledge and Technology Transfer in Biomedical Research - Status and Potentials of the Republic of Croatia

**PhD candidate:** Smiljka Vikić-Topić

**Part of the thesis:** Knowledge and Technology Transfer in Biomedical Research - Status and Potentials of the Republic of Croatia

**Mentor(s):** Academician Slobodan Vukičević, Assoc. Prof. Fran Borovečki, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** The success of the technology transfer (TT) of some institution is largely influenced by the innovation environment and, particularly the motivation of the researchers, as shown in all areas of research in many countries. TT in biomedicine is more complex than in other fields of research since research outputs need costly development, teams of highly educated experts respecting all the regulatory requirements and many intermediators on the way to market. Although the end users are patients in need for innovative treatments, the decisions are made up by others: doctors, insurance companies and industry. Biomedical scientists from academic institutions are very often main source of new ideas for inventing new medical interventions, but these ideas are useful only if the research results are successfully transferred to industry. Aims of this study are to (i) identify the status of knowledge and technology transfer activities among Croatian scientists in the field of biomedicine, (ii) determine their motivation for technology transfer and attitude towards commercial exploitation of the research results and (iii) to propose measures to increase the use of innovativeness of Croatian scientists in the field of biomedicine and their impact on the society.

**Materials and methods:** For the study of participation of biomedical scientists in technology transfer processes in the Republic of Croatia, their motivating and demotivating factors and their attitude towards technology transfer activities, we will perform the survey of the targeted population (sample) listed in the Register of Biomedicine and Health Sciences of the Republic of Croatia, led by the Ministry of Science and Education (MSE). Prior to the main survey, we will perform the qualitative exploratory research aiming at identifying problems, obstacles and motivations of scientists. This will be done through the in-depth, semi-structured interviews with excellent scientists and with TT professionals. For the analysis of the data distribution, we will use Smirnov-Kolmogorov test. Depending on the results, appropriate parametric and/or nonparametric tests will be applied. To identify factors that influence participants' involvement in TT we will use logistic regression. Interest for TT of different intensity by the different groups of scientists, and possible differences in quantitative values between individual groups will be analysed by analysis of variance.

**Results:** We have completed the preparatory work. The Ethical Board has approved the research and MSE provided the list of 2065 scientists recorded in the registry of the scientists working in the biomedical field. In-depth interviews were conducted with TTOs from Lithuania and Hungary, showing that the challenges for successful TT is lack of experience among TT professionals, insufficiently developed industry and lack of industrial interest for research. The questionnaire consists of 42 questions divided in 7 groups in order to collect information about researchers' employment, scientific activity, productivity, attitude toward work, experience in TT, potentials and motivation for TT, and personal questions. The survey will be conducted on-line after the in-depth interviews are completed and the questions adopted.

**Discussion:** The results of this study will bring new insight into the activities of transferring knowledge and technology in the Republic of Croatia. This will be the first time to collect the data about scientists' attitude and interest for technology transfer. We aim to propose the measures needed and requested by the researchers themselves to improve Croatian ranking in the European innovation scoreboard.

**Acknowledgments:** This PhD thesis has been supported by the EU H2020 project Alliance4Life, No 779303 and Scientific CoE for Reproductive and Regenerative Medicine ("Reproductive and regenerative medicine - exploration of new platforms and potentials", KK01.1.1.01.0008).

**MeSH/Keywords:** Technology Transfer/ innovation, translation, impact, entrepreneurial university, entrepreneurship

**Poster code:** R-01-05-150

**Poster Title:** Cell cycle arrest and monocytic differentiation by activating Checkpoint kinase 1

**PhD candidate:** Barbara Tomić

**Part of the thesis:** Signaling mechanisms and metabolic changes during differentiation and proliferation of leukemia cells

**Mentor(s):** Professor Dora Višnjić, MD PhD

**Affiliation:** University of Zagreb School of Medicine; Scientific Centre of Excellence for Basic, Clinical and Translational Neuroscience

**Introduction:** One of the most successful acute myeloid leukemia (AML) treatment modalities is differentiation therapy. However, in everyday clinical practice, this type of therapy is restricted to application of all-trans-retinoic acid (ATRA) in cases of acute promyelocytic leukemia (APL). 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 and inhibits proliferation of monocytic U937 leukemia cells. Furthermore, AICAr was demonstrated to inhibit pyrimidine synthesis at a step downstream of dihydroorotate dehydrogenase (DHODH), and AICAr-mediated effects on differentiation were prevented by the addition of uridine. Depletion of nucleotide pools is known to activate the DNA damage signaling pathway through activation of the ataxia telangiectasia and RAD3-related (ATR)/checkpoint kinase 1 (Chk1)-mediated checkpoint in S-phase of the cell cycle. This study is aimed to test for the role of ATR/Chk1 in AICAr-mediated effects.

**Materials and methods:** U937 cells were incubated in the presence of AICAr, brequinar, nucleosides (1x), uridine (30 and 300  $\mu$ M) and pharmacological inhibitors of ATR/Chk1, caffeine and Torin2. 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. siRNA transfections were performed using siRNA targeting Chk1 (Dharmacon) and Neon<sup>TM</sup> transfection system (Invitrogen). Total cell lysates were analyzed for the level of Ser345-phosphorylated Chk1 and actin by Western blot. Statistical analysis was performed using Student's t-test.

**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. Both effects were abolished by addition of either nucleosides or uridine. The activation of Chk1 was observed in the presence of DHODH inhibitor 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 caffeine and 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.

**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 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.

**Acknowledgments:** Funded by Croatian Science Foundation under the projects IP-2016-06-4581 and DOK-2018-01-9599 and project "Experimental and clinical research of hypoxic-ischemic damage in perinatal and adult brain"; GA KK01.1.1.01.0007 funded by EU

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

**Poster code:** R-01-06-098

**Poster Title:** Review of Research impact assessment frameworks in biomedical area

**PhD candidate:** Olja Ulični Nikšić

**Part of the thesis:** Impact factors of biomedical scientific projects of the Republic of Croatia

**Mentor(s):** Professor Srećko Gajović, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Relationship between financial value of the grant and the final project impact is complex. Researchers are frequently asked to prepare detailed budget plan and to justify the use of the financial resources. Funding agencies are under pressure to be savvy with their spending and are keen to allocate funds in the most efficient way. The societal and economic pay off coming from scientific research remains at top of goals and policies of many countries. Consequently, question of how to measure and assess investment returns in research, remains highly relevant. The researchers still mostly use bibliometric indexes instead of measuring more long-term and significant research impact. In recent years, there is a growing number of frameworks that try to better describe and understand such issues. Research impact assessment frameworks (RIAFs) are defined as a conceptual model/framework and/or collection of evidence designed for research evaluation beyond the traditional academic outcomes. RIAFs primarily work with the purpose of allocation of funding, future research orientation and speed of technology transfer.

**Materials and methods:** The search strategy included four elements: database searches, web searches, citation tracking and expert contacts. Online identification of existing RIAFs applicable to biomedical area was performed through Google Scholar, Scopus, WoS CC and PubMed electronic databases, and funding agency websites in April 2020. Systematic review search terms included 'research impact' OR 'policy and practice' OR 'research impact assessment framework' AND 'intervention research' AND 'translational research' AND 'health promotion' AND 'public health' AND 'biomedical research'. Systematic review included theoretical and opinion pieces, case studies, descriptive studies, frameworks and systematic reviews describing processes, gray literature, and conceptual models for assessing research impact. Systematic review is ongoing and will be conducted in two phases in spring/summer 2020. In phase 1, abstracts were retrieved and assessed against the review criteria. For abstracts that met the review criteria in phase 1, full papers will be retrieved and assessed for inclusion in the phase 2 and final review. Studies included in the review have to meet the following criteria: 1) published in English language till January 2020; 2) contain described processes, theories, or frameworks associated with the assessment of research impact applicable to biomedical area. Following PRISMA guidelines, two independent investigators will systematically screen for publications describing, evaluating, or utilizing a methodological research impact framework within the context of biomedical research.

**Results:** Our original search strategy identified 494 documents from Scopus, 211.826 documents from WoS CC, 71 documents from PubMed, and 301 documents from other sources (Google Scholar, funding agency websites and expert communication). Preliminary results of the baseline data have showed high increase in publications cooperated to general RIAF review conducted in 2013 where it listed 193.343 documents in total (Milat AJ, Bauman AE, Redman S. A narrative review of research impact assessment models and methods. Health Research Policy and Systems. 2015.) Also till May 2020, we spotted presence of 134 duplicates that had to be removed. Current preliminary results show RIAF "payback model" as the most frequently used. Five broad categories of impact are also emerging: a) advancing knowledge, b) capacity building, c) informing decision-making, d) health benefits, e) broad socio-economic benefits.

**Discussion:** This systematic review is trying to summarize all existing methodological impact frameworks in biomedical area for the first time using systematic methods. Research showed that project methodology beyond the initial citation analysis is neither a tidy nor linear process. This review will allow researchers and funders to consider pathways to impact at the design stage of a study and to understand the elements and metrics that need to be considered to facilitate prospective assessment of impact. Users do not necessarily need to cover all the aspects of the methodological framework, as every research project can impact on different categories and subgroups.

**Acknowledgments:** OUN was supported by FP7 project GlowBrain, H2020 project BioChip and Scientific Center of Excellence for Reproductive and Regenerative Medicine.

**MeSH/Keywords:** Research impact assessment framework, Research impact

**Poster code:** R-01-07-088



**Poster Title:** Adult upper cortical layers marker CUX2 is expressed in the transient cell populations of the human fetal brain

**PhD candidate:** Terezija Miškić

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

**Mentor(s):** Assoc. Prof. Željka Krsnik, MD PhD, Assoc. Prof. Mladen-Roko Rašin, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** CUX2 belongs to a family of homeobox genes expressed in the nervous tissue, considered as one of the distinctive molecular markers of the adult upper cortical layers. Until now, CUX2 expression pattern throughout human fetal cortical development was not profoundly studied, even though the human cortex is worthwhile to study due to the extraordinary resolution of laminar developmental events. Previous murine developmental studies did not show CUX2 reactivity in the subplate (SP) since SP in rodents has a narrow size throughout the embryonic development. Recently, CUX2 expression was depicted in the human SP during late midgestation.

**Materials and methods:** CUX2 spatio-temporal dynamics in the fetal cerebral cortex was followed, to get a better understanding of histogenetic interactions during neuronal migration, cellular fate commitment, and transient cortical lamination. CUX2 protein and RNA expression patterns are identified using immunohistochemistry, immunofluorescence, and RNA-scope on formalin fixed-paraffin-embedded sections of postmortem human brains from 10 to 38 post-conceptual weeks (PCW) of the Zagreb Neuroembryological Collection.

**Results:** In the early fetal 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 is shown in the cortical plate (CP). In the newborn, CUX2 protein was expressed in the cells of gyral white matter (WM), along with the cells of layer I. In addition, there was a distinct subcortical CUX2 reactivity.

**Discussion:** In the developing human neocortex, CUX2 was present (1) during the pre-subplate stage when associative neurons still didn't migrate through; (2) in the SP during midgestation; and finally, (3) in the gyral white matter of a newborn cortex when all associative neurons finished their migration; therefore, we suggest that CUX2 reactive nuclei belong to the postmigratory subplate neurons, prospective projection (transient associative) neurons. Moreover, CUX2 expression in the transient cell populations of developing fetal cortex, not only in cells destined for the prospective upper layers makes CUX2 a reliable indicator of distinct histogenetic events during corticogenesis, such as neuronal migration, differentiation, and synaptogenesis.

**Acknowledgments:** This work was supported in part by the Croatian Science Foundation and Adris Foundation. Research was co-financed by the Scientific Centre of Excellence for Basic, Clinical, and Translational Neuroscience (project Experimental and clinical research of hyp

**MeSH/Keywords:** human cortical development, layer markers, associative neurons

**Poster code:** R-01-08-024

**Poster Title:** Influence of splenectomy on the mouse brain damage after experimentally induced ischemic stroke

**PhD candidate:** Dominik Romić

**Part of the thesis:** Influence of splenectomy on the mouse brain damage after experimentally induced ischemic stroke

**Mentor(s):** Professor Srećko Gajović, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Molecular and cellular events in ischemic stroke are of crucial importance in understanding the evolution of brain damage. Molecular TLR pathway on microglia cells and macrophages from peripheral circulation is one of the most important in stimulating neuroinflammation. The spleen is a reservoir for circulating macrophages and our aim was to see whether splenectomy acts beneficiary on stroke lesion in wild type (WT) mice after middle carotid artery occlusion (MCAO) procedure.

**Materials and methods:** So far, for purpose of preliminary results, we used 16 WT mice that we subdivided into 4 groups - 3 sham operated for both procedures, 2 sham operated for splenectomy and with MCAO, 5 splenectomized and sham operated for MCAO, and 6 operated for both splenectomy and MCAO. After initial MRI scan and bioluminescence imaging before the procedures, mice were subjected to splenectomy the other day, and scanned with MRI and bioluminescence 13 days after. On 14th day mice were subjected to MCAO procedure, and again scanned with both MRI and bioluminescence on days 1, 2, 7, 14 and 28. Stroke size was calculated manually with two independent examiners for every mouse.

**Results:** There were no significant differences between the groups. However, we noticed a tendency of smaller stroke size in those splenectomized on day 1 and day 2. There were no other differences between the groups on neither bioluminescence nor MRI.

**Discussion:** Although no significant differences were shown, our study so far indicates a tendency towards decrease of stroke size when splenectomized. The possible mechanism could be diminished inflammation in the brain due to lesser amount of circulating macrophages that had their origin in spleen and consequent lesser brain tissue damage.

**MeSH/Keywords:** stroke, inflammation, wild type, bioluminescence, MRI, splenectomy, MCAO.

**Poster code:** R-01-08-067

**Poster Title:** The role of uroguanylin in ischemic stroke

**PhD candidate:** Martina Ratko

**Part of the thesis:** The role of uroguanylin in ischemic stroke development

**Mentor(s):** Assoc. Prof. 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. 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. Our aim was to investigate the potential role of uroguanylin (UGN), a well-known agonist of GC-C, in the development of ischemic stroke.

**Materials and methods:** Lesion volumes were characterized by MR imaging and neurological impairment testing 24 h after MCAO in male wild type (WT) mice and mice lacking the gene for either the hormone (UGN KO) or its receptor (GC-C KO). Two days after MCAO, intracellular Ca<sup>2+</sup> concentrations were measured in astrocytes in slices of ipsilateral and contralateral hemispheres following UGN (100 nM) administration. Astrocytes were determined by SR101 (5 μM) staining.

**Results:** GC-C KO, but not UGN KO, mice develop smaller lesions compared to their WT littermates. Lesion sizes correlate positively with oedema in all tested animals. The results of neurological impairment testing are in positive correlation with both lesion and oedema sizes in all tested animals except GC-C KO mice. On the other hand, even though the size of the lesion in UGN KO animals does not differ from their WT littermates, they have higher neurological deficits. WT animals exhibit a stronger Ca<sup>2+</sup> response to UGN stimulation in the ischemic penumbra than in similar regions of the cortex of the unaffected hemisphere. This stronger activation is gone in GC-C KO animals. When compared to their WT littermates there is a statistically significant reduction in Ca<sup>2+</sup> signalling in the ischemic penumbra. UGN KO animals show the same Ca<sup>2+</sup> response as WT animals in the area affected by stroke, but the signal is significantly longer (~ 15 s). As shown in WT animals, UGN KO animals exhibit a stronger Ca<sup>2+</sup> response to UGN stimulation in the ischemic penumbra than in similar region of the cortex of the unaffected hemisphere.

**Discussion:** The UGN signalling pathways are changed during stroke and affect the size of the lesion and severity of stroke symptoms. In astrocytes, the Ca<sup>2+</sup>-dependent signalling pathway is more active in the ischemic region but this effect is gone in GC-C KO mice who present smaller ischemic lesions, suggesting potentially harming effects of this activation. The hypothesis is supported also in UGN KO mice where the difference between ischemic and healthy hemispheres still exists and there is no difference in stroke volume when compared to WT littermates. The possible mechanism could be increased UGN activity on pH regulation in astrocytes via Ca<sup>2+</sup> signalling pathway and worsening of existing interstitial post ischemic acidosis. The involvement of cGMP dependent signalling pathway via GC-C will be further investigated.

**Acknowledgments:** Funded by project "Experimental and clinical research of hypoxic-ischemic damage in perinatal and adult brain" (GA KK01.1.1.01.0007) funded by the European Union through the European Regional Development Fund

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

**Poster code:** R-01-08-077

**Poster Title:** Dentate granule cells of TNF-deficient mice show alterations in dendritic spine density and spine head morphology

**PhD candidate:** Dinko Smilović

**Part of the thesis:** Morphological analysis of the dendritic tree and the expression and localization of actin-modulating protein synaptopodin in hippocampal granule cells of TNF-alpha KO mice

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

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Dendritic spines are sites of synaptic plasticity. The size of the spine head is tightly correlated with AMPA-R density and, thus, the strength of synaptic transmission. Since TNF, an inflammatory cytokine, influences synaptic transmission as well as synaptic plasticity and since synaptic strength and spine geometry are linked, we speculated that TNF-deficiency should cause structural changes. To address this question, we used perfusion fixed sections of adult male wildtype and TNF-/- mice and injected granule cells with the fluorescence dye. Sections were also immunolabeled for the actin-modulating protein Synaptopodin, which is an essential component of the spine apparatus.

**Materials and methods:** Adult male mice (10-26 weeks) lacking TNF- $\alpha$  (TNF-KO, C57BL/6J background, and their respective wild-type mice were used for the ex vivo analysis of granule cell spines in fixed slices. Animals were killed with an overdose of intraperitoneal Pentobarbital and subsequently intracardially perfused. Brains were taken out, post-fixed (18 h, 4% PFA in 0.1 M PBS, 4 C), washed three times in ice-cold 0.1 M PBS and sectioned (250 $\mu$ m) on a vibratome. Intracellular injections of granule cells in fixed slices were performed. Free-floating sections were washed several times, incubated in a blocking buffer for 30 min at room temperature (RT) and subsequently incubated with guinea pig anti-synaptopodin (SP, 1mg/mL, 1:2000, Synaptic Systems) for 3 days at RT. Sections were incubated with donkey anti guinea pig Alexa Fluor 488 (1mg/mL, 1:2000, Dianova) for 4 h at RT. Confocal imaging of fixed dendritic segments from identified, dentate granule cells in the outer molecular layer (OML) was done with an Olympus FV1000 microscope and a 60x oil-immersion objective (UPlanSApo, NA 1.35, Olympus). Image processing and data analysis were then performed using Fiji version 1.52h with spine analysis adapted from published criteria (Holtmaat et al. 2009). All statistical tests were performed using GraphPad Prism 6. If P values were less than 0.05 the null-hypothesis was rejected. Statistical values were expressed as mean  $\pm$  standard error of the mean (SEM) unless otherwise stated. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001.

**Results:** TNF-alpha-knock-out animals have a 20% lower density of dendritic spines (1.62 1/ $\mu$ m) when compared to their wildtypes (2.04 1/ $\mu$ m). \*p = 0.0166, Mann-Whitney U-test. Spine head sizes of TNF-WT and TNF-KO mice do not differ. n.s., not significant; p > 0.99, Mann-Whitney U-test. Number of segments n = 21 per genotype (7 animals; 3 cells per animal; 1633 TNF-WT spines, 1306 TNF-KO spines). Fraction of SP- and SP+ spines in TNF-KO animals (15% SP+) and their respective wildtypes (14% SP+). \*\*\*p < 0.001, Mann-Whitney U-test. SP- spines in TNF-WT: n = 1467, in TNF-KO: n = 1158; SP+ spines in TNF-WT: n = 165, TNF-KO: n = 147. Mean spine head size of SP+ and SP- spines. (TNF-WT 0.35  $\mu$ m<sup>2</sup> SP+, 0.15  $\mu$ m<sup>2</sup> SP-; TNF-KO 0.45  $\mu$ m<sup>2</sup> SP+, 0.15  $\mu$ m<sup>2</sup> SP-). Compared to wildtypes, SP+ spines are 33% larger in size in TNF-KO animals. \*\*\*p < 0.001, Mann-Whitney U-test. Correlation analysis between spine head size and SP cluster size reveals a tight positive connection. TNF-WT: Spearman r = 0.534, Linear regression analysis: R<sup>2</sup> = 0.286; TNF-KO: Spearman r = 0.344, Linear regression analysis: R<sup>2</sup> = 0.118.

**Discussion:** Compared to WT dendritic segments, TNF-/- segments had 20% fewer spines. These segments, however, also exhibited larger Synaptopodin-clusters and larger Synaptopodin-positive spines. This combination of structural changes is similar to the structural homeostatic adaptations observed in dentate granule cells following experimental denervation. In sum, we propose that TNF-deficiency results in fewer dendritic spines. This loss of spines is homeostatically compensated for by an increase in the head size of large Synaptopodin-positive spines. In all analyzed knockout animals and their wildtypes, we found that spine head size is highly (p < 0.001) correlated with SP cluster size, establishing a clear connection between the amount of SP in a dendritic spine and its size. Investigating granule cells of TNF-deficient animals will help to shed light on the role of the receptors involved in these structural alterations.

**Acknowledgments:** We thank Charlotte Nolte-Uhl and Anke Biczysko for technical support. We also thank Meike Hick and Michael Rietsche for methodological training and problem shooting.

**MeSH/Keywords:** Hippocampus, tumor necrosis factor-alpha, dendritic spine, granule cell, synaptopodin

**Poster code:** R-01-08-093

**Poster Title:** Do subthalamic and substantia nigra neurons share common neuronal lineage?

**PhD candidate:** Ema Bokulić

**Part of the thesis:** The developmental origin of subclasses of subthalamic neurons

**Mentor(s):** Assist. Prof. Goran Sedmak, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** The subthalamic nucleus (STN) is a small, biconvex nucleus in the diencephalon, lying rostrally from the internal capsule to substantia nigra caudally. This small brain structure gained new importance with the development of deep-brain stimulation as a surgical technique for alleviating symptoms of movement disorders. Interestingly, there is no consistent data regarding its development, molecular markers, and their spatial distribution. To this day, two theories have been postulated about the developmental origin of the STN. One theory proposes that the STN has diencephalic origin with its neurons arising from the separate longitudinal subthalamic zone between the ventral thalamus and the hypothalamus, while the other suggests that the nucleus originates from the hypothalamic germinative zone lying caudally from the mammillary recess. Novel studies investigating the development of mesencephalic dopaminergic (mesDA) neurons suggested, based on the expression profile of several transcription factors, that STN and mesDA neurons may have a common neuronal lineage. However, the majority of published studies were conducted using mouse brain, therefore leaving the question of possible interspecies differences unanswered. To further explore these developmental theories, we employed immunohistochemical staining to study the expression of several transcription factors in the STN and substantia nigra (SN) of adult mouse and rat brain.

**Materials and methods:** Our research was conducted on adult brains obtained from mouse (C57BL/6 strain) and rat (Wistar Han<sup>®</sup>( RccHan<sup>®</sup>WIST)). Adult male animals were anesthetized and transcardially perfused with phosphate buffer saline (1xPBS) followed by 4% paraformaldehyde (PFA). The brains were removed from the skull, postfixed, and dehydrated in an increasing series of alcohol and toluene. Whole-brain sections were embedded in paraffin and sectioned in coronal plane on a microtome at 10 µm for mouse and 12 µm for rat. Nissl staining was done to demonstrate the overall anatomy, and immunohistochemical staining was done to show the expression of specific neuronal markers: rabbit anti-Foxp2 (Abcam, ab16046, dilution 1:3000), rabbit anti-Barhl1 (NovusBiologicals, NBP1-86513, dilution 1:500), and rabbit anti-TH (Thermo Fisher, PA5-85167, dilution 1:100). Sections were digitalized using Hamamatsu NanoZoomer 2.0 RS.

**Results:** Firstly, BARHL1 immunoreactivity can be clearly observed in the STN of both mouse and rat, whereas the SN lacks BARHL1-immunoreactive cells. We observed FOXP2-immunoreactive cells in both the STN and SN of adult mouse and rat, but the expression pattern was different. The STN of both mouse and rat has a more dense population of FOXP2 positive cells.

**Discussion:** These preliminary results are in line with previous research on transcription factors regulating the development of mesDA and STN neurons. Our results propose at least two different sets of molecular markers, one that clearly defines glutamatergic fate (STN neurons) and the other set of mutual dopaminergic (SN neurons) and glutamatergic markers. However, these experiments should be carried out on a larger sample with more transcription factors analyzed. Further advancements in understanding human brain development could be made if these markers are analyzed in human fetal and adult brain tissue.

**Acknowledgments:** This research is supported by the Croatian Science Foundation grant UIP-2017-05-7578 and the Scientific Centre of Excellence for Basic, Clinical and Translational Neuroscience.

**MeSH/Keywords:** Dopaminergic neurons, neuronal lineage, transcription factors, subthalamic nucleus, substantia nigra

**Poster code:** R-01-08-096

**Poster Title:** The effects of diabetes mellitus on brain and retinal ischemia

**PhD candidate:** Anja Barić

**Part of the thesis:** Diabetes mellitus affects the development of edema and microglial response in a mouse model of brain and retinal ischemia by changing the expression of bradykinin receptors

**Mentor(s):** Assist. Prof. Marina Radmilović, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** One out of three patients suffering from diabetes mellitus (DM) develops brain or retinal ischemia due to stroke or diabetic retinopathy. Preexisting hyperglycemia exacerbates the ischemic lesion formation, slowing down recovery and increasing mortality in stroke patients with DM. Even though DM is a known risk factor for brain and retinal ischemia, it is rarely modeled in basic stroke research. The aim of the current study was to determine the effects of DM on brain and retinal ischemic lesion development in a mouse stroke model.

**Materials and methods:** Brain and retinal ischemia were induced in 2-4 months old male diabetic C57BL/6-Ins2Akita/J (Akita) and wild type C57BL6J (WT) mice by 30-minute intraluminal middle cerebral artery occlusion (MCAO) followed by reperfusion. Before the experiment, the presence of diabetes was confirmed by intraperitoneal glucose tolerance test. Seven days before MCAO and on the 1st and 3rd day after MCAO induction, the animals were subjected to fluorescein angiography in order to visualize the changes in retinal vasculature, and then scanned using a 7T BioSpec 70/20 USR magnetic resonance imaging system. The images obtained by a high-resolution T2-weighted anatomical scan of the brain and the ipsilateral eye were analyzed in ImageJ to determine the volume of the brain hemispheres and chorioretinal thickness while a T2-map scan was used to determine the size of the ischemic brain lesion. Both groups were scored for neurological deficit. At both post-MCAO timepoints, the animals were perfusion-fixed, after which the brains and eyes were isolated and processed for histological analysis.

**Results:** Our preliminary results showed no difference in survival or neurological deficit between the two groups after MCAO. However, both groups showed marked swelling of the ipsilateral hemisphere caused by the formation of vasogenic edema on the 1st and 3rd day after MCAO which was slightly more prominent in diabetic mice. Differences observed in brain edema development were also present in the eye, showing a more pronounced edema induced thickening of the ipsilateral chorioretinal layer in diabetic compared to WT mice. Images of retinal vasculature obtained by fluorescein angiography on the 1st day after MCAO failed to show fluorescein extravasation, however histological analysis confirmed that the ipsilateral retinas were more edematous in diabetic compared to controls. On the 1st day after MCAO a severe disruption in the layer of rods and cones and ganglionic layer where cells were reduced in numbers and affected by cytotoxic edema was evident only in diabetic Akita mice.

**Discussion:** Our preliminary data suggests that the presence of diabetes exacerbates both brain and retinal ischemia by promoting tissue swelling. Up to first three days after ischemia the presence of diabetes did not have any effect on survival or neurological deficit. In both Akita and WT mice the ischemic changes in the brain and retina were most severe on the 1st day after MCAO, however further experiments are needed to define if the differences observed in Akita mice were significant. Inclusion of comorbidities in animal models of brain and retinal ischemia may help us emulate their complexity in humans, where they usually arise as complications from a pre-existing condition such as diabetes.

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

**MeSH/Keywords:** diabetes mellitus, brain, retina, ischemia, edema

**Poster code:** R-01-08-112

**Poster Title:** The role of subplate neurons and white matter interstitial neurons in pathogenesis of epilepsy

**PhD candidate:** Petra Nimac Kozina

**Part of the thesis:** The role of subplate neurons and white matter interstitial neurons in pathogenesis of epilepsy

**Mentor(s):** Assist. Prof. Goran Sedmak, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Interstitial neurons of white matter are a large, but insufficiently explored group of neurons located beneath the cerebral cortex, between the bundles of white matter. The exact role of interstitial neurons in the functioning of the cerebral cortex has not been proven yet. Interestingly, in many neurological and psychiatric disorders the number, distribution, and density of interstitial neurons are altered. Epilepsy is one of the disorders where alterations of interstitial neurons have been observed. In the brain of people with epilepsy, it is often possible to find an increased number of interstitial neurons. According to classical authors, these neurons are cortical neurons that are not located in their correct position during migration. Although there is no single evidence that increased number of interstitial neurons in the white matter is the consequence of disorder during the migration of neurons, with the exception of clearly cited cortical malformations due to similar disorders (eg focal cortical dysplasia), today's prevailing opinion is that those are residual and aberrantly located cortical neurons.

**Materials and methods:** In the research, we are using post-mortem samples of the normal fetal and adult human brains which are part of versatile Zagreb Neuroembryological collection. All analyzed samples are without known neurological or psychiatric disorders and without a history of substance abuse or long-term use of psychoactive medications. Analysis of the epileptic samples is conducted on the post-operative tissue samples collected during indicated neurosurgical procedures for the treatment of pharmaco-resistant epilepsy. The samples are part of the University of Zagreb School of Medicine Department of Pathology and Clinical Hospital Centre Zagreb Department of Pathology. All used samples were collected with the prior ethical approval of IRB. We analyze three fetal brains per stage (15 – 18 PCW; 24 – 28 PCW and 35 PCW – Newborn) and five adult brains (age 30 – 60 years). We also analyze 10 adult samples from patients with epilepsy (age 30 – 60 years). To elucidate the molecular profile of neurons we are using classical histological methods (e.g. Nissl and NeuN), immunocytochemistry, in-situ hybridization, and RNAscope for different biochemical markers. The number of a subpopulation of subplate neurons (fetal brain) and white matter interstitial neurons in healthy controls (adult brain) and samples of patients with epilepsy is analyzed by a stereoscopic method using the StereoInvestigator computer program (MBF Inc.USA).

**Results:** Considering the characteristics of our research, as well as the limitations of neuroscientific histological research in general, our preliminary results cannot be expressed numerically. At this point, our results exist in the form of histological and immunocytochemical samples of the human brains. We are using classical histological methods (Nissl and NeuN), immunocytochemistry, in-situ hybridization, and RNAscope for different biochemical markers. The number of a subpopulation of subplate neurons using the StereoInvestigator computer program has not been conducted yet.

**Discussion:** The expected scientific contribution of the study will be a better understanding of the developmental origin of the interstitial neurons of white matter, the survival of the transient population of subplate neurons and their role in the pathogenesis of epilepsy.

**MeSH/Keywords:** subplate, interstitial neurons of white matter, epilepsy, human brain, cerebral cortex

**Poster code:** R-01-08-115

**Poster Title:** Tracing murine retinal vasculature using custom smartphone fluorescein angiography and automated image processing platform

**PhD candidate:** Marin Radmilović

**Part of the thesis:** The role of bradykinin in the development of diabetic macular edema

**Mentor(s):** Assoc. Prof. Aleksandra Dugandžić, MD PhD, Professor Zoran Vataavuk, MD PhD

**Affiliation:** University of Zagreb School of Medicine, Croatian Institute for Brain Research; Sestre milosrdnice University Hospital Center, Department of Ophthalmology

**Introduction:** Fluorescein angiography is a fundamental tool in retinal research due to its ability to provide qualitative and quantitative information on vascular morphology and function in real time and in vivo. The available commercial platforms for small animal fluorescein angiography are costly, and although regularly upgraded, usually still require additional customization for individual research-specific analyses. The aim of this study was to establish a simple, reliable, and affordable fluorescein angiography platform for mice and to develop automated image analysis software for detection and tracing of retinal vessels with quantification of their width, length and tortuosity.

**Materials and methods:** A table-mounted operating microscope was converted to a fluorescein angiography platform using a smartphone camera, a set of bandpass filters, and custom 3D-printed add-ons. The add-on with the excitation filter (483 nm) was mounted near the light source with a rotating mechanism. The smartphone camera was aligned with one of the ocular lenses of the microscope by an add-on which incorporated a sliding mechanism for the emission filter (535 nm). The mouse holder was carved from a 50 mL syringe, connected to a rubber tube for inhalational anesthesia, and placed on a separate unit for position adjustment. The image acquisition protocol starts with the anesthetized mouse being placed in the holder. A drop of tropicamide is applied to each eye for mydriasis, followed by a drop of lubricant to prevent drying of the cornea. The mouse is then positioned so that the retinal image is round, with the optic disc at its center and aligned with the optical axis of the microscope. A handheld 90 D lens is held over the eye while the microscope height is adjusted for fine focusing, after which the excitation and emission filters are applied and the mouse is injected with 10  $\mu$ l of 10% sodium fluorescein subcutaneously. The angiograms are then recorded on smartphone in video and photo format for both eyes subsequently. The images without gross artifacts are manually selected and analyzed using the custom image analysis software, which detects and tracks the retinal vessels. The precision of the detection algorithm is increased by preprocessing the image using the Perona-Malik anisotropic diffusion equation, where energy is allowed to flow only through a low-gradient change, creating equipotential areas. Radius-sensitive segmentation is done to remove further image shortcomings induced by an uneven light distribution. Final tracking and diameter estimation of retinal vessels is done using Gaussian process and Radon transform.

**Results:** Using our fluorescein angiography platform and the described standard operating procedure we were able to reliably obtain high quality images of the retinal vasculature. The duration of the procedure from the induction of anesthesia to image acquisition was under 20 minutes per animal. With the automated image analysis, which took seconds per image, we were able to consistently trace the vascular network and quantify the length, width and tortuosity of individual vessels.

**Discussion:** Our fluorescein angiography platform and image analysis software allow us to monitor and quantify the retinal vascular status. This could facilitate objective comparisons between animal groups or in the same-animal longitudinal studies for an array of retinal vascular disorders. Additionally, the software allows manual adjustment of a number of parameters which can be used for a tailored approach in case of differences in image quality stemming from animal pigmentation or future changes in image acquisition equipment.

**Acknowledgments:** The study is supported by the Croatian Science Foundation project BRADISCHEMIA (UIP-2017-05-8082). Image acquisition is performed at the Laboratory for Regenerative Neuroscience - GlowLab, University of Zagreb School of Medicine.

**MeSH/Keywords:** fluorescein angiography, computer-assisted image processing, retina

**Poster code:** R-01-08-146



**Poster Title:** Simultaneous induction of brain and retinal ischemia using the middle cerebral artery occlusion method

**PhD candidate:** Helena Justić

**Part of the thesis:** The role of bradykinin receptor type 2 in the development of brain and retinal ischemic lesion

**Mentor(s):** Assist. Prof. Marina Radmilović, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Middle cerebral artery occlusion (MCAO) is commonly used for brain ischemia induction in rodents. Several studies have shown the evidence of retinal dysfunction following MCAO, suggesting simultaneous brain and retinal ischemia. However, the disruption of the retinal blood supply via MCAO remains poorly investigated since there is no consensus on the origin of the ophthalmic artery (OA) in rodents. The aim of the study was to clarify this issue by longitudinal in vivo assessment of vascular perfusion and the resulting cerebral and retinal ischemic injury after MCAO.

**Materials and methods:** A 30-minute MCAO followed by reperfusion and sham operation were performed on male C57Bl/6J mice using short and long silicone-coated filaments. Seven days before and 2, 9 and 35 days after MCAO the animals were scored for neurological deficit and imaged by 7T Bruker BioSpec 70/20 USR magnetic resonance. The MR scans included a high-resolution T2-weighted anatomical scan of the brain and the ipsilateral eye, brain angiography and a T2-map scan of the brain. The obtained images were analyzed in ImageJ using standardized manual segmentation pipelines for brain hemisphere and lesion volume and chorioretinal thickness measurements. The animals were sacrificed after the last imaging session, and the brains and eyes were isolated and processed for histological analysis.

**Results:** Brain angiograms showed that MCAO procedure significantly reduced the blood flow to the middle cerebral artery (MCA) and completely reduced the blood flow through the pterygopalatine artery (PA), which gives rise to the OA in our C57Bl/6J mice. This effect was present regardless of the filament length. Sham procedure caused a partial reduction in blood flow through the internal carotid artery (ICA) and PA. Both filaments caused a severe cortico-striatal lesion development, characterized by vasogenic edema formation 2 days after MCAO and loss of tissue in the chronic phase. The anatomical scan of the eye had sufficient spatial resolution but inadequate contrast to distinguish between retinal and choroidal layers. Radially measured chorioretinal thickness changes correlated with the brain ischemia progression, showing significant thickening 2 days after MCAO, followed by edema resolution and retinal thinning in the chronic phase. Hematoxylin/eosin stained retinal slices demonstrated morphological disruptions of the ipsilateral retinas with pronounced loss of cells in the ganglion cell layer, thinning of the inner plexiform layer and complete loss of the outer plexiform layer 35 days after MCAO.

**Discussion:** Although there is evidence of OA occlusion during MCAO, none of the studies investigated the anatomical origin of the OA in mice or the status of vascular perfusion after occlusion. As showed by brain angiograms, MCAO leads to simultaneous occlusion of the MCA and the OA in C57Bl/6J mice, regardless of the filament length. The size of the filament used in our case did not produce a significant difference in lesion evolution, size or resolution. The chorioretinal thickness measurements correlated with the brain volumetric data, showing swelling in the acute phase due to edema development, followed by thinning and cell loss in the chronic phase. Histological analysis of the retinal slices confirmed the extensive damage to the ipsilateral retina. Partial reduction of the blood flow through the PA after the sham procedure suggests that the ligation of the common carotid artery (CA) alone might induce retinal ischemia. However, further experiments are needed to define the extent of the damage by CA ligation. Our preliminary data suggest that MCAO method could be used as a suitable model for simultaneous induction of brain and retinal ischemia.

**Acknowledgments:** The study is supported by the Croatian Science Foundation project BRADISCHEMIA (UIP-2017-05-8082) and the European Regional Development Fund. Multimodal imaging was done at Laboratory for Regenerative Neuroscience, University of Zagreb School of Medicine.

**MeSH/Keywords:** Ischemia, middle cerebral artery, brain, retina, ophthalmic artery

**Poster code:** R-01-08-159

Clinical medical sciences - preliminary research results

**Poster Title:** Comparison of efficacy of biofeedback, electrical stimulation and therapeutic exercise in patients with knee osteoarthritis

**PhD candidate:** Silvija Mahnik

**Part of the thesis:** Comparison of efficacy of biofeedback, electrical stimulation and therapeutic exercise in patients with knee osteoarthritis

**Mentor(s):** Assoc. Prof. Ivan Bojanić, MD PhD, Ana Aljinović, PhD, research associate

**Affiliation:** University of Zagreb School of Medicine, Department of Orthopaedic surgery, Clinical Hospital Center Zagreb

**Introduction:** Osteoarthritis is a chronic degenerative joint disease that can interfere with any joint in the body and knee is the most common localization. Knee OA treatment can be operative and non-operative. Non-operative treatment includes pharmacological treatment, changing life style and physical therapy. The goal of physical therapy in knee OA is to reduce pain and improve knee function through therapeutic exercise, especially by strengthening the quadriceps muscle. In addition to therapeutic exercise, muscle electrical stimulation is often used, and in the literature there is evidence of biofeedback therapy efficacy. Electrical stimulation refers to the application of electrical current in order to achieve muscle contraction. Biofeedback therapy is a method where muscle contraction is displayed as a sound or visual signal for the purpose of increasing or decreasing voluntary muscle activity. Strength training with biofeedback may be useful in maximising the recovery potential of patients with knee OA.

**Materials and methods:** 93 patients with knee OA according to ACR criteria and Kellgren and Lawrence radiological classification grades 1 and 2 will be included in study. Patients will be randomized in three groups. The first group will perform kinesiotherapy and biofeedback training, second group will perform kinesiotherapy and electrical stimulation of the quadriceps muscle and third group will only perform kinesiotherapy. All patients will complete: Visually Analogous Pain Scale (VAS), Western Ontario Universities Osteoarthritis Index (WOMAC), 36 Item Short Form Health Survey (SF 36), International Classification of Functioning, Disability and Health (ICF) osteoarthritis core set, and quadriceps muscle strength will be measured by EMG biofeedback device. Clinical examination will be performed on the first day, day 21, after 90 and after 180 days.

**Results:** From the planned final sample size of 93 subjects, in the initial phase of the study we included a pilot sample of 6 participants. Included were 3 female and 3 male participants with mean age of 68,8 years (range 55-75 years) and mean BMI of 33,7 kg/m<sup>2</sup> (range 27,9-38,1 kg/m<sup>2</sup>). Outcome of physical therapy in knee osteoarthritis was measured by means of VAS and WOMAC scales, ICF classification and SF-36 questionnaire. Additionally, we measured strength of quadriceps muscles using biofeedback device. Due to the blinded design of the study, here we discuss only the overall results in all three subject groups. A statistically significant improvement ( $p < 0,01$ ; Wilcoxon test) over the first three weeks of therapy was seen for all the mentioned parameters. Pain (VAS) decreased from mean of 5,7 +/- SD of 1,46 cm to 3,3 +/- 1,48 cm. Similarly, WOMAC decreased from the mean total score of 42,2 +/- 11,41 to 34,3 +/- 9,89 points. Over the same time period, muscle strength (BFB) increased from the mean of 37,5 +/- 2,72 mV to 41,0 +/- 4,27 mV. Regarding the ICF core set, we found that the biggest problems were in categories: sensation of pain, mobility of joint functions, muscle power functions, walking and dressing. Smallest problems were categories structure of upper extremity and products and technology for personal use in daily living. Finally, statistically significant differences in quality of life ( $p < 0,05$ ; Wilcoxon test) were found between first and second measurements, after 3 weeks of treatment. Analysed were physical and mental health values of the SF-36 scales.

**Discussion:** We expect to find that kinesiotherapy and biofeedback therapy are better than kinesiotherapy and electrical stimulation and kinesiotherapy alone in reducing pain, improving knee function and strengthening quadriceps muscle.

**MeSH/Keywords:** knee osteoarthritis, physical therapy, biofeedback, electrostimulation

**Poster code:** R-02-03-003

**Poster Title:** Diagnostic ultrasound in monitoring patients with rheumatoid arthritis compared to standard clinical disease activity indices

**PhD candidate:** Valentina Delimar

**Part of the thesis:** Diagnostic ultrasound in monitoring patients with rheumatoid arthritis compared to standard clinical disease activity indices

**Mentor(s):** Assoc. Prof. Porin Perić, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Rheumatoid arthritis (RA) is a chronic inflammatory disease that causes joint damage and severe functional disability. With the implementation of the treat-to-target strategy, disease control has substantially improved. The standard methods of RA monitoring include Disease Activity Index 28 (DAS 28), Simplified Disease Activity Index (SDAI) and Clinical Disease Activity Index (CDAI). Diagnostic ultrasound (US) is recommended as an additional tool in RA management, yet there are no official criteria for the needed number of assessed joints or the frequency of US evaluation. Also, there is a great heterogeneity of existing US scoring systems. Therefore, US is still not incorporated into disease activity indices or classification criteria for RA.

**Materials and methods:** One hundred and nine RA patients treated at the Clinic for Rheumatic Diseases and Rehabilitation, University Hospital Centre Zagreb, were included in the study after signing the informed consent. The study protocol comprised of initial and control examination after approximately 3 months. On study entrance, we collected patient history and demographic data and obtained plain radiographs of hand and feet. On initial and control examination we determined ESR and CRP, patients underwent clinical examination for tender and swollen joint count based on 28 joints, determined their pain level, general health and disease activity on a visual analogue scale (VAS) and filled the Health Assessment Questionnaire (HAQ) and Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F) questionnaire. US examination was done according to the standardized EULAR protocol. We examined elbows, radiocarpal joints, second and third metacarpophalangeal and proximal interphalangeal joints, knees, ankles, second and fifth metatarsophalangeal joints on both sides for signs of synovitis and erosions, as well as 2nd, 4th and 6th wrist extensor compartment, finger flexor digitorum superficialis and profundus tendons 3 and 4, peroneal tendons and tibialis posterior tendon for signs of tenosynovitis. Additionally, every clinically swollen or tender joint or tendon outside the initial set was examined. Synovitis and tenosynovitis were scored according to the latest EULAR/OMERACT scoring system and erosions according to the binary scoring system. DAS 28, SDAI and CDAI scores were calculated. Given the small sample processed, only descriptive statistics were used to present current results, using Shapiro Wilks for the assessment of the normality of data.

**Results:** So far we have processed the data of the initial examination of 33 patients, 30 female and 3 male, mean age  $55.06 \pm 10.13$  years. The majority of patients ( $n=25$ ) were on sDMARD therapy, mostly methotrexate, and eleven patients were on bDMARD therapy. The mean VAS pain score was  $4.12 \pm 2.4$ , the general health score was  $3.83 \pm 2.37$  and the patient disease activity score was  $3.91 \pm 2.51$ . HAQ score showed that only 2 patients had severe disability, while 15 and 16 patients had mild or moderate disability, respectively. Only 7 patients were in sonographic remission (no PD signal, no active erosions,  $SH < 2$ ), while 5 patients were in remission according to DAS 28 (ESR) score, 7 patients according to DAS 28 (CRP) score, 3 patients according to CDAI score and none according to SDAI score.

**Discussion:** In this research phase, we cannot comment on the discrepancy not only between the sonographic remission and remission according to standard clinical disease activity indices but also on the obvious discrepancy between the remission regarding standard indices themselves. Once we incorporate and analyze all the raw data we will be able to determine the value of diagnostic US in monitoring RA patients and the relation of US findings with the patient's functional status. Based on the obtained data we aim to propose a new composite scoring system based on US detected synovitis, HAQ and DAS 28 scores, and a model of CroUS joint count.

**Acknowledgments:** I would like to thank my mentor and all the staff at the Clinic for Rheumatic Diseases and Rehabilitation for support during the research.

**MeSH/Keywords:** rheumatoid arthritis, ultrasound

**Poster code:** R-02-03-078

**Poster Title:** PROGESTERONE LEVELS IN EARLY FOLLICULAR PHASE AND IVF OUTCOME IN POOR RESPONDERS

**PhD candidate:** Iva Pitner

**Part of the thesis:** The comparison of fixed and flexible antagonist protocol and IVF outcome in poor responders

**Mentor(s):** Professor Marina Šprem Goldštajn, MD PhD, Assoc. Prof. Krunoslav Kuna, MD PhD

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

**Introduction:** Based on Bologna criteria, patients who comply two out of three following criteria are defined as poor responders: age  $\geq$  40 years, no more than 3 oocytes retrieved in last cycle or abnormal ovarian test results (AFC $<$ 5-7, AMH  $<$ 0,7-1,3ng/ml). Evidence suggests that altered progesterone and estradiol levels during ovarian stimulation could predict poor pregnancy outcome. Majority of authors consider normal progesterone levels of  $\leq$ 1.6ng/ml ( $\leq$ 5.1nmol/L). Although in literature there is compelling evidence that connects estrogen, progesterone and LH levels with IVF outcome, patients with poor ovarian response present a special group in who more research needs to be conducted.

**Materials and methods:** In total, 92 patients receiving IVF treatment and meeting the criteria for poor responders defined by Bologna criteria, at the University Hospital Centre Zagreb from January 2015 to December 2018 were included in the study. Outcome measure was clinical pregnancy rate (CPR). Hormonal assessment of every patient was made on day 2 of stimulation. Patients in which progesterone was above 5,1 nmol/l were excluded from the study. Serum levels of progesterone were measured with the Ortho Diagnostics, Johnson&Johnson. For statistical analysis Chi-square test was calculated. Statistical significance was set at  $P<$ 0,05.

**Results:** Patients were classified into three groups according to progesterone concentrations on cycle day 2: group A (P4 0-0,9 nmol/L), group B (P4 1.0-1.9 nmol/L), group C (P4 2.0-> nmol/L). In each group clinical pregnancy rate was measured. Eleven patients met the criteria for group A, 65 for group B and 16 for group C. Clinical pregnancy rate in group A was 18% (2 out of 11 patients), in group B 21% (14 out of 65) and in group C 31% (5 out of 16). Although CPR was higher in group C, the difference was not statistically significant.

**Discussion:** Various protocols of ovarian stimulation in assisted reproductive technology are being used. Functional luteolysis at the end of the menstrual cycle is characterized by decreasing synthesis of progesterone in corpus luteum and consequential lower serum progesterone levels. In the long agonistic protocols, due to the suppression of gonadotropins and achievement of basal levels of steroid hormones prior to initiation of stimulation, most patients starting stimulation have normal progesterone levels. However, in the antagonistic and short agonistic protocols the efficacy of luteolysis and serum progesterone levels at the beginning of stimulation, are becoming more important. Elevated basal P levels probably caused by inefficient or incomplete luteolysis in those patients have been reported. Majority of authors consider normal progesterone levels of  $\leq$ 1.6ng/ml ( $\leq$ 5.1nmol/L). There are some doubts about the influence of basal progesterone levels in antagonistic protocols and results of IVF/ICSI procedures. Although it is known that progesterone levels above 5,1 nmol/l are associated with lower pregnancy rates, the significance of lower progesterone concentration is controversial. Most recent ESHRE guidelines state that assessment of progesterone level on day 2 of the cycle at the controlled ovarian stimulation is probably not recommended due to very low incidence of abnormal test results in general population, however they leave decision under consideration for patients  $>$ 39 years of age. A recent meta-analysis showed the estimated pregnancy rate for poor responders was 14,8% compared with 34,5% in normal responders. Significantly higher P levels in the early follicular phase of a spontaneous cycle have been demonstrated in women who had a poor response, possibly caused by continued production by the corpus luteum seen in aging ovaries. The question is, is there progesterone level in patients with poor ovarian response that could predict IVF outcome. At our knowledge, until today, no such a research has been conducted. The purpose of this study was to show if there is a threshold of progesterone on cycle day 2 of ovarian stimulation that could predict IVF outcome in poor responders. In this study, although the pregnancy rates were slightly higher in group C (P4 2.0-> nmol/L), the difference was not statistically significant. Further studies and larger sample are needed to show weather specific progesterone level could predict outcome in protocols of controlled ovarian stimulation in poor responders.

**MeSH/Keywords:** progesterone, poor responder, ovarian stimulation, female infertility

**Poster code:** R-02-05-099

**Poster Title:** Comparison of intracervical and intravaginal application of prostaglandin E2 for induction of labour in term pregnancies with unfavourable cervix

**PhD candidate:** Katja Vince

**Part of the thesis:** Comparison of intracervical and intravaginal application of prostaglandin E2 for induction of labour in term pregnancies with unfavourable cervix

**Mentor(s):** Professor Ratko Matijević, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Induction of labour is defined as initiation of labour before its spontaneous onset and is present in up to one in four deliveries in developed countries.<sup>1,2</sup> One of the most often used formulations for induction of labour in women with unfavourable cervix is prostaglandin E2 (PGE2, dinoprostone), which has been proven to be a safe and efficient formulation.<sup>3</sup> Dinoprostone can be applied as an intracervical (IC) or intravaginal (IV) formulation and studies remain indecisive which formulation is more efficient and safe. In a Cochrane Systematic Review from 2008, IC PGE2 was inferior to IV PGE2 with higher risk of not achieving vaginal delivery within 24 hours and the same risk of caesarean section or uterus hyperstimulation.<sup>4</sup> On the other hand, in a randomised controlled trial from 2014, IC application was shown to have a shorter induction to delivery time compared to IV application, while rates of adverse perinatal outcomes were similar.<sup>5</sup> The aim of this study was to compare IC and IV application of PGE2 for labour induction in term pregnancies with unfavourable cervix.

**Materials and methods:** This is a prospective randomised trial performed at University Hospital Merkur between December 2018 and April 2020. The trial included 212 pregnant women with term pregnancies, indication for labour induction and unfavourable cervix who were randomly assigned for labour induction with either IC (0.5mg) PGE2 or IV (2mg) PGE2. Main outcome assessed was existence of a difference of 4 hours or more from labour induction to delivery between two groups. Difference in adverse perinatal outcomes between two studied groups was also investigated. Statistical significance was defined as  $p < 0.05$ .

**Results:** Among 212 women randomised in this trial, drop-out rate was 8.5% and a total of 96 in IC group and 98 in IV group were analysed. The studied groups did not significantly differ regarding main characteristics such as age, parity, body mass index or Bishop score. Women in IV group compared to in IC group had a significantly shorter induction to delivery time ( $p < 0.001$ , Mann-Whitney U test), induction to active phase time ( $p = 0.001$ , Mann-Whitney U test), while there was no difference regarding active phase to delivery time ( $p = 0.934$ , Mann-Whitney U test). Median of induction to delivery time was 28 hours for IC group and 15 hours for IV group. Prevalence of vaginal delivery within 24 hours of labour induction was higher in IV group compared to IC group (63,3% vs 40,6%,  $p = 0.002$ , chi2 test) as well as prevalence of successful induction of labour (95,9% vs. 86,5%,  $p = 0.020$ , chi2 test). A total of 24 women in each group delivered by caesarean section, more women in IC group had instrumental vaginal delivery compared to IV group but difference in mode of delivery between groups was not significant ( $p = 0.453$ , chi2 test). There were no cases of uterine hyperstimulations or uterine ruptures in the study. More neonates in IV group had 5 minute Apgar score  $< 10$  compared to neonates in IC group, but this difference was not statistically significant.

**Discussion:** Results of this study indicate that IV application of PGE2 leads to shorter induction to delivery time compared to IC PGE2. Also, the two compared PGE2 formulations did not differ significantly regarding adverse maternal and neonatal outcomes suggesting their similar safety profile. IV application of PGE2 gel is more effective than IC application for induction of labour in term pregnancies with unfavourable cervix with no evident difference of adverse perinatal outcomes, mainly caesarean section rate, uterine hyperstimulation, uterine rupture, 5min Apgar score  $< 7$  or  $10$  or admission to neonatal intensive care unit.

**MeSH/Keywords:** induction of labour, unfavourable cervix, Bishop score, prostaglandins

**Poster code:** R-02-05-101

**Poster Title:** The use of lung ultrasound in the diagnosis of acute dyspnoea in emergency department

**PhD candidate:** Adis Keranović

**Part of the thesis:** The use of lung ultrasound in the diagnosis of acute dyspnoea in emergency department

**Mentor(s):** Assoc. Prof. Ivan Gornik, MD PhD

**Affiliation:** University of Zagreb School of Medicine; University Hospital Centre Zagreb Department of Emergency Medicine

**Introduction:** Dyspnoea due acute heart failure is a common presentation in the emergency department. Previous research has shown that acute heart failure is one of the leading causes of hospitalization and that affects 1-3% of the population and about 10% of the elderly (older than 70 years). Rapid and early diagnosis of acute heart failure is challenging, but treatment of heart failure primarily depends on fast diagnostics and the stage of disease. Today, patients are routinely diagnosed with chest X-ray, while NT-proBNP is becoming accepted as a biomarker of cardiac congestion. Unfortunately, lung ultrasound still did not find sufficient use in the emergency department. The most important ultra-sonographic sign of lung congestion are B lines which are reverberation artefacts. Three or more B lines per one intercostal space in one scan indicate the sub pleural component of interstitial syndrome. B lines verification is useful for the identification of a cardiogenic cause of dyspnoea with high sensitivity (94%) and with 92% specificity. There are protocols for using lung ultrasound and the most important is the BLUE protocol. In previous studies lung ultrasound is extremely rarely mentioned as an early method and use of lung ultrasound in congestive heart failure was generally not conducted in emergency medicine.

**Materials and methods:** This study has started at the beginning of 2019 in the Emergency department of Clinical Hospital Centre Zagreb and will include 120 patients both genders presenting with acute dyspnoea in whom congestive heart failure is a suspected cause. After clinical examination the blood sample should be additionally collected in order to determine the NT-proBNP value. Simultaneously investigator will perform a lung ultrasound and afterwards will perform a chest X-ray. The administration of therapy will follow immediately after the lung ultrasound. NT-proBNP will use standard age-dependent cut-off values for exclusion and conformation. Chest X-ray will be performed in the post-anterior projection. Interpretation and reports of the X-ray image will be done by the radiology specialist according to the previously standardized questionnaire. Standardized BLUE protocol for the lung ultrasound examination will be used for the diagnosis of heart failure. Lung ultrasound will be performed independently by two examiners: postgraduate student and another researcher (senior physician). Positive ultrasonic confirmation of acute heart failure is defined as the bilateral existence of 2 or more positive regions with 3 or more B-lines. After diagnostics, we will make a comparison of these three methods.

**Results:** In this preliminary report 17 participants have been included. There were 9 male and 8 female. Median patients age is 78.9. Considering the research objectives, lung ultrasound sensitivity and specificity was made, and the sensitivity of lung ultrasound versus lung X-ray and NT-proBNP was compared. Ultimately, the time to diagnosis was taken. The sensitivity of lung ultrasound is 94% while the specificity is 100%. The lung X-ray sensitivity is 64% while the sensitivity of NT-proBNP is 70%. The time to diagnosis in lung ultrasound is 9.05 minutes, while in lung X-ray is 59.41 minutes.

**Discussion:** The survey is slow to recruit respondents because of the inclusion criteria. So far, almost equal numbers of male and female respondents have been recruited, with median of 78.9 years. The sensitivity of lung ultrasound is expected and follows the previous literature, while the specificity is higher than the previous literature. Lung ultrasound has proven to be a more sensitive method compared to lung X-ray and NT-proBNP. A significantly shorter time to diagnosis than lung X-ray is required. With this results lung ultrasound has proven to be an excellent method of diagnosing dyspnoea and congestive heart failure.

**MeSH/Keywords:** acute dyspnoea, acute heart failure, lung ultrasound, N-terminal proBrain Natriuretic Peptide, X-ray

**Poster code:** R-02-06-002

**Poster Title:** Does your work ability change over-night? Effect of shift work on Work Ability Index in hospital healthcare professionals

**PhD candidate:** Maša Sorić

**Part of the thesis:** Impact of shift work on changes in arterial stiffness and work ability index among hospital healthcare workers

**Mentor(s):** Assist. Prof. Milan Milošević, MD PhD, senior research associate

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** It has been proven that shift work has many detrimental effects on health but shift work can also affect the work ability of workers. In order to preserve work ability of shift workers, it is necessary to evaluate factors that affect work ability and determine preventative measures for preservation of health and work ability of shift workers. Work ability can be self-assessed using a short validated questionnaire for Work Ability Index (WAI), developed by the Finnish Institute of Occupational Health, consisting of 7 questions. The answers are calculated into a Work Ability Index which (ranging from 7 to 49) is considered to be poor [7–27], moderate [28–36], good (37–43) or excellent (44–49). In this paper we will demonstrate how WAI changes after one night shift in hospital healthcare professionals.

**Materials and methods:** A prospective survey was carried out on all on-call physicians and nurses who work in the Emergency Department of an urban teaching hospital and give their informed consent. Respondents were divided into two groups: nurses who work 12-hour night shifts and residents who work in night shifts of 16 or 24 hours. Each group included at least 46 participants according to the power analysis. The research includes an analysis of sociodemographic and clinical data collected by a questionnaire compiled for this research. The questionnaire contains sociodemographic data (age, sex), information on habits, arterial hypertension, diabetes, dyslipidemia, and data related to the work environment. The working capacity will be assessed by a Work Ability Index Short Version questionnaire by World Health Organization consisting of seven questions - WAI short version (<http://www.arbeitsfaehigkeit.uni-wuppertal.de/index.php?wai-online-en>). Wai short questionnaire is extensively used in work ability research and quality of life assessments in hospital healthcare professionals as well as other shift workers. For linear correlation with normal distribution we used Pearson's correlation coefficient. Statistical analysis will be conducted with IBM SPSS Statistics version 25.0.

**Results:** We calculated Work Ability Index for 47 nurses and 48 doctors. There was no statistically significant difference in gender in those groups (nurses 46.8% male, doctors 46.9% male). Mean WAI for nurses was 40.98 before and 37.15 after a night shift which was a statistically significant decrease  $p < 0.000$  (95% CI 2.39–5.27). While in the doctor group, the mean WAI was 43.02 with a drop to 38.76 after a night shift,  $p < 0.000$  (95% CI 2.95–5.58). Overall, work ability is satisfactory since WAI above 37 is considered good. The decline in WAI after a single night shift for nurses and doctors combined correlates with more frequent diagnosis of dyslipidemia ( $r = -0.213$ ,  $p < 0.037$ ) and diagnosis of hypertension ( $r = -0.216$ ,  $p < 0.034$ ).

**Discussion:** Our results show that even one night shift performed by hospital healthcare professionals can negatively affect their self-assessed work ability. Overall Work Ability Index is similar to Emergency Department hospital healthcare professionals in other urban teaching hospitals. The same paper found that stress at work correlated with worse work ability. Furthermore, it found that night shift was identified as a significant stressor in the work place. In a previous study on nurses, assessing the impact of shift work on WAI and quality of life, there was no significant decrease in WAI due to shift work in general, and the mean WAI was scored as very good. We hope that the results of the research will help in the adoption of measures for preserving health, preventing diseases and preserving the working ability of healthcare workers.

**MeSH/Keywords:** Work Ability Index, Health Personnel, Shift Work Schedule

**Poster code:** R-02-06-018



**Poster Title:** The effects of fascia iliaca compartment block for hip fractures: Randomized controlled trial

**PhD candidate:** Anđela Simić

**Part of the thesis:** The effects of fascia iliaca compartment block in patients with hip fractures

**Mentor(s):** Assoc. Prof. Žarko Rašić, MD PhD, Assoc. Prof. Višnja Nesek Adam, MD PhD

**Affiliation:** University of Zagreb School of Medicine, University Hospital Sveti Duh

**Introduction:** Hip fractures in patients 65 years aged and older are a major personal and public health problem. Due to Croatian hospitals statistical reports, hip fractures are the third leading diagnose in people 65 years aged and older, with incidence 8,11/1000. Although the injury is not fatal due to the trauma itself, approximately every tenth patient dies in the first 30 days after hip fracture, and one-third of the patients die in the period of one year. Hip fractures are a painful injury. If the pain is not adequately treated it leads to prolonged stress response. Such stress response increases complications and mortality. For acute pain management, nonopioid analgesics are often not sufficient, and opioids have many adverse events. For these reasons fascia iliaca compartment block (FICB) could be the treatment of choice.

**Materials and methods:** A double-blind randomized controlled trial, FICB (40 ml 0.25% levobupivacaine) versus placebo, 80 participants, Emergency Department, University Hospital „Sveti Duh“ is being conducted. As standard care, all participants receive intravenous paracetamol 1 gram every 6 hours. If needed, rescue analgesic is given, intravenous tramadol, 100 mg to maximum 400 mg a day. Inclusion criteria are a hip fracture after minor intensity trauma, age 65 and older, and signed informed consent. Exclusion criteria are a pathological fracture, head injury, body mass less than 50 kg, cognitive impairment with less than 6 points on Abbreviated Mental Test Score -AMTS, permanent oral anticoagulants therapy, allergy to investigated medications, previous ipsilateral peripheral artery bypass surgery, ipsilateral cutaneous and subcutaneous infection in hip region, concurrent bilateral hip fracture, opioid analgesia prior to hospital arrival. Following outcomes are analyzed: copeptin and cortisol serum levels prior to therapy and 24 hours after therapeutic protocol, cognitive status measured by 11 points Abbreviated Mental Test Score -AMTS prior, 2 hours and 24 hours after therapeutic protocol, pain at rest and pain during passive leg elevation measured by 11 points Numerical Rating Scale-NRS prior to therapy and 30, 120, 240 minutes and 24 hours after therapeutic protocol, vital signs prior to therapy and 30, 120, 240 minutes and 24 hours after therapeutic protocol, the number of rescue analgesics and patient satisfaction with analgesia (Likert's scale) 24 hours after therapeutic protocol.

**Results:** There are 8 patients in the study, 4 FICB and 4 placebo, currently. FICB 3 women, mean age 80 years, (range, 71 to 92 years), placebo all women, mean age 81.75 years, (range, 69 to 93 years). Cognitive status impaired in 1 patient in the placebo group (AMTS 8 points before, and 2 points after 24 hours). Mean pain levels (NRS) at rest were FICB 8.5, placebo 8.75 (zero time), FICB 2.17, placebo 3.17, (30, 120, 240 minutes), FICB 2.25, placebo 3, (24 hours). Mean pain levels on movement were FICB 9.5, placebo 9.5 (zero time), FICB 4.17, placebo 6.41, (30, 120, 240 minutes), FICB 5.5, placebo 6.5, (24 hours). The number of rescue analgesics were FICB 3, placebo 9. Patient satisfaction average grade was 4.75 FICB and 4.00 placebo. Hypotension and bradycardia did not occur in any patient. Copeptin and cortisol serum levels will be measured after collection of all of 160 samples.

**Discussion:** Due to COVID - 19 pandemic we have only 8 out of 80 planned patients in this study, by now. In this small sample FICB has shown effectiveness in preoperative pain reduction, especially regarding pain on movement. FICB decreased the need for rescue analgesics, and there was no cognitive status impairment in any patient. Patient satisfaction was higher in the FICB group. However, we have to wait until the end of this study for definitive conclusions.

**MeSH/Keywords:** pain management, preoperative care, fascia iliaca compartment block, hip fractures

**Poster code:** R-02-06-127

**Poster Title:** The role of liver steatosis in the course and outcome of sepsis

**PhD candidate:** Juraj Krznarić

**Part of the thesis:** The role of liver steatosis in the course and outcome of sepsis

**Mentor(s):** Professor Adriana Vince, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Recent findings suggest the possibility of defining the point during liver steatosis where it becomes a susceptibility factor to bacterial infections. Certain clinical trials point out a possibility of establishing the diagnosis of liver steatosis using a combination of radiological procedures, biomarkers and scoring tests. The goal of this research is the evaluation of the relationship between the degree of liver steatosis and the course and outcome of sepsis.

**Materials and methods:** A prospective observational cohort study lasting three years involving 280 patients. The diagnosis of sepsis is made using the Sepsis-3 consensus guidelines while liver status is evaluated using abdominal ultrasound and transient elastography (liver stiffness measurement – LSM, controlled attenuation parameter - CAP). Routine biochemical and microbiological patient evaluation is performed upon admission. Biochemical markers of liver fibrosis (cytokeratine 18 and adiponectine) are be sampled upon admission. The levels of cytokeratine 18 and adiponectine will be measured using ELISA. Parameters specific to the intensive care unit are be taken into account. The data obtained will be analysed with the appropriate statistical tests in the program SAS v. 9.4.

**Results:** Most patients admitted for infectious events had CAP measurements above 220dB/m and/or LSM above 21kPa. Our cohort of patients with *S.agalactiae* sepsis had a mortality rating of 19% (with NAFLD) versus 5% (no pre-existing liver disease). We estimate that the difference in mortality in our cohort will be 10% (15% vs. 5%). Biomarker processing (cytokeratine 18 and adiponectine) has not been done so far and will be when the required number of samples (approx. 280) is collected. The research was halted during the COVID-19 pandemic.

**Discussion:** Although research is still at an early stage, initial results indicate that liver steatosis plays a role in predicting the course and outcome of sepsis.

**MeSH/Keywords:** liver steatosis, sepsis, LSM, CAP, cytokeratine 18, adiponectine

**Poster code:** R-02-07-121

**Poster Title:** Prevalence and molecular background of RHD allele with decreased or altered antigenic expression

**PhD candidate:** Hana Safić Stanić

**Part of the thesis:** RHD genotyping relevance in RhD negative blood donors

**Mentor(s):** Professor Ante Ćorušić, MD PhD, Assist. Prof. Irena Jukić, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Rhesus is one of the most polymorphic protein-based blood group system. Among the 54 Rh antigens, RhD antigen is the most immunogenic as it can strongly stimulate the immune response and formation of anti-D antibodies. Rh antigens are encoded by a pair of highly homologous genes (RHCE and RHD), on chromosome 1. Presence or absence of RhD protein differs the D+ (85% Caucasians) from D- (14%) phenotype. Up to 1% Europeans are carriers of aberrant RHD allele-that encodes different variants of D antigen: weak D, partial D, and DEL. Named variations in the expression of D antigen can result in D typing discrepancies: individuals may test D antigen negative, weakly positive, or positive by serology, and may or may not be at risk of alloimmunisation after exposure to D antigen. Identification of D variant allele is important because anti-D can occur in some but not all persons that express a variant RHD allele.

**Materials and methods:** The study was carried out at Croatian Institute of transfusion medicine, Zagreb. The blood specimen-6ml was collected from 6523 voluntary blood donors all typed as D negative on standard serologic testing including direct and indirect antiglobulin test. The samples were pooled in batches of 20, followed by DNA extraction and amplification, using commercially available QIAamp DNA Blood Mini kit (QiaGen, Germany) on QIAcube station (QiaGen, Germany). RHD screening among RhD negative blood donors, was performed by realtime PCR (qPCR) method (RT-PCR System 7500, Applied Biosystems, USA) with TaqMan chemistry used for exon 7 and 10 amplification. In case of qPCR-positive pool, we resolved the pool to individual samples and performed RHD genotyping from an individual sample using a PCR-SSP (sequence specific priming) method to define the RHD allele (Inno-Train, Germany). In those cases where RHD allele could not be classified, genomic DNA sequencing was performed for all ten exons of the RHD gene.

**Results:** Study revealed 23 (0.35%) RHD-PCR positive samples, in decreasing frequency: 11 hybrid RHD-CE (2-9) D-CE variant, 4 weak partial type 11, two weak D type 2. Six samples remained unresolved and were sequence. Novel mutation was identified in one sample, in exon 7 - 1027delT resulting in a premature stop codon at amino acid position 358 (M358\*), causing a truncated protein that would not be expressed at the cell surface. One sample was categorized as RHD\*01W.28- weak D type 28, with reported nucleotide alteration 1152A>C (T384T). For the remaining 4 samples no mutations were found in the RHD gene, indicating the genotype RHD\*D (normal D). All of the RHD-PCR-positive samples were also found to be C and/or E-positive. For 12 out of 23 samples (excluding large hybrids), absorption/elution of anti-D serum was performed, confirming that all twelve were D+. Calculated frequency of clinically significant D alleles was 1: 543.

**Discussion:** Widespread use of molecular genotyping methods enabled us to accurately predict RhD expression on RBC and reduce residual risk of RhD alloimmunization. In order to determine most D variants with RHD specific polymorphism we decided to screen for the presence of RHD exons 7 and 10, which correlates with similar European studies. We determined 0,35% RHD PCR positive blood donors amongst serologic RhD - individuals. Similar frequency has been reported to be 0.21, 0.4, 0.47% in German, Austrian, Swiss blood donor populations. The most prevalent hybrid RHD-CE (2-9)-D-CE is not clinically relevant, as carriers of this allele lack D antigen on the RBC. Second most prevalent allele RHD (M295I), (borderline DEL /weak D tip 11) can induce anti-D immunization, and such units should be redefined as RhD positive. The least prevalent weak D type 2 can be immunogenic as shown in other studies. The frequency of potentially alloimmunizing RHD variants in our Institute was determined as 1:543 (0.18%), or 1:53 in C/E blood donors which coincides with above-mentioned European studies. The most common DEL phenotype in Caucasians is RHD (M295I), which has also been identified in our country. This study is one of the first in this region verifying the frequency of RHD alleles on a much larger scale. The results of the identified D variants will help in introducing RHD molecular screening of serologically RhD negative blood donors into routine testing in Croatia leading to safer blood transfusions.

**MeSH/Keywords:** Rhesus, blood group, molecular genetics, transfusion, weak D

**Poster code:** R-02-09-005

**Poster Title:** Genetic background of innate immunity in chronic obstructive pulmonary disease and lung cancer

**PhD candidate:** Irena Sokolović

**Part of the thesis:** Genetic background of innate immunity in chronic obstructive pulmonary disease and lung cancer

**Mentor(s):** Assoc. Prof. Marko Jakopović, MD PhD, Jelena Knežević, PhD, research associate

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Toll-like receptors (TLRs) have a potential role in signaling tissue destruction, chronic organ injury, differentiation, and neoplastic disease (Hallman et al., 2001). Polymorphisms in TLR genes can move the balance between pro and anti-inflammatory cytokines, modulating the risk of infection, chronic inflammation and cancer development (Kutikhin, 2011). The aim of this study is to assess the prevalence of polymorphisms of genes encoding TLRs in COPD and lung cancer populations and analyze the impact of these SNPs on cancer risk.

**Materials and methods:** We enrolled 940 patients recruited at Department for respiratory diseases Jordanovac, UHC Zagreb and Department for Pulmonology, UHC Osijek, divided into three groups: COPD only (49,89%), COPD and lung cancer (29,89%) and lung cancer only (20,22%). We analyzed 13 SNPs that encode proteins of TLR family associated with dysfunctional inflammatory response regulation and tumor formation. DNA was isolated from white blood cells. KASP or TaqMan allelic discrimination methods were used for genotyping analysis of the selected SNPs. Serum concentration of interleukin-6 was determined by ELISA test. The association between the genotypes and LC risk was estimated by unconditional logistic regression.

**Results:** Unadjusted analyses showed that TLR5 rs2072493 minor allele was associated with increased risk of LC development (G/A+G/G OR=1.60 [1.08-2.36] p=0.02). Borderline association with increased risk of lung cancer was observed for heterozygous allele of TLR5 rs725084 (C/T OR=1.58 [1.00-2.49] p=0.05). Comparison of genotype distribution in the COPD+LC and COPD group showed that the presence of minor allele of TLR1 rs5743611 and TLR5 rs2072493 is associated with increased risk of LC development (rs5743611 C/G+CC OR=1.55 [1.05-2.28] p=0.03; rs2072493 G/G OR=2.82 [1.34-5.94] p=0.01). For the TLR9 we found borderline association of the rs352139 heterozygosity with protection in LC development (A/G OR=0.68 [0.46-1.00] p=0.05). When logistic regression data were adjusted on age and gender, only the frequency of the TLR5 rs2072493 minor allele showed statistically significant association with lung cancer development in COPD patients (G/A+G/G OR=1.76 [1.26-2.46] p<0.001; G/G OR=4.37 [2.03-9.45] p<0.001). Comparison of genotype frequency of selected SNPs between COPD and lung cancer patients showed that the minor allele of the same TLR5 SNP rs2072493 is significantly associated with lung cancer development (G/A+G/G OR=1.71 [1.20-2.43] p=0.003). Impact assessment of rs2072493 genotype on interleukin 6 serum concentration revealed that minor allele genotypes (G/G+A/G) are associated with statistically significant decrease of the serum IL-6 concentration (p=0.026).

**Discussion:** Our results indicate that there is a correlation between minor allele genotypes of TLR5 SNP rs2072493 and the risk for lung cancer development in COPD background. We found that the presence of the minor alleles is associated with decreased serum concentration of the IL-6 proinflammatory cytokine. Pathways that are activated by TLRs, including TLR5, culminate in the activation of the transcription factor NF- $\kappa$ B, which acts as a switch for inflammation, regulating the transcription of genes that encode proteins involved in immunity and inflammation (O'Neill, 2006). The NF- $\kappa$ B pathway is an important cancer signalling pathway that plays a crucial role in the induction of inflammatory response in lung cancer (Cho et al., 2011). We have shown that COPD patients harbouring minor allele genotype of TLR5 rs2072493 G/G exhibit decreased serum IL-6 concentration and are 4 times more susceptible to develop lung cancer. Therefore, the presence of mutated allele, and its association with lung cancer and decreased peripheral concentration of IL-6, may reflect the disturbed NF- $\kappa$ B activation. Most apparently, SNPs encoding variants of TLRs may alter their pathway proteins that eventually could be associated with risk of developing LC. According to our results, TLR5 rs2072493 might set forth an immunological link between LC and COPD that could lead to improved risk prediction, better understanding of disease mechanisms and enable future specific treatment.

**MeSH/Keywords:** Toll-like receptor, SNP, lung cancer, chronic obstructive pulmonary disease

**Poster code:** R-02-09-034

**Poster Title:** Serum activin A concentration in patients diagnosed with squamous cell lung cancer

**PhD candidate:** Lela Bitar

**Part of the thesis:** Significance of serum activin A concentration in patients diagnosed with squamous cell lung cancer

**Mentor(s):** Assoc. Prof. Marko Jakopović, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Lung cancer is among the most common malignant diseases in the world and the most common cause of cancer-related death. In Croatia every year 3000 people are diagnosed with lung cancer. In the selection of treatment strategy, prognostic and predictive markers are used to assess the prognosis and outcome. Activin A is a protein that regulates gene expression in many cellular functions. Mutations of signaling pathway components have been found in many types of malignant and chronic diseases. In the squamous cell carcinoma of the oral cavity, adenocarcinoma and squamous cell carcinoma of the esophagus and malignant mesothelioma increased expression of the activin A is associated with tumor aggressiveness. In previous studies in lung adenocarcinoma was shown that serum activin A level is associated with advanced stage of the disease and distant metastases.

**Materials and methods:** This study will include 110 patients with newly diagnosed squamous cell lung cancer and a control group of 60 volunteers without exclusion criteria (chronic obstructive pulmonary disease, asthma, diabetes mellitus type 2 and prior malignancies). Depending on the stage of the disease and the treatment strategy serum samples will be collected for activin A analysis. Serum samples of the control group volunteers will be collected once. Control group will be divided in two subgroups, one consisting of current smokers and one of non-smokers. Patients diagnosed with squamous cell lung cancer will be observed longitudinally over 2 years. In all patients an initial sample will be analyzed prior to initiation of treatment. In patients diagnosed with early stages of the disease (I-III A) the second sample will be analyzed during the first evaluation after surgery and the third at the time of the progression of the disease. In patients with advanced stages of the disease (III B and IV) the second sample will be analyzed on the first evaluation of the disease after two treatment cycles and the third one at the time of the disease progression.

**Results:** Sample collection started in January 2020 and until now samples from 24 patients were collected. 17 (70.8%) of them are male. Median age at the time of diagnosis is 64 years (min – max 37-90). 87% of the patients were current or ex—smokers with median pack/years of 41 (2-200). Most of the patients were in good performance status (ECOG 0-1), while only 16% had ECOG 2 or 3. Stage of the disease varied among them. 20 patients were diagnosed at the advanced stage, while the disease was locally advanced in only 4 patients. Activin A concentration was not yet assessed.

**Discussion:** The aim of this study is to determine whether there is a difference between serum activin A concentration in patients with squamous cell lung cancer and control group and whether there is a difference in serum activin A concentration in patients diagnosed in early stages of squamous cell lung cancer and in the advanced stages. Also, to ascertain whether serum activin A levels are correlated with disease progression and progression free survival in the first line of treatment. We will investigate if there is a correlation between serum activin A level and sex, age and smoking status of the patients. Until now our data are consistent with published world data of the patients diagnosed with squamous cell lung cancer. Activin A concentration analysis will give us the answer whether it could be used as a novel biomarker of the disease spread and disease progression in patients diagnosed with squamous cell lung cancer.

**MeSH/Keywords:** NSCLC, squamous cell carcinoma, activins

**Poster code:** R-02-09-041

**Poster Title:** TLR7 gene and protein in non-small cell lung carcinoma

**PhD candidate:** Fedža Džubur

**Part of the thesis:** TLR7 gene and protein in non-small cell lung carcinoma

**Mentor(s):** Academician Miroslav Samaržija, Jelena Knežević, PhD, research associate

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Lung cancer is the most common malignant disease in the world with high mortality rate. Around 3,000 patients are newly diagnosed every year in the Republic of Croatia. Numerous prognostic and predictive factors are used to select proper treatment strategy and also to estimate the prognosis or outcome of the chosen treatment. Several studies have shown that levels of TLR expression on tumour cells are associated with tumour progression. It has been shown that stimulation with TLR7 / TLR8 agonists leads to activation of NF- $\kappa$ B, enhanced expression of antiapoptotic proteins, enhanced tumour cell survival, and chemoresistance. In patients with non-small cell lung cancer, expression assays revealed that increased TLR7 expression was strongly associated with resistance to chemotherapy and poor clinical prognosis.

**Materials and methods:** For the needs of this study, approximately 450 samples of patients with a diagnosis of lung cancer and approximately 1200 samples of healthy subjects were collected. Samples of peripheral blood previously collected in EDTA anticoagulant tubes were used for DNA isolation and stored at -20°C until further analysis. All blood samples of patients and healthy subjects were treated the same way. After determining which of the tested polymorphic markers is associated with the tendency to develop lung cancer, its association with survival will be analyzed. In this section, the proposed study is prospective. For the purposes of analyzing the expression of the TLR7 and IFN $\gamma$  gene expression, the tissues of the subjects with known genotype will be used.

**Results:** Sample collection started in January 2016 and until now approximately 450 samples of patients with a diagnosis of lung cancer and approximately 1200 samples of healthy subjects were collected. Given that all respondents were collected in early 2016, we expect that the survival monitoring period, for the purposes of this research, would be until the end of 2020, which is approximately 5 years for most respondents. Median age at the time of diagnosis is 61 years (min – max 29-96). 89% of the patients were current or ex—smokers with median pack/years of 27 (1-175). Most of the patients were in good performance status (ECOG 0-1), while only 8% had ECOG 2 or 3. Stage of the disease varied among them. 89% of patients were diagnosed at the advanced stage, while the disease was locally advanced in only 11 patients. Expression of the TLR7 and IFN $\gamma$  gene expression was not yet assessed. We expect that results of this study is to partially illuminate the genetic basis of non-small cell lung cancer in the context of the association of promoter polymorphisms with the level of expression of the TLR7 gene and consequently the expression of IFN $\gamma$ , important regulators of inflammatory activation and immunological response modulators.

**Discussion:** The aim of this study is to contribute to understanding the role of TLR7 expression in patients with non-small cell lung cancer, which ultimately may contribute to the development of new therapeutic and prognostic strategies, in particular in the context of the immunotherapy.

**Acknowledgments:** \*

**MeSH/Keywords:** NSCLC, TLR7, SNP, promoter, expression, biomarker, immunotherapy

**Poster code:** R-02-09-044

**Poster Title:** Tracking the adherence to a gluten-free diet in newly diagnosed patients with celiac disease and its influence on nutritional status, the activity of the disease and the quality of life

**PhD candidate:** Cecilija Rotim

**Part of the thesis:** Tracking the adherence to a gluten-free diet in newly diagnosed patients with celiac disease and its influence on nutritional status, the activity of the disease and the quality of life

**Mentor(s):** Professor Željko Krznarić, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Celiac disease is a chronic, immunological disease of a small intestine caused by gluten hypersensitivity. Due to its chronic development, gluten enteropathy often leads to nutritional deficiencies, that is, to the deterioration of nutritional status and changes in tissue structure. Today, celiac disease therapy is based on a strictly gluten-free diet and in most of the patients leads to a satisfactory remission - a symptom, serological and histological remission.

**Materials and methods:** This cross-sectional research will include patients at the Centre for Clinical Nutrition, Clinical Hospital Center Zagreb. The study will include 80 adult patients with a diagnosed celiac disease. Excluding criteria will be age (<18 or >65 years of age), systemic infection, intestinal infections (bacteria, viruses, parasites), intestinal diseases (diverticulitis, enterocolitis, ischemic colitis), liver dysfunction (cirrhosis, active hepatitis), kidney dysfunction (chronic renal insufficiency), severe hypertension, coronary heart disease, peripheral artery disease, hematological, malignant and autoimmune diseases, pregnancy and rejection. All patients will sign the informed consent form, and the research will be approved by appropriate ethics committee. Demographic and anthropometric data will be collected from the prepared questionnaire, available medical documentation and measurements. Complete blood samples will be collected from the collected blood samples, and biochemical analysis will determine the routine parameters and parameters relevant for the assessment of the activity of the disease, the status of the drug and the estimation of systemic inflammatory activity (CBC, Fe, total and unsaturated iron binding capacity, acid, vitamin B12, 25-OH vitamin D, calprotectin, tissue transglutaminase, IgA, CRP). Evaluation of adherence to the unhealthy diet and nutritional quality will be evaluated by dietary and validated methods of eating habits and by measuring tissue transglutaminase. Nutritional status will be evaluated using validated methods (NRS-2002, anthropometry) to estimate nutrition. The patient's quality of life will be evaluated with a valid CDQ questionnaire. Measurements of GIP in urine and stool will be determined by a commercial test method.

**Results:** Data collection is still in progress (expected to be finalized in July 2020.) At the moment there are not any preliminary results available.

**Discussion:** The foundation of clinical success with these patients is in their cooperation and following the gluten-free diet. For this reason, it is extremely important to establish the nutritional status, tissue structure and relevant biochemical parameters at the diagnosis and at each following check-up of a patient on a gluten-free diet. In this way, it is possible to control if a patient is cooperative and if they follow a gluten-free diet and also to control the quality of this diet, which is often a neglected aspect in the treatment of celiac disease patients.

**MeSH/Keywords:** celiac disease, gluten-free diet, nutritional status, GIP

**Poster code:** R-02-09-046

**Poster Title:** Differences in extra cellular matrix remodeling in highly active Crohn disease and ulcerative colitis

**PhD candidate:** Viktor Domislović

**Part of the thesis:** Biomarkers of degradation and formation of collagen III, IV and V in serum as markers of inflammatory activity in patients with inflammatory bowel disease

**Mentor(s):** Professor Željko Krznarić, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Ulcerative colitis (UC) and Crohn disease (CD) require continuous evaluation of disease activity and response to therapy. Current gold standard is endoscopy, therefore it is important to investigate biomarkers associated with endoscopic disease activity. Extra cellular matrix (ECM) consists of basement membrane (BM) and interstitial matrix (IM). BM consists mainly of type IV collagen, while IM of types I, III and V collagen. Pathological environment leads to impaired remodeling, structure, quality and function of the ECM. Our aim was to investigate biomarkers of collagen degradation and formation and their association with endoscopic disease activity and in patients with CD and UC.

**Materials and methods:** In this cross-sectional study, we have measured five biomarkers of ECM remodeling in 94 IBD patients (60 with CD and 34 UC). Biomarkers of type III collagen degradation (C3M) and formation (PRO-C3), type IV collagen degradation (C4M), formation (PRO-C4) and type V collagen formation (PRO-C5) were measured in serum by ELISA. Inflammatory activity was measured using endoscopic scores; SES-CD for CD and modified Mayo endoscopic score (mMES) for UC which included extension of inflammatory activity. Patients were divided in remission and mild activity group vs. moderate-to-severe activity (SES-CD <7 vs. ≥7 and mMES <7 vs. ≥7) Student t-test and correlation analysis were applied in statistical analysis.

**Results:** Patients with CD and with moderate and severe disease had lower levels of PRO-C3 ( $14.7 \pm 10.5$  vs.  $9.9 \pm 4.6$ ,  $p = 0.04$ ), and higher levels of C4M ( $26.54 \pm 10.5$  vs.  $37.8 \pm 20$ ,  $p = 0.009$ ) compared with patients in remission and mild disease. Biomarker of type III collagen turnover (C3M/PROC3) showed higher levels in moderate and severe active disease in CD ( $1 \pm 0.5$  vs.  $1.8 \pm 1.1$ ,  $p = 0.006$ ). UC patients with moderate to severe activity showed higher levels of type III collagen turnover biomarker (C3M/PROC3) ( $1.1 \pm 0.4$  vs.  $1.97 \pm 1$ ,  $p = 0.049$ ). C4M correlated positively ( $r = 0.28$ ,  $p = 0.03$ ) and PRO-C3 correlated negatively ( $r = -0.29$ ,  $p = 0.03$ ) to SES-CD and combined in a multivariate regression model ( $r = 0.39$ ,  $p = 0.009$ ). C3M ( $r = 0.60$ ,  $p = 0.004$ ) and C4M ( $r = 0.36$ ,  $p = 0.039$ ) correlated positively with mMES.

**Discussion:** In highly active CD, there is increased type IV collagen degradation and reduced type III collagen formation. Both biomarkers significantly correlate with SES-CD, with increased correlation when combined together. Type III collagen turnover is increased in highly active CD and UC. Biomarkers of types III and IV collagen degradation correlate significantly with mMES. Biomarkers of types III and IV collagen could be used to differentiate highly active CD and UC from patients in remission. Our findings suggest that biomarkers of basement membrane and interstitial matrix remodelling may be used as surrogate markers for monitoring of disease activity for UC and CD. Further studies on the role of ECM in IBD are needed.

**MeSH/Keywords:** Extra cellular matrix, collagen, IBD, inflammation

**Poster code:** R-02-09-059



**Poster Title:** Influence of remission of Cushing's syndrome on parameters of metabolic syndrome

**PhD candidate:** Annemarie Balaško

**Part of the thesis:** Influence of remission of Cushing's syndrome on parameters of metabolic syndrome

**Mentor(s):** Assist. Prof. Tina Dušek, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Cushing's syndrome (CS) is a rare disorder of excessive exposure to glucocorticoids and it is associated with increased morbidity and mortality. Patients with CS experience a higher incidence of metabolic and cardiovascular complications and they have a worse perception of quality of life compared to healthy individuals. The purpose of this retrospective study was to evaluate the effect of remission of disease on different components of metabolic syndrome which include changes in glucose, blood pressure, and lipid profile as well as to evaluate the quality of life and differences between ACTH-dependent and ACTH-independent CS on these changes.

**Materials and methods:** A retrospective study was conducted at the University Hospital Zagreb. All patients with CS who were treated at the Department of Endocrinology in the period 2011-2017 were included in this study. Data were retrieved from available medical documentation. CS was defined as elevated free cortisol levels in 24-hour urine, no suppression of cortisol after overnight suppression test with dexamethasone (concentration of cortisol in blood > 138 nmol/L) and/or elevated cortisol levels at midnight (> 50 nmol/L while sleeping or > 207 nmol/L while awake). Measurement of ACTH distinguished ACTH-dependent from ACTH-independent CS. Other investigations to define the etiology of CS included magnetic resonance imaging of the pituitary gland and inferior petrosal sinus sampling (IPSS) if necessary as well as CT scan of adrenal glands. Remission of CS was defined as adequate suppression of cortisol after overnight dexamethasone suppression test (< 80 nmol/L). Improvement of metabolic parameters after remission of CS was defined with following parameters: lower levels of cholesterol, LDL and triglycerides as well as higher levels of HDL or reduction/exclusion of hypolipemic drugs; lower levels of fasting or postprandial glucose and HbA1c; reduction of waist circumference, body weight and BMI; better regulation of blood pressure and/or lowering of antihypertensive drugs.

**Results:** The study group comprised 77 patients (median age 43 years, range 25-71) of which 13 (16,9%) were males and 64 (83,1%) were females. There were 55 (71,4%) ACTH-dependent CS and 22 (28,6%) ACTH-independent CS. Regarding metabolic and cardiovascular comorbidities hypertension was found in 25 (32,5%), diabetes mellitus type 2 in 59 (76,6%) and dyslipidemia in 34 (44,2%) patients. Comparing metabolic changes and parameters there was a statistically significant reduction in glucose value, HbA1c, cholesterol, LDL-cholesterol, HDL-cholesterol, before and after remission of CS but no significant change in triglyceride values. There was also a statistically significant reduction in waist circumference, body weight, and body mass index ( $p < 0.001$ ) as well as in systolic and diastolic blood pressure ( $p < 0.001$ ). Regarding the etiology of CS, there was a statistically significant reduction in cholesterol and LDL cholesterol value after remission of ACTH-dependent CS compared to ACTH-independent CS.

**Discussion:** In our study group, remission of CS was associated with a significant improvement of metabolic parameters as well as in the reduction in blood pressure, body weight, and waist circumference. Besides the difference in the cholesterol and LDL cholesterol levels which were lower in the ACTH-dependant group, there were no significant differences in the metabolic parameters between the patients with respect to the etiology of CS. Extension of this study will further elucidate the possible differences in terms of hypothalamus-pituitary-adrenal axis recovery, cardiovascular risk assessment, and quality of life between the patients with ACTH dependent and ACTH independent CS.

**MeSH/Keywords:** Cushing's syndrome, ACTH-dependent, ACTH-independent, remission, metabolic parameters

**Poster code:** R-02-09-079

**Poster Title:** X-ray phase contrast imaging of endo-myocardial biopsies following heart transplantation - agreement with classical histology

**PhD candidate:** Ivo Planinc

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

**Mentor(s):** Assoc. Prof. Maja Čikeš, MD PhD, Patricia Garcia Canadilla, PhD

**Affiliation:** University of Zagreb School of Medicine; IDIBAPS, Barcelona, Spain

**Introduction:** Endo-myocardial biopsy is the gold standard for graft rejection surveillance following heart transplantation. Endo-myocardial biopsies are assessed by classical histology following sample slicing and staining, using a limited number of slices. Routinely, at least 3 samples of right ventricular (RV) myocardium are taken, and at least 10 sections are histologically analysed. Limited number of sections increases the possibility of missing important findings on non-analysed parts of the sample. X-ray phase contrast imaging (X-PCI) is a novel, synchrotron-based method to non-destructively and without tissue preparation image biological samples providing both 3D information on whole-organ level as well as on cellular level (virtual 2D and 3D histology). Purpose of this study is to show agreement of cardiac graft rejection detection by X-PCI in comparison to classical histology.

**Materials and methods:** Right ventricular endo-myocardial biopsies were taken from 23 heart transplantation recipients (20 males, mean age  $55 \pm 5$  y, median time from heart transplantation 33 months) as part of post-heart transplantation standard follow-up protocol. The clinical diagnosis of potential rejection was made in a standard fashion using classical histology (both ISHLT 1990. and 2004. grading systems). Additional endo-myocardial samples taken simultaneously were imaged by X-PCI with no prior tissue preparation, producing 3D datasets with 0.65 $\mu$ m pixel size, and approximately 2160-4320 images/sample, depending on the sample size. In off-line analyses, X-PCI images were prepared for comparative rejection analysis: 1) X-PCI 2D histology dataset (analysis of up to 10 consecutive images); 2) X-PCI 3D histology dataset, for the analysis of the entire 3D volume, enabling the analysis of the entire sample with great detail. Following X-PCI, these samples were also processed by classical histology. An experienced pathologist graded both histological and X-PCI images in a blinded fashion. In total, 4 sets of endo-myocardial biopsies gradings were obtained: classical histology grading for routine clinical diagnosis, X-PCI 2D virtual histology grades, X-PCI 3D virtual histology grades and repeat classical histology grades of these samples. Agreement between methods was assessed by weighted kappa.

**Results:** Overall, moderate to substantial agreement was shown when comparing X-PCI to classical histology (Table 1). The highest levels of agreement were achieved when comparing classical histology to X-PCI 3D histology. The weighted kappa reached 0.7051 and 0.7272 when comparing agreement between classical histology using ISHLT 2004 and 1990 classification with X-PCI 3D histology ( $p < 0.01$ ). The agreement between X-PCI 3D histology and classical histology was higher than that of repeat vs. initial classical histology analyses.

**Discussion:** X-PCI has shown high level of agreement with classical histology when used by an experienced pathologist in graft rejection detection. X-PCI 3D virtual histology enables thorough analysis of the whole tissue sample from infinite number of projections. This encourages further comparison of X-PCI to classical histology, linking to treatment choices and patient outcomes.

**MeSH/Keywords:** heart transplantation, heart failure, graft rejection, histology, synchrotron

**Poster code:** R-02-09-082

**Poster Title:** Occurrence and recurrence of nafld after liver transplantation

**PhD candidate:** Maja Mijić

**Part of the thesis:** Transient elastography in assessment of fibrosis and steatosis of donor livers

**Mentor(s):** Assoc. Prof. Tajana Filipec Kanižaj, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** NAFLD is the most rapidly growing indication for LT in western countries due to complications of end-stage liver (ESLD) disease or HCC. This growth closely mirrors the global epidemics of obesity, diabetes mellitus, metabolic syndrome and hyperlipidemia with negative impact on baseline recipients and donors characteristics. Recurrence or de novo occurrence of NALFD after LT is possibly very common but not well studied. In Croatia it is performed around 30 LTs per million population/year. Around 90% of all adult LT programme is performed in University hospital Merkur. In this retrospective study we aimed to evaluate time trends in LT for NAFLD, recipient and graft survival and rate of recurrence and de novo occurrence of NAFLD in adult LT recipients in University hospital Merkur, Zagreb, Croatia in 5 years (2012-2017).

**Materials and methods:** Data included in analysis were: indication for LT, 5- year's recipient and graft survival, pre-implantation bioptic finding of donor liver, bioptic finding of last liver biopsy performed at least 6 months after LT.

**Results:** In last 5 years 503 first LT from cadaveric donors were performed. ESLD due to NAFLD cirrhosis represents 7,4% of all indications and is stable in last 5 years. HCC is fastest growing indication especially due to NAFLD (7.1% to 41.7% in 5 years for NAFLD recipients). Twenty percent of all donors had steatosis before implantation (14% grade 5-29%, 6% grade 30-60%). Forty percent of all recipients had at least one bioptic sample, obtained later than 6 months after LT (42% with NAFLD and 39% non-NAFLD recipients). Overall 5-year graft and recipient survival were 90,1 and 80,9%, respectively. Primary indication (NAFLD vs non-NAFLD) and donor steatosis had no impact on recipient or graft survival. Comparison of basal donor bioptic sample and post-LT finding revealed that 9,5% NAFLD recipients and 11,4% with non-NAFLD indications had stable stage of liver steatosis, 4,8% and 24,7% regression or disappearance of steatosis and 23,8 and 12,4% recurrence/ de novo occurrence of steatosis, respectively (p 0.08). Only one patient with NALD had NASH in post-LT bioptic finding.

**Discussion:** NAFLD in Croatia represents 9% of all indications (ESLD and HCC). It is one of the fastest growing indication for LT due to HCC. LT recipients transplanted due to NAFLD or usage of liver grafts with steatosis had no impact on recipient and graft survival. Regression of graft steatosis is possible in up to 4,8 and 24,7% NAFLD and non-NAFLD recipients. Recurrence or de novo occurrence of NAFLD was detected in 23,8 and 12,4% recipients. This underlies higher rate and more rapid course of NAFLD recurrence than occurrence after LT.

**MeSH/Keywords:** non-alcoholic fatty liver disease, liver transplantation

**Poster code:** R-02-09-083

**Poster Title:** The role of NLRP3 inflammasome in systemic pro-inflammatory response of the patients with chronic obstructive pulmonary disease

**PhD candidate:** Ivona Markelić

**Part of the thesis:** The role of NLRP3 inflammasome in systemic pro-inflammatory response of the patients with chronic obstructive pulmonary disease

**Mentor(s):** Assist. Prof. Andrea Vukić Dugac, MD PhD, Professor Lada Rumora, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Chronic obstructive pulmonary disease (COPD) is a complex lung disease characterized by airflow limitation that is not fully reversible. The airflow limitation is usually both progressive and associated with an abnormal inflammatory response, both localised and systemic. The mechanisms for such amplified inflammation are not yet understood since there are many different coexisting mechanisms underlying its pathogenesis. More than three million people worldwide die annually of COPD, making this disease an important, unrecognized public health problem. COPD is projected to become the third leading cause of death by 2020.

**Materials and methods:** This study was a monitoring of systemic inflammatory biomarkers in groups of patients in stable phase of COPD and healthy subjects (smokers and non-smokers) during a four years period (2015.-2019.). All participants signed informed consent for scientific research and agreed to take part in it as volunteers. Present study included a total of 232 participants – 137 in stable phase of COPD and 95 in the control group. COPD patients were screened for eligibility and recruited during the outpatients control visit. With each visit, all relevant data and blood samples were taken. Patients were excluded if they had suffered from an exacerbation of COPD during the last three months, had severe liver or kidney diseases, malignant diseases, organ transplantations or coexisting pulmonary or systemic inflammatory diseases. Patients were also excluded if they were taking oral corticosteroids or recently changed inhalation therapy.

**Results:** A total of 232 subjects were included in the study – 137 patients with stable COPD and 95 healthy volunteers. COPD patients and healthy subjects had similar age with slightly different sex distribution. Median age of COPD patients was 65 years (44-86) and 86 (63%) of them were male. Median age of healthy subjects was 64 (46-83) and 49(52%) were mail. As expected, all lung function parameters were significantly lower in COPD patients. Measured inflammatory parameters CRP, Fbg and WBC showed significantly increased levels in COPD patients ( $P<0.001$ ), and increased number of monocytes was also found ( $P<0.001$ ). There are further on going subanalyses of collected blood samples in order to determine the role of NLRP3 inflammasome in systemic pro-inflammatory response, so we do not have complete data yet.

**Discussion:** Chronic low-grade inflammation is one of the underlying mechanisms in COPD pathogenesis. Inflammation involves many complex interactions led by immune-related cells, whose networking and activation result in respiratory tissue injury. Due to the inflammation, many cells are being recruited from blood into the lung and the presence of inflammatory cells in the airways is broadly reflected by increased numbers of the same cells in peripheral blood. Emerging scientific evidence suggests that persistent Nod-like Receptor 3 (NLRP3) inflammasome activation may be also involved in the onset of COPD pathogenesis. If our results reveal statistically significant increased expression of NLRP3 mRNA and higher concentration of IL-1 beta in COPD patients, we could have novel indicators that can contribute in diagnosing, classifying and monitoring the course of the disease which is extremely important given the growing mortality of COPD.

**MeSH/Keywords:** COPD, NLRP3 inflammasome, cytokine IL-1 beta, inflammatory parameters

**Poster code:** R-02-09-089

**Poster Title:** Impact of chronic ibrutinib therapy on left atrial function

**PhD candidate:** Matea Kolačević Zeljković

**Part of the thesis:** Impact of chronic Bruton's tyrosine kinase inhibitor ibrutinib therapy on left atrial function

**Mentor(s):** Assist. Prof. Nikola Bulj, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Ibrutinib is a well-established therapy of various B-cell malignancies with proven efficacy.

Different ibrutinib's side-effects are known, mostly with <3rd degree of severity and consequently not requiring dose adjustment or discontinuation. The most common cardiovascular side-effect is hypertension, followed by the atrial fibrillation (AFib). There are indices that ibrutinib inhibits phosphoinositide-3-kinase (PI3K)-Akt signal-pathway. However, the clinical effect of ibrutinib on the myocardium has not been studied. Hence, this study aimed to determine the impact of chronic ibrutinib therapy on echocardiographic parameters of the left atrium (LA).

**Materials and methods:** The study is conducted as a prospective, observational cohort study with a structured follow-up (FU) of 12 months. Consecutive patients with hemato-oncologic diseases prescribed with ibrutinib therapy were included in the study. Additional cardiology diagnostics was carried out including echocardiography, 24h-Holter-ECG and the laboratory findings. Diagnostic assessment was carried out before the introduction and repeated at 3, 6 and 12 month FU visits after the introduction of the ibrutinib therapy. Primary endpoint is a decrease in LA function measured by a decrease in total LA tissue strain by more than 10%. Secondary objectives are: systolic left ventricular function; LA function as a reservoir, conduit and pump; incidence of AFib; incidence of newly diagnosed or worsened hypertension. Signed informed consent for participation in the study was obtained from all enrolled patients. The Hospital Ethics Committee gave its approval of the study, which was conducted according to the Declaration of Helsinki. Standardized statistical methods and tests were done using SPSS Version 22.0.

**Results:** Total of 35 consecutive patients with hemato-oncologic disease prescribed with ibrutinib were included in the study. Patients were recruited from 4 University Hospitals: Zagreb (14 patients), Merkur (9 pts), Dubrava (4 pts) and Sestre milosrdnice (8 pts). Most common hemato-oncologic disease was chronic lymphocytic leukaemia diagnosed in 31 (89%) patients. Median age of the study group was 66 years (IQR 58-73), 54% were female. The most prevalent comorbidity was hypertension (45%) followed by smoking (29%). The remaining risk factors, including stroke and coronary artery disease had prevalence of <10%. Mean systolic arterial pressure was 131±15 mmHg, and diastolic 83±9 mmHg. Mean left ventricle ejection fraction was 60%, LA diameter 40±4 mm and volume 53±7 mL. So far, 27 patients completed 3-month follow-up (FU) and 17 completed 6 months FU. During 6 months FU, hypertension was newly diagnosed or worsened in 8 patients (23%), and 2 patients had paroxysmal AFib (5%). Ibrutinib was temporarily discontinued or dose adjusted in 3 patients due to non-cardiovascular side-effects. It was discontinued in 1 patient after 6 months due to congestive heart failure with preserved ejection fraction and uncontrolled hypertension. Patient inclusion as well as the FU timeline was altered by the epidemiological measures due to COVID-19, however no lost to FU occurred.

**Discussion:** To the best of our knowledge, this is the first study evaluating ibrutinib's impact on LA function. During study period, 2 patients (5%) had paroxysmal AFib which is less than in previous studies. However, incidence of worsened hypertension was 23% which is in line with previous data. Hypertension was strictly controlled with therapy correction and diet, and this could be the reason for lower incidence of AFib. In patients with worsening hypertension, LA diameter became higher with the increase of mean NT proBNP values. In conclusion, these data point to the importance of hypertension control in patients on ibrutinib therapy for the long-term freedom from AFib and highlight the potential of echocardiography in this context.

**MeSH/Keywords:** ibrutinib; echocardiography; left atrium; function; long-term

**Poster code:** R-02-09-123

**Poster Title:** Association between inflammatory markers, mean platelet volume and red blood cell volume distribution width in occurrence of postthrombotic syndrome after proximal deep venous thrombosis

**PhD candidate:** Jelena Bielen Rajić

**Part of the thesis:** Association between inflammatory markers, mean platelet volume and red blood cell volume distribution width in occurrence of postthrombotic syndrome after proximal deep venous thrombosis

**Mentor(s):** Assoc. Prof. Mislav Vrsalović, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Postthrombotic Syndrome (PTS) is a late chronic complication of deep vein thrombosis (DVT). It represents a set of symptoms and signs of chronic venous insufficiency, which consequently has a negative impact on the quality of life of an individual and a negative economic impact on the health system. About 40-50% of patients with proximal DVT develop PTS. Inflammatory indicators for the purpose of determining the population at risk of developing of post-thrombotic syndrome are insufficiently explored. Red blood cell volume distribution width (RDW) and mean platelet volume (MPV) have shown a prognostic role in patients with cardiovascular disease, but their role in the PTS formation has not been further explored. The aim of this study was to investigate the connection between inflammation parameters (leukocytes, leukocyte subpopulations, C reactive protein, neutrophil and lymphocyte ratio), platelet count, mean platelet volume (MPV) and red cell volume distribution width (RDW) and PTS formation after proximal DVT and evaluation of quality in PTS patients.

**Materials and methods:** This observational cohort study will include 110 patients hospitalized due to the first proximal DVT. Patients with active malignancy, renal failure IV and higher (eGFR <30mL / min / 1.73m<sup>2</sup>), heart failure, antiphospholipid syndrome, inflammatory conditions will be excluded from the study. Patients will be clinically monitored (follow-up) for one year at regular checkups after 3, 6, and 12 months. In the diagnosis of post-thrombotic syndrome, scoring systems will be used: the clinical-etiological-anatomic-pathophysiological scale (CEAP) and Villala scale in combination with the duplex ultrasound examination. Quality of life patients with post-thrombotic syndrome will be evaluated with the health status questionnaire consisting of 36 questions (SF-36).

**Results:** We have already examined 40 patients hospitalized due to the first proximal DVT and after 1 year, and 18 patients have PTS according to the CEAP classification and to the Villalta scale. We planned to examine more patients but COVID 19 pandemic interrupted us.

**Discussion:** The results of our study showed that inflammation parameters (leukocytes, leukocyte subpopulations, C reactive protein, neutrophil and lymphocyte ratio), platelet count, mean platelet volume (MPV) and red cell volume distribution width (RDW) might play a role in PTS formation after proximal DVT.

**MeSH/Keywords:** post-thrombotic syndrome, venous thrombosis, inflammation, mean platelet volume, erythrocyte indices, quality of life

**Poster code:** R-02-09-125

**Poster Title:** Cardiovascular risks and arterial stiffness in patients with haemophilia

**PhD candidate:** Petra Bubalo

**Part of the thesis:** Cardiovascular risks and arterial stiffness in patients with haemophilia

**Mentor(s):** Assoc. Prof. Silva Zupančić-Šalek, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Haemophilia is rare, recessive X-linked inherited bleeding disorder, and most commonly occurs as haemophilia A and haemophilia B. There are many mutations of factor VIII and IX genes that cause haemophilia. They are classified according to the level of FVIII/FIX activity into three groups: severe, moderate and mild. Although mortality of patients with haemophilia is 2-3x higher than in the general population, few studies have shown 50-80% lower cardiovascular mortality. The etiology has not yet been clarified.

**Materials and methods:** We plan to enrol 100 patients suffering of haemophilia A and haemophilia B. Until now, total of 40 patients have been included. The data of subjects is collected using standard laboratory methods. The blood pressure is measured 3x. The arterial stiffness is measured by non invasive Arteriograph.

**Results:** Every patient of 40 patients included had FVIII and FIX activity tested. For the patients with haemophilia A the mean FVIII activity value is 0.057 kIU/L. The minimum FVIII activity value is 0.02 kIU/L, while maximum FVIII activity value is 0.14 kIU/L. For the patients with haemophilia B the mean FIX activity value is 0.043 kIU/L. The minimum FIX activity value is <0.2 kIU/L, while the maximum FIX activity value is 0.09 kIU/L. The minimum blood pressure of patients is 95/55. The maximum blood pressure is 170/105. The mean blood pressure is 130/75. Other results are still needed to be included.

**Discussion:** The complete results will show if there is lower arterial stiffness in patients with severe haemophilia which could explain us higher cardiovascular risk, but lower cardiovascular mortality in patients with haemophilia.

**Acknowledgments:** None

**MeSH/Keywords:** haemophilia, bleeding, arterial stiffness, cardiovascular risk

**Poster code:** R-02-09-135

**Poster Title:** Association between fibroblast growth factor 23 and bone loss in liver transplant candidates

**PhD candidate:** Andrija Jurina

**Part of the thesis:** Association between fibroblast growth factor 23 and bone loss in liver transplant candidates

**Mentor(s):** Assoc. Prof. Mario Starešinić, MD PhD, Marijana Vučić Lovrenčić, PhD, research advisor

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Bone loss is the most frequent metabolic bone disease in patients with liver cirrhosis who are candidates for orthotopic liver transplantation (OLT). The most common risk factors for bone loss in cirrhosis are alcoholism, malnutrition, hypogonadism, and hyperbilirubinemia. Fibroblast growth factor 23 (FGF 23) is a hormone produced by osteoblasts and regulates phosphate, vitamin D and parathyroid hormone (PTH) metabolism. FGF 23 blood level is elevated in cirrhotic patients and correlates with the stage of cirrhosis, increased mortality until OLT and depletion of osteoprogenitor cells.

**Materials and methods:** Patients who are candidates for OLT due to liver cirrhosis at the Merkur University Hospital were included in the study. Patients with an acute inflammatory condition, chronic renal insufficiency, diabetes, malignant disease or those on corticosteroids and/or bone antiresorptive bone drugs were excluded. Informed consent was obtained before study enrollment. Patient's history and demographic data were collected and known risk factors for bone loss in cirrhosis recorded. Standard blood tests were obtained. Blood samples for the additional measurement of FGF 23, high-sensitivity C-reactive protein, interleukin 6, intact PTH, 25-hydroxyvitamin D and bone alkaline phosphatase were stored. Plain radiographs of thoracic and lumbar spine were obtained. Bone densitometry (Dual-energy x-ray absorptiometry, DEXA) of lumbar spine (L1-L4) and hips was used to assess bone mineral density (BMD). Descriptive statistics were used to describe continuous and categorical data and Shapiro Wilks for the assessment of the normality of data. Fisher's exact test was done for comparing categorical data. A value of  $p < 0.05$  was deemed statistically significant.

**Results:** So far, 38 patients, 23 (60.5%) male and 15 (39.5%) female, mean age  $58.97 \pm 8.52$  years were included in the study. DEXA results showed a total number of 22 (57.9%) patients with bone loss. Bone loss was characterized as osteopenia in 12 (31.6%) and as osteoporosis in 10 (26.3%) patients. Only three (7.9%) patients had previously confirmed bone loss and in 19 (50%) patients bone loss was newly discovered. Nine (23.7%) patients had vertebral fracture. In the processed sample bone loss was sex-dependent ( $p = 0.043$ ), given that 13 (34.21%) men and only 3 (7.89%) female patients had normal DEXA finding. (Figure 1)

**Discussion:** We confirmed bone loss in 22 (57.9%) patients, with 19 of them being newly discovered. In the current sample ( $n = 38$ ) bone loss was sex-dependent, with female predominance. However, this is a small representative sample considering that we plan to include a minimum of 80 patients (40 patients with normal BMD and 40 patients with bone loss) in the study. DEXA data of all patients will be paired with laboratory results and analyzed. Homeostatic Model Assessment 2 will be used for the evaluation of insulin resistance. Fracture Risk Assessment Tool will serve for the assessment of osteoporotic fracture risk. These findings will increase knowledge and could lead to better understanding of metabolic background of bone loss, which would enable more efficient prevention of fractures and improve quality of life and life expectancy in OLT candidates.

**MeSH/Keywords:** fibroblast growth factor 23, bone loss, cirrhosis, liver transplantation

**Poster code:** R-02-09-136



**Poster Title:** Functional properties of apoB-depleted serum are altered in patients with coronary artery disease

**PhD candidate:** Tomislava Bodrožić Džakić Poljak

**Part of the thesis:** Endothelial lipase and functional features of high-density lipoprotein in patients with different clinical presentations of coronary heart disease

**Mentor(s):** Professor Vesna Degoricija, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Clinical and epidemiological studies have demonstrated that low levels of high-density lipoprotein cholesterol (HDL-cholesterol) are associated with an increased risk of cardiovascular disease (CVD). However, using the Mendelian randomization it was not possible to show an association between genetic variants that raise HDL-cholesterol plasma concentrations and a lower risk of cardiovascular events, and a pharmacological HDL-cholesterol-raising therapy proved ineffective in reducing cardiovascular events. Therefore, the determination of HDL particle concentrations and HDL subclasses, as well as the assessment of the HDL functionality, have emerged as more appropriate metrics of the atheroprotective properties of HDL. Patients with coronary artery disease (CAD) have flow-limiting lesions in the coronary arteries and constitute a unique inflammatory milieu. Given that even small changes in the microenvironment can alter the structure and function of HDL, we hypothesized that HDL may have impaired functional properties in CAD patients and that patients with acute coronary syndrome (ACS) may differ from those with stable CAD (sCAD). We therefore aimed to examine functional properties of the apoB-depleted serum, a surrogate of HDL, as well as serum enzymes involved in the metabolism of HDL in sCAD and ACS patients in comparison to healthy controls.

**Materials and methods:** Serum samples of 23 healthy subjects, 34 sCAD and 26 ACS patients were analysed. Cholesterol efflux capacity of apoB-depleted serum was examined in cAMP-stimulated J774 murine macrophages and the anti-inflammatory activity in lipopolysaccharide-stimulated U937 monocytes. The antioxidative capacity was measured by inhibition of free radical-induced oxidation of dihydrorhodamine. Lecithin-cholesterol acyltransferase (LCAT) and cholesteryl ester transfer protein (CETP) activities were measured with commercial kits

**Results:** sCAD patients showed highest cholesterol efflux capacity whereas their anti-inflammatory activity was higher than in healthy controls, yet lower than in ACS patients who exhibited the lowest antioxidative capacity and serum LCAT activity. The serum CETP activity was significantly impaired in sCAD and ACS patients

**Discussion:** In the present study, we show that the functional features of HDL as well as activities of enzymes involved in HDL maturation are altered in patients with different clinical presentations of CAD. The most intriguing finding was that CAD patients showed a markedly increased anti-inflammatory capacity of the apoB-depleted serum and a decreased LCAT activity. Since the anti-inflammatory capacity of apoB-depleted serum was higher and the serum LCAT activity lower in ACS compared to sCAD patients, it seems that the severity of the underlying pathophysiology and not the chronicity of the disease is a principal determinant of LCAT activity, HDL maturation and HDL functionality in CAD. Indeed, the levels of IL-6 the parameter indicative of CAD severity were significantly higher in ACS compared to sCAD patients. In contrast to the increased anti-inflammatory capacity of the apoB-depleted serum in both, ACS and sCAD patients, the cholesterol efflux capacity was only increased in sCAD patients. This indicates that functional features of apoB-depleted serum in ACS are different from those in sCAD. Differences in the structural and compositional features of HDL in these two patients groups might explain this phenomenon. After all, we can conclude that complex alterations of the HDL function in CAD patients, exemplified by increased cholesterol efflux and anti-inflammatory capacities but a decreased antioxidative activity of apoB-depleted sera might be due to impaired HDL maturation.

**MeSH/Keywords:** HDL coronary artery disease, HDL functional properties

**Poster code:** R-02-09-147

**Poster Title:** Specific Phenotype of Inflammatory Bowel Disease Associated With Primary Sclerosing Cholangitis

**PhD candidate:** Mislav Jelaković

**Part of the thesis:** Nucleotide polymorphism rs531564 of pri-miR-124 gene in patients with primary sclerosing cholangitis and inflammatory bowel disease

**Mentor(s):** Assist. Prof. Silvija Čuković-Čavka, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Primary sclerosing cholangitis (PSC) is a chronic cholestatic disease with a high incidence of inflammatory bowel disease (IBD), ulcerative colitis (UC) and Crohn's disease (CD). UC and CD in combination with PSC have a specific phenotype and significantly increased risk of colorectal and other cancers, with unclear pathogenesis. MicroRNAs (miRs) are small, non-coding RNAs that play a role in post-transcriptional gene regulation and may have a tumor-suppressor role, but also inflammation modulating role which has been demonstrated in multiple studies. The general aim is to analyze the frequency of genotypes of single nucleotide polymorphism rs531564 of the pri-mir-124 gene, among patients diagnosed with PSC and/or IBD, and to compare it between groups. Furthermore, we will test for possible associations between SNP rs531564 and clinical characteristics of IBD patients and PSC/IBD patients. In this preliminary phase, we have aimed to compare clinical characteristics of IBD in our cohort of patients.

**Materials and methods:** We evaluated medical charts from patients diagnosed with IBD alone and PSC/IBD in our referral center for inflammatory bowel diseases from 2011-2020. Descriptive statistical analysis was conducted for comparison of clinical characteristics between two groups. A two-sided p-value <0,05 indicated independent statistical significance.

**Results:** In all, we have included 29 IBD/PSC, 30 UC and 28 CD patients. PSC/IBD patients were diagnosed at a significantly younger age (23,21 +/- 8,25 vs 29,17 +/- 1,99; p<0,05), more likely to be male and have colonic involvement or pancolitis (68,9% vs 50% vs 36,6%; 71,4% vs 46% vs 51%; p<0,05, respectively). As indirect markers of IBD severity, PSC/IBD patients were less frequently treated with biologics (38,1% vs 64,2% vs 66,6% p<0,05), less likely to require hospitalization due to IBD (44,8% vs 92,8% vs 57,6% p<0,05) or surgical resection (17,2% vs 50% vs 48,2%; p<0,05) and had a significantly lower number of IBD related hospitalizations (1,3 +/- 0,4 vs 2,4 +/- 1,6; p<0,05).

**Discussion:** In this preliminary analysis, we have demonstrated that, as hypothesized, PSC/IBD patients in our cohort represent a distinctive phenotype with male predominance, younger age at diagnosis, milder clinical course and colonic involvement in most patients, which is in line with the available literature. Pathophysiology behind this distinctive IBD phenotype in PSC/IBD patients is not clearly understood. In the next phase, we will analyze the frequency of genotypes of single nucleotide polymorphism rs531564 of the pri-mir-124 gene and test for possible associations between SNP rs531564 and clinical characteristics of IBD patients.

**MeSH/Keywords:** ulcerative colitis; Crohn disease; primary sclerosing cholangitis; single nucleotide polymorphism

**Poster code:** R-02-09-162

**Poster Title:** Extent of extranodal extension is a potential phenotype biomarker of survival in oral cavity cancer patients with occult neck setting

**PhD candidate:** Matija Mamić

**Part of the thesis:** Prognostic significance of the extent of regional metastasis extranodal extension in clinically negative neck in oral cavity squamous cell carcinoma patients

**Mentor(s):** Assoc. Prof. Ivica Lukšić, MD PhD, Danko Muller, PhD, research associate

**Affiliation:** University of Zagreb School of Medicine, University Hospital Dubrava, Zagreb

**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 139 OCC patients who were primarily surgically treated with tumor resection and elective neck dissection in time period from 2009 to 2013 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:** Sixty-five patients (46.8%) were identified with occult nodal disease, with 30 patients (21.6%) presenting with ENE. Receiver operating characteristics (ROC) curve analysis set threshold at 1.6 mm as an optimal ENE cutoff regarding both DFS and OS. Patients were divided by extent into minor ENE ( $\leq 1.6$  mm) and major ENE ( $> 1.6$  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. Preliminary results of our study determined prognostic extent of ENE with the cutoff value at 1.6 mm determined by ROC curve analysis. At this survival discriminatory cutoff microscopic extent, patients were divided into low-risk (minor ENE;  $\leq 1.6$  mm) and high-risk (major ENE;  $> 1.6$  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-02-10-015

**Poster Title:** Survival analysis of patient or graft after liver transplantation - preliminary research results

**PhD candidate:** Miran Bezjak

**Part of the thesis:** Survival analysis of patient or graft after liver transplantation by machine learning methods

**Mentor(s):** Assoc. Prof. Tajana Filipec Kanižaj, MD PhD, Professor Bojana Dalbelo Bašić, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Liver transplantation is a successful method of treatment of liver cirrhosis. Common indications are alcoholic cirrhosis, cryptogenic cirrhosis (of unknown origin), hepatitis C cirrhosis, and cholestatic liver diseases such as primary biliary cirrhosis and primary sclerosing cholangitis. Development of surgical techniques, immunosuppressive protocols and antimicrobial therapy brought better results regarding complications and overall survival. When all indications are considered 5 – year recipient survival is about 70%, depending on center of transplantation. In 2007 Republic of Croatia joined Eurotransplant, an organization for the coordination and exchange of transplant organs in Central Europe, as a full member. Over the years Croatia has successfully developed donor network, and in past few years has numbered 30 - 35 donors per one million inhabitants, which is the highest number of donors in this part of Europe. The disproportion between the number of patients in need of a new organ and the lack of donors has led to an increase in the use of grafts of marginal quality. Marginal quality donors have been defined by Eurotransplant manual. The aim of the research is to analyze pre-transplant data of patients with transplanted liver in University hospital Merkur, Zagreb and evaluate impact of different variables on survival of the graft and recipient during the defined study period.

**Materials and methods:** In this retrospective study we included 656 cases of liver transplantation in University hospital Merkur in period between April 2013 and December 2018. We analyzed set of donor, graft and recipient variables known prior to transplantation. Only patients with cadaveric liver transplantation were included. Each retransplantation was considered as a new case. Data was collected using Eurotransplant database and Clinical hospital Merkur database, as well as patient's medical history charts. To analyze data we used R programming language, open source software for statistical computing and graphics. Survival analysis was performed using Kaplan-Meier survival curves and Cox proportional hazardous models with forward stepwise variable selection. Stepwise variable selection procedure can be applied to obtain the optimal final Cox proportional hazards model.

**Results:** Recipient survival probability based on Kaplan-Meier curve is 85%, 80% and 73% at 1, 3 and 5 years after liver transplantation, respectively. Data analysis identified eight donor, graft and recipient covariates that are connected to graft failure or recipient death. The most influential variables are: recipient diagnosis acute hepatic failure, recipient diagnosis hepatitis C cirrhosis and number of transplantation. All variables were part of final Cox proportional hazardous model which was found statistically significant. Final model, as a result from forward stepwise procedure was: transplantation number + recipient age + recipient diagnosis + donor serum ALT >105 U/L + donor sex + cold ischemia time + ET-DRI + steatosis.

**Discussion:** Evaluation of mentioned variables and their interconnection is interesting regarding donor recipient matching and optimal allocation. This is first survival analysis on this dataset and further evaluation with different methods such as machine learning methods with attempt of creating pre-transplant prediction models will be carried out in the future.

**MeSH/Keywords:** liver transplantation, survival analysis

**Poster code:** R-02-10-047

**Poster Title:** Shear wave elastography in prediction of Achilles tendon rupture

**PhD candidate:** Domagoj Lemac

**Part of the thesis:** Sonoelastography of intact Achilles tendons and tendons after surgical reconstruction

**Mentor(s):** Assist. Prof. Gordana Ivanac, MD PhD

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

**Introduction:** Achilles tendon (AT) is at high risk for rupture due to its characteristics and subsequently applied load. It is characterized by relatively poor blood supply, specifically in the area 3-5 cm from its calcaneus origin. Rupture presents with local pain, palpable disruption of continuity, disability to stand on toes and lack of strength during plantar flexion. Ultrasound is considered as a gold standard for confirmation of AT rupture. Ultrasound shear wave elastography (SWE) is a tool with the ability to quantify the elasticity and stiffness of the tissue and it was established as a reliable technique for assessment of mechanical properties of tendon. The theory that individuals who already suffered the AT rupture are more prone to rupture of a contralateral tendon was published three decades ago. Therefore, we hypothesized that SWE could be used to predict which AT will be at higher risk to future rupture.

**Materials and methods:** A total of 36 subjects were recruited in the study, among which 24 patients who suffered AT rupture and were surgically reconstructed and 12 aged-matched healthy controls. Each participant was interviewed, filled validated questionnaire, and was subjected to B-mode ultrasound examination with additional SWE. SWE was performed by Supersonic Aixplorer system, using linear array transducer (4-15 MHz) in musculoskeletal preset. Because of heterogenous structure of reconstructed AT, instead of analyzing specific region of interest on SWE, we analyzed the whole area of elastogram by using in-house developed AnSilk software. Functional outcomes were assessed with American Orthopedic Foot and Ankle Society (AOFAS) scoring system. Data from B-mode ultrasound, SWE and AOFAS questionnaire were presented as mean  $\pm$  standard deviation (SD) and were analyzed using non-parametric Mann Whitney test. Differences were considered statistically significant when a P-value  $<0,05$  was detected.

**Results:** Upon the rupture, AT stiffness was markedly decreased (by 42%,  $P<0.0001$ ) when compared to contralateral tendon of the patient. AOFAS questionnaire confirmed deteriorated function of injured tendon. Appearance of tendons on B-mode didn't show difference between contralateral intact AT in patients and both tendons of healthy individuals. Additionally, according to AOFAS questionnaire both groups reported normal function and lack of pain. SWE was utilized to reveal exact stiffness of each analyzed tendon. Stiffness of contralateral AT in patients with AT rupture was 23% lower than healthy tendon ( $P<0.05$ ). AOFAS score positively correlated with ultrasound SWE values in healthy and ruptured AT. However, AOFAS underestimated state of contralateral AT of patients. Collectively, irrespective of the lack of difference in the subjective feeling assessed by AOFAS, the contralateral tendon in the patients with reconstructed Achilles tendon has significantly lower elasticity than healthy individuals. Therefore, contralateral tendons in patients who suffered from rupture are more prone to future ruptures.

**Discussion:** Despite extensive studies no predisposing factors for AT rupture were detected among healthy individuals. Additionally, only 10 percent of patients who sustain an AT rupture had preexisting Achilles tendon problems. We confirmed that individuals with history of AT rupture have increased risk for rupture of contralateral tendon. SWE was proved as highly reproducible method for assessing the mechanical properties of AT. Thus, we utilized SWE to provide evidence of significantly softer contralateral tendon in patients with ruptured AT in comparison to healthy individuals. Noteworthy, subjective questionnaire and B-mode ultrasound failed to recognize potentially more vulnerable AT. SWE was revealed as an appropriate tool for early detection of vulnerable AT, and thus could be used as a screening method to predict potential AT rupture.

**MeSH/Keywords:** Achilles tendon, shear wave elastography, rupture

**Poster code:** R-02-10-091

**Poster Title:** Immunohistochemical expression of MAGE- A10 and NY-ESO-1 in metastatic and nonmetastatic gastric cancer

**PhD candidate:** Zvonimir Misir

**Part of the thesis:** Immunohistochemical expression of MAGE- A10 and NY-ESO-1 in metastatic and nonmetastatic gastric cancer

**Mentor(s):** Assist. Prof. Monika Ulamec, MD PhD

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

**Introduction:** The most important features of gastric cancer are invasiveness and high metastatic potential. Metastasis is a complex process whose mechanisms remain poorly understood. There is growing evidence that MAGE antigens play an important role in the metastatic potential of tumors of different origin. In several studies, the association of MAGE antigen expression with a lower degree of tumor differentiation, higher metastatic potential, and poorer chemotherapy response was described. NY-ESO-1 and MAGE antigens belong to the "cancer-testis" antigen (CTA) group and are expressed in tumor tissues while in normal tissue their expression is limited to spermatogonia. In this study, the MAGE-A10 and NY-ESO-1 expression in the primary adenocarcinoma of the stomach with and without metastasis in regional lymph nodes was compared with TNM stage, age, sex, and survival.

**Materials and methods:** A retrospective research has been conducted in which the archival materials of paraffin-embedded tissue blocks was used together with all relevant patient clinical data. Tumor specimens have been obtained from patients operated on in the Department of Surgery, Sestre milosrdnice University Hospital Center, Zagreb, Croatia, from 2005 to 2014. It included 108 samples randomized into two groups: 55 with lymph node metastasis and 53 without lymph node metastasis. Standard immunohistochemistry was used to determine MAGE-A10 and NY-ESO-1 antigen expression in primary tumors. Descriptive and analytical statistical methods were used in the statistical analysis of the results. Five-year survival data were collected from the Croatian National Cancer Registry.

**Results:** Preliminary data analysis did not reveal a connection between analyzed antigens and lymph node metastasis. The NY-ESO-1 antigen emerged as an independent prognostic factor of survival. Further analysis is underway.

**Discussion:** According to these preliminary results, MAGE-A expression does not seem to be a powerful prognostic marker and does not seem to hold the potential for modifying treatment plans for patients with gastric cancer. Metastatic disease is common in gastric cancer and is the most important prognostic factor. It would have been interesting to investigate the expression of the analyzed antigens in the metastatic lymph nodes in our study.

**MeSH/Keywords:** Gastric Cancer, MAGE-A10, NY-ESO-1

**Poster code:** R-02-10-164

**Poster Title:** The association of interleukin 6 and disease activity with chronic fatigue, depression and quality of life in patients with primary Sjögren's syndrome

**PhD candidate:** Fanika Mrsić

**Part of the thesis:** The association of interleukin 6 and disease activity with chronic fatigue, depression and quality of life in patients with primary Sjögren's syndrome

**Mentor(s):** Professor Jasenka Markeljević, MD PhD

**Affiliation:** University of Zagreb School of Medicine, Clinical Hospital Center Sisters of Mercy

**Introduction:** Sjögren's syndrome (SS) is autoimmune disease of exocrine glands and internal organs characterized by dryness of the eyes, oral cavities and other mucous membranes. It manifests clinically as primary SS (pSS) with glandular and systemic manifestations or secondary (sSS) associated with other inflammatory diseases. Patients with Sjögren's syndrome may also have extraglandular symptoms and general symptoms such as chronic fatigue and depression. Proinflammatory cytokines play an important role in the emergence of systemic and exocrine manifestation of pSS. Interleukin 6 (IL-6) is one of the main proinflammatory cytokines in pSS and its increased level is associated with numerous extraglandular symptoms. Chronic fatigue as one of the main features of many chronic diseases is especially important in autoimmune diseases. Chronic fatigue is the most common symptom in patients with pSS. It affects many aspects of life and has a negative impact on the quality of life of patients.

**Materials and methods:** The study will include around 60 patients with pSS who meet the revised internationally recognized classification criteria for diagnosis of Sjögren's syndrome (American-European Consensus Classification Criteria-AECC). Patients will be of female sex at the age of 18-70 years. Exclusion criteria will include other autoimmune diseases, acute and chronic inflammatory diseases and patients with psychiatric diagnosis. For the assessment of disease activity, we will use two disease activity indexes; ESSPRI (EULAR SS Patient Reported Index) and ESSDA index (EULAR Sjögren's Syndrome Disease Activity Index). After they sign informed consent they will complete questionnaires on chronic fatigue (CFQ 11), depression and anxiety (HADS), presence of pain (VAS) and quality of life (SF36 QL). After completing the questionnaires, we will take blood samples for biochemical analysis (CBC, CRP, ESR, fibrinogen, haptoglobin, protein electrophoresis), immunological analysis (ANF, ENA) and for interleukin 6. To determine the concentration of IL 6 in the samples we will use the chemiluminescence method. In control groups we will have around 20 healthy individuals that are identical to age and sex without the presence of fatigue, sicca symptoms and other autoimmune diseases.

**Results:** The research is currently in data collecting phase. We are planning to include a total of 60 patients. Following inclusion and exclusion criteria, at the moment, data for 17 patients is collected, analyzed and partially assessed. All of them readily participated in this study. Up until now 17 patients filled out all required questionnaires. All of the patients are of female gender and with similar baseline disease activity (ESSDAI 1.3; ESSPRI 15.58). Median of age is 52.05 with range from 23 to 67 years. Majority of patients (64.7%) have reported increased fatigue and impaired quality of life. Blood samples are being analyzed for biochemical and immunological testing (CBC, CRP, ESR, ANF, ENA). In about 41% of enrolled patients we found increased markers of inflammation. Analyses of IL-6 concentration will be commenced when half of participants are enrolled.

**Discussion:** Previous studies have shown that the proinflammatory mechanism of cytokines plays a central role in the emergence of chronic fatigue. Disruption in the regulation of the cytokine network in patients with pSS is reflected by elevated levels of proinflammatory cytokines that manifests through systemic and exocrine manifestations including chronic fatigue, depression and impaired quality of life. This study is still in process and we do not have all data to make final conclusion. However, although this is a small sample of patients without controls to compare, by observing the given answers there are indicators that point out that patients with pSS have impaired quality of life and increased fatigue. Since in the majority of cases it is about middle-aged patients, assessment of chronic fatigue, depression and quality of life, would enable more accurate diagnosis and monitoring of disease activity with individualization of therapeutic approach in patients with pSS. This study will provide a better understanding of the relationship between the activity of the disease and proinflammatory interleukin 6 with chronic fatigue and depression in patients with pSS.

**MeSH/Keywords:** Sjögren's syndrome, interleukin 6, chronic fatigue, quality of life

**Poster code:** R-02-14-040

**Poster Title:** The frequency, characteristics and clinical significance of unusual indirect immunofluorescence patterns on HEp-2 cells

**PhD candidate:** Nada Tomić Sremec

**Part of the thesis:** The frequency, characteristics and clinical significance of unusual indirect immunofluorescence patterns on HEp-2 cells

**Mentor(s):** Professor Drago Batinić, MD PhD, Professor Branimir Anić, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Antinuclear antibodies (ANA) are antibodies targeting cell nuclear components, and are produced in many autoimmune diseases. Analysis of those antibodies is an essential step in laboratory diagnostic of systemic autoimmune rheumatic diseases (SARD). Golden standard for ANA analysis is indirect immunofluorescence on HEp-2 cells. Using that method, it is possible to visualize various immunofluorescence patterns, of which some more common, such as homogenous and speckled patterns, have been thoroughly investigated, as is their correlation to the presence of some specific autoantibodies, and their role in diagnostics and follow-up of certain diseases. Certain rare immunofluorescence patterns have not been sufficiently investigated, and their significance in diagnostics of autoimmune diseases is unknown. The aim of this study is to determine the frequency of particular rare immunofluorescence autoantibodies patterns on HEp-2 cells and to assess their association with certain clinical conditions and entities, and to associate them with specific autoantibodies.

**Materials and methods:** As of May 2020, we included in our study 3913 serum samples of patients referred for routine ANA assessment. Sera were handled according to routine procedures, and subsequently assessed for presence of ANA using indirect immunofluorescence on HEp-2 cells. All positive samples were classified according to current international guidelines (ICAP consensus), which distinguish between 29 separate ANA patterns. After this initial assessment, all positive samples have undergone assessment for the presence of specific autoantibodies, using a microbead based multiplex system (ZEUS AtheNA Multi-Lyte® ANA-II Plus). Rare or unusual ANA patterns are classified as those appearing in less than 3% of all ANA positive samples. Sex distribution of immunofluorescence patterns was assessed as well. Additionally, a number of concurrently present immunofluorescence patterns was noted.

**Results:** Of all samples assessed for the presence of ANA in this preliminary overview, rare or unusual immunofluorescence patterns were found in 1005 patients (25.68%). Patients with such immunofluorescence patterns were on average 53.24±17.07 years old. Female predominance was present, with 785 female patients (78.10%) and 220 male patients (21.90%). Most frequently noted patterns were AC-19 (cytoplasmic dense fine speckled), AC-2 (dense fine speckled), AC-15 (cytoplasmic linear fibrillar), and AC-29 (topo I). Apart from the rare patterns recognized by the ICAP initiative, there was a substantial number of samples where various until now non classified immunofluorescence patterns were observed, represented in our study as AC-99 (Figure 1). Additionally, apart from the most prominent immunofluorescence pattern, additional, background patterns were present in the same sample in 516 cases (51.34%), where one additional pattern was observed in 389 of such samples (75.38%), while the remaining samples had more than two concurrently present samples. The presence of specific autoantibodies was established in 469 (46.66%) of all sera with rare immunofluorescence patterns.

**Discussion:** Trends established in our interim data analysis point to few interesting facts. Firstly, there appears to be a large total proportion of patients assessed during routine ANA screening where immunofluorescence patterns observed are of unknown or dubious significance. Even amongst those patients, there are some patterns that are so rare or unusual that they haven't even been included in the official nomenclature to this moment. Since they are often accompanied by the presence of specific autoantibodies, one might conclude that they are potentially quite important as they might pertain to certain autoimmune diseases. After completing the assessment of the total number of patients' sera as per study protocol, we will perform additional comprehensive analyses that will aim to elucidate associations of these immunofluorescence patterns with particular specific autoantibodies, as well as their associations with clinical diagnoses, and patients' demographic specificities.

**MeSH/Keywords:** antinuclear antibodies, immunofluorescence, HEp-2, autoimmunity

**Poster code:** R-02-14-056



**Poster Title:** Clinical features of patients with rheumatoid arthritis and atrial fibrillation

**PhD candidate:** Melanie-Ivana Čulo

**Part of the thesis:** Association of systemic inflammatory activity and atrial fibrillation in patients with rheumatoid arthritis

**Mentor(s):** Professor Jadranka Morović-Vergles, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Atrial fibrillation (AF) is the most common arrhythmia in clinical practice strongly correlated with cardiovascular diseases (CVD). There are a lot of known risk factors for development of AF and new studies have shown that systemic inflammation is an independent risk factor. Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease that primarily affects joints. It is well known that cardiovascular morbidity and mortality is significantly increased in RA patients and RA is independent risk factor for development of CVD. For that reason, patients with RA may have an increased risk for development of AF. If the systemic inflammation is one of the risk factors for development of AF, patients with chronic systemic diseases might have an increased risk for development of AF regardless of CVD. Development of AF in RA patients could be due to increased prevalence of CVD and consequence of the direct effects of chronic systemic inflammation on AF pathogenesis. Two main conducted studies gave the opposite results. Lindhardtsen et al. showed that patients with RA have 40% increased risk for AF while Kim et al. showed that patients with RA do not have an increased risk.

**Materials and methods:** We conducted a multicenter, cross-sectional study on two group of patients, one with RA and other with osteoarthritis (OA). We used data from our existing data base of University Hospital Dubrava collected between 2009 and 2010. We collected data from 627 patients with RA and 352 with OA regarding duration of the disease, comorbidities, treatment, age and gender, smoking history, height, weight with body mass index (BMI) and arterial pressure and recorded ECG. The analysis of blood was done for CRP, RF, HDL, LDL and total cholesterol, triglycerides, creatinine and glucose level. The activity of RA was evaluated by DAS28CRP, a well known and validated index of disease activity in clinical practice that combines number of tender and swollen joints, CRP level and general health (GH) that is patient visual assessment of disease activity on visual scale from 0 to 100 mm. Activity of OA was measured with GH previously described, and visual analog scale (VAS) of pain on visual scale from 0 to 10. We used OA as control group because OA also affects joints like RA but without systemic inflammation that is characteristic for RA.

**Results:** Female predominance was observed in both groups, 83.3% in RA, and 83.8 % in OA patients. Patients with OA were older, had longer durance of arterial hypertension, higher values of systolic and diastolic arterial pressure, higher BMI, cholesterol, triglycerides, glucose level and lower CRP than RA patients. OA patients also had more CVD, such as angina pectoris, diabetes, arrhythmias, dyspnea, peripheral edema and metabolic syndrome. Overall, we recorded 24 AF cases in RA and 12 AF cases in OA patients. There was no statistical difference in prevalence of AF in RA (3.82%) and OA (3.41%) patients,  $p=0,738$ . Also, we did not show difference in prevalence of AF regarding disease activity in RA patients. AF was recorded in 6.67% of patients that were in remission, 0 % with low disease activity, 2.33 % with moderate and 4.21% with high disease activity. Analyzing clinical features of patients with RA who developed atrial fibrillation, we confirmed that older age and smoking history were significant risk factors for developing AF (OR=1.07, 95%CI,  $p=0.042$ ; OR=1.05, 95%CI,  $p=0.009$ ).

**Discussion:** This study showed that the prevalence of AF was not higher in RA patients compared with OA patients. If systemic inflammation plays an important role in pathogenesis of AF development in patients with RA, it may be due to increased risk for development of CVD, thus indirectly, not because of direct effect of chronic systemic inflammation. Also, patients in our data base with OA were older and with significantly more cardiovascular and other comorbidities than patients with RA which may explain negative results. There is a cohort study that found an independent association between CRP and AF risk in men, but not in women, since RA and OA are more common in women this may explain negative results.

**MeSH/Keywords:** Rheumatoid arthritis, atrial fibrillation, CRP, DAS28CRP, osteoarthritis

**Poster code:** R-02-14-155

**Poster Title:** Antibiotic susceptibility of fluoroquinolone-resistant uropathogenic *Escherichia coli* O25 strains in Zagreb outpatient population

**PhD candidate:** Maja Anušić

**Part of the thesis:** Characterization and estimation of clonal spread potential of fluoroquinolone-resistant uropathogenic *Escherichia coli* O25 ST131 strains in Zagreb outpatient population

**Mentor(s):** Professor Jasmina Vraneš, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** In the outpatient population with urinary tract infection, uropathogenic *Escherichia coli* (UPEC) may have reduced antibiotic susceptibility and can be expanded clonally. ARESC (Antimicrobial Resistance Epidemiological Survey on Cystitis) study confirmed the incidence of multiple-resistant UPEC in 10.3% of isolates over the period 2003–2006. Concurrent resistance to beta-lactam (ampicillin), sulfamethoxazole / trimethoprim and fluoroquinolones is the leading multiple resistance phenotype of UPEC strains from the ARESC study. Multiple-resistant *E. coli* isolates in the SENTRY and MYSTIC studies conducted in USA in 2007 at the proportion of 52% isolates belonged to the *E. coli* O25 ST131 clonal group. Throughout the world, the proportion of multiple resistance strains of UPEC is on the rise and is a serious public health problem. This study compares the antibiotic susceptibility of fluoroquinone-resistant uropathogenic strains of *E. coli* serogroup O25 observed at two time periods at intervals of 6 years with respect to the proportion of multiple-resistant phenotypes.

**Materials and methods:** *E. coli* O25 strains isolated in outpatients with proven urinary tract infection (significant bacteriuria, bacterial count  $\geq 10^5$  CFU/mL in pure culture-monoculture, and positive leukocyte esterase) and with resistance to fluoroquinolones (norfloxacin and ciprofloxacin) were included in the study and collected from March 2011 to January 2012 (47 strains total) and from October 2017 to August 2018 (122 strains total). Antibiotic susceptibility was tested according to EUCAST breakpoint table for Kirby Bauer disk diffusion method. The following antibiotics were tested: ampicillin (AMP), co-amoxiclav (AMC), cephalexin (CN), cefuroxime (CXM), cefixime (CFM), gentamicin (GM), cotrimoxazole (SXT), nitrofurantoin (FM) and ertapenem (ETP) in all strains of *E. coli* O25. The percentages of antibiotic resistance were calculated as well as the proportion of multiple resistant phenotypes in the both time periods observed.

**Results:** Between March 2011 and January 2012, there were 47 *E. coli* O25 strains, 16 (34%) of which did not produce ESBL and 31 (66%) of ESBL positive. Overall antibiotic resistance was 89% for ampicillin, 28% for co-amoxiclav, 66% for cephalexin, 66% for cefuroxime, 60% for cefixime, 21% for gentamicin, 64% for cotrimoxazole, 17% for nitrofurantoin and 0% for ertapenem. In *E. coli* O25 ESBL negative strains, the dominant multiple-resistant phenotype was concomitant resistance to ampicillin, cotrimoxazole and fluoroquinolones with a proportion of 19% (3 isolates). In *E. coli* O25 ESBL positive strains, the dominant multidrug resistance phenotype was resistance to the tested antibiotics with simultaneous sensitivity to 4 categories of antibiotics ( $\beta$ -lactam with inhibitor, aminoglycosides, nitrofurantoin and ertapenem) with a proportion of 42% (13 isolates). There were a total of 38 (81%) multiple resistant strains in this time period. Between October 2017 and August 2018, there were 122 *E. coli* O25 strains, of which 46 (38%) were ESBL negative and 76 (62%) were ESBL positive. Overall antibiotic resistance was 92% for ampicillin, 53% for co-amoxiclav, 65% for cephalexin, 63% for cefuroxime, 63% for cefixime, 43% for gentamicin, 68% for cotrimoxazole, 7% for nitrofurantoin and 0% for ertapenem. The dominant multidrug resistance phenotype in *E. coli* O25 ESBL negative strains was concurrent resistance to ampicillin, cotrimoxazole and fluoroquinolones with a proportion of 22% (10 isolates). In *E. coli* O25 ESBL positive strains, the dominant multidrug resistance phenotype was resistance to the tested antibiotics with simultaneous sensitivity to 4 categories of antibiotics ( $\beta$ -lactam with inhibitor, aminoglycosides, nitrofurantoin and ertapenem) with 32% (24 isolates). During this time period, there were a total of 106 (87%) multiple resistant strains.

**Discussion:** In concordance with results from the ARESC study, the dominant multidrug resistance phenotype of *E. coli* O25 ESBL negative strains (resistance to ampicillin, cotrimoxazole and fluoroquinolones) was also demonstrated in our study. Multiple resistant strains of *E. coli* O25 were recorded over 80% in both observed time periods, some of which certainly belong to the clonal group of *E. coli* O25 ST131 according to the results of multiple resistant isolates of *E. coli* O25 ST131 from SENTRY and MYSTIC study.

**MeSH/Keywords:** *Escherichia coli* O25, urinary tract infection, multipleresistance

**Poster code:** R-02-16-038

**Poster Title:** Surgical site infections-first Croatian national study

**PhD candidate:** Ana Gverić Grginić

**Part of the thesis:** Correlation of risk reduction procedures and surgical site infections

**Mentor(s):** Professor Ana Budimir, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Surgical site infections represent important part of health care-associated infections (HCAI). Their epidemiological burden varies, depending on healthcare system development and resources. European Centre for Disease Prevention and Control's point prevalence survey conducted in European states in 2011 and 2012 showed that prevalence of HCAs was 6% (95% CI 5.7–6.3%), with relative frequency of surgical site infections 19.6% (8.8%– 29.0%). Aims of this research were to determine prevalence of surgical site infections in Croatia and frequency among overall HCAs, and to establish association between risk reduction features and surgical site infections. Additional aims of the study were to determine SSIs' bacterial causative agents, their antibiotic susceptibility and to assess rational use of surgical antibiotic prophylaxis.

**Materials and methods:** Multicentre cross-sectional point prevalence study of healthcare-associated infections and antimicrobial use was conducted in May 2017. This study was a part of European Centre for Disease Control's study. National coordination was conducted from Ministry of Health's Referral Centre for Nosocomial Infections in University Hospital Centre Zagreb. Web-based closed questionnaire was addressed to participating institutions to identify applied risk reduction procedures of surgical site infections prevention. Study participants were acute hospitals in Croatia with surgical wards. Instructions for study conduction and training material were enclosed in participation invitation in the study. Data were extracted from medical records without contact with patients and/or healthcare workers and were collected in a single day for each ward by hospital infection control personnel, and were recorded in standardized hospital, ward and patient ECDC forms. HELICS/IPSE definitions of healthcare-associated infections were used. Hospital data included institution's structure and process indicators. Hospital data included structure and process indicators. Structure indicators were hospital categorization, number of surgical beds, and number of discharges in surgical wards in previous year, and infection prevention doctor and nurse/250 beds ratio. Process indicator was alcohol hand hygiene antiseptics consumption in surgical wards (L/year). Data about rational antibiotic use included generic name of the antibiotic, way of application, and indication. Duration of surgical antibiotic prophylaxis was compared to Croatian national guidelines for antimicrobial prophylaxis in surgery.

**Results:** A total of 32 (32/34, 94.1%) acute care hospitals with surgical departments were included in the study. Prevalence of overall healthcare-associated infections was 5.3% (95% CI 4.8-5.7). Prevalence of surgical site infections was 0.9% (95% CI 0.7-1.1), and they were third most common infection with relative frequency of 15.9% (0%-50%). In university hospitals prevalence ranged from 0% to 2.4%, in regional hospitals from 0% to 1.1%, in county hospitals from 0% to 3.2%, respectively. Most common bacterial causative agents were gram positive cocci (44.6%), followed by Enterobacteriaceae (26.2%). In 79% of patients surgical antibiotic prophylaxis was prolonged.

**Discussion:** Prevalence and relative frequency of surgical site infections in Croatia are comparable to those in other European countries. Highest prevalence and frequency were reported in county general hospitals and hospitals with lowest hand hygiene alcohol antiseptics consumption and infection prevention nurse/bed ratio. Surgical antibiotic prophylaxis prolongation was high in all hospital categories. Further data analysis will give the answers about the correlation of surgical site infections burden with risk reduction procedures. Conclusions can be drawn from preliminary results that additional infection prevention staff were lacked, and education on surgical site infection prevention are needed.

**MeSH/Keywords:** surgical site infection, prevention, risk factors, drug resistance, microbial

**Poster code:** R-02-16-051

**Poster Title:** Pseudophakic cystoid macular edema in patients with non-proliferative diabetic retinopathy.

**PhD candidate:** Anđela Jukić

**Part of the thesis:** The effect of topical bromfenac in prevention of pseudophakic cystoid macular edema in patients with non-proliferative diabetic retinopathy.

**Mentor(s):** Assist. Prof. Miro Kalauz, MD PhD

**Affiliation:** Ophthalmology Clinic, University Hospital Center Zagreb ; Department of Ophthalmology, University Hospital Dubrava

**Introduction:** Pseudophakic cystoid macular edema (PCME) occurs as a complication 6-10 weeks after cataract surgery. It is the main cause of vision loss after cataract surgery and could lead to permanently impaired central vision owing to altered outer photoreceptor features.

**Materials and methods:** 90 patients with cataract and non-proliferative diabetic retinopathy (Mild and Moderate NPDR by ETDRS Classification, LOCSIII-2 and 3) Group 1: 7 days preoperatively and 3 weeks postoperatively: topical bromfenac 2x daily (0.9 mg / mL) Group 2: 7 days preoperatively and three weeks postoperatively: topical dexamethasone 3x daily (1mg / mL) Group 3: 7 days preoperatively and 3 weeks postoperatively: topical placebo 2x daily All three groups: topical antibiotic (tobramycin 3mg / mL) 4x daily three weeks postoperatively. On the day of the cataract surgery aqueous humor samples from anterior eye chamber will be collected and analyzed for interleukin 6 (IL6) concentrations. Ophthalmologic examination and OCT scan (optical coherence tomography) will be performed 7 days before the surgery, on the day of the surgery, and 1, 7, 30 and 90 days after the surgery.

**Results:** So far we enrolled 20 patients with diabetes mellitus type II (10 male, 10 female) with a mean age of M=69.55 (Medijan=69); 8 patients in bromfenac group, 5 patients in maxidex group, and 7 patients in placebo group.. There was no significant difference in average score of central foveal thickness (CFT) between the groups regardless of the observed measuring point. Slightly higher results are visible at CFT 30 measurements for all three groups of participants. OCT showed cystoid macular edema (CME) in one patient in placebo group.

**Discussion:** Conclusions were made on a small sample. The study should be continued and IL6 concentration should be analyzed in aqueous samples to make definitive conclusions.

**MeSH/Keywords:** pseudophakic cystoid macular edema (PCME), cataract, central retinal thickness (CRT), OCT (optical coherence tomography), diabetic retinopathy, interleukin 6 (IL6)

**Poster code:** R-02-18-028

**Poster Title:** Structural and functional changes of retina and optic nerve in patients with dysthyroid orbitopathy and elevated intraocular pressure

**PhD candidate:** Petra Kristina Ivkić

**Part of the thesis:** Structural and functional changes of retina and optic nerve in patients with dysthyroid orbitopathy and elevated intraocular pressure

**Mentor(s):** Assist. Prof. Jelena Juri Mandić, MD PhD

**Affiliation:** Department of Ophthalmology and Optometry, Clinical Hospital Center Zagreb, University of Zagreb School of Medicine

**Introduction:** Dysthyroid orbitopathy or Graves orbitopathy (GO) is an organ-specific autoimmune orbital disease associated with autoimmune thyroid disease, in which IgG antibodies bind to TSH receptors causing inflammation of the extraocular muscles (EOM) and infiltration of interstitial tissue, orbital fat tissue and lacrimal gland with inflammatory cells associated with glycosaminoglycan accumulation and retention of fluid. Due to these changes, volumetric and contraction changes occur to the EOM. In the vast majority of patients with GO regardless the stage of the disease, we found elevated intraocular pressure (IOP) values.

Etiopathogenesis of the secondary elevated IOP in GO is not fully clarified. It is generally accepted thesis that increasing the volume of orbital tissue disables normal venous drainage, causing additional congestion of orbital tissue. The elevated IOP at various stages of the newly detected GO can lead to specific changes in the structure of the optical nerve fibers, retina and macula which is different from the damage found in patients with primary elevated IOP.

**Materials and methods:** We divided respondents into two groups of patients with newly diagnosed GO and different clinical stage of the disease, and one control group of respondents. First group consists of patients with GO and IOP up to 22 mmHg. Second group consists of patients with GO and elevated IOP above 22 mmHg. Third control group consists of patients with glaucoma, without GO, comparable age and sex distribution. In this study we excluded participants with ophthalmic comorbidities that are not a consequence of the underlying disease which affects on biomechanical changes in eye structures: high myopia (> -5 dpt), high hypermetropia (> +3 dpt), optical disk disease, macular disease, vascular and degenerative retinal disease, blur of optical media, amblyopia, other orbital diseases and patients with neurological disorders who have pathologically changed perimeter findings due to underlying disease, and respondents with clinical activity score from 8 to 10. We are in progress of performing optical coherence tomography and recording of the optical nerve fibers, retinal and ganglion cell complex with Octopus field 900 in G2 software with trend analysis.

**Results:** The study is still ongoing, currently it is in the phase of data collection.

**Discussion:** Data collected from study participants are still not sufficient for proper statistical analysis, therefore no clear conclusions can be drawn at this time. We expect that the analysis of specific structural and functional changes in the nerve fibers of the retina, optical nerve and ganglion cell complex will lead to a better prevention of the structural and functional damages in patients with newly diagnosed GO in different stages of disease and secondary elevated IOP, and right selection of appropriate treatment modalities for earlier and better health and social rehabilitation.

**MeSH/Keywords:** dysthyroid orbitopathy, intraocular pressure, optical coherence tomography, visual field, retinal nerve fibers, optic nerve and macula

**Poster code:** R-02-18-118

**Poster Title:** Visual outcome after implantation of multifocal intraocular lenses

**PhD candidate:** Mateja Jagić

**Part of the thesis:** Visual outcome after implantation of multifocal intraocular lenses

**Mentor(s):** Assoc. Prof. Smiljka Popović-Suić, MD PhD, Professor Iva Dekaris, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Over the past few decades, cataract surgery has greatly improved, in surgical procedure itself and in the technology of intraocular lenses (IOLs). With progress improvement methods, the rate of cataract surgery is increasing. Active lifestyle and need for good vision at all distances leads to an increase in number of patients approaching the surgery. First generations of multifocal IOLs, along with providing good vision at all distances, also exhibited some of negative side-effects, such as decreased contrast sensitivity, visual impairment in poor light (scotopic) conditions and impaired vision at intermediate distances. New generation of multifocal IOLs, should significantly reduce disadvantages of previous generations of multifocal IOLs. Unlike multifocal, standard monofocal IOLs cannot enable good vision at all distances, but no side-effects are observed as in first generation of multifocal IOLs.

**Materials and methods:** In prospective randomized trial, 4 groups of 50 patients are bilaterally embedded: a) one of the 3 types of multifocal IOL (trifocal intraocular lens, intraocular lens with extended range of vision (EROV) and diffractive intraocular lens with minimal, low addition) and b) monofocal intraocular aspherical lenses (control group). Follow up is planned for a period of 1 year Postoperatively. For objective analysis of visual outcome, uncorrected and best corrected visual acuity for all distances distances, contrast sensitivity, values of high order aberrations (HOAs), rate of opacity of posterior capsule, dysphotopsia level under reduced (scotopic) light condition and the subjective level of patient satisfaction with the result will be measured.

**Results:** UDVA and CDVA values were significantly better in EROV IOL group than other 3 IOLs ( $P = 0.002$ ;  $P = 0.014$ ;  $P = 0.001$ ;  $P < 0.001$ ). UNVA between the 4 IOLs were statistically significant ( $P < .001$ ), where trifocal IOL had the best outcomes compared with other 3 IOLs ( $P < .001$ ). EROV IOL ( $P = 0.011$ ) and Low add IOL ( $P = 0.019$ ) showed slightly but significantly better CNVA results when compared with trifocal IOL. UIVA and DCIVA in trifocal and EROV IOL were better than in monofocal and LowAdd IOL ( $P < 0.001$ ), with superior results in trifocal IOL. Contrast sensitivity values for monofocal and EROV IOL were significantly better than those in trifocal and LowAdd IOL ( $P < 0.001$ ). Total and intraocular HOAs at a 3.0 mm pupil diameter were not statistically different in multifocal IOL group comparable to monofocal IOL. At 5.0 mm diameter, EROV IOL had significantly higher values than other 3 IOLs. There was no statistically significant difference in posterior capsule opacification appearance between the IOLs. Dysphotopsia scores were significantly higher in all multifocal IOLs than the monofocal IOL, particularly halo and glare, mostly reported in Low add and trifocal IOL. Trifocal IOL resulted in significantly better spectacle independence than other 3 IOLs ( $P < 0.001$ ).

**Discussion:** Since there is an increase in the use of computers and smartphones, near and intermediate vision is becoming more relevant. There were obvious limitations in intermediate vision with bifocal IOL so trifocal IOL design was introduced, with additional focus for intermediate vision and associated with increased background halos and worse distance visual quality, according to optical bench analyses conducted by Madrid Costa et al. Another strategies for obtaining good intermediate vision is designing lower addition in diffractive IOL, mix-and-match technique (Jacobi et al., Mastropasqua et al. The current literature shows an excellent UDVA/CDVA for EROV IOL in several studies (Pedrotti et al., Cochener et al., Menucci et al.). At UIVA/CDIVA multifocal IOL fared well and better than the monofocal IOL, with the trifocal and EROV IOL achieving better results than the Low add IOL, comparable with results in a optical bench analysis (Lee et al.). Some of the studies reported incidences of halos and glare in multifocal IOL (Monaco et al.) The results of our study were consistent with various results obtained in the aforementioned studies. The evaluated IOL, monofocal and multifocal, provide a successful restoration of the distance vision, however, trifocal IOL provided better near visual outcomes, EROV and trifocal IOL provided significantly better intermediate visual outcomes, but with better quality of vision levels with EROV IOL.

**MeSH/Keywords:** multifocal intraocular lens, visual quality, cataract surgery, refractive lens exchange

**Poster code:** R-02-18-144

**Poster Title:** Correlation of PPAR $\gamma$  gene and BDNF protein in glaucoma patients

**PhD candidate:** Tena Križ

**Part of the thesis:** Correlation of biomarkers BDNF, MYOC, IL-6 i PPAR $\gamma$  with optic nerve damage in glaucoma patients

**Mentor(s):** Professor Jadranka Sertić, MD PhD, Assist. Prof. Mia Zorić Geber, MD PhD

**Affiliation:** University of Zagreb School of Medicine; Clinical Institute of Laboratory Diagnosis, University Hospital Center Zagreb, Zagreb, Croatia; Department of Ophthalmology, University Hospital Center Sestre milosrdnice, Zagreb, Croatia

**Introduction:** Glaucoma is a chronic, slowly progressive optic neuropathy that consequently leads to damage to the visual field. It is one of the major causes of irreversible blindness in the world. The most common type of glaucoma is Primary Open Angle Glaucoma (POAG), and despite numerous studies, the exact pathomechanism has not yet been determined. POAG is most often an asymptomatic disease and often goes unnoticed until damage has already progressed. Currently, the only treatment option is to lower intraocular pressure, and other treatment options, including neuroprotection, are still being explored. Brain Derived Neurotrophic Factor (BDNF) is a protein whose role is to preserve neurons. Its serum concentration is reduced in people with glaucoma. BDNF gene polymorphisms, and in particular polymorphism (Val66Met), have been associated with neurodegenerative diseases and glaucoma. PPAR $\gamma$  is a nuclear transcription factor for multiple genes and proteins involved in neurodegenerative diseases, among other disease, and its agonists have a neuroprotective role. The most common genotype in healthy subjects is CC, and the GG genotype is associated with pathological biological processes. PPAR $\gamma$  is found in the retina and slows the progression of degeneration and inflammation by inhibiting microglia activation. The aim of this study was to examine, for the first time, the correlation between BDNF protein and PPAR $\gamma$  gene as possible predictors of disorders leading to glaucoma development.

**Materials and methods:** The study included 90 examinees from the Referral Center for Glaucoma, UHC Sestre milosrdnice, aged between 35-80 years with a diagnosis of glaucoma. After a complete ophthalmological examination, two additional examinations were performed on all subjects; visual field and OCT angiography to make an accurate diagnosis of glaucoma damage. For the purposes of molecular analysis, 2 test tubes of venous blood (with and without anticoagulants of 3 mL each) were taken immediately before surgery, and a sample of aqueous humor at the beginning of glaucoma surgery. The concentration of BDNF in aqueous humor and serum was determined by ELISA method. Genotyping of PPAR $\gamma$  (Pro12Ala) was performed using the PCR-RFLP method. A multivariate regression analysis was performed for the analyzed variables. The level of statistical significance is the value of  $p < 0.05$ .

**Results:** The difference between the concentration of BDNF protein and PPAR $\gamma$  genotype in serum and aqueous humor was tested. The multiple comparison test showed that the PPAR $\gamma$  CC:GG and CG:GG genotypes differed statistically significantly, while there was no statistically significant difference between the CC and CG genotypes. This study revealed statistically significantly higher concentrations of BDNF protein in aqueous humor in patients with the PPAR $\gamma$  GG genotype. Elevated values are associated with a compensatory neuroprotective response. In serum, the differences between BDNF protein concentration and PPAR  $\gamma$  GG genotype were not statistically significant.

**Discussion:** We did not find any study examining the association between PPAR $\gamma$  genotype and brain neurotrophic factor (BDNF) in glaucoma patients reviewing the literature. To best of our knowledge this is the first one which compares the above mentioned. The PPAR $\gamma$  GG genotype could be a predictive biomarker for early identification of at-risk glaucoma patients. Further research on a larger number of examinees is needed to determine the role of PPAR $\gamma$  GG polymorphism in the pathogenesis of glaucoma and the molecular mechanisms responsible for glaucoma development.

**MeSH/Keywords:** Glaucoma, BDNF, PPAR gamma

**Poster code:** R-02-18-160

**Poster Title:** Prognostic significance of and lymph node ratio (LNR) in clinically node-negative (cN0) oral cancer: subgroup analysis of preliminary results

**PhD candidate:** Iva Nikles

**Part of the thesis:** Prognoštička vrijednost udjela pozitivnih limfnih čvorova u klinički negativnom vratu oboljelih od karcinoma usne šupljine

**Mentor(s):** Assoc. Prof. Ivica Lukšić, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** With an estimated 354, 800 new cases and 177, 300 deaths per year, oral squamous cell carcinoma (OSCC) is among the most common malignant tumours and a significant cause of morbidity. Although the incidence of OSCC has decreased in most developed countries over the past decades, it remains a common cancer for both men and women in south–central Asia and in central and Eastern Europe. According to AJCC/UICC, the presence of lymph node metastases has been associated with poor outcome. However, nodal stage by itself was not shown to reliably predict prognosis. Lymph node ratio (LNR) has been shown to be an independent predictor of recurrence risk and survival in different types of cancer. Previously we have shown that LNR is reliable predictor of final outcome in patients with clinically node-negative oral cancer. The aim of our study was to additionally evaluate characteristics of LNR in this clinical scenario.

**Materials and methods:** Inclusion criteria were histologically proven OSCC, no clinical evidence of regional metastases (cN0), pathological lymph node metastases (pN+) and no prior treatment for head and neck cancer. The study included patients with OSCC who were primarily surgically treated between 2000 and 2004 at the Department of Maxillofacial Surgery, University Hospital Dubrava, Zagreb, Croatia. Neck was considered being cN0 when there were no palpable lymph nodes on physical examination and the size of the lymph node was <1 cm determined by computed tomography (CT) without area of central necrosis (central low density or inhomogeneity). Patients were separated into a low and high number of positive lymph nodes, and LNR groups using ROC curve analysis.

**Results:** A total of 61 patients were primary surgically treated using intraoral excision and elective neck dissection. Neck metastases were confirmed in 26 neck dissection specimens (42.6%). Nineteen patients had sublingual cancer, while 21 and 18 patients had tongue cancer and cancer of retromolar space, respectively. There were 19 selective neck dissections and 42 radical neck dissections with respect to the extent of surgical treatment. Median number of dissected lymph nodes was 10 (range 10-75). In most of the cases (24/26=92.3%) regional metastases were confined to the neck levels I-III, while in 2 cases (6.7%) metastatic spread occurred in region IV and/or V. Half of the patients had involvement of one region, while other half experienced neck failure in multiple regions. Fourteen patients had extranodal extension, while 12 patients had intact lymph node capsule. Patients with high LNR experienced more recurrences and shorter disease-free survival (DFS) period compared to those having low LNR (12 vs. 6 disease relapses, 31 vs. 45 months DFS period)

**Discussion:** LNR is reliable predictor of final outcome and disease recurrence in clinically node-negative oral cancer subjects. Additionally, high LNR is associated with shorter DFS period. It seems that lymph node metastases occur more often in clinically negative neck than is generally appreciated and that extranodal extension is frequent event in such setting. Further investigations and prospective trials are needed in order to assess prognostic significance of LNR in head and neck cancer.

**MeSH/Keywords:** oral cancer, lymph node ratio, disease-free survival

**Poster code:** R-02-19-006



**Poster Title:** Epigenetic modifications of IL17 gene in early invasive breast cancer

**PhD candidate:** Ljubica Radmilović Varga

**Part of the thesis:** Epigenetic modifications of IL17 gene in early invasive breast cancer

**Mentor(s):** Assist. Prof. Natalija Dedić Plavetić, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Significant progress has been made in understanding the invasive breast cancer biology in recent 10 years, as well as developing new and more successful therapeutic approaches. However, in approximately 30% of patients with optimal curative treatment of early invasive breast cancer, the distant disease occurs. IL-17 is a product of T helper 17 lymphocytes (Th17). Previous research has shown an IL-17 overexpression in the breast cancer microenvironment, as a result of epigenetic changes in the IL17 gene and together with the predominant Th2 immunosuppression pattern, appears to have a pro-oncogenic effect.

**Materials and methods:** Breast cancer paraffin-embedded tissue specimens and blood samples will be obtained from a series of 150 female patients with primary operable invasive breast carcinomas referred to the University Hospital Center Zagreb, between January 2012 and January 2017. As a control group, we will analyze serum samples from 50 healthy women volunteers. The DNA IL17 methylation degree will be measured by pyrosequencing. IL-17 serum levels will be measured by the enzyme-linked immunoadsorbent assay (ELISA) and the IL-17 tissue expression will be determined immunohistochemically. The tumor size, histological type, histological and nuclear grade, estrogen and progesterone receptor status, the involvement of axillary lymph nodes, HER-2 status, and lymphovascular invasion will be obtained for all patients. All of these patients are followed-up prospectively for two years according to routine standard institutional practice with local recurrence, distant metastases, or death as primary outcome end-points. According to this information, the data on two-year disease-free survival will be collected.

**Results:** Blood samples and tumor tissue from 150 women diagnosed with early invasive breast cancer were taken. A blood samples from 50 healthy women volunteers were taken also. We collected data on tumor size, histological and nuclear grade, estrogen and progesterone receptor status, the involvement of axillary lymph nodes, HER-2 status, and lymphovascular invasion for 118 patients.

**Discussion:** IL-17 overexpression in breast cancer microenvironment appears to have a pro-oncogenic effect, and IL-17 is overexpressed by epigenetic changes in IL17 genes (DNA methylation). As far as we know, this is the first study to investigate the correlations between the methylation IL17 gene levels and IL-17 protein serum concentration in patients with early invasive breast cancer. In further research, we will determine and compare the IL17 gene methylation degree with the IL-17 concentration in the serum of women with an early invasive breast cancer (different surrogate biological subtypes) and in the serum of healthy women. Also, we will determine the correlation of the degree of methylation DNA (IL17) isolated from the blood of women with early breast cancer with the expression of IL-17 in tumor tissue and with other clinical and pathohistological characteristics. The correlation of the degree of methylation DNA (IL17) isolated from the blood of the women with early breast cancer and two-year disease-free survival will be analyzed. Our results may lead to clarifying the role of epigenetic changes in IL17 genes and expression of prominent cytokine IL-17 that can play an important role in breast cancer etiopathogenesis but also affect the clinical course and disease outcome.

**Acknowledgments:** I would like to thank my mentor Assistant Professor Natalija Dedić Plavetić, MD, PhD, for scientific guidance and support.

**MeSH/Keywords:** DNA methylation, epigenetic modification, IL-17, breast cancer.

**Poster code:** R-02-19-103

**Poster Title:** Local application of recombinant human Bone Morphogenetic Protein 6 to osteoporotic rats pretreated systemically with bisphosphonates

**PhD candidate:** Petra Jurina

**Part of the thesis:** Local application of recombinant human Bone Morphogenetic Protein 6 to osteoporotic rats pretreated systemically with bisphosphonates

**Mentor(s):** Academician Slobodan Vukičević

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Atypical femoral fractures are rare fractures associated with long-term bisphosphonate therapy in osteoporosis patients. Often, fractures appear bilaterally with high nonunion rate and development of pseudoarthrosis. Despite advanced osteosynthesis methods, fractures represent significant disability for the patients. Studies suggest that synergy of BMPs anabolic effect and bisphosphonates antiresorptive effect achieves better and faster bone healing with firmer callus.

**Materials and methods:** Experimental research is conducted using animal model of Sprague-Dawley rats, 4 months of age, weighing 250-300 g. Research was divided into experiment A and B. By the time of publishing this poster experiment A was concluded, showing further results in tables and pictures. In experiment A rats were divided in 5 groups (A-E). Group A were SHAM, while group (B-E) were ovariectomized. Groups C-E were receiving alendronate in doses 0.5, 1 and 2 mg/kg orally, imitating intake of alendronate in postmenopausal women with osteoporosis. Experiment A was to conclude the most effective dose and duration of alendronate exposure measuring bone mineral density with densitometry.

**Results:** Experiment A showed that alendronate dose of 2 mg/kg is resulting in significant difference in bone mineral density, both in trabecular (spine) and cortical bone (right femur), in comparison to lower applied doses of 0.5 and 1 mg/kg.

**Discussion:** Alendronate dose of 2 mg/kg will be used in experiment B, applied in combination with recombinant human Bone Morphogenetic Protein 6 to evaluate synergetic effect of bisphosphonates and BMP in order to achieve better and faster bone healing with firmer callus.

**MeSH/Keywords:** atypical femoral fractures, rhBMP 6, alendronate, osteoporosis

**Poster code:** R-02-20-021

**Poster Title:** Effect of usage of topical anesthesia on level of pain and discomfort during high-speed digital imaging

**PhD candidate:** Juraj Slipac

**Part of the thesis:** Effect of topical anesthesia on phonation process during high-speed digital imaging

**Mentor(s):** Assoc. Prof. Mario Bilić, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** High-speed digital imaging is contemporary method of the phonation process assessment. Recording of laryngeal images at rate up to 4000 frames per second is performed transorally with rigid laryngoscope. The most common indication for high-speed digital imaging is hoarseness which is often caused by laryngopharyngeal reflux, retrograde movement of gastric contents into the laryngopharynx. According to current literature, usage of topical anesthetic in oral cavity, oropharynx and laryngopharynx during examination is optional, with no proven benefit from usage of topical anesthetic on level of pain and discomfort. There are also no evidence that patients who are suffering from laryngopharyngeal reflux have different levels of pain and discomfort during high-speed digital imaging compared to healthy subjects.

**Materials and methods:** This single-blinded randomized controlled study included 30 subjects (15 healthy and 15 suffering from newly endoscopically and anamnesticly diagnosed laryngopharyngeal reflux), aged from 18 to 45. Complete medical history was obtained, otorhinolaryngologic and fiberendoscopic examination was performed and, in case of meeting criteria, informed consent was signed. Depending on results of examination subjects were stratified in two groups (healthy and those suffering from laryngopharyngeal reflux). All subjects were examined with high-speed digital imaging in 2 consecutive days, with and without usage of topical anesthesia, by the same experienced examiner. Order of examination type was predetermined with random number generator. After each examination subject was asked to fill out visual-analog scale (VAS) 100 mm regarding pain and discomfort level during examination.

**Results:** Usage of topical anesthetic during high-speed digital imaging doesn't statistically significant influence on level of pain and discomfort in healthy subjects and in subjects suffering from laryngopharyngeal reflux. There are no statistically significant difference between healthy subjects and those suffering from laryngopharyngeal reflux regarding level of pain and discomfort during examination.

**Discussion:** Benefits and indications for usage of topical anesthetic during high-speed digital imaging still remain unclear. Further investigations on larger number of subjects and various laryngeal pathology should be performed to define advantages, disadvantages and indication for usage of topical anesthetic during high-speed digital imaging.

**MeSH/Keywords:** high-speed digital imaging, topical anesthesia

**Poster code:** R-02-21-064

**Poster Title:** Identifying a novel biomarker of papillary thyroid carcinoma metastases

**PhD candidate:** Filip Matovinović

**Part of the thesis:** Prognostic value of the proteomic profile of well-differentiated papillary thyroid carcinomas

**Mentor(s):** Professor Vladimir Bedeković, MD PhD, Professor Lovorka Grgurević, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Papillary thyroid carcinoma is the most common cancer of the thyroid gland as it represents about 85% of all cancer types of the thyroid gland. Most often, it affects patients between 40 and 50 years of age. Its mortality rate, although low, is still at an unacceptable rate of 0,5 per 100 000. The biggest factors of mortality are regional and distant metastases. Papillary thyroid carcinoma has a high incidence of dormant regional metastases at the time of primary surgery of the thyroid gland, which then requires additional surgery procedures (i.e. neck dissections) when the metastases become clinically noticeable on an already operated neck, with subsequently higher rates of morbidity and mortality. So, ideally, it would be of great information and importance to preoperatively identify patients who have papillary thyroid carcinomas which have a greater chance of providing regional metastases so an elective neck dissection could be done synchronously with the primary surgery of the carcinoma bearing thyroid gland, resulting in better surgical outcomes and lowering morbidity and mortality. The aim of this thesis is to use the relatively new method of proteomic analysis to identify potential biomarkers which would identify carcinomas with a higher rate of regional metastases.

**Materials and methods:** This is a retrospective analysis of 35 papillary thyroid carcinomas which did not provide regional metastases at the time of surgery and 35 specimens of papillary thyroid carcinomas that have provided regional lateral neck metastases at the time of surgery. The carcinoma specimens, which are initially enclosed in paraffin, will be put through liquid chromatography - mass spectrometry analysis in order to obtain detailed protein components of the specimens. Protein information will then be further analysed by means of recursive partitioning, a method based on computer learning. A thorough research of literature will also be performed in order to identify certain proteins that deserve more attention and further analysis, which can be helpful in the potential identification of a novel biomarker of metastatic disease.

**Results:** By searching the PubMed database with the keywords "papillary thyroid carcinoma", "papillary thyroid cancer", "metastases", "proteomic", "proteomics" a total of 14 papers were found. A study by Polish authors found that there are bigger differences in the proteome profile of cancer and metastatic disease in the same patient than there are differences in primary and metastatic cancers between different patients. Proteins that helped differentiate between the two are proteins from the immunity-related, chromatin and cytoskeleton families. Multiple studies from Chinese, Korean, German and Canadian researchers have found differences in the proteomic profile of primary and metastatic disease. This includes several proteins of interest in developing a biomarker, namely LAMC2, LAMB3, ATP5A1, MYO1G, S100-A4, S100-A6, S100-A10, FAS, SRC, TLN1, ITGB2, CAPNS, MTS1, Thioredoxin, cyclin D1, ER $\beta$  and Annexin A3.

**Discussion:** Various studies have found that there exists a vast difference and heterogeneity in papillary thyroid carcinoma and its metastatic disease. Past and current investigations definitely show progress in identifying and quantifying these differences in the hope of developing a novel biomarker. This thesis will further focus on finding a potential biomarker that will differentiate more aggressive carcinomas from more benevolent ones.

**MeSH/Keywords:** papillary thyroid carcinoma, proteomics, metastases

**Poster code:** R-02-21-113

**Poster Title:** Proteomic profile of oropharyngeal squamous cell carcinoma and prediction of disease prognosis

**PhD candidate:** Boris Ivkić

**Part of the thesis:** Proteomic profile of oropharyngeal squamous cell carcinoma and prediction of disease prognosis

**Mentor(s):** Professor Vladimir Bedeković, MD PhD, Professor Lovorka Grgurević, MD PhD

**Affiliation:** Department of otorhinolaryngology, Department of pathology and cytology, Clinical hospital Centre "Sestre milosrdnice" Zagreb. Section of Anatomy, Proteomics department of translation and clinical researches for University of Zagreb School of Medicine

**Introduction:** Histopathologic and morphologic markers are the main factor in classification and prognosis of oropharyngeal carcinoma. This study represents the connection between proteomic profile in tumorous tissue and prognosis of the disease. Prognosis of patients with oropharyngeal cancer is still unclear and depends on many factors, such as localization, age and gender, HPV infection, functions, habits and nutritional status. Today, there is not so many studies in literature which are analysing correlation between proteomic profile of oropharyngeal squamous cell carcinoma and disease specific survival rate. Hopefully, our research will help understanding the connection between these two parameters and possibly contribute in discovering of some new proteomic biomarkers.

**Materials and methods:** By researching proteomic profiles of 60 tissue samples of patients with oropharyngeal squamous cell carcinoma we will follow the expression of proteomic tissue biomarkers. Ten palatal mucosa tissue samples of patients who were not diagnosed with squamous cell carcinoma will be used as a control sample. Also, every of tumorous tissue sample will be tested for HPV infection by using polymerase chain reaction (PCR) kit. Patients will be followed postoperatively, or, till the date of the patient's death due to primary diagnosis. After the follow-up period, we will search for the connection between proteomic profile of tumorous tissue and disease specific survival, which will hopefully help us to expand our knowledge in proteomics and biomarkers of oropharyngeal squamous cell carcinomas.

**Results:** The study is still ongoing, currently it is in the phase of data collection. Till this moment analyzed 39 out of 60 tumorous sample for now by PCR for HPV infection and the results came that 26 out of 39 samples came positive on HPV infection.

**Discussion:** We are in progress of collecting pathohistologic specimens of squamous cell carcinoma tumorous tissue. Till this moment we analyzed 39 of our 60 tumorous sample by PCR for HPV infection. Some of the samples are distributed for the analysis at Proteomics department for translational and clinical research Institute, but there is no results for now. We expect to show the expression of known tumor biomarkers and hopefully to discover some new potential proteins which can be of significant importance in prognosis of patients with oropharyngeal carcinoma. Also, we will try to contribute for the better understanding of the protein biomarkers behavior in oropharyngeal squamous cell carcinoma tissue.

**MeSH/Keywords:** oropharynx, squamous cell carcinoma, biomarkers, proteomics, tumors, head and neck, prognosis

**Poster code:** R-02-21-116

**Poster Title:** E-cadherin and  $\beta$ -catenin protein and mRNA expression in Ewing sarcoma and primitive peripheral neuroectodermal tumors

**PhD candidate:** Aleksandra Bonevski

**Part of the thesis:** E-cadherin and  $\beta$ -catenin expression in diagnosing and monitoring patients with Ewing sarcoma and primitive peripheral neuroectodermal tumor

**Mentor(s):** Professor Sven Seiwerth, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Ewing sarcoma (EWS) and primitive peripheral neuroectodermal tumors (pPNET) are part of Ewing sarcoma family of tumors with very aggressive biology. They have common „small round blue“ tumor cells histologic phenotype which shows predominantly undifferentiated sheets of cells with relatively little stroma. The lack of differentiation has led to difficulty in understanding the tumor cell origin. In some cases however, EWS have limited neural differentiation suggested that EWS may arise from neural crest, and recently it was suggested that tumor has mesenchymal stem cell origin. But, it was also shown that EWS and pPNET could frequently express cytokeratins, suggesting the partial epithelial differentiation indicative of intercellular junctions, including tight cell-cell junctions.

**Materials and methods:** We have analysed 20 specimens of primary tumor tissue – paraffin embedded blocks of patients with EWS and pPNET at the time of diagnosis. The patients age interval was from 2-14 years with median of 10,5; 12 boys and 8 girls. The specimens were obtained by diagnostic biopsy. At the time of diagnosis 11 patients had localised disease, 4 had locally disseminated and 5 had distant metastases. 2 of 5 metastatic patients had pulmonary metastasis and bone marrow infiltration at the time of diagnosis. For e-cadherin and  $\beta$ -catenin expression analysis we used: immunohistochemistry method with specific antibodies (Dako) and RT-PCR method (Qiagen One step RT-PCR Kit) using the G6 control gen and nanodrop method for establishing the density of mRNA. Immunohistochemistry also included the analysis of pan-cytokeratin (AE1/AE3), epithelial marker. All the specimens were previously analysed by standard histopathology methods, including the immunohistochemistry positive expression of membrane glycoprotein CD99+, and molecular analysis of specific translocation t(11;22)(q12;q24) EWS-FLI1, which provided the definitive diagnosis and possible prognostic value.

**Results:** Immunohistochemistry: 5 specimens had low positivity for pan-cytokeratin – several cells (25%). 18 specimens were positive for membrane  $\beta$ -catenin (90%), and 2 were positive for membrane e-cadherin (10%). One specimen showed nuclear staining for all 3 markers (15%). RT-PCR: 18 specimens (90%) were positive for  $\beta$ -catenin (density interval from 3-5) and 2 for e-cadherin (10%).

**Discussion:** Immunohistochemistry and RT-PCR methods were used to determine protein and mRNA expression. Also, we wanted to compare and correlate the results of these two methods. There was a strong correlation between immunohistochemistry method used for determination of e-cadherin and  $\beta$ -catenin protein expression and mRNA expression of e-cadherin and  $\beta$ -catenin made by RT-PCR method. Also, mRNA expression of  $\beta$ -catenin by RT-PCR and mainly non-expression of e-cadherin are in accordance to existence of cell-cell adhesive receptors in these tumors, and partial epithelial differentiation was determined by positive expression of pan-cytokeratin obtained by immunohistochemistry in 25% of primary tumor specimens.

**MeSH/Keywords:** Ewing sarcoma, e-cadherin, beta-catenin, protein expression, mRNA

**Poster code:** R-02-23-094

**Poster Title:** Early detection of diabetic nephropathy in children

**PhD candidate:** Ivana Trutin

**Part of the thesis:** Combination of glomerular, vascular and tubular biomarkers of renal damage in early detection of diabetic kidney disease in children with diabetes mellitus type 1

**Mentor(s):** Assist. Prof. Mario Laganović, MD PhD, Assist. Prof. Gordana Stipančić, MD PhD

**Affiliation:** University of Zagreb School of Medicine, University of Zagreb School of Dental Medicine, Sestre milosrdnice University Hospital Center

**Introduction:** The early detection of diabetic kidney disease (DKD) has a pivotal role in the prevention of end-stage renal failure in young people with type 1 diabetes mellitus (T1D). Kidney biopsy done 1.5 to 2.5 years after the onset of the disease, and in children who were still normoalbuminemic, exhibit structural changes in the sense of glomerular and tubular basement membrane thickening, leading to the issue of albuminuria as an early indicator of DKD development. Serum cystatin C is removed by glomerular filtration and represents a sensitive indicator of impaired renal function. Tubular damage is important in the developing of DKD and kidney injury molecule-1 (KIM 1) in urine a promising tubular marker. Microvascular complications may be present early in the course of the disease so the aim of this study was to compare Doppler renal resistive index (RI), as an indicator of increased vascular resistance, in children with T1D who are normoalbuminuric with normal age-matched controls.

**Materials and methods:** A total of 76 children with T1D (10-18 years) and 76 healthy age-matched controls were enrolled. All participants had normal renal function and normoalbuminuria. Inclusion criteria for patients with T1D were: both sexes, age 10-18, stages of pubertal development  $\geq$  II by Tanner (for girls of  $\geq$  II degree, for boys testicle size  $\geq$  4 ml per Prader), duration of T1D  $\geq$  3 years regardless of whether it was diagnosed before puberty and duration of T1D  $\geq$  2 years if diagnosed in puberty. The exclusion criteria were acute urinary tract infection, glucocorticoid therapy, other kidney diseases, orthostatic proteinuria, thyroid disease, diabetic ketoacidosis, renal artery stenosis, and parvus-tardus spectrum on Doppler ultrasonography, malignant diseases. In the control group there were 76 healthy children. They all respond by age and gender to the respondents. Anthropometric parameters, blood pressure (BP), serum creatinine (sCr), eGFR, serum uric acid (SUA), urine albumin/creatinine ratio (ACR) fT3, fT4, TSH, urinary KIM1, serum cystatin C, Doppler RI and degree of pubertal development was determined in all participants. HbA1c was done in patient group.

**Results:** Lower sCr ( $<0.001$ ) and SUA (0.014) and higher ACR ( $p=0.004$ ) were observed in T1D group. Higher eGFR was also observed in T1D group although not statistically significant. Higher mean RI (mRI) was found in T1D group ( $p < 0.001$ ). No differences were found in age, gender distribution, BP or pubertal development. An unsupervised machine learning approach was used to identify a possible marker for renal impairment within the diabetic group. With the expectation maximization method 2 groups were identified, the so-called clusters of patients with common characteristics. Common features of Cluster 2, compared to Cluster 1, are higher RI values, ACR, eGFR, lower sCr and lower diastolic BP.

**Discussion:** Based on this analysis, two categories of diabetic patients were identified and therefore a new hypothesis can be extrapolated and generated - patients with higher RI values will be more prone to albuminuria. Renal RI is increased in the hyperfiltration phase of DKD in children with T1D. Renal RI may be helpful as a marker in diagnosing DKD in the early preclinical course of the disease in normoalbuminuric children with type 1DM.

**MeSH/Keywords:** Doppler renal resistive index, Type 1 Diabetes Mellitus, children

**Poster code:** R-02-24-001

**Poster Title:** Arterial stiffness measured with oscillometry considering dynamic or static type of exercise in children athletes

**PhD candidate:** Maja Vugrinec Mamić

**Part of the thesis:** Arterial stiffness measured with oscillometry considering dynamic or static type of exercise in children athletes

**Mentor(s):** Assist. Prof. Milan Milošević, MD PhD, senior research associate, Assist. Prof. Vesna Herceg-Čavrak, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Arterial stiffness is important cardiovascular risk factor in adults. Increased arterial stiffness is one of the first indicators of atherosclerosis that starts already in childhood. Exercise training has been shown to improve arteries compliance in middle-aged and older adults, but evidence regarding the associations of physical activity, arterial stiffness, structure, and function in children is limited.

**Materials and methods:** One hundred children athletes age 13-18 years will be included in this study. We divide them into two equal groups according on type of exercise (high level of dynamic and low level of static load, and vice versa). Measurements are performed before and after exercise and will be repeated two years after the first one. Including criteria will be that the children athletes in previous period practice the same sport. Parameters arterial stiffness are measured with validated device called Arteriograph. Before the measurements children and their parents fulfilled questionnaire, and physical examination and anthropometric measurements are performed.

**Results:** We performed measurements of arterial stiffness parameters on 83 children (42 boys and 41 girls), 29 of them were athletes, 30 football players and 24 gymnasts. 59 of them (71,1 %) practice sport with high level of dynamic and low level of static load, and 24 (28,9 %) sport with high level of static and low level of dynamic load. Twelve children (20%) also practice sport in school sport club. Gymnasts practices sport the longest  $9,22\pm 1,96$  years at the time of measurements were performed. Athletes practice the same sport for  $3,90\pm 1,47$  years, the duration of practicing football was  $5,46\pm 1,94$  years. Every group work out 3-4 times a week. Highest PWVaortic was measured in a group of children athletes during the rest and before workout (average  $5,99\pm 0,92$  m/s). In a group of football players PWVaortic was  $5,34\pm 0,73$  m/s and in a group of gymnast's  $5,32\pm 0,72$  m/s. Comparing those results athletes had significantly higher values of PWVaortic in relation to football players ( $P=0,002$ ) and gymnasts ( $P=0,003$ ). Values of PWVaortic in 60 th. minute of training were also highest in group of athletes average  $6,45\pm 1,00$  m/s, in gymnasts  $6,04\pm 0,86$  m/s, while in football players values were the lowest  $5,85\pm 0,89$  m/s, but without significant difference.

**Discussion:** Our study showed highest values of PWV aortic in children athletes compared to football players and gymnasts before training, while there is no difference in 60 minutes of training between groups. Considering anthropometrical measures football players were the shortest with average of 160 cm (but also the youngest average 13 years old), athletes and gymnasts were equal in height 164 cm, but athletes had the longest jugulum symphysis distance 49.55 cm (football player 44.73 cm, and gymnasts 46.57 cm). There are only a few studies that describe the normal value of arterial stiffness in young healthy population. The positive effects of regular exercise training on cardiovascular performance have been documented. It has been published that intervention strategies with physical activity could improve arterial function in youth with risk factors. Different exercise intensities play a decisive role in the adaptation mechanism of vascular function and vascular structure. At this point our study enrolls small sample of children athletes to form valid conclusion about correlation between arterial stiffness and type of exercise.

**MeSH/Keywords:** arterial stiffness, children athletes, dynamic and static type of exercise

**Poster code:** R-02-24-036



**Poster Title:** Dysautonomia in children with irritable bowel syndrome and inflammatory bowel disease

**PhD candidate:** Antonella Geljić

**Part of the thesis:** Disorders of the autonomic nervous system in children with chronic inflammatory bowel disease and irritable bowel syndrome

**Mentor(s):** Iva Hojsak, PhD, senior research associate

**Affiliation:** University of Zagreb School of Medicine, Childrens Hospital Zagreb, Department of Pediatrics

**Introduction:** Inflammatory bowel disease (IBD) is a group of chronic disorders that consists of ulcerative colitis, Crohn's disease, and undetermined IBD. Irritable bowel syndrome (IBS) is a functional abdominal pain disorder characterized by chronic abdominal pain and altered bowel habits. It is well known that psychological effects, such as stress, affect the brain-gut axis and contribute to different gastrointestinal disorders, mainly functional, like IBS, but also contribute to the symptoms even in quiescent IBD. Part of this interaction could be at least partially explained by the influence of autonomic nervous system (ANS). There are very few studies on the incidence of ANS dysfunction in children and adolescents with IBD and IBS. The aim of the study is to evaluate the presence of autonomic nervous system abnormalities (ANS) in children with irritable bowel syndrome (IBS) and quiescence inflammatory bowel disease (IBD) comparing to controls.

**Materials and methods:** Consecutive children with quiescence IBD, IBS and aged and sex matched healthy controls (HC) were referred for the evaluation of dysautonomia (IBD: N=24, mean age 15.7 yrs, 16 females; IBS: N=18, mean age 14.8 yrs, 9 females; HC: N=18, mean age 14.2 yrs, 9 females). Dysautonomia was evaluated subjectively with the Composite Autonomic Symptom Score (COMPASS 31), and objectively with the following autonomic tests: heart rate (HR) and blood pressure (BP) responses to the Valsalva maneuver, heart rate response to deep breathing (RSA), blood pressure response to passive tilt, and quantitative sudomotor axon reflex test (QSART). Additionally, heart rate variability (HRV) analysis was performed by Kubios HRV 2.2. Following HRV parameters were compared between the groups in supine and tilted positions: total power of low (LF) and high frequency domain components (HF), normalized HF (HFnu), low-to-high frequency ratio (LF/HF), standard deviation of normal-to-normal intervals (SDNN) and percentage of successive RR intervals that differ by more than 50 ms (PNN50).

**Results:** Children with IBS scored highest on COMPASS-31, followed by patients with IBD and HC (median 15.6, 8.7 and 2.3, respectively,  $p < 0.001$ ). Similar differences were observed in the orthostatic intolerance and gastrointestinal domains of the COMPASS-31. No differences between groups were observed in HR and BP responses to the Valsalva maneuver, RSA and BP response to passive tilt. Children with IBS had higher sweat volumes on proximal lower leg on QSART (median IBD 0.9, IBS 1.5, HC 0.8  $\mu\text{L}$ ;  $p = 0.039$ ). There was no difference in the HRV parameters between groups. However, children with IBS had significantly higher drop in LF ( $p = 0.01$ ) and SDNN ( $p = 0.03$ ) and lowest drop in PNN50 ( $p = 0.01$ ) during tilt test compared to children with IBD and HC.

**Discussion:** We found significant subjective and objective ANS abnormalities in children with IBS compared to children with IBD and HC.

**MeSH/Keywords:** inflammatory bowel disease, irritable bowel syndrome, brain-gut axis, dysautonomia

**Poster code:** R-02-24-045

**Poster Title:** Troponin in urine as a marker of cardiac damage in children

**PhD candidate:** Matija Bakoš

**Part of the thesis:** Troponin in urine as a marker of cardiac damage in children

**Mentor(s):** Assist. Prof. Daniel Dilber, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** The prevalence of congenital heart disease in Croatia ranges from 7 to 8 in 1000 live births, with ventricular septal defect (VSD) being the most frequent one. Cardioselective enzymes that are examined in University Hospital Centre Zagreb are Troponin T and NT-proBNP. Troponin is an intracellular protein that is important for regulating muscular contraction. Troponin T is a larger molecule than troponin I; its molecular weight is 43 kDa, while the mass of troponin I molecule is approximately 22.5 kDa. As the molecules become smaller, there is a greater likelihood of their filtration through the glomerular membrane in kidneys and the possibility of troponin or part of the troponin molecule being detected in the urine. There are only few studies which show the presence of troponin in the urine.

**Materials and methods:** The subjects will be infants after cardiosurgical closure of the ventricular septal defect. Using the residual blood and morning urine (2ml), we will determine troponin T and Troponin I. Around 200 measurements of both troponin T and I will be performed. Complete echocardiography will be performed several times. We will value troponin T and I from plasma and urine in six measurements: 1) on the day of admission in hospital, 2) on admission into the Pediatric Intensive Care Unit, 3) on the first postoperative day, 4) on the third postoperative day, 5) on the fifth postoperative day, and on the 6) tenth postoperative day. Data was analyzed using the Statistical Package for the Social Sciences (SPSS) program. In the data analysis, Fisher exact, Mann-Whitney, and Pearson Chi-square test were used to compare parametric and nonparametric variables, while some data was analyzed descriptively. A p-value < 0.05 was considered to be statistically significant.

**Results:** In the period from May 2017 to February 2020 a total number of 20 patients were analyzed. Levels of cardiac troponins were evaluated in all the participants before and after surgical correction of ventricular septal defect. All of the participants were infants with mean age of 5.8 months (interquartile range; IQR 3-11) with no diseases accompanied. Twelve patients were female (60%) and the average weight was 4.8 kg (IQR 3.2 – 8.8 kg). The mean PICU stay was 3.8 days, while the mean in-hospital stay was 11 days. The troponin T and I values were highest immediately after the cardiac surgery with detected values in urine both before and after cardiac surgery.

**Discussion:** This research has shown that it is possible to determine the level of troponin T and I in urine. The average value of troponin T in urine is 6.36 ng/L. There is no statistically significant difference in troponin T values in urine before and after cardiac surgery which is different than what was expected. Furthermore, comparing these results to healthy controls, there is no difference in troponin levels in urine nor before nor after the surgery. According to preliminary results obtained on a 20 patients no statistically significant association was found between cardiac damage and levels of troponin T in first morning urine sample. This finding needs to be further investigated.

**Acknowledgments:** We would like to thank all the doctors and nurses at the pediatric intensive care, pediatric cardiology and cardiac surgery units and all others that have worked and are still working with patients before and after cardiac surgery of ventricular septal de

**MeSH/Keywords:** Ventricular septal defect, troponin T, troponin I, urine, cardiac surgery

**Poster code:** R-02-24-050

**Poster Title:** The variance of different subtypes of Escherichia coli in stool of children with juvenile idiopathic or reactive arthritis at the first occurrence of symptoms

**PhD candidate:** Ivana Radoš

**Part of the thesis:** The variance of different subtypes of Escherichia coli in stool of children with juvenile idiopathic or reactive arthritis at the first occurrence of symptoms

**Mentor(s):** Lovro Lamot, PhD, research associate, Assist. Prof. Mario Cindrić, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Juvenile idiopathic arthritis (JIA) is the most common rheumatic disease in childhood with a prevalence of 4 cases per 1000 children. By definition, it is characterized by arthritis of unknown origin persisting for more than 6 weeks and starting before the age of 16. Seven different types of JIA can be defined according to the International League of Associations for Rheumatology (ILAR). JIA is one of the main reasons for short term and longterm disability in children. On the other hand, reactive arthritis (ReA) is a heterogeneous group of self-limited arthritides that develop as a response to prior extraarticular infection. The reasons why some children develop JIA are still not fully understood. It is thought to be a consequence of an interplay between genetic and environmental factors. The microbiota has recently received increasing attention as a potential contributing factor to the development of immune-mediated diseases, including JIA. Microbiota is a joint term for microorganisms that colonize the human body. The development of the gut microbiota occurs primarily during infancy and is essential for the healthy development of the immune system and for protecting the host against colonization, overgrowth, and invasions of pathogens. Changes in composition and function of gut microbiota, which is known as dysbiosis, may be associated with several clinical conditions. Among the bacteria which undergo changes in terms of diversity, resistance, and resilience in the state of dysbiosis are also the members of Escherichia coli (E.coli) species. E. coli is a dominant aerobic bacteria in the intestine and is one of the first bacteria that colonize the intestine shortly after birth.

**Materials and methods:** Stool samples of 14 patients with joint swelling were collected during their first visit to Pediatric Rheumatology Clinic in Sestre milosrdnice University Hospital Center in Zagreb, Croatia. Three months later, the diagnosis of JIA was made in seven patients while the other seven patients had been classified as ReA. All of the samples were analyzed by mass spectrometry on nanoLC-Synapt G2 Si instrument. To identify the most abundant E. coli subtypes, specialized software named \*Protein Reader with implemented Dust algorithm have searched through NCBI nr database, which contains the records of more than 400 E. coli subtypes. The median age of patients was 7.14 and 7.11, respectively.

**Results:** Various E. coli subtypes (P0301867.1-10, O104:H4, O103:H25, O111:H11, KTE and K) were three times more abundant in patients with JIA, while increased abundance of diarrheagenic E. coli (DEC) was detected in children with ReA.

**Discussion:** This research has shown differences in the subtypes of E. coli present in the stool of children with ReA and JIA at the beginning of the disease. Since E. coli is one of the paramount bacteria in gut microbiota, it is reasonable to assume that the differences described in this research can have a potential impact on the gut environment, contributing to the development of the chronic disease in JIA patients, or the resolution of symptoms in children with ReA. A better understanding of the interactions between the host and microbiome may help to establish alternative ways in which to treat, or even prevent disease.

**MeSH/Keywords:** juvenile idiopathic arthritis, reactive arthritis, Escherichia coli, gut microbiota

**Poster code:** R-02-24-140

**Poster Title:** Morphological and quantitative magnetic resonance evaluation of healing of osteochondral defects after treatment with cellular and tissue therapy

**PhD candidate:** Rudolf Vukojević

**Part of the thesis:** Morphological and quantitative magnetic resonance evaluation of healing of osteochondral defects after treatment with cellular and tissue therapy

**Mentor(s):** Assist. Prof. Alan Ivković, MD PhD, Assist. Prof. Dijana Zadavec, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Articular cartilage, unlike most tissues, does not have blood vessels, lymphatics or nerves so her capacity for self repair is minimal. The development of a full-thickness articular cartilage defect will cause joint dysfunction and with time develop into osteoarthritis. MRI provides excellent visualization of the repair tissue and its relationship to adjacent native articular cartilage and bone. dGEMRIC is sensitive to the concentration of glycosaminoglycan in cartilage whereas T2 mapping is sensitive to the integrity of the collagen network structure, collagen concentration, and water concentration in cartilage. The aim is to use this non-invasive powerful diagnostic tool to evaluate and compare the healing of osteochondral defects after treatment with tissue therapy with that of a cellular therapy.

**Materials and methods:** A sheep animal model was designed with “kissing lesions” that include cartilage damage created on the articular surfaces of the femoral trochlea and patella in the sheep right patellofemoral joint. In total 26 animals will be used. Three study are created: N-CAM (kissing lesions treated with grafts made from nasal chondrocytes (NC) seeded on a scaffold and cultured for 2 days-10 sheeps), N-TEC (treated with NC seeded on a scaffold and cultured for 2 weeks-10 sheeps) and control group with scaffold only-6 sheeps. Six weeks and six months after the implantation animals will be euthanized and specimens obtained for ex vivo MRI analysis: pre-contrast T1 and post-contrast T1 measurements for dGEMRIC (Delayed Gadolinium-enhanced Magnetic Resonance Imaging of Cartilage) and a pre-contrast T2 measurement for T2mapping will be performed. Also native high-resolution anatomical images will be obtained using a PD-weighted spin-echo sequence and modified MOCART (Magnetic Resonance Observation of Cartilage Repair Tissue) score will be used to evaluate healing of the defects. MR imaging will be performed with 7T MR imaging system.

**Results:** Preliminary results of the baseline data are reported for 12 sheeps-10 sheeps time point six weeks ( N-CAM and N-TEC) and 2 sheeps time point six months after the implantation ( also N-CAM and N-TEC). Data are presented as the mean  $\pm$  standard deviation. Mean modified MOCART score for cartilage layer N-CAM 6 weeks and 6 months are  $60.83 \pm 15.30$  and  $60.00 \pm 16.01$ . Mean modified MOCART score for cartilage layer N-TEC 6 weeks and 6 months are  $48.75 \pm 9.97$  and  $63.33 \pm 7.31$ . T2 values (msec) measured in chondral part of patella and trochlea osteochondral defects for N-CAM and N-TEC 6 weeks and 6 months are  $54.02 \pm 20.87$ ,  $46.78 \pm 19.69$  and  $68.10 \pm 18.54$ ,  $31.89 \pm 14.54$ .  $\Delta R$  values of cartilage layer for N-CAM and N-TEC 6 weeks and 6 months are  $1.72 \pm 0.23$ ,  $1.08 \pm 0.35$  and  $1.92 \pm 0.18$ ,  $1.06 \pm 0.27$ .

**Discussion:** Collected data from preliminary study show significant increase in the mean modified MOCART score from 6 weeks to 6 months in both patella and trochlea of N-TEC group. In comparative, we do not see that trend in N-CAM group.  $\Delta R$  values in the chondral part of the defect in trochlea and patella significantly decrease in N-CAM and N-TEC group from 6 weeks to 6 months which means more GAG content, without significant differences present between groups. T2 values significantly decrease in N-TEC group from 6 weeks to 6 months in both patella and trochlea, more than group N-CAM what is associated with maturity of repaired tissue. Preliminary study results show positive trend in accordance with hypothesis that healing of kissing lesions is showing better results when treated with tissue therapy than cellular therapy treated lesions. Once we collect all data we can discuss the final results.

**Acknowledgments:** Funding for this research has been received from European Union's Horizon 2020 research and innovation programme under grant agreement No. 681103, BIO-CHIP

**MeSH/Keywords:** MRI, dGEMRIC, kissing lesion, cartilage tissue engineering

**Poster code:** R-02-25-009

**Poster Title:** Correlation between choline peak and apparent diffusion coefficient at multiparametric magnetic resonance and calcium sensing receptor expression in breast cancer - preliminary findings

**PhD candidate:** Iva Bušić Pavlek

**Part of the thesis:** Correlation between choline peak and apparent diffusion coefficient at multiparametric magnetic resonance and calcium sensing receptor expression in breast cancer

**Mentor(s):** Assist. Prof. Maja Prutki, MD PhD, Zlatko Marušić, PhD, research associate

**Affiliation:** University of Zagreb School of Medicine, KBC Zagreb

**Introduction:** Breast cancer is the most frequent malignant disease of women in Croatia and the leading cause of death from malignant diseases. Magnetic resonance imaging (MRI) of the breast is a diagnostic method in preoperative breast cancer staging and management planning. Calcium-sensing receptor (CaSR) is found on the breast cancer cells and has previously been associated with the development of bone metastases.

**Materials and methods:** Twenty-five female patients underwent multiparametric MRI with spectroscopy in the preoperative examination for staging of breast cancer proven by core needle biopsy. Breast cancer tissue specimens were stained for the immunohistochemical analysis of CaSR expression using commercially available antibodies and analyzed with a reference visual scale used in published papers.

**Results:** Mean age of the patients at diagnosis was 57.16 years. Mean tumor diameter was 2.1 cm. Of 25 patients, infiltration of the skin was observed in 2 (6%) patients, infiltration of the mamilla in 4 (12%) and infiltration of the pectoralis muscle in 3 (8%) patients. Sixteen (70%) patients had no infiltration of the skin or surrounding structures. Twenty-two tumors were ER positive (88%), 20 were PR positive (80%) and 7 (28%) were Her positive. Proliferation index measured by Ki 67 had a median of 36.34. There were 6 (24%) Luminal A subtypes, 17 (68%) Luminal B subtypes and 1 (4%) of triple negative subtype and Her+ subtype. Median value of CaSR was 4. Mean value of Cho integral was 69.4. Mean ADC value was 610,5. There was no correlation between apparent diffusion coefficient (ADC) or Cho integral and CaSR expression. No significant correlation has been found between morphologic criteria of a tumor on MRI and CaSR expression, except for the kinetic curve type which exhibited a weak positive correlation. Morphological characteristics were uniform across tumor types; 90% of the tumors analyzed were irregularly shaped masses with spiculated borders, and 78,6% of the tumor had a type 3 curve of post contrast imbibition. There is a positive correlation between the Ki 67 total value and CaSR expression. CaSR correlates positively with the size of the tumor as well as the expression of Her+ receptor. There is a negative correlation between the expression of ER and PR and CaSR expression. Tumors with distant metastases at the time of detection correlated positively with CaSR expression.

**Discussion:** CaSR is a cellular receptor that has been found in cultures of breast cancer cells, and the expression of this receptor has been shown to be directly related to the occurrence of bone metastases. MRI is a radiological method for detecting tumors and monitoring breast disease. The diagnostic value of in vivo spectroscopy is based on the fact that it is possible to measure elevated levels of Cho components in tumors, compared to healthy tissue. ADC is a measure of the diffusion of water molecules within tissues, and is obtained by software calculation that allows diffusion signal quantification. Two meta-analysis evaluated the possibility of quantification of diffusion values of breast lesions and found an overall sensitivity of 84% and a specificity of 79%. Our preliminary results showed no correlation between the cellularity of the tumor measured using ADC (measuring Brownian movement of water molecules) and CaSR. Triple-negative cancers were found to have more total choline than other forms of breast cancer. The lack of positive correlation between Cho peak integral as a marker of cell turnover and CaSR might be due to the fact that there is a lack of triple negative tumors in the research so far. These tumors are known for their high cell turnover, so the results might differ once a higher number of this tumor type is included as opposed to a high rate of idler tumors. Our preliminary results confirm a positive relation between CaSR expression and Ki 67 value. Since Ki 67 measures proliferation value it can be stated that tumors expressing CaSR have a higher expression of the CaSR if they are more active in terms of mitosis. Lack of correlation between morphological criteria on the MRI and CaSR expression, might be explained by a rather uniform distribution of morphological criteria across tumor type in the research. Further enrolment might disperse the morphological criteria making a correlation more visible. A larger sample is required for further analysis.

**MeSH/Keywords:** Breast Cancer, Multiparametric Magnetic Resonance, Calcium-Sensing Receptor

**Poster code:** R-02-25-102

**Poster Title:** Correlation of the cross-sectional area of the vastus medialis obliquus muscle and the value of the lateral patellar tilt in patellofemoral instability

**PhD candidate:** Marko Šimunović

**Part of the thesis:** Correlation of the cross-sectional area of the vastus medialis obliquus muscle and the value of the lateral patellar tilt in patellofemoral instability

**Mentor(s):** Assoc. Prof. Mislav Jelić, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Patellofemoral (PF) instability is a common condition in pediatric and adolescent patients. The cause of PF instability is multifactorial, and includes abnormalities of bone morphology and soft-tissue structures resulting in improper sliding of the patella during knee flexion and extension, which subsequently can lead to dislocation of the patella. Using computed tomography (CT) scans, Dejour et al. defined the objective factors of PF instability as: trochlear dysplasia, pathological lateral patellar tilt, which contributes to atrophy of the musculus vastus medialis obliquus (VMO), abnormal patellar height, and excessive tibial tuberosity – trochlear groove displacement. In current literature, there is a clear belief that the pathological lateral patellar tilt is a result of weakness of the VMO, and a predictor of patellar instability. There are different variants or angular modifications for the measurement of patellar tilt, in current practice most often use the modified measurement procedure by Fulkerson, and measurement by Sasaki-Yagi. To the latest findings, as far as we know, there are only two studies in which researchers have measured the cross-sectional area (CSA) of VMO at magnetic resonance imaging (MRI) in patients with PF instability, their results have shown that VMO is not significantly associated with PF instability. The CSA of the muscle is a strong indicator of muscle strength and can be measured reliably by multislice CT.

**Materials and methods:** This preliminary results performed retrospectively on axial CT scans of the knee at UHC Zagreb. After exclusion criteria, value of patellar tilt obtained by standard measurements according to Fulkerson and Sasaki-Yagi as well as the new method to assess the correlation of the impact of morphology (cross-sectional area) of VMO. Statistical processing of electronically stored data was performed using MedCalc (version 18.10.2, MedCalc Software bvba, Ostend, Belgium; <http://www.medcalc.org>; 2018) with a statistical significance of 5%, statistical strength of 0,80. The symmetry of the distribution was examined by the Shapiro-Wilk test, the correlation of the variables with Pearson (rp) and Spearman (rs) coefficients, and the differences between them by one-way analysis of variance (ANOVA) with sporadic logarithmization in the case of statistically significant Leven test.

**Results:** Measuring methods are related to each other (rs between the 0,49 and 0,91,  $p < 0,0001$ ) and inversely with age (rs between  $-0,26$  and  $-0,18$ , p between 0,01 and 0,03), but not with the cross-sectional area of the VMO (rs between  $-0,10$  and 0,03, p between 0,48 and 0,96), while they differ considering to instability (F between 9.39 and 13.90, p between  $<0,001$  to 0,003) and presence of trochlear dysplasia (F between 3.85 and 9.79, p between 0,003 and 0,055), but not considering to sex (F between 0.45 and 1.93, p between 0.17 and 0.50) and laterality (F between 0.03 and 1.50, p between 0.22 and 0.87). Sasaki-Yagi and Fulkerson measurement procedures were inversely related to FR (order rs =  $-0,36$ ,  $-0,44$ ; both  $p < 0,001$ ) and FR – TR (order rs =  $-0,41$ ,  $-0,52$ ; both  $p < 0,001$ ), but not with TR (respectively rs =  $-0,11$ ,  $-0,15$ ;  $p = 0,19$ , 0,06), while, on the contrary, the new method is inversely related to TR (rp =  $-0,20$ ,  $p = 0,02$ ), but not FR (rp =  $-0,16$ ,  $p = 0,06$ ) and FR – TR (rp = 0,02,  $p = 0,86$ ).

**Discussion:** The results of this preliminary research indicate that the cross-sectional area of VMO is not associated with the patellar tilt. This may be important because conservative therapy in PF instability is based on strengthening the VMO. It should be noted that the new method for patellar tilt distinguishes stable from unstable knees and is not dependent on femorotibial rotation. Further results are pending.

**Acknowledgments:** Acknowledgments: I would like to thank my mentor for his guidance, my family for their support and dr. Dinko Nizić for his kind assistance.

**MeSH/Keywords:** knee; computed tomography, patellar tilt angle; patellofemoral instability, VMO

**Poster code:** R-02-25-117

**Poster Title:** Diagnostic value of digital breast tomosynthesis in patients with nipple discharge - preliminary research results

**PhD candidate:** Sanja Baršić Ostojić

**Part of the thesis:** Diagnostic value of digital breast tomosynthesis in patients with nipple discharge

**Mentor(s):** Assist. Prof. Maja Prutki, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Nipple discharge could be physiologic or pathologic. Pathologic nipple discharge (PND) is defined as a clear, serous, or bloody secretion (not green or milky), spontaneous, discharging from a single duct and unilateral. It is frequently caused by a benign lesion, such as intraductal papilloma(s) (35–56% of cases) or ductal ectasia (6–59%), but an underlying malignancy can be present in a percentage of cases reported to be variable from 5 to 33%. Breast imaging in patients with nipple discharge includes mammography, sonography, and breast magnetic resonance imaging (MRI). Digital breast tomosynthesis (DBT) is a variant of standard digital mammography that allows the creation of thin-section reconstructed images that decrease the lesion-masking effect of overlapping tissue and increase specificity and sensitivity of mammography. The purpose of our prospective study is to determine the diagnostic value of DBT for the evaluation of patients with nipple discharge.

**Materials and methods:** The study is prospective and approved by the Ethical Committee of the University Hospital Centre Zagreb. 53 patients with nipple discharge were enrolled in the study and examined in our Breast care department. All the patients underwent DBT, which was compared to digital mammography (DM), breast sonography (US) and discharge cytology. Patient with pathologic nipple discharge underwent breast MRI. Morphologic characteristics of all detected lesions were analysed in all three diagnostic modalities, and their kinetic characteristics (enhancement curves and peak enhancement) were analysed using breast MRI. All detected lesions were classified according to the American College of Radiology (ACR) BI-RADS lexicon criteria. The BI-RADS category of each lesion, of each diagnostic method, was compared with the histopathology report. BI-RADS categories 1 and 2 were considered negative, while categories 3, 4 and 5 were considered positive. The final diagnosis was established by histopathological analysis of core biopsy, vacuum-assisted stereotaxic biopsy or surgical excision specimen. Benign (negative) categories will have an annual follow-up. Based on the collected data we analyzed sensitivity, specificity, predictive values and accuracy for all three diagnostic methods.

**Results:** All patients were female. The average age of patients was 54 years (ranging from 31-84 years). 40 patients (75%) had PND. Five lesions were histopathologically verified as malignant lesions, all of them among patients with PND (12,5% of patients with PND). The sensitivity and the negative predictive value, for the detection of carcinoma in the patients with nipple discharge, of DM and MRI were 100%. The sensitivity of DBT was 100%, the specificity of DBT was 82,98%, while the sensitivity and specificity of breast MRI were 100% and 61,90%, respectively. DBT and breast MRI had the same positive predictive value (0.429), while DM and the US had lower positive predictive values (0.314 and 0.167). DBT had higher specificity (82,9%) than breast MRI (61,9%) in the detection of carcinoma in patients with nipple discharge. The sensitivity of DM was 83,3%, the specificity of DM was 76,6%, while the sensitivity and specificity of the US were 66,7% and 57,5%, respectively.

**Discussion:** In patients with nipple discharge breast carcinoma may be confidently excluded at DBT with NPV of 100% making invasive procedures redundant. DBT and MRI were superior to DM and US in the detection of breast carcinoma, and therefore DBT should be considered as an alternative to MRI in the evaluation of patients with nipple discharge.

**Acknowledgments:** Assist. Prof. Maja Prutki, MD PhD; Vlatko Duspara, MD; Marko Petrovečki, MD; Lucija Kovačević, MD; Prof. Ivica Sjekavica, MD PhD.

**MeSH/Keywords:** Breast cancer, Nipple discharge, Digital breast tomosynthesis

**Poster code:** R-02-25-151

**Poster Title:** Cell Free DNA Methylation of OCT3/4 and NANOG Gene in Semen of Patients with Testicular Seminoma

**PhD candidate:** Miroslav Tomić

**Part of the thesis:** Cell Free DNA Methylation of OCT3/4 and NANOG Gene in Semen of Patients with Testicular Seminoma

**Mentor(s):** Professor Božo Krušlin, MD PhD, Assist. Prof. Nino Sinčić, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Testicular tumors are the most prevalent malignant tumors in adolescent age and young adults (15.-35.y). 98% of these tumors are of stem cell origin and pure form of seminoma comprise more than half of testicular germ cell tumors. Carcinogenesis and mechanisms of origin are still largely unexplained but there are researches supporting theories that (micro) environmental and (EPI) genetic factors lead to its development. Epimutation of DNA methylation as tumor biomarker is emphasized because of its distinctive chemical and biological stability, which gives them an advantage in developing non-invasive diagnostics. A large area of research is oriented to biomarkers from body fluids, as non-invasive screening methods. Potential biological markers in ejaculate can be identified for pure seminoma group of tumors. This research is based on the methylation of cell free DNA (cfDNA) of promotor regions of genes OCT3/4 and NANOG in ejaculate of the patient, as potential biological molecular markers of testicular seminoma.

**Materials and methods:** This was a prospective study for a period of 3 years, from 2016.-2019. The power test was performed according to the epidemiological study rules and for a strength of 0.90, 14 samples per group would be enough, ie 14 patients and 28 samples and we recruited 21 patients and 42 samples. The patient population was recruited from a male population with suspicion of a testicular tumor from University Hospital Centre "Sestre milosrdnice", Department of Urology, Zagreb. Ejaculate was taken from patients with seminoma pre and postoperatively. Excluding criteria was any histopathological diagnosis other than pure seminoma. cfDNA was isolated at the Laboratory for Epigenetics and Molecular Medicine, Department of Medical Biology, School of Medicine Zagreb, University of Zagreb, Croatia. DNA methylation analysis was performed according to the usual methodology. The pathohistological diagnosis of seminoma and tumor tissue sampling was done at Department of Pathology and Cytology Ljudevit Jurak, University Hospital Centre "Sestre milosrdnice", Zagreb, immediately after orchidectomy. Morphometric analysis of the IHC signal was performed on a light microscope according to standard protocols. Using a light microscope, the whole tumor was examined and IHC reaction was evaluated on one sectional tumor with highest reaction, "hot spot". The statistical analysis was conducted with statistical tests such as Fisher's exact test, Fisher-Freeman-Halton's exact test, Mann-Whitney U test and Spearman correlation coefficients. All P values less than 0.05 was considered significant. The analysis was also using statistical programs such as Statistics 12.0 ([www.statsoft.com](http://www.statsoft.com)) and StatsDirect version 3.0.171 ([www.statsdirect.com](http://www.statsdirect.com)).

**Results:** So far we evaluated preoperative and postoperative concentrations of cfDNA in ejaculate samples of 12 patients with seminoma. In our preliminary results the median concentration of cfDNA in preoperative samples is higher than in postoperative. Methylation analysis of cfDNA OCT3/4 and NANOG genes was performed with pyrosequencing. Pyrogram and histogram for OCT ¾ and NANOG genes matches so we concluded that the analysis is successful.

**Discussion:** In our results preoperative cfDNA concentrations were higher in ejaculate samples which is expected and associated with an increase in tumor volume and a higher rate of cell necrosis and apoptosis. After tumor removal there is no source for those cells. On control patients pyrogram and histogram for OCT ¾ and NANOG genes matches which means analysis is so far successful. Next step in our plan is to analyze samples from seminoma patients for OCT ¾ and NANOG genes methylation.

**Acknowledgments:** This research is implemented within the project "Epigenetic biomarkers in blood and ejaculate of the patients with testicular seminoma", which is approved by the Croatian science foundation, IP-06-2016.

**MeSH/Keywords:** seminoma, methylation, cell free DNA, genes, OCT3/4, NANOG

**Poster code:** R-02-28-010



**Poster Title:** Biochemical changes in the composition of perfusion fluid as a predictor of kidney graft function

**PhD candidate:** Zoran Zimak

**Part of the thesis:** Biochemical changes in the composition of perfusion fluid as a predictor of kidney graft function

**Mentor(s):** Professor Željko Kaštelan, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Chronic kidney disease is a global health problem and represents a major financial burden for any health care system. There are five stages of chronic kidney disease according to the KDOQI (Kidney Disease Outcomes Quality Initiative) classification, and each is associated with cardiovascular comorbidities, premature mortality, and reduced quality of life. Kidney transplantation is the best method of treating end-stage chronic kidney disease, but before we can enter the transplantation procedure we need to procure the kidney from a donor. Perfusion solutions are special fluids used for organ perfusion, preservation and transport, and play a critical role in successful kidney transplantation.

**Materials and methods:** We have performed a prospective study on 13 samples of perfusion solutions (University of Wisconsin (UW), Histidine-tryptophan-ketoglutarate (HTK)) with whom the donor kidney was perfused and stored in after the explantation. The samples were obtained in the procedure of kidney transplantation at University hospital centre Zagreb. They were analyzed in the biochemical laboratory of UHC Zagreb. We excluded patients with kidney graft failure within the first month after kidney transplantation, and samples of perfusion fluid whose composition differed from UW or HTK solution. We compared the declared composition of perfusion fluids with the composition of samples of perfusion fluid and correlated the results with delayed graft function (DGF), kidney graft failure and overall graft survival.

**Results:** Median age of patients was 55.4 years. Median cold ischemia time was 15.8 hours. Overall graft survival in the period of one year was 95 %. Delayed graft function appeared in 4 patients (30.7%), this was correlated with concentration changes in the components of perfusion fluids analyzed. Composition changes in the analyzed solutions showed a positive predictive value for delayed graft function.

**Discussion:** This prospective pilot study confirmed the hypothesis that composition changes of the perfusion fluids, in which the kidney was stored, affect the delayed graft function and by that the overall graft survival. However this is a small sample and further investigations and a longer follow up is needed to draw certain conclusions.

**MeSH/Keywords:** kidney transplantation, perfusion fluid, University of Wisconsin (UW), Histidine-tryptophan-ketoglutarate (HTK), cold ischemia

**Poster code:** R-02-28-149

**Poster Title:** Immunohistochemical expression of PTEN and ERG proteins in prostate samples obtained with targeted, cognitive, mpMRI guided biopsy in patients with prostate cancer

**PhD candidate:** Ivan Pezelj

**Part of the thesis:** Immunohistochemical expression of PTEN and ERG proteins in prostate samples obtained with targeted, cognitive, mpMRI guided biopsy in patients with prostate cancer

**Mentor(s):** Professor Božo Krušlin, MD PhD, Assist. Prof. Igor Tomašković, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Multi-parametric (mpMRI) prostate MRI has become a standard diagnostic procedure for detecting and staging prostate cancer. mpMRI guided biopsies have greater accuracy, especially with clinically significant prostate cancer and have become a standard in a repeat biopsy setting. Images are graded according to the Prostate Imaging Reporting and Data System (PI RADS score) on a scale from 1 to 5. PTEN (Phosphatase And Tensin Homolog) is a multi-functional tumor suppressor that is very commonly lost in human cancer and correlates with poor prognosis in prostate cancer. Recurrent gene fusion between the androgen-regulated gene TMPRSS2 and ETS transcription factor ERG is present in about 50% of prostate cancer cases. In multivariate Cox regression analysis presence of TMPRSS2-ERG in the tumor was significantly associated with poor prognosis.

**Materials and methods:** Samples of peripherally located tumors will be obtained retrospectively and prospectively from the archives of the Clinical department of pathology and cytology "Ljudevit Jurak", which were obtained from patients who had received a targeted mpMRI guided prostate biopsy in the Clinical hospital center "Sestre Milosrdnice". For the immunohistochemical analysis ERG and PTEN protein antibodies will be used.

**Results:** 59 out of 90 necessary patients have been enrolled in the study. PIRADS score and tumor grade distribution is as expected, based on the sample used to perform the power test (30,9 % PIRADS 3, 42,5% PIRADS 4 and 26,6 % PIRADS 5). No clinically significant prostate cancer was found in the PIRADS 3 group. 52,5% of all PIRADS 4 patients as well as 82.0 % of all PIRADS 5 patients had clinically significant prostate cancer.

**Discussion:** Final patient enrollment is expected by the end of the year when PTEN and ERG protein analysis will commence. Considering that multiple studies have shown a positive correlation between PTEN loss and tumor grade as well as ERG expression and tumor grade we expect a higher incidence of PTEN loss and ERG expression in patients with higher PIRADS scores. The study should be finished by April 2021 based on the current data. Final findings are expected to further elucidate the role of PTEN and ERG in prostate cancer diagnosis and treatment outcome prediction.

**MeSH/Keywords:** PTEN, ERG, mpMRI, prostate cancer

**Poster code:** R-02-28-157

**Poster Title:** Connection of diagnostic parameters of metabolic syndrome with severity and outcome of ischemic stroke

**PhD candidate:** Ivana Kern

**Part of the thesis:** Connection of diagnostic parameters of metabolic syndrome with severity and outcome of ischemic stroke

**Mentor(s):** Professor Zdravka Poljaković, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Metabolic syndrome (MetS) is defined as a cluster of interrelated factors that significantly increase the risk of cardiovascular diseases such as stroke. The aim of this study was to investigate connection between MetS and its diagnostic criteria with severity and outcome of stroke.

**Materials and methods:** Ninety four patients with acute ischaemic stroke were included in the study. Patients were categorized into two groups: with (n=47) or without MetS (n=47). Their demographic data were analysed, medical history, individual criteria for MetS, anthropometric characteristics, stroke severity (NIHSS, neuroimaging criteria, laboratory), recanalization treatment method (thrombolysis and/or thrombectomy), recanalization rate and in hospital complications rate. Late outcome (in 12-months follow up) was measured by modified Rankin scale (mRs), followed by functional evaluation of plegic hand, walking assessment, selfcare ability, physical therapy days and major adverse cardiovascular events. MetS was determined if the participant had three of the five criteria using National Cholesterol Education Program (NCEP) guidelines.

**Results:** 92,6% of all patients had hypertension and 63,8% were obese. In spite of the fact there were no statistically significant differences in stroke severity and/or recanalisation rate between two groups, MetS patients had to use devices more while performing hand functions ( $P<0.027$ ). Patients without MetS walked more non-assistive ( $P<0.020$ ). MetS patients were less able for selfcare (44.4% vs. 75%,  $P<0.031$ ) and spent more days in physical therapy (median 30.0 vs. 16.5,  $P<0.043$ ).

**Discussion:** Results from this study show that 64% of all post-stroke patients were centrally obese. 37.7% of adult Croatian population is overweight, reported by Croatian institute of public health. Central obesity has been strongly linked to cardiovascular diseases. More concerning is the fact that 20.9% of children in Croatia are overweight, which emphasizes that the problem begins early. As expected, MetS group had higher levels of CRP, which is concordant with proinflammatory state biochemically created within patients having MetS. Even though not all overweight or obese individuals are metabolically disturbed, the majority are. Central obesity is thought to be an early step, as visceral adipose tissue secretes a variety of different cytokines leading to inflammation and pathogenesis of MetS. Patients without MetS obtained independent walking in higher percentage than MetS patients (81.3% vs. 48.1% of survivors). Returning to prior level of function, most importantly independent ambulation, remains a priority to stroke survivors. Affected arm function significantly improved in non-MetS patients, 68.8% vs. 40.7% of survivors used the arm without assistive devices. Our results show that selfcare ability is obtained in 75% of non-MetS surviving patients vs. 44.4% of MetS survivors which is a statistically significant difference. Even though there is no statistically significant difference in mortality between groups, MetS patients die earlier. Overall mortality in this study is 37.2%. Stroke is the second leading cause of death in Croatia. This study confirmed that hyperglycemia positively correlates with previously having myocardial infarction, recurrent stroke, higher number of acute in-hospital complications after stroke, poorer modified Rankin score and death. Obesity was shown to negatively correlate with TICI score thus implying poorer thrombectomy result. Since the burden of stroke is serious, modifiable criteria for MetS such as obesity should be prevented.

**MeSH/Keywords:** metabolic syndrome, stroke, obesity, hyperglycemia, outcome measures

**Poster code:** R-02-30-007

**Poster Title:** Markers of apoptosis in the serum and cerebrospinal fluid of patients with convulsive status epilepticus

**PhD candidate:** Lejla Ćorić

**Part of the thesis:** Markers of apoptosis in the serum and cerebrospinal fluid of patients with convulsive status epilepticus

**Mentor(s):** Assoc. Prof. Željka Petelin Gadže, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Epilepsy is a chronic neurological disease with basic feature is prolonged predisposition to generating epileptic seizures that arise as a consequence of abnormal excessive or synchronised neural brain activity. Epileptic status (ES) is the most serious instance of epileptic seizures. The prolonged excitation activity in brain cells during epileptic seizures, can lead to brain damage and death of cells, by activating apoptotic and/or necrotic pathways. Apoptosis is a process of programmed cell death that occurs intracellularly or extracellularly. Our hypothesis was that the levels of extrinsic ( FAS/CD95) and intrinsic ( Bcl 2) markers of apoptotic pathways in the cerebrospinal fluid and serum of patients with convulsive epileptic status, is significantly higher, compared to a control group of subjects.

**Materials and methods:** The purposes of this study is to analyse collected data of study samples - 15 adult patients with history of convulsive status epilepticus, and an appropriate number of subjects who will form the control group. Inclusion criteria will be diagnosis of convulsive status epilepticus (according the Commission of the International League Against Epilepsy from 2015), set up by a neurologist. The following criteria are the age between 18-55 years of life, the absence of clinical signs of terminal stage malignancies and chronic autoimmune diseases. The control group will seem like subjects who viewed TIA neurological ambulance in an emergency, and to exclude acute infectious disease of the central nervous system or suspected subarachnoid hemorrhage were observed, and the diagnostic method underwent lumbar puncture, drawing blood and other necessary procedures. Level SFAS / APO 1 in serum and cerebrospinal fluid, Bcl protein will be commercially available enzyme-specific linked immunosorbent assay (ELISA) values.

**Results:** Data from 8 patients (5 male, 3 female) were collected from cohort of patients with ES, referred to two neurology divisions in Zagreb. Also, data from 10 patients ( 6 male, 4 female) were collected from cohort of control group. Three patients had ES as a first sign and symptom of disease, and five of patients with ES were patients with early diagnosed epilepsy. All the patients had to have undergone neurologic examination, brain MRI, EEG recording and long term clinical and EEG follow up. Age at onset of seizures, seizure semiology, EEG characteristics, brain MRI findings and therapy with antiepileptic drugs were analysed for each case. Seizures were classified according to revised criteria of the International League Against Epilepsy ( ILAE). Generalized tonic-clonic seizures were encountered in 3 patients as a beginning of convulsive ES; focal seizures with generalization in 5 other patients with ES. Diffuse anomalies were recorded in three patients with generalized seizures, two patients had normal EEG activity; three patients showed a combination of focal and diffuse anomalies

**Discussion:** This research aims at establishing the presence of markers of apoptotic pathways in the serum and cerebrospinal fluid of patients with convulsive ES, and at exploring the difference in the concentration of markers in extrinsic apoptotic pathways (FAS/CD95) and markers of intrinsic apoptotic pathways (Bcl 2) in the cerebrospinal fluid and serum of patients with convulsive ES. The study of biochemical markers in ES in humans, will contribute to a better understanding of the domination certain pathogenic mechanisms of prolonged epileptic activity to draw attention to the possible further development of epileptogenesis, but also new possibilities of therapeutic action.

**MeSH/Keywords:** epileptic status, apoptosis, Bcl-2, sFAS.

**Poster code:** R-02-30-025

**Poster Title:** Antinuclear antibodies and apoptotic factors in multiple sclerosis

**PhD candidate:** Josip Sremec

**Part of the thesis:** Antinuclear antibodies and apoptotic factors in multiple sclerosis

**Mentor(s):** Assoc. Prof. Nataša Kovačić, MD PhD, Assist. Prof. Sanja Tomasović, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Multiple sclerosis (MS) is an illness of a proposed, but not yet fully understood autoimmune origin, with a marked neurodegenerative component that assumes a primary role as the disease advances. Apoptotic processes have a significant role in pathogenesis of this disease, as they represent a method using which the immune system has the ability to control autoreactive lymphocyte clones on the peripheral level. These processes are malfunctioning in MS which enables autoreactive cells to migrate into the CNS and exert adverse effects on the neural tissues. There are many studies that noted an elevated frequency of ANA positivity in MS patients, which hasn't been adequately explained pathogenetically. The aim of this study was to determine the possible association between ANA positivity and variations in apoptotic processes.

**Materials and methods:** 44 RRMS patients currently in a relapse were included in this study, with additional 14 patients with a diagnosis of CIS and 12 patients with RRMS currently in a remission. Subjects were assessed for ANA positivity, and its indirect immunofluorescence (IIF) pattern was determined, as well as presence of specific autoantibodies. Concentrations of soluble apoptosis markers sFas and sFasL were measured in patients' sera. CSF concentrations of those markers were measured in a minority of cases as well.

**Results:** Serum concentrations of both analyzed apoptosis markers were higher in ANA-positive patients, and correlated well with each other. ANA-positive patients had shorter disease duration, fewer experienced relapses, and a lower EDSS score. There was no clear association between a particular IIF pattern or the presence of specific autoantibodies and either of the assessed disease characteristics, with a notable exception of sex distribution, as specific autoantibodies were found solely in female patients. sFasL concentrations correlate inversely with disease duration, number of experienced relapses and EDSS scores. Concentrations of both sFas and sFasL are lower in patients currently in a relapse, while only sFas concentrations are lower in patients diagnosed with CIS. CSF concentrations of analyzed apoptosis markers correlate with each other, but show no clear correlation with their serum concentration. No associations were observed between analyzed parameters and patients' other disease characteristics.

**Discussion:** The results of this study imply a link between ANA positivity and differences in apoptotic processes, for which there is a possibility of stemming from changes in abundance of cellular nuclear and cytoplasmic antigens (a byproduct of autoreactive lymphocyte elimination via apoptotic processes) presented to the immune system, which interprets them as foreign and starts the production of antibodies. The decline of apoptotic mediator levels in the course of the disease, mirrored in a diminishing proportion of ANA-positive patients, could be a result of changing levels of apoptotic activity as the disease advances, possibly due to a greater immune system tolerance for autoreactive processes. This could be responsible for the accumulation of persistent neurological impairments with subsequent relapses, parallel with advancing neurodegenerative processes. Further investigations are warranted in order to elucidate the role of apoptotic processes in MS, as well as the dynamic of their change. This could lead to better understanding of disease pathogenesis, as well as to discovery of potential therapeutic targets.

**MeSH/Keywords:** multiple sclerosis, antinuclear antibodies, autoimmunity, apoptosis

**Poster code:** R-02-30-031

**Poster Title:** THE ROLE OF TRANSCRANIAL SONOGRAPHY IN PATIENTS WITH PARKINSONS DISEASE

**PhD candidate:** Mislav Budišić

**Part of the thesis:** Correlation of substantia nigra and raphe nuclei echogenicity in transcranial brain sonography with characteristics of Parkinson's disease.

**Mentor(s):** Professor Arijana Lovrenčić-Huzjan, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Since there is no widely accepted test to help us confirm Parkinson's disease (PD), clinical examination of well-established symptoms is the still most accurate way of PD diagnosis. However, at such point the misdiagnosis rates for PD in the early stages is as high as 20–30%. Neuroimaging examinations like MSCT or MR lack sensitivity for the diagnosis of idiopathic Parkinson's disease and are just helping in differentiating atypical Parkinsonism. Recent studies reported that transcranial sonography of brain parenchyma (BPS) detects in 90% patients with Parkinson's disease alterations in the echogenicity of substantia nigra (SN). Prevalence of such hyper echogenic SN finding in healthy population is around 10% and at this point its clinical significance is unknown. Recent reports have revealed that such increase in echoic signal might be due to elevated tissue iron level and increased gliosis. Also, few BPS studies showed that disruption of echogenic midbrain line corresponding to basal limbic system and raphe nuclei (RN) within are frequently found in patients with depression disorders including Parkinson's disease related depression and also with suicidal ideations. Recent meta-analysis showed that BPS yield high sensitivity (84%) and specificity (85%) in diagnosis of Parkinson's disease, however further research are endorsed by latest MDS-PB guidelines. In addition, there is inconsistency of correlation of BPS findings with clinical aspects of the Parkinson's disease.

**Materials and methods:** We will include 50 patients with diagnosis of Parkinson's disease (MDS diagnostic criteria) and 50 healthy controls. Informed consent is obtained before entering the study. Two independent physicians blinded on the results of each other performed BPS and complete neurological examination. Severity of the disease and presence of depression is measured with UPDRS III part scale, DSM-IV and Hamilton depression scale. BPS is applied bilaterally transtemporal by standardized protocol; SN displayed, manually circled and measured. The echogenicity of the RN is rated semi quantitatively on a three-point scale (not visible, slightly echogenic/interrupted RN, normal RN echogenicity). Appropriate statistical analysis is used. Study is ongoing and due to current epidemiological status, at this point 20 patients and 20 healthy controls are included in the study.

**Results:** In the control group, bilateral combined mean SN size was 0.15cm<sup>2</sup> (±0.07). In the PD group of patients, we found mean bilateral SN size of 0.27 cm<sup>2</sup> (±0.06). In PD group 18/20 (90%) patients had hyperechogenic SN (>0.20cm<sup>2</sup>) while 6/20 (30%) PD patients who also reported depressive symptoms (Hamilton depression scale) had disrupted RN line. In control group only one individual (5%) had hyperechogenic SN size and 2 controls (10%) also had slightly echogenic/interrupted RN line.

**Discussion:** Our results so far confirmed previous finding that SN hyperechogenicity in BPS is a highly specific finding of PD. Our results also suggest that alteration of raphe nuclei is more frequent BPS finding in PD patients with depressive symptoms. Routine usage of BPS in evaluation of Parkinson's disease patients might add to clinical accuracy in establishing diagnosis. Its practical value might be in confirming PD diagnosis in doubtful clinical cases or in the early stages (prodromal/ preclinical) of the disease when symptoms are overlapping or are not fully developed. Because of fine resolution, portability, lack of invasiveness, and low cost, assuming appropriate temporal bone window, BPS may serve as practical and sufficiently sensitive neuroimaging in evaluation of patients with Parkinsons disease.

**MeSH/Keywords:** transcranial sonography, brain parenchyma sonography, Parkinsons disease

**Poster code:** R-02-30-053

**Poster Title:** Influence of smoking and genetic polymorphisms of UGT enzymes and drug transporters on lamotrigine concentrations

**PhD candidate:** Ivana Šušak Sporiš

**Part of the thesis:** Influence of genetic polymorphisms of UGT1A4 and UGT2B7 enzymes and drug transporters ABCB1 and ABCG2 lamotrigine concentrations in serum

**Mentor(s):** Assoc. Prof. Nada Božina, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Lamotrigine (LTG) is a modern antiepileptic drug that has been widely used for the treatment of patients with epilepsy. Pharmacokinetics of LTG is characterized by considerable interindividual variability. Studies have shown that this is in part due to the presence of some non-genetic factors such as age, smoking, pregnancy and concomitant medications. The coadministration of an inhibitor such as valproic acid (VPA) significantly reduces the elimination of LTG. Along with clinical and environmental factors, genetic predisposition has been recognized to be a relevant factor for interindividual variability in drug response, but available data are insufficient and contradictory. Therefore, therapeutic drug monitoring is often necessary to prevent the occurrence of unwanted reactions or toxicity as well as changes in drug efficacy. The aim of this study is to investigate the role of genetic polymorphisms of metabolic enzymes (UGT1A4 142T>G; UGT2B7 -161T>C), in addition to transporters (ABCB1 1236C>T; ABCG2 421C>A) on steady-state disposition of LTG and on the LTG-VPA interaction. Also, smoking habit will be analyzed as important parameter for evaluation of the serum LTG levels.

**Materials and methods:** The study will include at least 150 patients 18-70 years old with diagnosis of epilepsy undergoing LTG monotherapy or LTG polytherapy with VPA. So far, 98 patients have been included in the study. Patients are divided into two groups according to pharmacotherapy and subdivided according to smoking habit. The therapeutic drug monitoring is performed by high-performance liquid chromatography-diode array detector and immunoassay. Genotyping of ABCB1 1236C>T, ABCG2 421C>A, UGT2B7 -161T>C will be performed using TaqMan Drug Metabolism Genotyping assays, while genotyping of UGT1A4 142T>G will be performed using Custom TaqMan® SNP Genotyping assay by real-time polymerase chain reaction genotyping method.

**Results:** Of the 98 patients included in the study, mean age 41, 76 patients are in the monotherapy group, while 22 patients are in the polytherapy group. 38, out of 76 patients in the monotherapy group, are smokers, while in polytherapy group only 7 patients were smokers. We analysed the effect of smoking on LTG concentrations. The mean LTG serum level in smoker's monotherapy group was 10.65 µmol/L and in non-smoker's 16.02 µmol/L, while mean serum LTG level in smoker's polytherapy group was 19.46 µmol/L, and in non-smoker's polytherapy group 41.33 µmol/L. After dose adjustment, LTG serum average level-to-dose ratio in monotherapy group of smokers was 0.06 and non-smokers 0.08. LTG serum average level-to-dose ratio in polytherapy group of smokers was 0.08 and non-smokers 0.18. Smokers showed a significantly lower LTG serum level-to-dose ratio than non-smokers in both groups, monotherapy and polytherapy group (monotherapy:  $p = 0.00359$  and polytherapy:  $p = 0.0004083$ ). Upon completion of patients enrollment, concentrations of LTG in mono- and polytherapy will be analyzed in smokers and non-smokers in relation to the genetic variants of metabolic enzymes UGT1A4 142T>G and UGT2B7 -161C>T, as well as transporters ABCB1 1236C>T and ABCG2 421C>A since genetic analysis should be done at once because of the technical reasons.

**Discussion:** Our data indicates significant effects of smoking on serum levels of LTG. Since LTG is metabolized by glucuronidation via UDPGT1A4 and UDPGT2B7 present data indicate that the demonstrated effect of smoking on LTG metabolism is likely to be mediated via UDPGT2B7, as LTG is not a substrate of cytochrome P450 isoenzymes and UDPGT1A4 activity may not be affected by nicotine. After analysis of the genetic polymorphism impact on serum concentrations of lamotrigine in smokers and non-smokers we expect this study to provide evidence of the contribution of pharmacogenetic markers in individualization and therapy optimization, which represents a scientific contribution that could be applied to clinical practice.

**MeSH/Keywords:** Lamotrigine, valproic acid, epilepsy, pharmacogenetics, smoking

**Poster code:** R-02-30-072

**Poster Title:** Prognostic value of laboratory, clinical and neuroradiological findings in ischemic stroke outcome

**PhD candidate:** Ana Sruck

**Part of the thesis:** Correlation between serum concentration dynamics of Suppression of Tumorigenicity 2 and ischemic stroke outcome

**Mentor(s):** Assoc. Prof. Daria Pašalić, MD PhD, Assist. Prof. Hrvoje Budinčević, MD PhD

**Affiliation:** Sveti Duh University Hospital, University of Zagreb School of Medicine

**Introduction:** Early outcome prediction after ischemic stroke (IS) is of great importance. Prognosis is usually based on clinical variables and neuroradiological findings, while serum biomarkers and laboratory tests may contribute to prognostic accuracy. The aim was to investigate prognostic values of laboratory, clinical and neuroradiological findings in IS outcome.

**Materials and methods:** We prospectively enrolled acute IS patients who presented to our hospital 24 h within an onset between September and December 2019. Initial neurological assessment was performed using the NIH Stroke Scale (NIHSS); patients scored  $\geq 8$  were included. Standard laboratory tests from serum (concentration of C-reactive protein, glucose, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, urate, and count of leucocytes) were measured at admission while prognostic nutritional index (PNI) and Glasgow prognostic score (GCS) were calculated from the values obtained. Infarct volume was calculated based on control brain CT at intervals of at least 20-24 hours after the initial one. The primary outcome was 90-day modified Rankin score. The secondary outcome was 90-day all-cause mortality.

**Results:** We studied 20 patients (median age 83.5 years, 0.65 women). All of the patients had unfavourable outcomes (mRS 3-6), and 8 (0.4) of them died. Spearman's rank correlation confirmed that 90-day mRS was positively correlated with blood glucose concentration ( $r=0.513$ ,  $p=0.02$ ), initial NIHSS ( $r=0.754$ ,  $p<0.001$ ) and infarct volume ( $r=0.752$ ,  $p<0.001$ ), while negative correlation was found with PNI ( $r=-0.855$ ,  $p<0.001$ ). Mann-Whitney test showed significant differences between the groups of survivors and deceased, in blood glucose concentration ( $p=0.04$ ), PNI ( $p=0.01$ ), initial NIHSS ( $p=0.003$ ) and infarct volume ( $p=0.004$ ). C-reactive protein, leucocytes, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, urate values and Glasgow prognostic score were not associated with outcome.

**Discussion:** Our findings show that higher blood glucose concentration, lower PNI, higher initial NIHSS and larger infarct volume can assist the clinician in predicting unfavourable IS outcome.

**Acknowledgments:** I would like to thank my mentors for their guidance.

**MeSH/Keywords:** ischemic stroke; prognostic value; laboratory; NIHSS; outcome

**Poster code:** R-02-30-133



**Poster Title:** Role of chemokines CCL20, IL-8 and CXCL10 as prognostic and diagnostic markers in multiple sclerosis

**PhD candidate:** Jelena Koščak Lukač

**Part of the thesis:** Association of chemokines CCL20, IL-8 and CXCL10 with Epstein Barr virus, and their role as prognostic and diagnostic markers in multiple sclerosis

**Mentor(s):** Assoc. Prof. Nataša Kovačić, MD PhD, Koraljka Bačić Baronica, PhD, research associate

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Multiple sclerosis (MS) is an immune-mediated inflammatory and demyelinating disease of the central nervous system (CNS). Studies have shown that chemokines CCL20, IL-8 and CXCL10 have an important role in inflammatory damage of CNS in experimental autoimmune encephalomyelitis (EAE) and patients with MS. Studies performed on serum and cerebrospinal fluid (CSF) levels of CCL20, IL-8 and CXCL10 in patients with MS are limited in their number and their results are contradictory, which requires further investigations. The aim of this preliminary study was to investigate role of chemokines CCL20, IL-8 and CXCL10 in pathogenesis of MS and their role as markers in MS.

**Materials and methods:** Patients with relapsing-remitting MS (RRMS) and control group were enrolled in the study. Measurement of levels of chemokines CCL20, IL-8 and CXCL10 in serum was performed using bead-based immunoassay with flow cytometric analyte quantification. Serum levels of chemokines were compared between patients and controls. Distribution of the variables was analysed considering clinical characteristics of the disease, age and sex of the patients and neuroradiological findings. Selection of the statistical tests that were used depended on distribution of the results. Differences were considered significant if  $p < 0.05$ .

**Results:** Serum levels of CXCL10 and IL-8 were significantly lower in MS patients than in controls;  $p < 0,0001$  and  $p=0,0416$ . There were no significant differences between serum levels of CCL20 in patients and controls. We did not find significant correlation between serum levels of IL-8, CXCL10, CCL20 and EDSS, disease duration, number of experienced relapses or age of the patients. Concentrations of chemokines were not different in subgroups divided by presence of gadolinium enhancing lesions and sex of the patients.

**Discussion:** In preliminary study we observed significantly lower serum levels of CXCL10 and IL-8 in MS patients than in controls. Results suggest that chemokines CXCL10 and IL-8 play role in pathogenesis of MS and possibly could be used as markers in MS.

**MeSH/Keywords:** multiple sclerosis, chemokines, markers

**Poster code:** R-02-30-143

Public health and healthcare - preliminary research results

**Poster Title:** Risk Factors for Alcohol Abuse among Sixteen-Year-Olds in Croatia

**PhD candidate:** Diana Jovičić Burić

**Part of the thesis:** Risk Factors for Alcohol Abuse among Sixteen-Year-Olds in Croatia

**Mentor(s):** Assoc. Prof. Sanja Musić Milanović, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Alcohol consumption is one of the main causes of disease and death in adults. According to the World Health Organization, worldwide 3 million deaths every year result from harmful use of alcohol which represents 5.3 % of all deaths. Despite the known harmful effects of alcohol consumption, it is still acceptable form of behaviour in many societies. Adolescents are particularly prone to engage in risky behaviours, among which alcohol drinking is especially common. This study aims to explore differences between sixteen-year-olds prone to alcohol addiction and sixteen-year-olds who are not prone to alcohol addiction.

**Materials and methods:** The data was collected as part of European School Survey Project on Alcohol and Other Drugs 2015 (ESPAD) in Croatia conducted on nationally representative sample of sixteen-year-old students (n=2558). A population of students prone to alcohol addiction was defined by the criteria: drinking at least once in their lifetime, drinking at least once in the last 12 months, drinking 5 or more drinks in the last 30 days, drinking 30 grams or more of alcohol at the last occasion they drank and onset of drinking before age 13. Examined individual and environmental factors included gender; school achievement; the perception of the risk of drinking 1 or 2 drinks almost every day, drinking 4 or 5 drinks almost every day, drinking 5 or more drinks on the weekends; active involvement in sports; taking sedatives prescribed by doctors; satisfaction with relationship with mother, father and friends; parental monitoring; perception of how many friends they have who drink; the perception of how many friends they have who get drunk; mother's education, father's education; material status; family structure; drinking of a close person. The difference between students prone to alcohol addiction and other students was calculated with the  $\chi^2$  test and analysed at  $p < 0.01$ .

**Results:** Results showed that the proportion of students prone to alcohol addiction is 7.2% (n=183), statistically more boys (10.5%) comparing to girls (3.6%). Statistically higher proportion of students who are prone to alcohol addiction than those who are not perceive lower parental monitoring (29.6% to 12.3%), are not satisfied with their relationship with mother (19.3% to 11.4%), have lower school performance (43.6% to 27.4%), perceive that all or almost all of their friends drink alcohol (81.8% to 62.2%) and get drunk (66.1% to 31.6%), perceive that someone close to them drink (55.6% to 40.7%), have taken sedatives prescribed from their doctors (15.9% to 7.3%), have a lower perception of risk of drinking on health (for drinking 1 or 2 drinks a day 31.3% to 14.2%; for drinking 4 to 5 drinks a day 18.2% to 9.3%; drinking 5 or more drinks on weekends 26.1% to 14.6%).

**Discussion:** Our study provides information about characteristics of the group of adolescents prone to alcohol addiction in a national representative sample. Students prone to alcohol addiction have lower parental monitoring, relationship with mother, school performance, lower perception of risk on health, with higher influence of friends and close person who drinks and higher prevalence of taking sedatives comparing to students who are not prone to alcohol addiction. Future research is needed to explore gender differences and predictive value of these factors. These findings would be beneficial for preventive programs aimed at reducing alcohol drinking and risk for addiction development among adolescents.

**MeSH/Keywords:** alcohol, alcohol drinking, adolescents, risk behaviour, Croatia

**Poster code:** R-03-01-030

**Poster Title:** Prevalence of neuroinvasive arboviral infections in Croatia (2017-2019)

**PhD candidate:** Maja Bogdanić

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

**Mentor(s):** Assist. Prof. Tatjana Vilibić Čavlek, MD PhD, Assist. Prof. Vladimir Savić, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Arboviruses cause systemic and neuroinvasive infections with worldwide distribution. The main reservoirs of these viruses are vertebrates and the vectors are arthropods (mosquitoes, ticks, sandflies). Due to a low-level viremia, humans represent accidental 'dead-end' hosts for majority arboviruses. In the last few decades, an increasing trend of arboviral zoonoses has been observed. Although mostly asymptomatic, in some patients arboviral infections are presented with a severe neuroinvasive disease. In Croatia, tick-borne encephalitis (TBEV), West Nile (WNV), Usutu (USUV) and Toscana (TOSV) infections have been detected as well as a serologic evidence of Tahyna (TAHV) and Bhanja (BHAV) infections.

**Materials and methods:** A total of 424 patients with neuroinvasive infection (meningitis, encephalitis, myelitis) were tested for the presence of arboviruses during a three consecutive transmission seasons (2017-2019). TBEV, WNV, USUV, TOSV, TAHV and BHAV RNA were detected in cerebrospinal fluid (CSF) and urine samples using a real time reverse transcriptase-polymerase chain reaction (real-time RT-PCR). IgM and IgG antibodies were detected in serum and CSF samples using commercial enzyme-linked immunosorbent (ELISA) assays (TBEV, WNV, USUV) or indirect immunofluorescence (IFA) assay (TOSV). Demographic (age, gender, profession) and epidemiological (place of residence, history of mosquito/tick/sandfly bites, travelling to areas with documented virus circulation in the last year) data were collected using a questionnaire.

**Results:** Neuroinvasive arboviral infections were confirmed in 24.5% (104/424) patients. WNV and TBEV were the most common arboviruses detected in 59.6% (62/104) and 34.6% (36/104) patients, respectively. In three (2.9%) patients, USUV infection was detected and in three (2.9%) patients TOSV infection. TAHV and BHAV infections were not detected. Arboviral infections were detected from April to December, with majority occurred in July/August/September (83/79.8%). First cases of TBEV infections have been detected in April, while majority of patients were notified from June to September (21/20.1%). In Gorski Kotar, a small outbreak, with six cases of TBEV infection was detected in 2019. WNV infections were detected in July, with majority reported from July to September (67/64.4%). USUV and TOSV infections were detected in August. Males were affected more common (69/66.3%) than females (39/33.7%). Patients in age group >60 years had increased number of arboviral infections. Most patients with TBEV infections (27/75.0%) were in age groups 40-59 and >60 years (14/38.9% and 13/36.1%, respectively), while majority of WNV infections (48/77.0%) were reported in age group >60 years. Infections were detected in 14/21 Croatian counties. The highest prevalence was recorded in north-western and eastern counties. TOSV infections have been reported at the Croatian littoral.

**Discussion:** In 2018, Croatia experienced the largest outbreak with more than 60 confirmed human cases of WNV neuroinvasive disease and WNV fever. In summer and autumn 2018, absolute maximum air temperatures were above averages (0.7-3.3°C in eastern and 2.2-4.4°C in north-western counties) which probably influenced the vector competence by accelerating the virus replication within mosquitoes and prolonged their breeding season. High temperatures in summer did not have significant impact on TBEV infections.

**Acknowledgments:** This study was supported by the project, Croatian Science Foundation, IP 2016-06-7456: Prevalence and Molecular Epidemiology of Emerging and Re-emerging Neuroinvasive Arboviral Infections in Croatia; CRONEUROARBO.

**MeSH/Keywords:** Neuroinvasive infections, arboviruses, prevalence

**Poster code:** R-03-02-032

**Poster Title:** Knowledge and attitudes of general population, students of medicine and nurses about nursing as a profession in Zagreb

**PhD candidate:** Sanda Franković

**Part of the thesis:** Knowledge and attitudes of general population, students of medicine and nurses about nursing as a profession in Zagreb

**Mentor(s):** Professor Miroslav Mastilica, MD PhD

**Affiliation:** School of Nursing Mlinarska, Zagreb

**Introduction:** Nursing encompasses autonomous and collaborative care of individuals of all ages, families, groups and communities, sick or well and in all settings. Modern understanding of nursing specifies nursing as a professional discipline which is equally based in science as it is in practical skills based on research (evidence) of its own practice. Goal of this thesis is to determine to what extent its autonomy and professional status of nurses recognized by the tested groups Three group will be tested: general public, sixth year students of medicine and employed nurses.

**Materials and methods:** Research will use a questionnaire containing questions about demographic characteristics of participants and their attitudes towards nursing and questions about nursing professional autonomy. This research is using a questionnaire regarding attitudes towards nursing (Nursing image questionnaire), seventh version (NIQ-7), Čukljek linguistically and culturally modified the questionnaire and prepared it for use on a Croatian population. To question the knowledge about the scope of autonomous nursing work a list of questions was created based on prescribed competencies by the Nursing Act and Croatian Nursing Council. Participants of this research are students on the final year of School of Medicine in Zagreb, nurses working in University Hospital Centre Zagreb, University Hospital Centre Sestre Milosrdnice, Clinical Hospital Dubrava, Clinical Hospital Sveti Duh, Clinical Hospital Merkur, Health Centres Zagreb Centar, East and West and the council members of the Local Committees and City Districts in Zagreb. Preliminary results are based on the analysis of 68 participants from the nursing sample. The collected data were processed by methods of descriptive statistics. Variance analysis was used to test significant differences of variables between the groups based on their level of education. The level of significance was  $\alpha=0,05$ .

**Results:** Preliminary results present attitudes of 68 participants from one Clinical Hospital and a Health Center which makes less than 10% of planned sample. 92,6% of participant were female. Average age of the participants is  $40,4\pm 10,4$  with the average work experience of  $20,5\pm 11,4$  years. Most of them are married (66,2%), 17,6% are single, 10,3% are divorced and 5,9% are widows. Most of them have a undergraduate degree (44%) and 38% have a high school diploma. 17.6% have a graduate degree. Most common work area are surgical (36,8%), internal medicine (25%) and community nursing (16,2%). In the analysis of agreement with the statements from the questionnaire regarding the attitudes toward nursing, 4 statements showed statistically significant difference based on education. Participants with a graduate degree more fervently agree with the statement that nurses protect patients in the healthcare system ( $F=3,429$ ;  $p=0,038$ ), that intelligence is important for the profession ( $F= 4,381$ ;  $p=0,017$ ), and that finishing a university degree significantly contributes to better care for patients ( $F = 5,928$ ;  $p=0,004$ ). Results of the questionnaire about autonomous nursing work shows that 34,3% of participants think that in the case of insufficient number of nurses their duties can be performed although in a more limited scope by paramedics, 53% of participants state that nursing diagnosis are a vital part in diagnosis of a disease. Only 24.2% of participants would recommend their profession to their child.

**Discussion:** Since up until now only a small portion of participants results have been reviewed and only one of the planned three groups was represented further analysis is needed before making any conclusions.

**MeSH/Keywords:** attitudes, nursing, profession, general population, students of medicine

**Poster code:** R-03-02-154

**Poster Title:** Physicochemical properties of bottled waters

**PhD candidate:** Rea Janda

**Part of the thesis:** Factors of migration of bisphenol A and phthalate in water from plastic bottles

**Mentor(s):** Professor Ksenija Vitale, MD PhD, Assoc. Prof. Šime Ukić, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Bottled waters on the Croatian market can be natural mineral, natural spring and table waters, and are divided according to the Ordinance on natural mineral, natural spring and table waters (NN 85/19). Natural spring and natural mineral waters that are recognized in the Republic of Croatia are in the List of natural mineral waters and natural spring waters recognized in the Republic of Croatia (NN 39/20), which is usually updated once a year. For the doctoral dissertation, in addition to the concentrations of bisphenol A and phthalate, I will determine how physico-chemical parameters change over time and at different temperatures of bottled water storage: pH value, electrical conductivity, CO<sub>2</sub> concentration and Solids, residue on evaporation at 180 degrees C, total, gravimetric (dry matter at 180 ° C). The weight of an empty bottle without a label and without a cap will also be considered. pH value is a measure of how acidic/basic water is. The range goes from 0 to 14, with 7 being neutral. pH values of less than 7 indicate acidity, whereas a pH value of greater than 7 indicates a base. By definition, the pH value is negative logarithm of concentration of hydrogen ions in water. Since pH can be affected by chemicals in the water, pH is an important indicator of water that is changing chemically. The pH value determines the solubility and biological availability of chemical constituents such as nutrients and heavy metals. Conductivity is a measure of the ability of water to pass an electrical current. It is affected by the presence of inorganic dissolved solids, anions or cations. Conductivity is also affected by temperature: the warmer the water, the higher the conductivity. For this reason, conductivity is reported as conductivity at 25 degrees Celsius (25 °C). The gas CO<sub>2</sub> is quite soluble in water in which more than 99% exists as the dissolved gas and less than 1% as carbonic acid H<sub>2</sub>CO<sub>3</sub>, which partly dissociates to give H<sup>+</sup>, HCO<sub>3</sub><sup>-</sup>, and CO<sub>3</sub><sup>2-</sup>.

**Materials and methods:** Materials: oven Instrumentaria ST-01/02, pH meter Mettler Toledo SevenEasy, conductivity measuring apparatus Mettler Toledo MC226, scale Mettler Toledo AG245, laboratory glassware (burettes, pipettes, beakers, volumetric flasks, Erlenmeyer flasks, porcelain bowls), standard buffer solutions pH values 7.00 and 9.21, electrical conductivity standard 1413 uS/cm, HCl, concentrated acid (w = 36%), NaOH, granules, sodium carbonate, methyl orange, phenolphthalein, potassium sodium tartrate, sodium citrate; Methods: pH value determination HRN ISO 10523:2012 Water quality – Determination of pH; Conductivity determination: HRN EN 27888:2008 – Water quality - Determination of electrical conductivity; Determination of CO<sub>2</sub>: PSK 7.0/41 Determination of CO<sub>2</sub>, method of Laboratory for water analysis and balneology; Determination of dry matter at 180 °C: USGS-NWQL I-3750:1985

**Results:** Waters are named by numbers from 1 to 7. First four waters are natural mineral waters and last three are natural spring waters. Masses of empty bottles without label and cap are: 21,8533 g, 22,6407 g, 21,5903 g, 18,8633 g, 19,9970 g, 18,0183 g and 18,9545 g. pH values of examined waters are: 5,6, 5,5, 5,5, 6,6, 6,7, 7,0 and 7,0, while conductivity of these waters are: 3540 uS/cm, 3290 uS/cm, 3300 uS/cm, 531 uS/cm, 512 uS/cm, 587 uS/cm and 462 uS/cm. Determined values of dry matter at 180 °C are: 2479 mg/L, 1856 mg/L, 1860 mg/L, 277 mg/L, 246 mg/L, 330 mg/L and 197 mg/L. Concentrations of CO<sub>2</sub> in examined samples are: 3765 mg/L, 3601 mg/L, 3776 mg/L, 186 mg/L, 143 mg/L, 143 mg/L and 88 mg/L.

**Discussion:** Results show that waters with higher concentrations of CO<sub>2</sub> have lower pH values, as expected, because CO<sub>2</sub> in reaction with water produces carbonic acid, which contributes to the acidity of such waters. It can also be noticed that carbonated waters are bottled in bottles with higher weight of empty bottle and have higher conductivity values. Because carbonic acid consists of hydrogen and carbonate ions, water with a higher concentration of CO<sub>2</sub>, carbonated waters also have higher values of dry matter at 180 °C. The results also show that, although water number 4 is characterized as mineral, its physicochemical properties are more similar to the properties of spring waters. Taking everything into account, it can be concluded that the physico-chemical properties of water do not depend on the type of water but on the CO<sub>2</sub> content.

**MeSH/Keywords:** water, physicochemical properties, water types, bottles, Croatia

**Poster code:** R-03-02-161

## Thesis proposals

Basic medical sciences - thesis proposals



**Poster Title:** Dentin matrix protein 1 role in bone formation and regeneration

**PhD candidate:** Kristian Bakić

**Part of the thesis:** Dentin matrix protein 1 role in bone formation and regeneration

**Mentor(s):** Academician Slobodan Vukičević

**Affiliation:** University of Zagreb School of Medicine, Center of Excellence for Reproductive and Regenerative Medicine

**Introduction:** Dentin matrix protein 1 (DMP1) regulates phosphate metabolism and plays a key role in regulating bone and tooth mineralization. Bone morphogenetic protein 1 (BMP1) cleaves DMP1 to generate fragments deposited in extracellular matrix. Bone morphogenetic protein 6 (BMP6), in combination with autologous blood clot, is a novel osteoinductive drug that is being tested in the third phase of clinical trials.

**Hypothesis:** Dentin matrix protein 1 alone or in combination with bone morphogenetic protein 1 and bone morphogenetic protein 6 contributes to the process of bone formation and regeneration.

**Aims:** General: Investigate the influence of dentin matrix protein in bone formation, monitoring DMP1 activity in cell lines, and its regeneration at ectopic and orthotopic sites in animals. Specific: to examine the genetic expression of DMP1 and BMP1 in MC3T3-E1 cells; to determine the of ectopic bone formation by analyzing histological specimens, after implantation of osteoinductive implants in animals; to determine the contribution of DMP1 in the process of bone formation in ectopic sites; to quantify the process of formation and remodeling of bone tissue, formed at the ectopic and orthotopic site in animals

**Materials and methods:** (1) MC3T3-E1 cells will be treated with DMP1 in combination with BMPs and their antibodies, after which gene expression will be monitored by polymerase chain reaction (PCR). (2) The osteoinductive implant will be prepared by drawing blood from the rat vein to mix it with a solution of DMP1, BMP1 and BMP6 proteins and allowing it to coagulate. Bone formation will be monitored at different time points and implants will be removed after termination, scanned with a  $\mu$ -CT device and histologically processed. In addition to bone volume, the number, thickness, and distance between the trabeculae will be determined for the newly formed bone. Immunohistochemical analysis will be performed on selected histological specimens, showing the prescribing factors RUNX2, SOX-9, osteocalcin, type II collagen and the enzyme alkaline phosphatase. (3) Rats will have a transverse closed fracture of the tibia under general anesthesia. Before the fracture is performed, a needle will be inserted into the medullary cavity of the tibia as a means of intramedullary fixation. Bone callus formation at the fracture site will be recorded ex vivo. The following combinations will be used: 1. DMP1 alone; 2. DMP1 + BMP6; 3. BMP6 alone; 4. control group. Samples will be processed by the same methods as in step 2.

**Expected scientific contribution:** The scientific contribution of this research will be shown in a better understanding of the mechanisms of dentin matrix protein 1 in osteogenesis and bone regeneration and in its interaction with BMP 1 and BMP 6.

**MeSH/Keywords:** Dentin matrix protein 1; bone formation; bone regeneration; Bone morphogenetic protein 6; Bone morphogenetic protein 1

**Poster code:** T-01-01-054

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

**PhD candidate:** Ivona Matić Jelić

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

**Mentor(s):** Academician Slobodan Vukičević

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Bone morphogenetic protein (BMP) 1 is a secreted, glycosylated, zinc-dependent metalloproteinase. Administration of the BMP1-3 (BMP1 isoform) neutralizing antibody reduced renal fibrosis, preserved renal function and increased survival in rat renal fibrosis model. BMP1-3 antibody therapy reduced fibrosis progression in a rat liver cirrhosis model. Congenital muscular dystrophy (CMD) belongs to a group of neuromuscular disorders and is characterized by muscle weakness, muscle wasting. Merosin deficient congenital muscular dystrophy type 1A (MDC1A) is the second most prevalent CMD form. The responsible gene is *lama2*, important for production of laminin-211 which contributes to the protein called merosin, extracellular matrix protein that is strongly expressed in the basement membrane of skeletal muscle. The most commonly used animal models for MDC1A are mouse strains *dy* (dystrophia muscularis) which present a muscle pathology due to a complete and partial deficiency in the  $\alpha 2$  chain of laminin. It has been reported that fibrosis is an early signature of MDC1A pathology and there is a significant upregulation in collagen in muscle tissues isolated from young *dy* mice.

**Hypothesis:** The application of neutralizing antibody to BMP1 and its isoforms has a therapeutic effect in a mouse model of MDC1A.

**Aims:** Examine the role of BMP1, its isoforms and the therapeutic effect of the corresponding neutralizing antibodies in MDC1A.

**Materials and methods:** Neutralizing polyclonal antibodies to isoforms of BMP1 were prepared in Laboratory for Mineralized Tissue, School of Medicine using White New Zealand Rabbit. The prepared antibodies will be given to 2 weeks old *dy* and *wt* (wildtype) mice for a period of 3 weeks, twice a week (optimal dose) in order to examine the effect of given antibodies on muscle histology, muscle regeneration as well as the locomotor activity and muscle strength of the mice.

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

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

**Poster code:** T-01-01-057

**Poster Title:** Association of life habits and environmental factors, sperm quality and seminal fluid N-glycans in men

**PhD candidate:** Tihana Marić

**Part of the thesis:** Association of life habits and environmental factors, sperm quality and seminal fluid N-glycans in men

**Mentor(s):** Assist. Prof. Ana Katušić Bojanac, MD PhD, Aleksandra Fučić, PhD, research advisor

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Infertility is defined as inability of a couple to conceive a child after 1 year of unprotected intercourse according to World Health Organization (WHO) and affects 10-15% of couples. Medical diagnosis of male infertility relies on sex hormone status and semen analysis that evaluates sperm count, motility and morphology. Although semen analysis is performed on daily basis in clinical and research laboratories, the etiology causing male reproductive disorders often remains unclear. Infertility status is also highly susceptible to lifestyle habits and environmental risk factors. Therefore, our aim is to investigate association of semen quality and seminal fluid N-glycans with life and environmental risk factors

**Hypothesis:** Abnormal semen parameters are associated with higher DNA fragmentation index, higher histone to protamine ratio, altered N-glycans in seminal fluid and with life habits and environmental risk factors (smoking, alcohol, diet, urban/rural area).

**Aims:** Main aim is to determine association of semen parameters (motility, count and morphology), DNA integrity, histone to protamine ratio and seminal fluid N-glycans with life habits and environmental risk factors. Specific aims are: 1. Determine association of semen parameters with DNA fragmentation index, histone to protamine ratio in men with normal and abnormal semen parameters 2. Determine association of N-glycans in men with normal and abnormal semen parameters 3. Determine association of DNA fragmentation index, histone to protamine ratio with N-glycans in seminal fluid in men with normal and abnormal semen parameters 4. Determine association of life habits and environmental risk factors with DNA fragmentation index and histone to protamine ratio in men with normal and abnormal semen parameters 5.

Determine association of life habits and environmental risk factors with N-glycans in men with normal and abnormal semen parameters 6. Evaluate N-glycans as biomarkers of life habits and environmental risk factors and semen parameters

**Materials and methods:** Study participants will be recruited during regular semen examination. Patients willing to join the study will sign informed consent and answer questionnaire, which includes general information, medical history and questions about life habits such as smoking, drinking, diet and living area. Healthy participants (80) with normal sperm parameters and participants with abnormal sperm parameters (65) according to WHO classification will be enrolled. Sperm parameters that include sperm count, motility and morphology will be determined according to WHO with computer aided sperm analysis (CASA) system. DNA integrity will be assessed by DNA fragmentation index with commercial halosperm assay, which determines fragmentation according to DNA halo around sperm head. Sperm chromatin condensation will be determined by histone to protamine ratio with aniline blue assay. N-glycans in seminal fluid will be determined by ultra-performance liquid chromatography that detects the most abundant N-glycan forms in the sample. Multivariate statistical analysis will be conducted to determine association of several variables - sperm parameters, DNA fragmentation index, histone to protamine ratio and N-glycans in seminal fluid with life habits and environmental factors.

**Expected scientific contribution:** Scientific contribution of this study is innovative association of semen parameters, DNA integrity and chromatin condensation level with N-glycans in seminal fluid, which could contribute to male infertility diagnostics. Comparative analysis of these parameters, especially seminal fluid N-glycans with life habits and environmental risk factors (smoking, alcohol, diet, urban/rural area) will significantly straighten understanding and preservation of male fertility.

**Acknowledgments:** This publication was co-financed by the European Union through the European Regional Development Fund, Operational Programme Competitiveness and Cohesion, under grant agreement No.KK.01.1.1.01.0008, Reproductive and Regenerative Medicine - Exploring New P

**MeSH/Keywords:** male infertility, sperm, environment, DNA fragmentation, protamine, N-glycans

**Poster code:** T-01-04-124

**Poster Title:** Cell-free DNA methylation of RASSF1 and CAV1 genes in blood and ejaculate of patients with prostate cancer

**PhD candidate:** Lucija Škara

**Part of the thesis:** Cell-free DNA methylation of RASSF1 and CAV1 genes in blood and ejaculate of patients with prostate cancer

**Mentor(s):** Assist. Prof. 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.

**Hypothesis:** RASSF1A and CAV1 are hypermethylated in cfDNA in blood and ejaculate of patients with prostate cancer in regard to RASSF1A and CAV1 in cfDNA in blood and ejaculate of patients with BPH.

**Aims:** 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. This will be accomplished by: 1.

determining and comparing the pattern and degree of methylation of RASSF1 and CAV1 promoter regions in cfDNA of blood and ejaculate from PC and BPH patients. 2. determining and comparing the pattern and degree of methylation of RASSF1 and CAV1 promoter regions in genomic DNA (gDNA) from tissue of PC and BPH patients 3. determining protein expression of RASSF1 and CAV1 in prostate cancer and BPH tissue 4. intergroup and intragroup comparison of gDNA methylation pattern in tissue with protein expression and cfDNA methylation pattern in blood and ejaculate 5. comparing obtained molecular results with collected clinical and anamnestic data.

**Materials and methods:** In this study 40 prostate cancer and 40 BPH patients will be included. Ejaculate and blood samples are centrifugated and separated from cells to avoid releasing their content in samples. From blood and semen plasma cfDNA will be isolated while from paraffin embedded tissues gDNA will be isolated. DNA will be bisulfite-treated, PCR amplified and promoter regions of RASSF1 and CAV1 will be pyrosequenced. Paraffin embedded tissues will be used for immunohistochemical staining with antibodies raised against RASSF1 and CAV1 protein.

**Expected scientific contribution:** Scientific contribution is expected in the development of epigenetic biomarkers which could be useful for discrimination prostate cancer from benign prostatic hyperplasia in liquid biopsy samples. Furthermore, contribution to a deeper understanding of such minimally invasive and precise diagnostic, as well as its biological, technical and financial advantages and limitations is expected.

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

**Poster code:** T-01-05-058

**Poster Title:** Cell free DNA methylation of APC and LGALS3 in blood and ejaculate of patients with 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):** Assist. Prof. Nino Sinčić, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Prostate cancer (PCa) is the most diagnosed neoplasm among men in Croatia, with expected rise in incidence and mortality. New biomarkers are being investigated to improve current diagnostic sensitivity and specificity. Among them, changes in genomic DNA (gDNA) methylation in promoter region of the APC and LGALS3 genes were detected in PCa tumor tissue. Research is pointing to the cell free DNA (cfDNA) in liquid biopsies as potential biomarker source that could lead to better PCa and benign prostate hyperplasia (BPH) discrimination, and further improve diagnosis and prognosis.

**Hypothesis:** Promoter regions of the APC and LGALS3 genes in cfDNA isolated from blood and ejaculate of early-stage prostate cancer patients are hypermethylated compared to the ones in benign prostate hyperplasia patients.

**Aims:** The aim is to identify methylation degree and pattern in promoter region of the APC and LGALS3 genes, by analyzing cfDNA isolated from blood and ejaculate of early-stage PCa patients; and compare it with the ones in BPH patients.

**Materials and methods:** This prospective study will include participants directed to prostate biopsy due to clinical suspicion of PCa within Urology departments of Clinical Hospital Centers Zagreb and Sestre milosrdnice. Their blood (EDTA tube, 12 mL) will be taken, as well as their semen samples collected by masturbation. Inclusion criteria are BPH diagnosis or PCa diagnosis in stage I or II, according to the American Joint Committee on Cancer (AJCC) 8th Cancer Staging Manual. Upon histopathological diagnosis, patients will be divided into two groups: PCa and BPH patients. Each group will contain 40 subjects which is the number greater than minimal number of subjects required for adequate study power, as determined by using software ClinCalc and published data. Collected specimens will be processed into blood and seminal plasma, cfDNA will be isolated and quantified by commercially available kits. Genomic DNA (gDNA) will be isolated from formalin-fixed paraffin-embedded biopsy (FFPE) tissue specimens from PCa patients. The DNA methylation of promoter regions of the APC and LGALS3 genes will be analyzed by pyrosequencing using PyroMark Q24 Advanced System, after PCR amplification of bisulfite converted gDNA and cfDNA fragments. Protein expression of APC and LGALS3 genes in FFPE prostate biopsy tissue will be analyzed using morphometric immunohistochemical analysis. Data will be statistically analyzed to investigate the difference in methylation degree and pattern of APC and LGALS3 genes in cfDNA isolated from blood and ejaculate between two patient groups. Moreover, cfDNA methylation and gDNA methylation of mentioned genes will be compared within PCa patients, as well as correlated with protein expression of chosen genes. Finally, sensitivity and specificity of cfDNA methylation of APC and LGALS3 genes in blood and seminal plasma for PCa and BPH patient discrimination will be determined.

**Expected scientific contribution:** Feasibility of cfDNA from liquid biopsies and robustness of DNA methylation set ground for the direction of cancer biomarker development. Ejaculate potentially represents global prostatic pathophysiology and could be a source of PCa specific biomarkers. Therefore, the results would contribute to the studies of changes in cfDNA methylation in PCa in comparison to BPH, as well as to the research of PCa epigenetic biomarkers in liquid biopsies, especially in the case of ejaculate that has not been previously used in such diagnostics.

**Acknowledgments:** This study will be done as a part of the project "Epigenetic biomarkers of prostate cancer" (epiPro, UIP-2017-05-8138) funded by Croatian Science Foundation.

**MeSH/Keywords:** Prostate Cancer, DNA Methylation, Biomarkers, Cell-Free DNA

**Poster code:** T-01-05-074

**Poster Title:** Methylation of cell free DNA of the RASSF1A gene in blood and semen as an epigenetic biomarker in patients with testicular seminoma

**PhD candidate:** Dora Raos

**Part of the thesis:** Methylation of cell free DNA of the RASSF1A gene in blood and semen as an epigenetic biomarker in patients with testicular seminoma

**Mentor(s):** Professor Davor Ježek, MD PhD, Assist. Prof. Nino Sinčić, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Testicular germ cell tumors (TGCT) affect the young male population with a high incidence and mortality rate in Croatia. Seminomas (SE) arise under genetic and (micro)environmental influences. DNA methylation are most investigated epigenetic aberrations (epimutations) and have great potential as tumor biomarkers due to high chemical and biological stability. Nowadays, cell-free DNA (cfDNA) methylation pattern in liquid biopsies is investigated as a platform for noninvasive diagnostics, but these investigations are very rare especially in ejaculate samples. RASSF1A is a tumor suppressor gene, which is located within the region of 3p21.3 chromosome and codes for RASSF1A scaffold protein. RASSF1A responds to different stimuli by regulating the progression of the cell cycle, apoptosis, and microtubule stability. Currently, several tumor suppressor genes are investigated as potential tumor biomarkers and RASSF1A represents one of the strong emerging biomarkers for TGCT. Methylation of RASSF1A in gDNA was discriminative between SE and healthy tissue but its methylation pattern in cfDNA was not investigated neither in blood nor in ejaculate samples. In this study, pattern and degree of RASSF1A methylation in cfDNA as well as in gDNA from liquid (blood and ejaculate) and tissue samples of seminoma patients will be investigated.

**Hypothesis:** RASSF1A promotor region in cfDNA from blood and ejaculate of seminoma patients is hypermethylated regards to healthy controls.

**Aims:** The main aim of this study is to determine methylation pattern and degree of RASSF1A promotor region in cfDNA from blood and ejaculate of seminoma patients and to determine its potential as a seminoma biomarker.

**Materials and methods:** According to the power test analysis, 24 samples per group will be enough for Fisher's exact test, obtaining thereat a strength of 95%. Healthy volunteers, mostly students, will be recruited from a healthy population. The patient population will be recruited from a male population with suspicion of a testicular tumor. Excluding criteria will be any histopathological diagnosis other than pure seminoma. From healthy volunteers, one set of blood and ejaculate will be taken. From patients, two sets of liquid samples, i.e. preoperative and postoperative together with tumor tissue will be taken. All participants will provide their informed consent and the study was approved by competent Ethical committee. The pathohistological diagnosis of seminoma and tumor tissue sampling will be done at UHCs "Sestre milosrdnice" and Zagreb, immediately after orchidectomy. CfDNA will be isolated from blood and ejaculate, and gDNA will be isolated from tumor tissue according to optimized protocols. After isolation, cfDNA and gDNA concentration will be measured. DNA methylation analysis will be done by pyrosequencing. Morphometric analysis of the immunohistochemical signal will be performed according to the standard protocol on the light microscope in collaboration with pathologists. Surrounding tumor-free tissue with preserved normal spermatogenesis will be considered as healthy tissue. The statistical analysis will be conducted with statistical tests such as Fisher's exact test, Fisher-Freeman-Halton's exact test, Mann-Whitney U test, and Spearman correlation coefficients. All p values less than 0,05 will be considered significant. The analysis will be performed using statistical program GraphPad Prism 7.

**Expected scientific contribution:** Serum biomarkers used in routine diagnosis of testicular seminoma are not sensitive and specific enough. Investigation of DNA methylation of gene's promotor region will determine if the methylation of RASSF1A promotor region is significant enough to be a potential biomarker for testicular seminoma and can it contribute to the development of specific epigenetic biomarkers for testicular seminoma. Investigation of cfDNA methylation from blood and seminal plasma would contribute to the earlier, simpler and non-invasive diagnosis of testicular seminoma as well as easier monitoring of patients with testicular seminoma.

**Acknowledgments:** This study will be done as a part of the project "Epigenetic biomarkers in blood and ejaculate of patients with testicular seminoma" (epiSem, IP-06-2016) funded by Croatian Science Foundation.

**MeSH/Keywords:** seminoma, RASSF1A, DNA methylation

**Poster code:** T-01-05-076

**Poster Title:** The role of Notch signalling in murine models of hepatic fibrosis

**PhD candidate:** Dino Šisl

**Part of the thesis:** The role of Notch signalling pathway in murine model of liver fibrosis

**Mentor(s):** Assoc. Prof. Tomislav Kelava, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Chronic liver diseases are a major health burden associated with severe morbidity and mortality. Hepatic fibrosis is a common feature of various liver diseases, which are all characterized by the accumulation of extracellular matrix. Progressive fibrotic processes may lead to liver cirrhosis. Recent fate mapping studies have suggested that activated hepatic stellate cells (HSC) are a major source of myofibroblasts, alpha-smooth muscle actin ( $\alpha$ SMA) positive cells that produce extracellular matrix during fibrogenesis. However, signals that activate quiescent HSC are still not elucidated, recent evidence suggests possible role of Notch signalling pathway.

**Hypothesis:** Activation of Notch signalling pathway in  $\alpha$ SMA-positive cells leads to exacerbation of hepatic fibrosis, while its inhibition ameliorates it.

**Aims:** To characterize the effect of Notch signalling pathway modulation in  $\alpha$ SMA positive cells on the process of hepatic fibrogenesis, we will use a transgenic mouse model in which expression of certain genes can be modulated in said cells, and in which those cells can be identified and followed. In order to get this transgenic model, we will employ a breeding protocol that will lead to mice in which Notch signalling pathway can be selectively activated or inhibited in  $\alpha$ SMA-positive cells.

**Materials and methods:** In order to determine baseline expression, expression during different stages of fibrogenesis and expression of Notch signalling pathway-related genes during recovery, we will use transgenic mice of SMACreERT2 strain, where we will label the  $\alpha$ SMA-positive cells and trace them during the process of fibrogenesis. In order to determine the role of Notch signalling pathway overexpression in  $\alpha$ SMA-positive cells on development of fibrosis, we will use SMACreERT2/RosaNotch transgenic mice, in which fibrosis will be induced by two different modalities, toxic (intraperitoneal application of carbon tetrachloride – CCl<sub>4</sub>) and diet-induced model (food containing DDC - 3,5-diethoxycarbonyl-1,4-dihydrocollidine). For determination of the role of Notch signalling inhibition in  $\alpha$ SMA-positive cells on development of fibrosis, mice of SMACreERT2/ $\Delta$ Rbpjk $\Delta$ / $\Delta$ Rbpjk $\Delta$  strain will be used, and the same two previously mentioned models of fibrosis induction will be used. The degree of fibrosis will be assessed by collagen staining (Sirius red staining) and by PCR (COL1A1,  $\alpha$ SMA expression). Expression of Notch receptors on various liver cells (hepatocytes, stellate cells, activated stellate cells, leukocytes) will be determined by flow cytometry. Expression of Notch signaling related genes will be determined from whole liver tissue and from isolated hepatic stellate cells by PCR.

**Expected scientific contribution:** This research has the potential to identify cellular and molecular signalling pathways that are crucial for development of hepatic fibrosis, which can lead to identification of potential targets for development of antifibrotic therapy necessary to ameliorate or halt the fibrosis progression in patients that are high-risk for development of liver cirrhosis. Finally, these results would be beneficial to scientific community, pharmaceutical industry and healthcare workers in the field of regenerative medicine.

**Acknowledgments:** This work is supported by Croatian Science Foundation project number UIP-2017-05-1965

**MeSH/Keywords:** hepatic fibrosis, hepatic stellate cells, Notch signalling pathway, murine model of liver fibrosis

**Poster code:** T-01-06-145

**Poster Title:** Molecular characteristics of interneurons in the human frontal cortex

**PhD candidate:** Ivan Banovac

**Part of the thesis:** Molecular characteristics of 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:** Over 80 percent of cortical GABAergic (gama aminobutyric acid) neurons express one of three calcium-binding proteins: calretinin, calbindin and parvalbumin. Although the rat frontal cortex shows a high level of colocalization between parvalbumin and calbindin, no such colocalizations were found in the monkey dorsolateral prefrontal cortex. Furthermore, parvalbumin neurons are the predominant population of GABAergic neurons in rodents, while in the primate prefrontal cortex, calretinin neurons are the most numerous. Systematic research on GABAergic neurons in humans is still lacking. Preliminary data on the calretinin population in humans suggest ventrodorsal differences in the proportions of calretinin neurons, and recent research on primates indicates a relatively low proportion of somatostatin neurons, which are, in rodents, as numerous as calretinin and parvalbumin neurons. Additionally, the relation and degree of colocalization between the calbindin and somatostatin populations in primates are unclear. Since interneurons are the key regulators of the cortical network, determining their specific characteristics in humans is crucial for understanding the complexity of human cognitive processes and various neuropsychiatric disorders.

**Hypothesis:** There are ventrodorsal differences in the proportion of different GABAergic neuron populations in the human frontal cortex.

**Aims:** The aim of this research is to determine the molecular characteristics of GABAergic neurons in the human frontal cortex and to examine the differences in the molecular profile and proportions of different interneuron populations between Brodmann areas 9 and 14.

**Materials and methods:** Postmortem frontal lobe sections of the adult human brain (5 brains) archived in the Zagreb neuroembryological collection will be used in this research. The sections will be stained using immunofluorescence and RNA (ribonucleic acid) in situ hybridization (RNAscope) with the goal of determining the molecular markers in different populations of GABAergic neurons in the supragranular layers (I, II and III). Analysis and systematic scanning of cortical areas will be performed using a laser confocal microscope. The quantification will be performed using specialized software (Neurolucida, MBF Bioscience). The acquired quantitative data will be statistically analyzed (analysis of variance, t test) to determine the areal differences (BA 9 and 14) in the proportions of GABAergic neuron populations.

**Expected scientific contribution:** The areal specificities in the structure of the GABAergic neural network, significant for understanding the functional properties of dorsal and orbital areas of the prefrontal cortex, will be determined in this research. The disorganization of the GABAergic neural network is present in a number of neuropsychiatric disorders (schizophrenia, autism, sleep disorders) and data providing its characteristics in the normal brain represent the foundation for future research on the pathophysiology of such disorders.

**Acknowledgments:** This research is supported by the supported by the Croatian Science Foundation (project: IP 3182) and the Scientific Centre of Excellence for Basic, Clinical and Translational Neuroscience (project: GA KK01.1.1.01.0007).

**MeSH/Keywords:** interneurons, GABA, calretinin, parvalbumin, calbindin, somatostatin, cerebral cortex, human

**Poster code:** T-01-08-004



**Poster Title:** The role of neural stem cells in controlling regulated cell death after ischemic stroke in mouse brain

**PhD candidate:** Damir Lisjak

**Part of the thesis:** The role of neural stem cells in controlling regulated cell death after ischemic stroke in mouse brain

**Mentor(s):** Assoc. Prof. Dinko Mitrečić, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Brain stroke is one of the most common causes of death and disability in the world, with ischemic stroke being the most common variant. A decrease or complete interruption of blood flow due to blockage of a brain artery causes hypoxia and hypoglycaemia leading to oxidative stress, excitotoxicity, inflammation and regulated cell death. Stem cells are cells that are capable of continuous dividing and differentiating into a vast number of different cell types. Although neural stem cells (NSC) have been proven to have neuroregenerative and neuroprotective effects, use of stem cells in the treatment of human stroke requires knowledge of the molecular mechanisms underlying negative and positive events of NSC transplantation.

**Hypothesis:** Transplantation of neural stem cells into the mouse brain affected by ischemic stroke reduces the intensity of regulated cell death.

**Aims:** The main goal is to analyse the activity of regulated cell death by monitoring apoptosis, pyroptosis and necroptosis in mouse brain affected by ischemic stroke and to determine the extent to which transplanted stem cells affect the regulated cell death in ischemia-affected mouse brain. In order to achieve the main goal, three specific goals are designated: 1-Determine the intensity of regulated cell death in healthy mouse brain and brain affected by ischemia by monitoring specific markers for apoptosis, pyroptosis, and necroptosis. 2-Determine incidence of certain regulated cell death type in neurons, astrocytes, and microglia. 3-Determine the impact of NSC transplantation on the intensity of regulated cell death in the area affected by ischemia.

**Materials and methods:** For the purposes of this study C57BL/6 mice will be used. NSCs will be isolated from telencephalic wall of 14 days old embryos and cultured until neurospheres are formed. Stroke will be caused by standardized method of a middle cerebral artery occlusion (MCAO) for 30 minutes. NSC transplantation will be performed with a stereotaxic transplant device (Kopf 9000LS) into the striatum. Lesion volume and edema monitoring will be performed in vivo by MRI imaging system (Bruker BioSpec 70/20 USR). Animals will be divided into four groups: 1st group-Sham, 2nd group-MCAO, 3rd group-MCAO + NSC, 4th group-MCAO + complete medium for NSC. Each group will be split in half and brain tissue will be sampled at time points on second and fifth day after MCAO. The presence of certain types of regulated cell death in certain cell types will be monitored by immunohistochemistry using specific markers: for neurons (MAP2), astrocytes (GFAP) and microglia (CD11b), and for cell death, apoptosis (CASP3), pyroptosis (CASP1 and GSDMD) and necroptosis (RIPK3 AND MLKL). The specimens will be labelled with fluorescent secondary antibodies and imaged on a confocal microscope. Gene expression will be measured by the q-PCR and protein expression by the Western blot using previously mentioned markers for regulated cell death of interest. For immunohistochemistry, the whole brain will be fixed and cut on a cryo-cutter. For western blot and q-PCR fresh tissue will be removed, brain will be divided into contralateral and ipsilateral hemispheres, lysed and stored at -20°C, prior to analysis.

**Expected scientific contribution:** This research will provide an insight into the intensity of regulated cell death after stroke and cells which are involved in this process. Moreover we will be able to conclude about the molecular events behind the already proven beneficial effect of transplantation of the neural stem cells. The results of this study will open possibility for safer translation of stem cell technology into clinical practice.

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

**Poster code:** T-01-08-014

**Poster Title:** Influence of stem cells on necroptosis in the cells of nervous system in vitro caused by hypoxia

**PhD candidate:** Valentina Hribljan

**Part of the thesis:** Influence of stem cells on necroptosis in the cells of nervous system in vitro caused by hypoxia

**Mentor(s):** Assoc. Prof. Dinko Mitrečić, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** One of the main consequences of brain hypoxia-ischemia is cell death of necrotic morphology which is characterized by loss of cell membrane integrity and leakage of cell content in extracellular space which leads to inflammation. Necrosis was for a long time considered as an unregulated form of cell death caused by physical or chemical stress. Today, it is known that most of the cell death of necrotic morphology is regulated, i.e. mediated by certain molecular pathways. This gives the opportunity to target those pathways and thereby reduce cell death in diseases where cell death is a negative factor. This research will focus on the effect of stem cells on necroptosis, one of the types of regulated necrosis caused by hypoxia.

**Hypothesis:** Exogenous neural stem cells decrease the intensity and duration of necroptosis of the cells of nervous system and thereby they act positively on cell survival following hypoxic damage.

**Aims:** The main goal is to investigate the activity of necroptosis in the cells of the nervous system, in normoxic and hypoxic conditions, and the effect of exogenous neural stem cells (NSC) on decreasing death of the cells of the nervous system caused by necroptosis following hypoxia. The specific goals are: 1. To determine the activity of necroptosis during NSC differentiation and in in vitro culture of mature cells of the nervous system, in the conditions of ambient and tissue normoxia, 21% and 5% of oxygen, respectively; 2. To determine the activity of necroptosis in in vitro culture of the cells of nervous system following hypoxia (1% of oxygen); 3. To investigate hypothesized effect of exogenous NSC on the reduction of death of the cells of nervous system caused by necroptosis following hypoxia; 4. To compare the effect of NSC with the effect of chemical inhibitor of necroptosis, Necrostatin-1, on necroptosis in in vitro culture of the cells of nervous system exposed to hypoxia.

**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. Differentiation will be performed in 21% and 5% of oxygen, ambient and tissue normoxia, respectively. During differentiation we will analyse cells in multiple time points using markers for NSC (Nestin, Sox2), neurons (Map2, MapT), astrocytes (Gfap) and necroptosis (Ripk1, Ripk3, Mlkl), both on RNA (real-time PCR) and protein level. Once we determine which of the two concentrations of oxygen is better for cell survival, we will repeat the differentiation protocol in that concentration, until the point we get differentiated neurons and astrocytes (after day 5). Differentiated neurons and astrocytes will be exposed to hypoxia, 1% oxygen, for 24 h. After hypoxia, cells will be treated with: NSC, conditioned medium in which NSC grew, or with the inhibitor of necroptosis Necrostatin-1. Then we will investigate if, and to which extent, some of these treatments reduce necroptosis. Cells will be analysed immediately after hypoxia and 24 and 48 h following treatment using markers for neurons (Map2, MapT), astrocytes (Gfap) and necroptosis (Ripk1, Ripk3, Mlkl) using real-time PCR for relative gene expression, immunocytochemistry for colocalization of necroptosis marker (MLKL) with markers of astrocytes (GFAP) and neurons (MAP2, MAPT) and western blot to relatively quantify marker of necroptosis (MLKL) on protein level.

**Expected scientific contribution:** This work will contribute to better understanding of pathophysiological mechanisms involved in dying of the cells of nervous system following hypoxia. Also, it will clarify when and in which cells necroptosis occurs. If stem cells act positively and reduce necroptosis, discovery of molecular mechanism responsible for this would represent potentially novel treatment for brain ischemia.

**Acknowledgments:** Funded by Croatian Science Foundation (IP-2016-06-9451 and PhD grant for VH) and the Scientific Centre of Excellence for Basic, Clinical and Translational Neuroscience (project GA KK01.1.1.01.0007).

**MeSH/Keywords:** stem cells, necroptosis, hypoxia, cells of the nervous system

**Poster code:** T-01-08-019

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

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

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Biological membranes, characterized by highly diverse lipid environment, are housing incredibly elaborated protein architecture. Many of molecular processes vital for intercellular communication are associated with membrane proteins. For instance, ion homeostasis is maintained by P-type ATPases and in case of receptors, information received extracellularly is translated, refined and transduced via signalling hubs composed of intracellular adaptor proteins. Proteins neuroplastin (Np) and Toll-like receptor 2 (TLR2) are examples of such a phenomenon. Np, expressed as ubiquitous Np55 and neuron-specific Np65 isoform, is highly glycosylated membrane resident displaying various actions: 1) positioning of and cross-talk with receptors, as shown for Np55 which interacts with plasma membrane calcium ATPase (PMCA), while Np65 positions GABA A receptor; 2) participating in the molecular mechanism of learning and memory, supported by finding of associative memory deficits in Np65 knock-out mice; 3) involvement in neuroprotective molecular events during recovery following ischemic insult, and tissue compensatory response in neurodegeneration, as evidenced for Np65. TLR2, on the other hand, is involved in: 1) activation of the immune system; 2) neurodegeneration and hypoxic stress; 3) neurodevelopment and cell differentiation. Although functions of Np as a cell-adhesion molecule and TLR2 as a pattern recognition molecule differ, recent data from literature revealed that they share the same adaptor protein, TRAF6.

**Hypothesis:** Absence of TLR2 leads to changes in expression and sub-membrane localization of Np and P-type ATPases in the murine brain.

**Aims:** To investigate the potential functional relationship between Np and TLR2, this study focuses on a mouse model, B6.129-Tlr2tm1Kir/J, deficient in TLR2 protein (TLR2D). A detailed analysis of protein and gene expression, synaptic proteome, (glyco)lipidome and submembrane localization of Np and P-type ATPase will address the potential changes in biochemical cellular make-up in brain tissue derived from TLR2D mice in comparison to wild-type (WT) animals.

**Materials and methods:** Brain tissue and samples of selected brain regions will be dissected from TLR2D and age- and sex-matched WT mice. Proteome analysis by LC-MS will give insight into the response of membrane and synaptic proteins to TLR2 deficit. Western blot will be used to detect the expression pattern of Np and ATPases in isolated brain regions, and immunofluorescence by confocal microscopy will depict the distribution and position of Np and ATPases throughout the brain regions. Localization of Np and ATPases immunoreactivity within different cell populations will be tracked by specific cell markers. Gene expression pattern of Np and ATPases will be analyzed by RT-PCR method. Finally, quantitative and qualitative analysis of gangliosides will be used for investigating membrane lipid composition in brain tissue of TLR2D vs WT mice. Possible shifts in submembrane distribution of Np and ATPases in TLR2D brain tissue will be analyzed in membrane fractions using lipid raft isolation method.

**Expected scientific contribution:** Systematically collected data will clarify biochemical phenotypic differences between TLR2D and WT mice, with a focus on gene and protein expression and multi-level localization of Np and P-type ATPases in brain tissue. Provided information will untangle connections within Np and TLR2 signalling pathways, and clarify molecular and cellular consequences of TLR2 deficiency. TLR2D mouse model is frequently used in neuroinflammation research, but its biochemical and molecular phenotype is understudied. It is to be expected that deciphering relationship of here investigated membrane proteins involved in cell adhesion, neuroplasticity and inflammation may open new perspectives for utilization of TLR2D mouse model in investigating specific molecular aspects of neurodevelopment, neurodegeneration and behavioural disorders.

**MeSH/Keywords:** Neuroplastin, P-type ATPases, TLR2, brain, cell membrane, cell-cell communication

**Poster code:** T-01-08-071

**Poster Title:** Influence of deep brain stimulation on structural changes of central nervous system and blood-brain barrier in patients with Parkinson's disease

**PhD candidate:** Petar Marčinković

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

**Mentor(s):** Assist. Prof. Darko Chudy, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** DBS (Deep Brain Stimulation) is established as a very effective therapy option for advanced Parkinson's disease (PD). DBS surgery comprises of neurostimulator (battery) implantation which in turn, via subcutaneously placed extensions and electrodes implanted in specific brain regions, sends impulses to those brain regions/ targets and afterwards alleviates the symptoms of the PD. Despite significant symptom improvements with the use of DBS, the exact mechanism of DBS functioning as well as its effect on the structures of the central nervous system (CNS) remains unknown.

**Hypothesis:** Deep brain stimulation induces structural reorganization (volumetric and diffusion changes) of parts and/or regions of the central nervous system alongside changes in the permeability of the blood-brain barrier (BBB) in patients with movement disorders, i.e. Parkinson's disease.

**Aims:** To determine the structural changes or more precisely volume and diffusion changes of specific brain areas/regions caused by DBS in patients with PD using volumetric and tractographic analysis of preoperative and postoperative magnetic resonance imaging (MRI) alongside analysis of the blood-brain barrier permeability prior and during DBS.

**Materials and methods:** Each of the 25 subjects with PD included in this study will perform a preoperative magnetic resonance imaging (MRI) and two postoperative MRIs. These MRIs will afterwards be used in volumetric analysis via automated programs (CIVET, volBrain, pBrain) which will determine any changes in designated regions of the CNS (supratentorial volumes of white and grey matter, cerebrospinal fluid, alongside with the quantification of regional cortical thicknesses and volumes of individual brain lobes, volumes of nucleus caudatus, putamen, globus pallidus, amygdala, thalamus, hippocampus, nucleus accumbens, nucleus ruber, substantia nigra and nucleus subthalamicus) comparing volumes prior and after a 12 month period of DBS. Using manual delineation and analysis of individual regions of interest (ROI) in a tractographic analysis software tool (TrackVis), fraction anisotropy (FA) parameters and diffusion coefficients will be monitored preoperatively and postoperatively, in regions known for their characteristic microstructural degradation in patients with PD (precentral gyrus, postcentral gyrus, supplementary motor area, substantia nigra, thalamus, putamen). Analysis of the concentrations of the blood-brain barrier permeability indicators NSE (neuron specific enolase) and s100b protein prior and during DBS, will determine if there is any DBS impact on the permeability of the BBB. Statistical analysis will be performed using MedCalc.

**Expected scientific contribution:** Clarifying DBS effect on CNS structures, their volume changes alongside eventual diffusion alterations and BBB permeability modifications which will further elucidate more precise indications for DBS in patients with PD.

**MeSH/Keywords:** DBS, PD, volumetric analysis, tractographic analysis, blood-brain barrier, NSE, s100b

**Poster code:** T-01-08-081

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

**PhD candidate:** Paula Josić

**Part of the thesis:** The effect of inflammation on apoptotic cell death after ischemic lesion of the mouse brain

**Mentor(s):** Professor Srećko Gajović, MD PhD

**Affiliation:** University of Zagreb School of Medicine, Laboratory for Regenerative Neuroscience - GlowLab

**Introduction:** Ischemic stroke is a leading global health problem with an existing need for understanding pathogenic mechanisms and improvement of therapeutic strategies. Using imaging of living mice, it has been shown that activation of the innate immune response after stroke is characterized by substantial and long-term induction of the Toll-like receptor 2. It is unclear whether the induction of immune response after ischemia is protective or detrimental; therefore, the extent of apoptosis in Tlr2-deficient mice will be compared to that of wild type mice with functional Tlr2 response. This study aims to elucidate in vivo the time course of molecular events during mouse brain recovery.

**Hypothesis:** Reduction of inflammation after ischemic stroke will increase the extent of apoptosis. Tlr2 knock-out mice will express an amplified apoptotic response to ischemic injury compared to wild type mice.

**Aims:** The general aim of presented research is to measure in vivo post-stroke apoptosis and determine the influence of inflammation mediated by Tlr2 on apoptosis. Specific aims of presented research are to (1) establish the procedure of in vivo bioluminescence imaging with caged DEVD-luciferin in wild type and Tlr2 knock-out mice; (2) visualize and determine the temporal sequence of apoptosis at time points of 2, 4, 7, 10, 14 and 28 days post stroke; (3) compare the results obtained by apoptosis imaging in wild type and Tlr2 knock-out mice.

**Materials and methods:** In this study, 2-4 months old male mice will be used. Sixty animals will be used; 30 wild types and 30 Tlr2 knock-outs. Neurological score will be determined for each mouse at baseline and after ischemic lesion in a series of time points. Ischemic brain lesion will be induced with transient middle cerebral artery occlusion. To measure the extent of apoptosis, in vivo bioluminescent imaging (BLI) with caged DEVD-luciferin released only by apoptotic cells will be used. To measure stroke volumetry, magnetic resonance imaging (MRI) will be implemented. T2-weighted and T2 map sequence scans will be performed. On days 7 and 28, the designated mice will be subjected to perfusion and brain isolation. Brain samples will be cut on a cryostat. To inspect the extent of apoptosis, immunohistochemistry will be performed using neuronal and apoptosis markers. For statistical analysis the Shapiro-Wilk normality test, two-way ANOVA, repeated measurement ANOVA and Bonferroni post-hoc analysis will be performed. In case of non-parametric distribution, Kruskal-Wallis test with Mann-Whitney post-hoc analysis. Statistical significance will be defined as  $p < 0.05$ .

**Expected scientific contribution:** The aim of this research is to elucidate the relationship between inflammation and apoptosis during post-ischemic brain recovery, which will be determined by MRI stroke volumetry and the extent of apoptosis via BLI during 28 days post ischaemia. Obtained results will help in differentiating detrimental and reparative processes in the acute and chronic phases of ischemic brain injury, as well as potentially contribute to the development of standardized evaluation of ischemic brain injury. Furthermore, obtained results will serve as a base for evaluation of neuroprotective therapies in development.

**Acknowledgments:** Supported by EU European Regional Development Fund, Operational Programme Competitiveness and Cohesion, grant agreement No.KK.01.1.1.01.0007, Croatian Science Foundation project RepairStroke (IP-06-2016-1892) and CoRe-Neuro

**MeSH/Keywords:** ischemia, immune response, stroke volume, apoptosis, magnetic resonance imaging, bioluminescence imaging

**Poster code:** T-01-08-090

**Poster Title:** Changes of perineuronal nets in the rat brain after controlled short-term perinatal hypoxia

**PhD candidate:** Sara Trnski

**Part of the thesis:** Changes of perineuronal nets in the rat brain after controlled short-term perinatal hypoxia

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

**Affiliation:** University of Zagreb School of Medicine, Scientific Centre of Excellence for Basic, Clinical and Translational Neuroscience

**Introduction:** In this study, perinatal mild hypoxic brain injury will be performed, which has not been described in the literature so far in terms of the type of occurrence and intensity. Previous research has described stronger hypoxia or a combination of hypoxic-ischemic injury and consequent extensive inflammatory processes, motor, and other deficits. In this study, the goal is to achieve mild hypoxic brain injury and to investigate changes in the distribution and number of perineuronal nets, and behavioral and learning changes using immunohistochemical methods and behavioral testing. Also, the demonstration of the transcription factor of hypoxia, apoptosis, and activation of microglia and oligodendrocyte after mild hypoxic injury in the perinatal age has not been investigated. Disorders of perinatal developmental processes as well as reorganization processes after hypoxia injury in perinatal age have also not been investigated. The impact of mild hypoxia on anxiety, hyperactivity or hypoactivity, social and learning skills, and spatial memory in adolescence and adulthood after a mild perinatal hypoxic injury has not been investigated. Therefore, we believe that this model will provide new insights in the field of perinatal brain injury research.

**Hypothesis:** The expression of the perineuronal nets is qualitatively and quantitatively altered in the brain of adult rats after controlled short-term perinatal hypoxia.

**Aims:** This study aims to discover changes of perineuronal nets in the rat brain, at the molecular and histological level (qualitatively and quantitatively), and to correlate them with changes in behaviour (from the motor to cognitive functions) in the rats that were exposed to controlled short-term perinatal hypoxia.

**Materials and methods:** For this study, 144 Wistar Han (RccHan: WIST) rats of both sexes will be used. Newborn rats will be separated from dams at the age of first postnatal day (P1) and exposed to controlled normobaric hypoxia (8% O<sub>2</sub>, 92% N<sub>2</sub>) for 2 hours, or to control conditions (21% O<sub>2</sub>, 78% N<sub>2</sub>, 2 hours). At 30 and 70 days of age, the same rats will be tested for behavioral tests: open field, hole board, social choice, and T – maze. Post mortem brain samples of rats aged P1, P45, P85 will be taken to analyze changes in the brain: (I) At the level of histoarchitectonics (Nissl staining); (II) Developmental cellular and molecular markers; and (III) At the level of perineuronal nets (histochemical, immunohistochemical methods) using light, fluorescence or confocal microscopy and the FIJI/ImageJ program.

**Expected scientific contribution:** This study is expected to contribute to the establishment of a new animal model of controlled perinatal hypoxic brain damage in the rat that will complement current animal models. Since these models are used in human prematurity and neonatal brain damage research, we will provide additional answers to open-ended questions.

**Acknowledgments:** Research funded by project "Experimental and clinical research of hypoxic ischemic damage in perinatal and adult brain" GA KK 01 1 1 01 0007 funded by EU and Croatian Science Foundation (DOK-2018-01-3771 and HRZZ IP-2019-04-3182)

**MeSH/Keywords:** chondroitin sulfate proteoglycans, interneurons, microglia

**Poster code:** T-01-08-097

**Poster Title:** Psychosocial effects of COVID-19 pandemic on individuals in Croatia, Italy and worldwide

**PhD candidate:** Vanja Kopilaš

**Part of the thesis:** Effects of COVID-19 pandemic on psychological status and everyday activities of individuals in Croatia, Italy and worldwide.

**Mentor(s):** Professor Srećko Gajović, MD PhD, Assist. Prof. Lovorka Brajković, MD PhD

**Affiliation:** 1. Faculty of Croatian Studies University of Zagreb 2. University of Zagreb School of Medicine

**Introduction:** The continuing outbreak of coronavirus disease (COVID-19) has spread all over the world and has become a global pandemic. Due to its relatively non-specific symptoms that could easily be attributable to already existing diseases, COVID-19 was not detected as a new disease and its outbreak went unnoticed for almost two months. It has been evident throughout history how psychological factors are related to almost any significant event. Whether the event is positive or negative, there are equivalent psychological effects. For the purpose of this research, focus will be on the impact of negative events. There is no much previous research related to the COVID-19 pandemic. Some findings show negative mental health outcomes caused by COVID-19, such as elevated symptoms of depression and anxiety. These findings are very similar to the recent pandemics but more research is needed.

**Hypothesis:** Participants who have been in contact with COVID-19 infected people, and participants who have been in areas of high COVID-19 impact, will score higher on measures compared to participants who have not been in contact with COVID-19 infected person and are in areas of low COVID-19 impact.

**Aims:** GENERAL: Explore the impact of the COVID-19 pandemic on mental health and daily activities SPECIFIC: Analyze the presence and intensity of symptoms of depression, anxiety, stress and loneliness Analyze the positive and negative affect Analyze the degree of adherence to the recommended epidemiological measures Analyze participants' digital and physical activity Analyze participants' perception of the COVID-19 pandemic

**Materials and methods:** Study will be carried out in two phases. In the first phase, 4 groups will be examined. The first test group consists of postgraduate students who were informed by the Croatian Institute for Public Health about being in contact with a person diagnosed with COVID-19, and were issued with precautions. The second test group consists of people who, due to the specific situation and the declaration of a red zone throughout Italy, found themselves in isolation. The third, control group consists of postgraduate students who have not been in contact with the person diagnosed with COVID-19. The fourth, control group consists of graduate students who also have not been in contact with the person diagnosed with COVID-19. In the second phase, the Qualtrics link will be spread globally by "snowballing" method. Participants will receive an email invitation to fill out the questionnaire. After reading the consent form and agreeing to participate in the study, they will be able to start with the questionnaires. The study will consist of validated psychological measures and ad hoc questions created for this specific situation to assess participants' digital and physical activity. Validated psychological measures include: The UCLA Loneliness Scale, Impact of Event Scale-Revised, Positive and Negative Affect Schedule and Depression Anxiety Stress Scale-21 .

**Expected scientific contribution:** Due to the fact that during our lifetime we have not encountered an event with such a devastating global impact, our finding should be valuable in assessing how this pandemic has affected our lives, particularly in terms of the psychological effects, and how this can inform us of how to recover faster.

**MeSH/Keywords:** COVID-19, Depression, Anxiety, Loneliness

**Poster code:** T-01-08-139

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

**Mentor(s):** Assoc. Prof. 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 induces the functional connectivity. The modulatory effect of music is evident in limbic and paralimbic structures, particularly the amygdala, hippocampus, ventral striatum and nucleus accumbens, and midbrain. 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 levels of IL-8 have 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 the pathophysiology of anxiety and depression disorders. High levels of neurofilaments represent a general index of axonal damage. Their plasma levels can be used to monitor or predict the state of neurological diseases or the effectiveness of a particular therapeutic procedure. Therefore, plasma EGF, IL-8, or NfL levels may reflect an underlying mechanism affecting some molecular pathway(s) involved in anxiety and depression symptoms in women with breast cancer.

**Hypothesis:** Receptive music therapy alleviates the symptoms of anxiety and depression in women with breast cancer, improves the quality of life, and reduces the concentrations of epidermal growth factor, interleukin-8, and neurofilament light chain in plasma during treatment, compared to the control group.

**Aims:** This doctoral thesis aims to investigate the effect of receptive music therapy on the relief of anxiety and depression symptoms, improving quality of life and on the levels of epidermal growth factor, interleukin-8, and neurofilament light chain in plasma during the perioperative period and chemotherapy 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 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 - anthracyclines or taxanes). In the control group, there will be regular medical care applied. At four 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, as well as measuring the plasma levels of EGF, 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 determinants: tempo 60-80 bpm, continuous rhythm, gentle melodic lines, and consonantly composed harmonic progressions.

**Expected scientific contribution:** This research will investigate the effect of receptive MT on 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. If proven effective, receptive MT might be more widely used in breast cancer patients, and also investigated in other populations of patients with cancer. In addition, this study would determine if plasma levels of EGF, IL-8, and NfL might be potential biomarkers of depression and anxiety in those patients and whether their levels change with treatment.

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

**Poster code:** T-01-08-148



**Poster Title:** The effect of proteotoxic stress on human tau protein aggregation and toxicity expressed in yeast *Saccharomyces cerevisiae*

**PhD candidate:** Klara Zubčić

**Part of the thesis:** The effect of proteotoxic stress on human tau protein aggregation and toxicity expressed in yeast *Saccharomyces cerevisiae*

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

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** One of the main neuropathological hallmarks of Alzheimer's disease (AD) are neurofibrillary tangles (NTFs), large aggregates of microtubule-binding protein tau that form within the affected neurons. The progression of the disease, as well as the degree of cognitive impairment, are directly correlated with the number of NTFs. The formation of NTFs is preceded by early-stage tau oligomers, however the molecular pathways that initiate tau aggregation are unclear. Disturbed protein homeostasis is considered to be one of the possible causes of neurodegenerative diseases characterized by protein aggregates. The aim of this thesis is to investigate the factors that lead to the aggregation and toxicity of tau proteins in cells, especially the influence of proteotoxic stress. To better understand early steps in tau pathology, we will construct a tau-tau protein interaction reporter based on the complementation of the enzyme luciferase. Since molecular pathways of protein aggregation are largely evolutionarily conserved, we selected a simple cell model, yeast *Saccharomyces cerevisiae*. Luminescent reporter for the use in yeast will enable performing genetic screens for the modifiers of tau aggregation and toxicity, thus contributing to our understanding of the molecular mechanisms of AD development.

**Hypothesis:** Proteotoxic stress leads to increased aggregation and toxicity of human tau protein in yeast cells.

**Aims:** This doctoral thesis aims to define the effect of proteotoxic stress on aggregation and toxicity of human tau protein in a yeast cell model *Saccharomyces cerevisiae*.

**Materials and methods:** This research will be conducted on yeast strains of *Saccharomyces cerevisiae*, isogenic to wild-type strain S288C. The required gene constructs will be obtained by molecular cloning by standard recombinant DNA techniques (PCR, enzymatic restriction and ligation) and by homologous recombination in yeast. The expression level of fusion proteins will be examined in total cell lysates by the Western blot method, using antibodies to epitope tags (HA and V5) and to tau protein (Tau5). The luminescence intensity will be measured in living yeast cells using a microplate reader and NanoGlo luciferase assay (Promega). The signal will be normalized according to cell density, measured using OD600. Proteotoxic stress will be induced using yeast strains with impaired ubiquitin-protease system. Tau oligomerization will be examined using tau-NanoBIT reporter system. Tau toxicity will be determined by measuring the viability and reproductive capacity of cells that express tau protein under the inducible promoter (CUP1 gene promoter induced by copper ions, or GAL promoter induced by galactose). The viability of cells that inducibly express tau protein and are under conditions of proteotoxic stress will be measured.

**Expected scientific contribution:** This research will help to elucidate how proteotoxic stress affect protein aggregation and toxicity. The construction and establishment of the luminescent reporter for the use in yeast will enable performing genetic screens for the modifiers of tau aggregation and toxicity in future research.

**MeSH/Keywords:** Alzheimer's disease, protein aggregation, tau, protein-protein interaction, luciferase, yeast *Saccharomyces cerevisiae*

**Poster code:** T-01-08-156

Clinical medical sciences - thesis proposals

**Poster Title:** Investigation of the skin microbiome changes in patients with periocular dermatitis

**PhD candidate:** Iva Ferček

**Part of the thesis:** Skin microbiome in patients with periocular skin disorders

**Mentor(s):** Assist. Prof. 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 and is a source of genetic diversity, modulates diseases, is a fundamental component of our immune system and an entity that affects metabolism and modulates the interaction between medications. According to newer studies performed by molecular methods, the skin microbiome is even more diverse than previous studies have shown. The older studies, which were based on conventional methods of microbial cultures, had underestimated the diversity of certain groups of bacteria. Periocular dermatitis 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. The occurrence of periocular skin changes is linked to a few factors, such as 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, which is in accordance with findings in other skin regions. Despite numerous scientific findings, there are still no clinical studies conducted on patients with periocular dermatitis that would examine composition of microbiome using molecular methods.

**Hypothesis:** The change in the periocular skin microbiome in patients with periocular dermatitis compared to healthy individuals is manifested by the depletion of bacterial diversity

**Aims:** The aim of this research is to investigate periocular skin microbiome, identify its changes in periocular dermatitis and compare microbiome between patients and healthy subjects using molecular methods

**Materials and methods:** The proposed case-control research will include 100 patients: 50 patients with periocular dermatitis and 50 healthy subjects. The composition of microbiome will be analyzed from visible skin changes in the periocular region in patients and from the intact upper eyelid skin in healthy subjects. Sampling will be performed by taking a skin swab with a sterile swab tipped with synthetic material that is immediately stored at -80°C after sampling. Analysis of microbiome will be performed using T-RFLP (Terminal Restriction Fragment Length Polymorphism) and NGS (Next Generation Sequencing) method. DNA will be extracted manually using commercial kit specific for microbial DNA purification, followed by PCR amplification of the 16S rRNA gene. After purification of the PCR products, both T-RFLP and NGS will be performed. Restriction will be performed in parallel with the MspI and HhaI enzymes. The following step is desalination of the restriction product with EDTA, sodium acetate and ethanol, capillary electrophoresis in the automated sequencing device (ABI PRISM 310 Genetic Analyzer). In NGS, microbiome will be analyzed using targeted next-generation sequencing of V1-V3 variable regions of 16S rRNA gene using Illumina MiSeq sequencer. Reagents, primers and protocols will be used according to the manufacturer's instructions. The results will be bioinformatically and statistically analyzed.

**Expected scientific contribution:** Possible changes in the composition of microbiome will potentially contribute to the understanding of the etiopathogenesis of periocular dermatitis. Gaining further knowledge about variations in the microbiome during periocular skin changes would also most likely contribute to more efficient treatment of skin lesions

**MeSH/Keywords:** periocular, dermatitis, microbiome, T-RFLP, NGS

**Poster code:** T-02-02-026

**Poster Title:** Pharmacoeconomic aspect of treatment moderate and severe psoriasis with ustekinumab

**PhD candidate:** Ante Orbančić

**Part of the thesis:** Pharmacoeconomic aspect of treatment moderate and severe psoriasis with ustekinumab

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

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

**Introduction:** Psoriasis is a chronic, recurrent, autoimmune disease that affects 1-3% of the world population, 20% of whom are diagnosed with moderate and severe psoriasis. In addition to the physical aspect of the disease, many patients develop psychological problems such as shame, unwillingness to display body parts, fear of stigmatization in society, depression, etc. All of these affect the quality of life of patients. Based on International Psoriasis Council data, total direct and indirect health care costs of psoriasis for patients are calculated at \$11.25 billion annually, with work loss accounting for 40 percent of the cost. All these numbers shows us that psoriasis is not just an individual, but it is also global problem. It is therefore necessary to find appropriate therapy with reasonable cost to help patients maintain a high quality of life as soon as possible.

**Hypothesis:** Using ustekinumab in treatment of severe and moderate psoriasis shows pharmacoeconomic non inferiority compared to apremilast and acitretin.

**Aims:** The general aim of this research is to evaluate direct cost of treatment of moderate and severe psoriasis with ustekinumab as new drug treatment compared to apremilast and acitretin. Its specific aims are to investigate whether ustekinumab increases quality of life compared to apremilast and acitretin and to show that quality of life of patients who received biological treatment increase faster than quality of life of patients on other systematic therapy.

**Materials and methods:** This is a retrospective study including data from all patients having moderate and severe psoriasis that were treated with ustekinumab, apremilast and acitretin in Sestre milosrdnice University Hospital Center in Zagreb in the years 2016.-2018. Included patients will be 18 years old and older, with diagnose moderate or severe psoriasis and have no significant medical condition except psoriasis. Excluded criteria will be patients who have other diagnose that could affect their quality of life score. Severity of psoriasis was determined by dermatologist. Data will be collected by analyzing medical records of patients. For assessment quality of life Dermatology Quality of Life Index (DQLI) questionnaire was used. Data about outpatient direct costs included: number and type of visits to specialists, number of laboratory blood tests and the data about pharmacotherapy will be calculated by using data of Croatian Health Insurance Fund. All data derived from medical documentation will be collected and incorporated into an Excel spreadsheet. All results will be expressed as mean  $\pm$  95% confidence interval, standard deviation, median and range. We will use Spearman's coefficient to evaluate the strength of correlation between independent or confounding with dependent variables.

**Expected scientific contribution:** Treatment of severe psoriasis with conventional systematic therapy is associated with low costs for patients. In contrast, biological medicines are financial burden for both healthcare system and patients. Additionally, access to these treatments is often restricted because patients are eligible to receive biological therapy only if they do not respond to conventional therapy. This is the first time to compare three prescription medicines for moderate and severe psoriasis (ustekinumab, apremilast and acitretin) and to analyze cost-effectiveness using method which takes into consideration cost of each treatment while using quality of life as measurable outcome.

**MeSH/Keywords:** psoriasis, pharmacoeconomic, ustekinumab, apremilast, acitretin

**Poster code:** T-02-02-029

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

**PhD candidate:** Nika Franceschi

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

**Mentor(s):** academic Mirna Šitum, Assist. Prof. Ivan Šamija, PhD

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

**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.

**Hypothesis:** A higher BRAF mutant allele percentage in tumor tissue is associated with a better response to BRAF and MEK inhibitor treatment in metastatic BRAF-mutated melanoma patients.

**Aims:** The aim of this study is to investigate the association between BRAF mutant allele percentage in melanoma tumor samples and treatment response to BRAF and MEK inhibitor treatment (dabrafenib + trametinib, vemurafenib + cobimetinib). Specific objectives include determining the presence of a BRAF mutation in patients with stage IV metastatic melanoma, assessing the percentage of BRAF-mutated alleles in melanoma tissue samples obtained from patients with metastatic BRAF-mutated melanoma, investigating whether there is a difference in response to BRAF and MEK inhibitor treatment between patients with BRAF V600E and patients with BRAF V600K mutations, and investigating whether there is an association between BRAF mutant allele percentage and treatment response to BRAF and MEK inhibitor treatment during 2 years of follow-up.

**Materials and methods:** The study will be conducted on 80 patients with stage IV metastatic BRAF-mutated 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 existence and proportion of BRAF mutant alleles. Patients will undergo follow-up for 2 years after initiation of BRAF and MEK inhibitor treatment and will be monitored using clinical, imaging and laboratory methods to determine response to therapy, using progression-free survival as clinical outcome measure.

**Expected scientific contribution:** This study will evaluate the BRAF mutant allele percentage as a predictor of response to treatment with BRAF and MEK inhibitors in patients with stage IV metastatic melanoma. The results may lead to the potential identification of a predictive marker for response to targeted BRAF and MEK inhibitor therapy in metastatic melanoma patients, contributing to an individualized approach to each patient. Additionally, this research may help to better understand response and resistance mechanisms to BRAF and MEK inhibitor treatment.

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

**Poster code:** T-02-02-122

**Poster Title:** The effect of administration BPC 157 in the treatment of psoriasis-like lesions in experimental laboratory animals

**PhD candidate:** Marija Šola

**Part of the thesis:** The effect of administration BPC 157 in the treatment of psoriasis-like lesions in experimental laboratory animals

**Mentor(s):** Professor Predrag Sikirić, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Psoriasis is a chronic recurrent inflammatory skin disease characterized by the appearance of typical erythematous, scaly skin lesions that occur in genetically predisposed individuals under the influence of endogenous and/or exogenous factors. The disease is immunologically mediated, with the key role of T lymphocytes, dendritic cells which excrete cytokines IL 12, IL 23, IL 17, TNF alpha and induce the formation of erythematous scaly skin lesions. The disease typically affects skin, nails, scalp and when the joints are affected we are talking about psoriatic arthritis. Severity of the disease is measured by the PASI (psoriasis area severity) index. The best model for the study of psoriasis in laboratory animals is the induction of psoriasis-like lesions using 5% imiquimod cream. Gastric pentadecapeptide BPC 157 is a synthetic 15-amino acid peptide used in a variety of experiments on various experimental models with well proven benefits.

**Hypothesis:** Treating psoriasis-like lesions using BPC 157 in laboratory animals will lead to significantly faster regression of psoriasis like lesions without any side effects of BPC 157 use when compared to control group. It is expected to see significant histological difference between BPC 157 treated lesions and control group in favour of BPC 157.

**Aims:** Aim of this experiment is to prove effectiveness of BPC 157 use in the treatment of psoriasis -like lesions both topically in a cream and orally in drinking water, without any side effects of its use.

**Materials and methods:** Experiment is done on male laboratory rats Wistar Albino who live in controlled laboratory environment. At the beginning of experiment hair at their back in the field size 3x4 cm will be shaved. 5% imiquimod cream will be applied in the amount of 125 mg per application (6,25 mg imiquimod) daily for 7 consecutive days, which will induce psoriasis-like lesions. BPC 157 is 15 amino acid peptide, part of human gastric juice protein with beneficial effects on wound healing, especially skin burn lesions, bones, tendons, muscles healing. In the process of healing skin lesion it was effective when applied topically as well as orally. There were no side effects, no toxicity, lethal dose is still not established. On the 8th day of the experiment the treatment of laboratory animals using BPC 157 will start. There will be 6 animals per group: 2 groups will be taking BPC 157 orally in drinking water in concentration 0,016mcg/ml; and 0,16ng/ml; 2 groups will be treated only topically using BPC 157 in a Belobaza cream in concentration 1mcg/g and conc. 10ng/g. Cream will be applied in thin layer once a day, intake water containing BPC 157 will be ad libitum. Lesions will be observed daily, filmed using videocamera. Modified PASI score will be calculated daily for every animal. At predetermined day animals will be sacrificed (8.10.14.21. day). Skin samples will then be histologically examined and scored.

**Expected scientific contribution:** It is expected to observe significantly faster regression of psoriasis-like lesions using BPC 157 without any side effects. This could be foundation for further experiments in developing novel drug containing BPC 157 for treating psoriasis since psoriasis has incidence of approximately 1 to 4% of world population and it is chronic disease with huge impact on lowering quality of life.

**MeSH/Keywords:** BPC 157, psoriasis, imiquimod

**Poster code:** T-02-02-163

**Poster Title:** Biomechanical gait analysis of patients with ankylosing spondylitis

**PhD candidate:** Vedran Brnić

**Part of the thesis:** Biomechanical gait analysis of 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 Center

**Introduction:** Ankylosing spondylitis (AS) is a chronic inflammatory rheumatic disease affecting predominantly sacroiliac joints and axial skeleton, often resulting in spinal deformity and postural changes, which can lead to an altered gait pattern. Considering the importance of gait in functional independence there is a need for objective gait assessment which can be accomplished through instrumental biomechanical analysis with pedobarography and kinematic analysis.

**Hypothesis:** There are differences in the kinetic pedobarographic and kinematic parameters of gait between patients with AS and controls without disease or condition that could affect gait.

**Aims:** The aims of this study are to assess alteration of gait pattern through kinetic pedobarographic and kinematic parameters in AS patients compared to controls and to establish correlations of this 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. Participants will be aged 18-70. Pedobarographic and kinematic gait parameters of all participants will be recorded. Demographic and clinical characteristics, disease duration, BASFI, BASDAI, BASMI and mSASSS scores of AS patients will be assessed.

**Expected scientific contribution:** The results of this study will contribute to a better understanding of gait alteration in AS patients and to the design of targeted gait kinesitherapy program as part of the rehabilitation process. To the best of our knowledge, this study is the first to correlate radiographic spinal structural changes measured by mSASSS with kinetic pedobarographic and kinematic gait parameters in AS patients.

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

**Poster code:** T-02-03-037

**Poster Title:** Personality and psychopathology in rheumatoid arthritis

**PhD candidate:** Bernarda Škegro

**Part of the thesis:** Personality traits measured by the Minnesota Multiphasic Personality Inventory in rheumatoid arthritis

**Mentor(s):** Assoc. Prof. Porin Perić, MD PhD, Assist. Prof. Milan Milošević, MD PhD, senior research associate

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** The Minnesota Multiphasic Personality Inventory (MMPI) is a frequently used questionnaire to assess personality and psychopathology in patients with rheumatoid arthritis (RA). The hypothesis that certain personality traits have a significantly higher incidence in the RA patients than in the healthy population was set more than 100 years ago. Different research have found clinically significant elevations on the MMPI scales hypochondria, depression and hysteria in patients with RA, which was not found in healthy subjects. These elevations are common in diseases characterized by chronic pain, but significant differences in MMPI profiles have been found between patients with RA, fibromyalgia, chronic headache and others. Studies examined whether there is a typical MMPI personality profile of patients with RA and whether it differs from those in healthy subjects. Contradictory results are the reason that there is still no consensus between clinicians and researchers on this hypothesis, so this systematic review and meta-analysis will attempt to answer that.

**Hypothesis:** There will be statistically significant elevations on the hypochondria, depression, and hysteria scales on the MMPI inventory in patients with RA compared with healthy subjects.

**Aims:** 1. To identify, evaluate and summarize observations on personality and psychopathology of RA patients on all MMPI scales. 2. To compare the MMPI profiles of patients with RA with healthy subjects and identify the main differences in profiles.

**Materials and methods:** We will conduct a systematic literature review and meta-analysis to check whether certain personality traits exist more often in patients with RA than in the healthy population. In order to answer the set hypothesis, a larger number of individual observations is needed, and with the help of meta-analysis, the results of a larger number of studies are summarized and general conclusions can be drawn.

**Expected scientific contribution:** This is the first systematic review and meta-analysis of the personality and psychopathology of patients with RA assessed by the MMPI inventory, which will contribute to the clarification of previous contradictory findings. The aim of the study is to provide clear evidence (evidence based medicine) to answer the question whether there is a difference in the MMPI profiles of RA patients compared with healthy individuals.

**MeSH/Keywords:** rheumatoid arthritis, MMPI, personality

**Poster code:** T-02-03-138



**Poster Title:** Association of cigarette smoking with the concentration of metallothionein and MT2A gene methylation in the mother-infant pairs

**PhD candidate:** Iva Miškulin

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

**Mentor(s):** Assist. Prof. Lana Škrkatić, MD PhD, research associate, Jasna Jurasović, PhD, research advisor

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

**Introduction:** Metallothioneins (MT) are cysteine-rich low molecular weight proteins that bind metals in the body. MTs are involved in homeostatic regulation of essential elements, while exposure to xenobiotics as toxic metals, results in increased synthesis of MT proteins, which play a protective and detoxifying role. Cigarette smoking is one of the most important sources of exposure to toxic concentrations of cadmium (Cd) and lead (Pb). Pregnant women who smoke have a higher concentration of Cd in the blood and the placental tissue which may interfere with the transfer of essential elements to the fetus. Exposure of the fetus to Cd and Pb from tobacco smoke during intrauterine life may lead to changes in epigenetic programming of gene expression via DNA methylation.

**Hypothesis:** Cigarette smoking before and /or during pregnancy due to exposure to toxic metals Cd and Pb from cigarette smoke induces a hypomethylation of MT2A gene and increases metallothionein protein synthesis and changes in concentration elements in the mother, placenta and fetus.

**Aims:** Investigate the association between cigarette smoking, MT protein concentration and MT2A methylation in maternal blood, placenta, and umbilical cord blood. Assess the impact of smoking habits on exposure to toxic metals Cd and Pb, concentrations of essential elements, antioxidant protection and possible MT2A polymorphism.

**Materials and methods:** This cross-sectional study includes 90 mothers (couples mother - child), approximately half of women who smoke cigarettes. Inclusion criteria in the study were: healthy women with a full term birth ( $\geq 37$  weeks of pregnancy) without serious health problems or chronic illness, without pathology in pregnancy and vaginal delivery. A survey questionnaire was used in this study to collect the following data: gain, socioeconomic conditions, possible sources of environmental and workplace exposure, smoking shift (number of cigarettes / day) and newborn data, sex, birth weight and length, APGAR vitality assessment in 1st and 5th minutes after birth. The assessment of exposure and the effect of smoking as a source of exposure to metals will be analyzed and compared between: a) group of smokers - women who smoked during any period or pregnancy or stopped smoking within a period not exceeding 12 months before pregnancy and b) non-smokers - women who have never smoked or have stopped smoking more than 12 months before pregnancy. A single urine sample was taken before, and the entire placenta and blood samples from the umbilical cord and maternal venous blood within one hour after delivery. Methods: DNA isolation, methylation state of the MT2A gene, metallothionein concentration, metal concentration (Cd, Pb, Zn, Cu i Fe), cotinine in urine, activity of antioxidant enzymes SOD i GPX, polymorphisms rs28366003 i rs1610216.

**Expected scientific contribution:** New insights into the association of epigenetic indicators of DNA methylation on the MT2A gene with biological markers of tobacco smoke metal exposure and total metallothionein (MT) concentration in biological samples of maternal and fetal origin.

**MeSH/Keywords:** MT2A methylation, metallothionein, toxic and essential elements, epigenetic marker, placenta

**Poster code:** T-02-05-065

**Poster Title:** Biomarkers in the urine of patients with recurrent urinary tract infections caused by the uropathogenic *Escherichia coli*

**PhD candidate:** Ivana Puškarić

**Part of the thesis:** Biomarkers in the urine of patients with recurrent urinary tract infections caused by the uropathogenic *Escherichia coli*

**Mentor(s):** Assoc. Prof. Fran Borovečki, MD PhD, Professor Alemka Markotić, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Urinary tract infections (UTIs) are one of the most common and important health problems in women of reproductive age. About 60% of all women will develop UTI, and about 25% of these women will have recurrent UTI. Uropathogenic *Escherichia coli* (UPEC) is responsible for about 90% of UTI. The high frequency of recurrent UTI, as well as the increase in antibiotic resistance of microorganisms indicate the importance of understanding the immune response of UTI and acting on the immune response for the prevention and treatment of UTI. UPEC attacks bladder epithelial cells (BECs) and develops into intracellular bacterial communities (IBCs), and after maturation, spreads to neighboring BECs by repeating this cycle. In the case of a strong acute inflammatory response in the early stage of infection, there is a greater chance of developing future chronic cystitis caused by UPEC, which then creates a predisposition for recurrent cystitis. Urine has been shown to be a good source of various biomarkers. Interleukin 8, Nerve growth factor, Neutrophil gelatinase-associated lipocalin, cellulose have been shown to be potential biomarkers from urine in recurrent UTI. Numerous studies have shown an association between changes in serum IgG glycosylation and the immune response.

**Hypothesis:** There are differences in immunoreactions at the level of gene expression of soluble biomarkers of urinary sediment epithelial cells and glycosylation of IgG molecules in the urine of patients with single UTI, healthy subjects and patients with recurrent UTI.

**Aims:** General aim is to detect biomarkers in urine samples at the level of nonspecific and specific immunity that could play a role in the development of recurrent UTI in women. Specific aim is to detect changes in gene expression in urine sediment cells, detect soluble biomarkers and analyze changes in glycosylation of IgG molecules in urine of women with single UTI and healthy subjects compared to women with recurrent UTI.

**Materials and methods:** This study will be performed on urine samples of 30 patients with single acute pyelonephritis, 30 patients with recurrent pyelonephritis caused by UPEC and 20 healthy subjects of appropriate age (18 years and older). The patients will be hospitalized or treated during 2019-2021 at the University Hospital for Infectious Diseases "Dr. Fran Mihaljević" in Zagreb. The research will be conducted in the Department for Urogenital Infections and the Scientific Unit of the University Hospital for Infectious Diseases "Dr. Fran Mihaljević" in Zagreb, partly in the Center for Translational and Clinical Research, School of Medicine, University of Zagreb, and GENOS DNA laboratory d.o.o. Urine and urine sediment samples will be used for testing. Urine sediment samples will be isolated by RNA and the expression of genes responsible for the immune response will be analyzed by the Real-time PCR array method, which contains 84 genes associated with innate and acquired immune reactions. After detection of immune parameters important for recurrent urinary tract infections caused by UPEC at the gene expression level, relevant parameters will be detected at protein level using Enzyme-linked immunosorbent assay (ELISA) or Luminex immunoassay in urine samples. IgG will be isolated from urine and analyzed by using MALDI-TOF mass spectrometry, which includes determination of percentage of galactosylation, fucosylation, sialylation, etc. Analysis of clinical and laboratory parameters will be made based on data from the history of the disease. Gene expression, soluble immune parameters, and changes in urinary IgG glycosylation will be further evaluated in correlation with clinical symptoms and patient parameters.

**Expected scientific contribution:** Supplementing current knowledge on immunoreactions in recurrent IMS that may be important for disease immunopathogenesis.

**MeSH/Keywords:** *Escherichia coli*, urinary tract infection, immunoreactions, biomarkers.

**Poster code:** T-02-07-152

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

**PhD candidate:** Nikolina Marić

**Part of the thesis:** Safety and efficacy of alteplase compared to heparin in patients with pulmonary embolism

**Mentor(s):** Assoc. Prof. Robert Likić, MD PhD

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

**Introduction:** Pulmonary embolism (PE) is an acute cardiorespiratory disease classified in three risk categories: high, intermediate and low. While therapeutic strategies for high and low-risk PE patients are well determined, optimal therapeutic strategy for intermediate-risk patients is still a topic of debate. Majority of patients in this category are candidates for anticoagulant therapy, while those with severe right ventricular dysfunction and clinical signs of poor prognosis along with low bleeding risk may be considered for fibrinolytics.

**Hypothesis:** Alteplase with no concomitant administration of heparin in treatment of intermediate-high risk PE patients in comparison to heparin alone is not associated with higher risk of bleeding (non-inferiority) and it results in better patient survival and shorter hospital stay.

**Aims:** The main aim is to compare safety and efficacy of alteplase vs heparin in treatment of intermediate-high PE patients. Specific aims are: to analyse the factors that contribute to selection of alteplase; to determine the incidence, severity and location of bleeding; to determine mortality rate; to determine the length of hospital stay.

**Materials and methods:** This prospective cohort study will include patients with intermediate-high risk PE. The patients will be divided in two groups: 40 patients in the intervention group and 40 in the control group. The intervention group patients will receive alteplase at a total dose of 100 mg given as 10 mg bolus followed by a 90 mg intravenous infusion over 2h. During alteplase infusion patients are not going to receive heparin. 2h after the end of alteplase slow intravenous infusion of unfractionated heparin without the bolus dose will be started. The control group will receive intravenous infusion of unfractionated heparin according to the Raschke protocol with bolus dose of 80 IU/kg and infusion at rate of 18 IU/kg/h. Main end points of the study are: mortality rate, clinical deterioration requiring escalation of therapy and appearance of major bleeding.

**Expected scientific contribution:** In this trial PE treatment will be conducted without concurrent administration of alteplase and heparin. We expect that this study will provide additional information regarding the safety profile of alteplase as well as clarify this drug's role in the treatment of intermediate-risk PE patients.

**MeSH/Keywords:** pulmonary embolism, fibrinolytic drugs, unfractionated heparin

**Poster code:** T-02-08-105

**Poster Title:** BAFF, CXCL9 and CCL15 in cGVHD phenotypes

**PhD candidate:** Ana Zelić Kerep

**Part of the thesis:** Proinflammatory cytokines in different clinical manifestations of chronic graft versus host disease

**Mentor(s):** Assist. Prof. Dražen Pulanić, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Chronic graft-versus-host disease (cGVHD) is a multisystem immune disorder. It occurs in patients who underwent an allogeneic hematopoietic stem-cell transplant (alloHSCT), which is a method of choice for an array of hematological malignancies. The clinical presentation is heterogeneous, and the pathophysiological three-phase model has only recently been established (inflammatory phase, immune dysregulation and sclerotic phase), largely based on pre-clinical research. Therefore, it is necessary to research the feasibility of this model in humans. This study will examine the association of clinical phenotypical manifestations and the underlying immunological mechanisms, using indicators of inflammation in the peripheral blood and potential biomarkers (BAFF, CXCL9, CCL15) according to the proposed pathophysiological model

**Hypothesis:** Inflammatory clinical phenotypical presentation of cGVHD has higher levels of laboratory markers of inflammation in the peripheral blood, as well as cytokines BAFF and CXCL9, whereas sclerotic clinical phenotypical manifestations of cGVHD have lower levels of laboratory markers of inflammation and higher levels of the cytokine CCL15 in the peripheral blood.

**Aims:** The main aim is to determine the difference in the concentrations of different cytokines in different clinical phenotypical presentations of cGVHD. Other aims are: To determine the association of clinical phenotypes with pre-transplantation factors; to determine the impact of clinical phenotypes on overall survival; to determine the impact of clinical phenotypes on health-related quality of life and to compare laboratory markers and cytokines between the cGVHD group and control group (patients who received alloHSCT, but without cGVHD).

**Materials and methods:** The population is comprised of both adult and pediatric patients who received an alloHSCT. They are assessed by a multidisciplinary team, along with cGVHD NIH staging and diagnosis confirmation (pathohistological confirmation, other diagnostic methods as indicated, including HRCT, spirometry, and endoscopy). The patients were examined by a hematologist, dermatologist, dental medicine specialist, psychiatry specialist, neurologist, nutritionist, gynecologist (females), and other specialties were involved as indicated. Patients were also asked to complete the Short Form 36 questionnaire (validated in Croatian). Comprehensive laboratory analysis was done as well and additional plasma aliquots were stored for future analyses. Patients will be sorted into three groups: inflammatory, sclerotic, or mixed phenotype. Inflammatory phenotype is considered to be the following: any erythematous skin manifestation, lichenoid skin changes, erythematous or lichenoid changes of outer genitalia and oral mucous membrane, oral ulcerations, fasciitis, eye erythema and elevated liver enzymes or bilirubin (if they are unequivocal of cGVHD etiology). Sclerotic manifestations are sclerotic skin manifestations, limited joint range of motion, sclerotic changes of mouth and genitalia, an obstructive pattern of lung function tests. The mixed phenotype includes patients with both inflammatory and sclerotic manifestations. Patients after alloHSCT without cGVHD are controls. Patients with overlap cGVHD and active infection at the time of evaluation (positive blood culture, urine culture, pneumonia, or ongoing active antimicrobial treatment) are excluded from this study. After grouping the patients in the aforementioned groups, laboratory markers, autoantibodies, cytokines, and immune cells profiles in the peripheral blood will be compared.

**Expected scientific contribution:** This study will bring new knowledge about biomarker profiles in different cGVHD clinical phenotypes, as well as enrich the body of knowledge about cGVHD physiology. Also, this study will facilitate the utilization of a clinical phenotypical model of cGVHD in future research of cGVHD biology.

**MeSH/Keywords:** chronic graft-versus-host disease, clinical phenotypes, biomarkers

**Poster code:** T-02-09-016

**Poster Title:** Pulmonary rehabilitation outcome in relation to the nutritional status of patients with chronic obstructive pulmonary disease

**PhD candidate:** Davorka Muršić

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

**Mentor(s):** Assist. Prof. Andrea Vukić Dugac, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** 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 recommended method for assessing nutritional status in any patient, including patients with COPD. 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. One of the objective methods for assessing nutritional status is the use of bioelectrical impedance analysis (BIA), a simple and non-invasive method which indirectly estimates body composition and biochemical methods for measuring serum albumin and prealbumin.

**Hypothesis:** Initial nutritional status in COPD patients affects the outcome of pulmonary rehabilitation.

**Aims:** To determine if there is a difference in the results of pulmonary rehabilitation depending on the initial nutritional status of COPD patients divided into three groups: A, B and C according to the SGA questionnaire. Secondary, to assess if there is a difference between groups in the values of different nutritional assessment methods (BIA, prealbumin, albumin), pulmonary rehabilitation outcomes (6-minute walk test, spirometry, quality of life, disease and symptom severity questionnaire), values of markers of systemic inflammation (leukocytes, fibrinogen, CRP), lipid profile and in the duration of the positive effect of pulmonary rehabilitation 3 months after.

**Materials and methods:** The study will be prospective, COPD patients who were referred for pulmonary rehabilitation to the Clinic for Lung Diseases Jordanovac, University Hospital Centre Zagreb will be included in the study. Using power analysis, the planned total number of patients is 100, of which at least 30 patients per group according to SGA questionnaire (A – well-nourished, B - mildly/moderately nourished or C - severely malnourished). All patients will meet 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 will be 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 locomotor system. All patients will have the same pulmonary rehabilitation program for 4 weeks. Patients will be classified into one of three groups by the SGA questionnaire, measured by BIA (FFM, FFMI and phase angle values), they will fill in questionnaires of the degree and severity of symptoms (CAT), the degree of dyspnea (mMRC) and questionnaire of quality of life (SGRQ-C). Lung function testing, 6-minute walk test (6MWT) and blood sampling for required laboratory tests will be performed. At the end of the program and after 3 months, all measurements performed before the start of the pulmonary rehabilitation program will be repeated (spirometry, 6MWT, CAT, mMRC, SGRQ-C, blood markers of systemic inflammation and lipid profile).

**Expected scientific contribution:** The obtained results would contribute to defining the impact of nutritional status on the outcome of pulmonary rehabilitation in patients with COPD as well as the personalization of pulmonary rehabilitation programs and therefore better and more successful comprehensive treatment of patients with COPD.

**MeSH/Keywords:** COPD, pulmonary rehabilitation, nutritional status

**Poster code:** T-02-09-023

**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):** Assist. Prof. Dražen Pulanić, MD PhD

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

**Introduction:** Chronic Graft-versus-Host Disease (cGvHD) is a late complication of allogeneic hematopoietic stem cell transplantation (alloHSCT) occurring among 50% of long-term alloHSCT survivors. It is known that cGvHD is complex alloimmune and autoimmune disease characterized by inflammation, endothelial dysfunction and fibrosis, but its utter pathophysiology remain elusive until today. Clinical presentation varies from mild single site involvement to severe multi-organ failure causing debilitating morbidities and 25% mortality rate among long-term alloHSCT survivors. Standard therapy for cGvHD are high doses of steroids and for 50% of patients who eventually develop steroid refractoriness, there is no defined second line therapy. Major obstacle of investigational progress is absence of biomarkers causing evaluation of cGvHD being based exclusively on appearance and dynamics of clinical manifestations defined by NIH consensus criteria. Von Willebrand Factor (vWF) and Factor VIII (FVIII) are coagulation factors whose activity and concentration can be laboratory evaluated in any larger hospital, but also known indicators of endothelial dysfunction and inflammation in different settings. It has been confirmed that 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. High levels of vWF have been described among alloHSCT survivors, but its biomarker potential has not been evaluated. Interestingly, high levels of FVIII in those reports have been attributed to its physiological connection to vWF, but recent study outlined prognostic importance of FVIII:vWF ratio suggesting different mechanism of their regulation.

**Hypothesis:** Activity and concentration of von Willebrand Factor and Factor VIII are elevated among patients with chronic Graft-versus-Host Disease and are connected to clinical manifestations of disease.

**Aims:** Comparison of vWF and FVIII levels between cGvHD patients non-cGvHD long-term alloHSCT survivors and connection of their levels to clinical manifestations, severity, demographic characteristics and laboratory determinants of cGvHD.

**Materials and methods:** This research will be conducted as part of the Croatian Science Foundation project (IP-2016-06-8046) entitled New biomarkers of chronic graft-versus-host disease that is held from 2017 to 2021 and includes patients who underwent alloHSCT in University Hospital Centre Zagreb, developed cGvHD, do not have active infection and are willing to participate in this research that is approved by the Ethical committee. Within inclusion, detailed history and comprehensive laboratory check-up is obtained and patients are evaluated by the Multidisciplinary team for cGvHD and staged according to established NIH consensus criteria. Each cGvHD patient is re-evaluated regularly every 6-8 months and blood samples are collected in the same visit, while control group consisted of non-cGvHD long-term alloHSCT survivors is evaluated only by the inclusion. In this study will be used standardized laboratory methods for detecting levels of von Willebrand antigen, von Willebrand Ristocetin Cofactor and Factor VIII activity from the plasma of at least 61 cGvHD and 10 non-cGvHD patients together with laboratory check-up containing routine acute phase reactants. Statistical analysis will be focusing on differences between groups, correlation of factors and cGvHD clinical and laboratory characteristics, and dynamics of factors in regard to change of cGvHD severity. Multivariate logistic regression analysis will be used to identify most predictive determinants of vWF and FVIII in cGvHD. P values below 0.05 will be considered significant.

**Expected scientific contribution:** Establishing vWF and FVIII levels on well-defined cohort of long-term alloHSCT survivors. Correlating vWF and FVIII levels with clinical manifestation of cGvHD estimated by multidisciplinary clinical and laboratory evaluation will contribute to understanding of cGvHD biology and could result with available biomarker of cGvHD.

**Acknowledgments:** This work was supported by the Croatian Science Foundation project IP-2016-06-8046 entitled New biomarkers of chronic graft-versus-host disease

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

**Poster code:** T-02-09-033

**Poster Title:** The possible synergistic effect of sex hormones and glucagon-like peptide 1 (GLP-1) on body mass decline in patients with type 2 diabetes mellitus

**PhD candidate:** Tanja Režić

**Part of the thesis:** The possible synergistic effect of sex hormones and glucagon-like peptide 1 (GLP-1) on body mass decline in patients with type 2 diabetes mellitus

**Mentor(s):** Assist. Prof. Srećko Marušić, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Obesity is one of the key factors in the development of type 2 diabetes mellitus (T2DM) and one of the major targets in T2DM treatment. Sex hormone deficiency in both men and women contributes to metabolic dysregulation, while hormone replacement therapy improves metabolic parameters. Glucagon-like peptide 1 (GLP-1) is a gut peptide that has hypoglycaemic effect mediated by several different mechanisms including appetite suppression. The development of antidiabetic drugs that demonstrate efficacy on both body mass decline and gluoregulation are in the focus of scientific interest with glucagon-like peptide-1 receptor agonists (GLP-1 RAs) as the most recognized. Nevertheless, around 30% of patients fail to achieve adequate gluoregulation and/or expected weight reduction with this type of treatment. Recent research has suggested potential therapeutic effects of combination therapies with GLP-1 RA and sex hormones in reducing body mass and improving metabolic parameters.

**Hypothesis:** Higher endogenous GLP-1, estradiol and/or testosterone plasma levels result in greater weight loss and better glycaemic control in obese patients with T2DM.

**Aims:** To investigate if there is an association between endogenous GLP-1, estradiol and testosterone plasma levels and body weight and appetite in obese T2DM patients.

**Materials and methods:** Sixty obese (body mass index (BMI)  $\geq 30$  kg/m<sup>2</sup>) T2DM patients are included in this prospective observational clinical study. Patients with type 1 diabetes mellitus, those with hematologic, autoimmune and other inflammatory diseases, those already treated with GLP-1 RA or dipeptidyl peptidase-4 (DPP-4) inhibitor, patients with primary and secondary hypogonadism (with or without hormonal replacement therapy (HRT)), postmenopausal women receiving HRT and patients taking hormone suppression therapy in the treatment of hormone-dependent cancer are not included in the study. All patients will be given GLP-1 RA up to 1,2 mg sc. for 6 months. All patients will be asked to fill in Eating Habits Questionnaire before starting GLP-1 RA. Patients will undergo anthropometric (weight, height, waist circumference, BMI), metabolic (serum glucose, LDL, HDL, total cholesterol, triglycerids, HbA1c, creatinine, AST, ALT, GGT, urine albumin to creatinine ratio) and hormonal (serum total testosterone, estradiol, endogenous GLP-1) assessment at the baseline. Endogenous GLP-1 plasma levels will be determined using Human Active GLP-1 (7-36) ELISA Kit (ALPCO, US) at the beginning of the study. Anthropometric measurements, metabolic parameters, serum total testosterone and estradiol will be reassessed after 6 months of treatment, at the end of the study. Statistical analysis will be performed using SAS 9-3 Statistical Package. The normality of distribution will be accessed by Shapiro-Wilk test. Nominal variables will be presented with absolute number and percentage while numeric variables will be presented with mean (standard deviation) or median (range). All the variables will be log-transformed to correct for their skewed distribution. Baseline characteristics of the groups will be compared using t-test or the chi-square test. The correlations will be assessed with Pearson's correlation test. Significantly different variables will be assessed in the binary logistic regression equation. A P value less than .05 will be considered as statistically significant.

**Expected scientific contribution:** The reduction of body weight is one of the basic effects of GLP-1 RA in the treatment of obese T2DM patients. However, our knowledge on how GLP-1 regulates appetite is still limited. Better understanding of different biological factors possibly involved in the regulation of energy balance has the potential to maximize the effectiveness of GLP-1 based therapies for the treatment of obesity and T2DM.

**MeSH/Keywords:** Obesity, sex hormones, diabetes mellitus, glucagon-like peptide 1

**Poster code:** T-02-09-035

**Poster Title:** Serum chitinase-3-like-1 protein (YKL-40) in patients with acute pancreatitis as a potential novel biomarker of disease severity and need for local complications management.

**PhD candidate:** Nina Blažević

**Part of the thesis:** Serum chitinase-3-like-1 protein (YKL-40) in patients with acute pancreatitis as a potential novel biomarker of disease severity and need for local complications management.

**Mentor(s):** Assist. Prof. Tajana Pavić, MD PhD, Professor Dunja Rogić, MD PhD

**Affiliation:** University of Zagreb School of Medicine, School of Pharmacy and Biochemistry

**Introduction:** Acute pancreatitis (AP) is acute inflammatory disease of the pancreas with multiple etiologies. Severity of disease is defined by the presence of organ failure and/or local complications. The management of patients with AP is complicated by the difficulty to differentiate the severity of disease on admission. There are several indicators of disease severity (multiple factors systems, biochemical markers), but none of them are accurate. YKL-40 (chitinase 3-like 1 protein) is glycoprotein that plays mayor role in inflammation and angiogenesis. A study on small group of patients with AP showed that YKL-40 is better indicator of severity of disease on admission compared with other markers.

**Hypothesis:** Serum concentrations of YKL-40 in patients with acute pancreatitis are elevated compared with control group and the value of YKL-40 is proportional to disease severity. High serum concentrations of YKL-40 can be predictor of occurrence of pancreatic necrosis and symptomatic peripancreatic collections that require management.

**Aims:** The aim of this study is to determine diagnostic sensitivity and specificity of YKL-40 in evaluation of AP severity and development of local complications. This study will compare serum concentrations of YKL-40 in patients with mild, moderate and severe AP, determine connection between YKL-40 and AP severity and formation of pancreatic necrosis and peripancreatic collections that require management and investigate serum concentrations of proinflammatory cytokines (IL-6, IL-8 and TNF-alfa) in patients with AP and it's correlation to disease severity.

**Materials and methods:** This prospective research will include patients (number: 150) with AP that are hospitalised in Clinical Hospital Center Sestre milosrdnice, Zagreb, Croatia in one-year period. The study protocol will be approved by the ethics committees and written informed consent will be obtained from each subject (including control group). The diagnosis of AP is based on the presence of acute upper abdominal pain associated with a raised serum amylase concentration and/or radiological evidence (ultrasonography (US) or contrast-enhanced computed tomographic (CT) scan) compatible with AP based on latest revision of Atlanta classification from 2012. In all patients, US will be performed within 24 hours of admission. In majority of patients, CT scan will be performed after 3rd day of the disease, providing information about presence of pancreatic necrosis and acute peripancreatic collections. In all patients the APACHE II score will be calculated on admission and 48 hours thereafter, and the Ranson score will be calculated after 48 hours. In all patients on admission, 2nd, 7th, 14th and 28th day of the disease, routine laboratory tests (including CRP and PCT) will be measured. Furthermore, on admission, 2nd, 7th, 14th and 28th day of the disease, serum concentrations of YKL-40, IL-6, IL-8 and TNF-alfa will be determined by standard enzyme-linked immunosorbent assay (ELISA). The clinical course of the patients will be followed prospectively until discharge, withdrawal of consent or death. In those patients with symptoms and acute peripancreatic collections showed on early CT scan, additional CT scan after 28th day of the disease will be performed. Decision regarding endoscopic or surgical drainage of the collection will be made afterwards. Control group will be consisted of healthy subjects (number: 200). In all subjects, serum concentrations of YKL-40, IL-6, IL-8 and TNF-alfa will be determined once. Data collected in this study will be displayed graphically and tabular. Comparisons of marker levels will be done using student's t-test or Mann-Whitney U-test. The significance level will be set to 5%.

**Expected scientific contribution:** The results of the study will facilitate the identification of patients with severe AP and the ones requiring management of local complications.

**MeSH/Keywords:** acute pancreatitis, disease severity, YKL-40, complications, peripancreatic collections

**Poster code:** T-02-09-048



**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 Hospital Centre Zagreb, Croatia

**Introduction:** It is known that sleep breathing disorders (OSA, CSA, mixed type of apnea) are complexly associated with increased cardiovascular risk in these patients. Epidemiological studies reported increased incidence of arterial hypertension, heart failure, coronary and cardiovascular disease. Main part of disorders is based on hypoxemia and hypercapnia induced sympathetic hyperactivity (joined with parasympathetic dysfunction) as a causal factor in the development of hypertension. Arterial stiffness is valuable predictor of cardiovascular morbidity and mortality.

**Hypothesis:** 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 decrease of arterial stiffness during the night.

**Aims:** MAIN AIM: 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). SUBSIDIARY AIMS: 1. Compare pulse wave velocity values and 24-hour stiffness values between patients with OSA and CSA and mixed type of sleep apnea. 2. Analyze the presence of damage to target organs. 3. Determine whether there is an independent association of an adequate nocturnal fall in arterial stiffness with a milder degree of damage to target organs, degree of vascular aging, cardiovascular risk and mortality. 4. Determine the relationship between the severity of sleep apnea with the stiffness of the great arteries.

**Materials and methods:** Subjects included in the study will be patients admitted and treated at the Clinic for Internal Medicine, Clinical Hospital Center Zagreb, Croatia. Inclusion criteria - age  $\geq 18$  years, patients with a previous diagnosis of sleep apnea regardless of the duration of the disease, signed consent. Exclusion criteria - history of chronic kidney disease (eGFR  $< 60$  ml / min /  $1.73\text{m}^2$ ), personal history of myocardial infarction or stroke, diabetes, pulmonary hypertension, COPD, chronic inflammatory diseases, secondary and / or resistant hypertension, patients undergoing biological therapy, patients with a previous diagnosis of sleep apnea on CPAP therapy. 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 of 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.

**Expected scientific contribution:** The results of this study will provide insight into the etiology of central sleep apnea and its association with possible sympathetic hyperactivation. It will help to determine the percentage of patients with pre-existing but unrecognized vascular aging and increased CV risk. Furthermore, the role of pulse wave velocity in sleep apnea will be investigated and for the first time, according to the literature, the stiffness of the large arteries between individual types of sleep apnea will be compared. The results of the study will show the percentage of absence of nocturnal fall of large artery stiffness in patients with sleep apnea and the association with target organ damage, vascular aging, and cardiovascular risk.

**MeSH/Keywords:** sleep apnea, pulse wave, arterial stiffness, cardiovascular risk

**Poster code:** T-02-09-108

**Poster Title:** Association of single nucleotide polymorphisms for PNPLA3, NOTCH3 and EGF with hepatocellular carcinoma in alcoholic cirrhosis

**PhD candidate:** Ana Bainrauch

**Part of the thesis:** Association of single nucleotide polymorphisms for PNPLA3, NOTCH3 and EGF with hepatocellular carcinoma in alcoholic cirrhosis

**Mentor(s):** Assoc. Prof. Anna Mrzljak, MD PhD, Assoc. Prof. Tomislav Kelava, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Alcoholic cirrhosis (AC) is a risk factor (RF) for the development of hepatocellular carcinoma (HCC) and one of the most common indications for liver transplantation (LT). HCC does not develop in all patients with AC, rather depends on the existence of risk factors (RF). Increasing incidence of HCC, especially among elderly and lack of sensitive diagnostic tools for early HCC diagnosis, are some of the reasons for investigations concerning the estimation of individual genetic RF for HCC development. Single nucleotide polymorphisms (SNP) of the PNPLA3 gene is one of the known genetic RF for liver steatosis as well as for HCC development in AC. According to the investigation of cell lines and tissues, polymorphisms for NOTCH3 signalling pathway and the EGF gene might influence HCC development in AC. The synergistic effect of those polymorphisms on HCC development or effect on HCC recurrence after LT has not been explored previously. Identification of genetic RF for HCC development might be used for patient risk stratification and individualised follow-up in order to diagnose HCC early and improve survival.

**Hypothesis:** SNPs of PNPLA3 (rs738409) gene, NOTCH3 (rs1043996) signalling pathway and EGF (rs4444903) gene are RF for the development of HCC as well as for the recurrence of HCC after LT.

**Aims:** GENERAL AIM: to explore the association between polymorphisms of PNPLA3, NOTCH3 and EGF genes polymorphisms and the risk for HCC development among patients transplanted due to AC SPECIFIC AIMS: 1. to determine the frequency of PNPLA3, NOTCH3 and EGF gene polymorphisms among patients with AC and patients with AC and HCC 2. to analyse the association between PNPLA3, NOTCH3 and EGF gene polymorphisms and HCC development among patients with AC 3. to determine the association of other known RF (diabetes, obesity) with HCC development among patients with AC and determine whether those polymorphisms are independent RF 4. to explore whether the combination of PNPLA3, NOTCH3 and EGF gene polymorphisms increases the risk for HCC development 5. to explore if there is any association between PNPLA3, NOTCH3 and EGF gene polymorphisms and the risk for HCC recurrence after LT 6. to determine the association between known RF (number and size of tumor lesions, alpha-feto protein, histological characteristics) and HCC recurrence after LT and determine whether those polymorphisms are independent RF

**Materials and methods:** This case-control study will include 250 LT patients due to AC in the University Hospital Merkur. Patients are divided in two groups adjusted for age and sex (125 pts with AC and 125 pts with AC and HCC) and followed-up at least two years after LT. Blood sample (3-5 ml) is taken during routine follow-up and stored at -80°C until DNA isolation (PureLink® Genomic DNA Mini Kit) and gene polymorphisms analysis (TaqMan SNP assays) is done on machine AB7500 (Applied Biosystems). Blood sample storage, DNA isolation and polymorphisms analysis will be done in the Laboratory for molecular immunology at the Croatian Institute for brain research. Patient demographic and laboratory data will be taken from the hospital information system. For HCC confirmation, explanted liver histology data is used. SNPs PNPLA3, NOTCH3 and EGF were chosen from SNP database (<https://www.ncbi.nlm.nih.gov/snp/>) with MAF (minor allele frequency) >20% in the European population. Hardy-Weinberg equilibrium and odds ratio for HCC would be expressed in dominant, codominant, overdominant and recessive model by using SNPstats (<https://www.snpstats.net/start.htm>).

**Expected scientific contribution:** SNP analysis of PNPLA3, NOTCH3 and EGF gene among patients with AC might be useful for better HCC risk stratification. Precise genetic risk assessment for HCC may be used for individualised patient follow-up, timely referral to specialized centres, early development and treatment of HCC with consecutive better survival. Confirmation of this association may positively influence post-transplant follow-up.

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

**Poster code:** T-02-09-110

**Poster Title:** Effects of inhaled corticosteroids on markers of osteoporosis in patients with asthma

**PhD candidate:** Sanda Dokoza Terešak

**Part of the thesis:** Effects of inhaled corticosteroids on markers of osteoporosis in patients with asthma

**Mentor(s):** Marija Gomerčić Palčić, PhD, research associate

**Affiliation:** University Hospital Centre "Sestre milosrdnice", Zagreb, University of Zagreb School of Medicine

**Introduction:** Asthma is a global health problem affecting all age groups. It is defined as a chronic inflammatory disease of the airways characterized by occasional and reversible airflow obstruction caused by bronchial hyperreactivity, which, if not treated, can lead to bronchial remodeling and an irreversible decline in lung function. Inhaled corticosteroids are the most efficient anti-inflammatory drugs used to treat asthma. Several studies link long-term use of high doses of inhaled corticosteroids with faster development of osteoporosis, which is characterized by decreased bone density. Currently, the golden standard for detection of osteoporosis is dual-energy x-ray absorptiometry (DXA) of the hip and lumbar spine. Bone turnover markers are useful tools in assessment of bone metabolism. Cathepsin K is cysteine protease involved in the degradation of collagen and other non-collagenous proteins which form bone matrix. Several studies found cysteine protease inhibitors efficient in the prevention of bone resorption, suggesting cysteine protease has a significant role in bone metabolism. Cathepsin K is a highly selective biochemical marker of osteoclasts activity. Its increased levels might indirectly suggest development of osteoporosis in patients with asthma treated with inhaled corticosteroids. It could be used as a fast and simple laboratory test for early diagnosis of osteoporosis.

**Hypothesis:** There is a negative correlation between the level of cathepsin K values in asthma patients taking inhaled corticosteroid therapy and "T-scores" and "Z-scores" obtained by densitometry.

**Aims:** The aim of this study are as follows: 1. to determine the levels of cathepsin K, osteocalcin and CTX in a group of patients with asthma treated with inhaled corticosteroids; 2. to explore whether treatment with inhaled corticosteroids in patients with asthma, regardless of the dose and type of inhaled corticosteroid, has influence on prevalence of osteoporosis and level of cathepsin K 3. to explore the relationship between cathepsin K and other bone turnover markers (osteocalcin and CTX), and densitometry (T score and Z score); 4. to compare densitometry in a group of patients with asthma treated with inhaled corticosteroids with the densitometry of the control group.

**Materials and methods:** In this case control study at the Sisters of Charity University Hospital Centre, 200 adult patient with asthma treated with inhaled corticosteroids will be divided into three groups depending on the dose used (low, medium and high dose). Inhaled corticosteroids will contain one of the following active substances: ciclesonide, beclomethasone, budesonide, fluticasone propionate and fluticasone furoate. Patient history shall be recorded and physical examination performed. In addition to standard laboratory tests, biochemical bone turnover markers level will be measured. Spirometry with bronchodilator test will be used to assess pulmonary function. Bone mineral density measurements will be obtained by Hologic Delphi C Bone Densitometer. Diagnosis of osteoporosis will be based on T-scores. In case of persistent hip, shoulder or knee pain, bone X-ray will be performed in order to rule out osteonecrosis. The control group will consist of 80 participants without asthma, significant comorbidity or history of significant comorbidity, corticosteroid and other significant drugs use. All data will be shown using tables and graphs. A  $P < 0.05$  will be considered significant.

**Expected scientific contribution:** Cathepsin K level in a serum in patients with asthma treated with inhaled corticosteroids will be investigated for the first time. We consider that the cathepsin K, as a novel bone turnover marker, might be a useful tool in the follow-up of patients with asthma treated with inhaled corticosteroids as well as the assessment of their bone turnover, reducing the need for densitometry.

**MeSH/Keywords:** Asthma, osteoporosis, cathepsin K, inhaled corticosteroids, densitometry

**Poster code:** T-02-09-119

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

**PhD candidate:** Dino Kasumović

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

**Mentor(s):** Professor Krešimir Galešić, MD PhD

**Affiliation:** University of Zagreb School of Medicine; University Hospital Dubrava;

**Introduction:** Cardiovascular diseases are the leading cause of morbidity and mortality worldwide. Changes of the arteries in the form of arteriosclerosis are directly related to certain cardiovascular risk factors and they are associated with certain cardiovascular diseases. Main cardiovascular risk factors are older age, male gender, arterial hypertension, diabetes mellitus, dyslipidemia and smoking. Arterial changes can be detected indirectly by using noninvasive diagnostic methods, such as Doppler ultrasound. Yet, arteriosclerosis can be directly assessed pathohistologically, as in case of kidney biopsy.

**Hypothesis:** For the same level of cardiovascular risk, pathohistologically determined moderate and severe arteriosclerotic changes of kidney arteries occur with signs of arteriosclerosis in other parts of the body, determined by noninvasive diagnostic methods.

**Aims:** The aim of this study is to relate the level of arteriosclerotic changes of kidney arteries that are determined on pathohistological examination of kidney biopsy specimens with signs of arteriosclerosis in other arteries determined by noninvasive diagnostic methods, depending on cardiovascular risk factors.

**Materials and methods:** Examinees are hospitalized patients in whom a kidney biopsy is indicated. The kidney biopsy is the main inclusion criteria of this study. The patients are hospitalized in the Department of Nephrology and Dialysis in Clinical Hospital Dubrava, Zagreb. They will be divided in three groups according to their cardiovascular risk level which will be assessed by European Society of Cardiology score and Framingham 10-year risk score for cardiovascular disease. Separate risk factors' presence and influence will be also analyzed. Two arteriosclerotic changes will be inspected in kidney biopsy specimens - fibromuscular intimal hyperplasia of the arteries and hyalinosis of the arterioles. Those changes will be graduated by their severity. Parameters and methods that will be used to verify changes in other arteries are following: intima-media thickness and presence of plaques in carotid arteries measured by Doppler ultrasound, ankle-brachial index measured by Doppler ultrasound, aortic pulse wave velocity measured by oscillometric method and arterial changes of the retina inspected by ophthalmoscope.

**Expected scientific contribution:** Biopsy is a unique opportunity to directly detect arteriosclerotic changes present in the body, and the goal is to relate them (and their level of severity) to such changes in other arteries of the body which are more commonly detected by noninvasive diagnostic methods. Thus providing a more complete and deeper understanding of vascular pathology and cardiovascular diseases.

**MeSH/Keywords:** cardiovascular diseases, arteriosclerosis, risk factors, kidney, biopsy

**Poster code:** T-02-09-120

**Poster Title:** Analysis of selected laboratory and echocardiographic indicators of myocardial microtrauma before and after the game in rugby players

**PhD candidate:** Petra Radić

**Part of the thesis:** Analysis of selected laboratory and echocardiographic indicators of myocardial microtrauma before and after the game in rugby players

**Mentor(s):** Assist. Prof. Zdravko Babić, MD PhD

**Affiliation:** School of Medicine, University of Zagreb; Department of Cardiology, University Hospital Sisters of Charity, Zagreb

**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 impacts, 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 other cardioselective markers. Athlete's myocardium physiologically adapts to chronic exposure to training and competition which is also echocardiographically visible. Research to date has not yet fully elucidated the clinical significance of frequent thoracic injuries in contact sports, such as rugby, on myocardial function.

**Hypothesis:** High static-dynamic loading during rugby match is associated with laboratory, but also echocardiographic signs of microtrauma and decreased left ventricular function, especially a decrease in total global longitudinal left ventricular deformity by more than 0.5%.

**Aims:** The general aim of this study is to determine the effect of rugby as a contact sport on cardiac microtrauma through laboratory and echocardiographic parameters and to compare these results with data from the literature. Specific objectives of this study are to evaluate left ventricular systolic and diastolic function, left ventricular deformity and values of hs-troponin I, creatinine kinase and NT-proBNP before and after a rugby match as well as to correlate the increase in hs-troponin I, creatinine kinase and NT-proBNP with changes in left ventricular deformity in rugby players.

**Materials and methods:** This prospective research 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, recording 12-channel ECG, performing 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 which the subject played at least 60 minutes. Echocardiographic examination will include analysis of the following parameters: left ventricular ejection fraction, left ventricle 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.

**Expected scientific contribution:** Scientific contribution of this research can 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 cardiac morbidity. More frequent measurements of common laboratory and echocardiographic parameters may lead to an objective assessment of myocardial microtrauma.

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

**Poster code:** T-02-09-142

**Poster Title:** Is the extent of lymphocytic infiltration of primary tumor predictive of extranodal extension in regional metastases?

**PhD candidate:** Mia Lorencin

**Part of the thesis:** Prognostic significance of tumor-infiltrating lymphocytes in oral cavity squamous cell carcinoma

**Mentor(s):** Assist. Prof. 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 malignancies of the head and neck, and cervical lymph node involvement is a very important prognostic indicator. One third of patients already have metastases in regional lymph nodes at the time of diagnosis, while patients with clinically negative neck after elective dissection in 40-50% of cases have proven occult micrometastases in regional lymph nodes. Extranodal tumor extension through the lymph node capsule is an additional poor prognostic indicator, and in the new edition of the TNM classification it is included as a factor in categorizing disease stages in regional lymph nodes. 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.

**Hypothesis:** The percentage of lymphocytic infiltration of primary squamous cell carcinoma of the oral cavity is lower in patients with tumor extranodal extension in the affected cervical lymph nodes, compared to those in whom extranodal extension is not present.

**Aims:** General aim of this research is analysis of the percentage of lymphocytic infiltration in tumor tissue of patients with primary oral squamous cell carcinoma with cervical lymph node metastasis. Specific aims are: 1. to determine whether there is an association between the percentage of lymphocytic infiltration of the primary tumor and tumor extranodal extension in affected cervical lymph nodes; 2. to determine whether there is an association between the percentage of lymphocytic infiltration of the primary tumor and pT category, pN category, and patient age, sex and 5-year survival.

**Materials and methods:** Research will be 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, University of Zagreb School of Medicine, from 2009 to 2014 with a minimum of a 5-year follow-up. Inclusion criteria: pathohistological diagnosis of carcinoma of the oral cavity, including all anatomic subsites according to AJCC, 8th Edition; pathohistological diagnosis of regional lymph node metastasis after either elective or curative neck dissection; tumor material available for analysis - paraffin cubes from the archives of the Clinical Institute of Pathology, University Hospital Dubrava; 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 have previously been treated for a malignant tumor in the head and neck area will be excluded from the study. pT and pN category will be reevaluated. The degree of lymphocytic infiltration will be 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. In the regional lymph nodes, the presence or absence of extranodal extension of the tumor will be reevaluated. According to power analysis for binary logistic regression according to the following parameters: the assumed probability ratio of belonging to the group with extranodal extension OR = 2.5, significance level  $\alpha = 0.05$  and test strength of 0.90, at least 90 subjects should be included in the study.

**Expected scientific contribution:** This study will show whether there is an association between the extent of lymphocytic infiltration of the primary tumor and extranodal extension in metastatic squamous cell carcinoma of the oral cavity. Because extranodal extension is considered a reflection of tumor aggressiveness and the worst prognostic indicator for patient survival, better knowledge of factors associated with extranodal extension will contribute to better understanding and more accurate prediction of tumor behavior.

**MeSH/Keywords:** Oral cavity, squamous cell carcinoma, tumor-infiltrating lymphocytes, extranodal extension

**Poster code:** T-02-10-087

**Poster Title:** Morphological characteristics of intracranial aneurysms

**PhD candidate:** Dragan Janković

**Part of the thesis:** Morphological and hemodynamic characteristics of intracranial aneurysms

**Mentor(s):** Assoc. Prof. Krešimir Rotim, MD PhD, Assoc. Prof. Marko Radoš, MD PhD

**Affiliation:** Department of Neurosurgery, Clinical Hospital Center Sestre Milosrdnice; University of Zagreb School of Medicine

**Introduction:** The prevalence of unruptured intracranial aneurysms in the general population is 2–5%, however only 1% of all intracranial aneurysms rupture. Aneurysm rupture is manifested by a sudden, potentially fatal subarachnoid hemorrhage. Aneurysmal subarachnoid hemorrhage (SAH) occurs at a rate of 6 to 16 cases per 100,000 people. Approximately 10% of patients with SAH die before reaching the hospital, while only a third of patients report “good results” after the treatment. Given the great importance of morphometric parameters in the hemodynamics of intracranial blood vessels and aneurysms as well as their ruptures, this research will analyze ruptured and unruptured intracranial aneurysms in detail.

**Hypothesis:** Certain morphological criteria of intracranial aneurysms belonging to three groups of radiological indicators are associated with an increased risk of rupture.

**Aims:** Identify morphological factors related to an increased risk of rupture by performing intracranial radiological analysis of aneurysms.

**Materials and methods:** The research is organized as a prospective-historical cohort study. The research will include patients treated at the Clinical Institute for Diagnostic and Interventional Radiology and Department of Neurosurgery of the Clinical Hospital Center Sestre Milosrdnice with the diagnosis of unruptured or ruptured intracranial aneurysm, treated from 1st January 2015. The examined parameters will be divided into three groups: ratios, angle, shape. Ratios include aneurysm size, the shape ratio, size ratio of the aneurysm and the parent artery, and bottleneck index. The angles calculated will include the angle of the blood vessel, flow angle and the inclination angle of the aneurysm, while shape characteristics of the aneurysm include ellipticity index, nonsphericity index, and undulation index. Measurements will be performed using the IMPAX system for the analysis of neuroradiological images (in this study, three-dimensional (3D) computed tomography, angiography (CTA) and 3D rotational digital subtraction angiography (DSA). The 3D reconstruction of the aneurysm and parent arteries will be performed with the help of algorithms programmed in MATLAB R2007a (MathWorks, Inc., Natick, MA). MedCalc Statistical Software version 19.0.5 and SPSS 16.0 will be used for statistical analysis.

**Expected scientific contribution:** The expected scientific contribution of this research consists of the wider application of morphological parameters in assessing the risk of aneurysm rupture as well as the inclusion of the mentioned parameters into algorithms for assessing the risk of intracranial aneurysm rupture.

**MeSH/Keywords:** Intracranial aneurysm, Morphology, Rupture risk, Size ratio, Vessel geometry

**Poster code:** T-02-10-126

**Poster Title:** Association of BRAF V600E mutant allele proportion with the dissemination stage of papillary thyroid cancer

**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):** Assist. Prof. Tomislav Jukić, MD PhD, Assist. Prof. Ivan Šamija, PhD

**Affiliation:** Department of Oncology & Nuclear Medicine, University Hospital Centre Sestre milosrdnice; University of Zagreb School of Medicine;

**Introduction:** Thyroid cancer is the most common endocrine malignancy representing a significant burden on the health system. Papillary thyroid cancer (PTC) is considered the least aggressive type of thyroid cancer and has, in general, an excellent prognosis. The clinical significance of metastases to local lymph nodes is still not completely clear, while the presence of distant metastases decreases the 10-year survival rate by over 50%. Therefore, it is extremely important to have good prognostic factors for disease development. The currently used disease marker for malignancy is thyroglobulin (Tg) measurement in the aspirate of suspected nodes. However, Tg is not an indicator of tumor aggressiveness and metastatic potential, but only a marker of the current state of the disease. Therefore, the development of other molecular markers could significantly improve postoperative monitoring of patients. Mutations in the BRAF gene have been demonstrated in several types of solid tumors, including PTC and are a potential indicator of disease aggressiveness and an unfavorable prognosis in patients. The most common BRAF variant is the V600E transversion. Although the association between the presence of the BRAF V600E mutation and clinical-pathological prognostic factors of PTC has been extensively investigated in the last decade, a clear association between this mutation and the outcome of the disease has not yet been demonstrated and the role of this mutation as a prognostic factor in PTC is still questionable. However, most studies determined only the presence or absence of the BRAF V600E mutation, without its further quantification. One of the proposed explanations for the unclear relationship between the presence of the BRAF V600E mutation and tumor aggressiveness of PTC that we want to further investigate is that the heterogeneous distribution of cells with the BRAF V600E mutation within tumor tissue affects its impact on disease course.

**Hypothesis:** The percentage of tumor cells with the mutant BRAF V600E allele in papillary thyroid cancer is associated with disease dissemination.

**Aims:** The aim of this study is to investigate the association between the percentage of BRAF V600E mutant alleles in pathohistological samples (PH) of PTC with respect to disease dissemination.

**Materials and methods:** The study will include 150 PTC patients who underwent thyroidectomy, divided into three groups depending on disease dissemination stage. The first group will include patients without disease dissemination, the second with local metastases and third with distant metastases. The percentage of malignant cells in histological slices will be estimated and the presence and frequency of BRAF V600E mutant alleles will be determined by competitive allele-specific TaqMan real-time PCR. Since the assessment of genetic heterogeneity in tumor samples may be inaccurate due to contamination with non-tumor tissue cells, the data obtained will be normalized with respect to the previously determined percentage of tumor cells in the sample. Verification of results at protein level will be performed by immunohistochemistry on selected BRAF V600E positive and negative samples.

**Expected scientific contribution:** Analysis of the BRAF mutational status has been highlighted as a potential marker of PTC aggressiveness, but further research is needed before applying this analysis in clinical practice. One suggested explanation for the unclear relationship between the mutation presence and tumor aggressiveness is the heterogeneous distribution of the mutation. We expect that the results of this study will show the possible existence of an association between the proportion of BRAF V600E tumor cells in relation to the disease aggressiveness. Determining the frequency of the BRAF V600E mutant alleles may contribute to a personalized approach to PTC patients, for predicting disease aggressiveness.

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

**Poster code:** T-02-17-049



**Poster Title:** Corneal biomechanics and pseudoexfoliative syndrome

**PhD candidate:** Kata Čulina

**Part of the thesis:** The influence of corneal biomechanics on surgically induced astigmatism in cataract surgery in pseudoexfoliative syndrome

**Mentor(s):** Assoc. Prof. Tomislav Jukić, MD PhD

**Affiliation:** University of Zagreb School of Medicine, Department of Ophthalmology, University hospital Centre Zagreb

**Introduction:** Corneal biomechanical properties are the corneal hysteresis (CH) and corneal resistance factor (CRF). A device that can measure them is Ocular Response Analyzer (ORA). Corneal biomechanical properties can influence the surgically induced astigmatism after cataract surgery. Patients with pseudoexfoliative (PEX) syndrome have different, relatively lower value of corneal biomechanical properties compared to patients without pseudoexfoliative syndrome. This study will attempt to clarify the behavior of the cornea in patients with clinically manifest PEX syndrome after cataract surgery, that is the impact of corneal biomechanical properties on surgically induced astigmatism after cataract surgery.

**Hypothesis:** Patients with clinically manifest PEX syndrome have lower CH and CRF values before cataract surgery and greater surgically induced astigmatism after cataract surgery compared to patients without PEX syndrome.

**Aims:** General aim is assessment of the effect of corneal biomechanical properties on postoperative astigmatism after cataract surgery in patients with PEX syndrome. Specific aims are determination of: - average CH values preoperatively and postoperatively on days 1, 7 and 30 in patients with clinically manifest PEX syndrome and without it - average CRF values preoperatively and postoperatively on days 1, 7 and 30 in patients with and without clinically manifest PEX syndrome - average values of astigmatism preoperatively and postoperatively on days 1, 7 and 30 in patients with clinically manifest PEX syndrome and without it - correlation of preoperative CH values and surgically induced astigmatism at day 30 in patients with and without clinically manifest PEX syndrome - correlation of preoperative CRF values and surgically induced astigmatism at day 30 in patients with and without clinically manifest PEX syndrome

**Materials and methods:** The study is planned as a prospective study and it will be conducted at the Department of Ophthalmology, University Hospital Centre Zagreb. The target population are patients planned for cataract surgery. Inclusion criteria: cataract, pseudoexfoliation syndrome, both genders, 50-80 years. Non-inclusion criteria: eye surface disease, corneal pathology, glaucoma, ocular hypertension, high refractive errors, previous eye surgery, systemic diseases (diabetes, autoimmune diseases), unilateral PEX syndrome. Exclusion criteria: intraoperative and postoperative complications. A detailed ophthalmic examination will be performed for all patients ( UCVA, BCVA, IOP, slit lamp examination and fundus examination with dilated pupils) as well as tear break-up time (TBUT) and Schirmer test. Based on the preoperative examination, the subjects will be classified into 2 groups: 1. subjects without PEX syndrome (control group), and 2. Subjects with clinically manifest PEX syndrome. Preoperatively, subjects will have measuring of corneal hysteresis (CH) and corneal resistance factor (CRF) as well as corneal topography. These measurements will be repeated postoperatively on the first, seventh and thirtieth day. Corneal topography will be performed on a WaveLight Oculyzer II device and the strength and axis of the corneal cylinder will be measured. Corneal hysteresis (CH) and corneal resistance factor (CRF) will be measured on an Ocular response analyzer (ORA). All cataract surgeries will be performed by the same surgeon by the method of phacoemulsification and implantation of the intraocular lens into the capsular bag through a 2.75 mm corneal incision, which will be positioned at 12 hours. All patients will receive the same postoperative therapy. Statistical analysis will be performed using the statistical software package STATISTICA ver. 12. A probability level of  $p < 0.05$  will be considered as statistically significant for all performed analyzes.

**Expected scientific contribution:** This research will contribute to a better understanding of the corneal response to cataract surgery in patients with PEX syndrome.

**MeSH/Keywords:** corneal biomechanics, pseudoexfoliative syndrome, cataract surgery, surgically induced astigmatism

**Poster code:** T-02-18-013

**Poster Title:** Macular perfusion analysed by OCT-A after cataract surgery by phacoemulsification

**PhD candidate:** Ana Križanović

**Part of the thesis:** Macular perfusion analysed by optical coherence angiography after cataract surgery by phacoemulsification

**Mentor(s):** Assoc. Prof. Nenad Vukojević, MD PhD, Assist. Prof. Mirjana Bjeloš, MD PhD

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

**Introduction:** Retina is one of the tissues with the highest metabolic requirements in the body, and photoreceptors are the most metabolic active layer of the retina. The delivery of metabolic substrates and oxygen to the retina depends not only on the blood vessels located in the retina itself, but also on the choroidal blood vessels, which, in addition, have the highest tissue flow in human body in order to efficiently remove the heat released from the absorption of light energy in the retina. Histologically defined, there are three layers of the retinal blood vessels: superficial, deep, and intermediate layer, which is thought to intertwine with the deep layer. The superficial layer contains arterioles, venules, and capillaries, while the deep layer consists of capillary-sized blood vessels only. Optical coherence angiography, available in everyday praxis, is a novel, non-invasive method based on the detection of erythrocyte movement through blood vessels that allows monitoring changes of macular vasculature: superficial, deep and avascular retinal complex as well as choriocapillaris (CC) and deep choroid. The exact impact of cataract surgery, one of the most common surgical procedures in the world, on macular perfusion is unknown. If phacoemulsification affects macular perfusion, it is necessary to define whether these changes are favourable or adverse and thus to provide an evidence-based recommendation for the indication of cataract surgery if patients can reach further benefits beyond improvement in visual acuity.

**Hypothesis:** Uncomplicated cataract surgery by phacoemulsification alters the blood supply of the macula

**Aims:** To investigate the changes of vascular parameters of the macular blood vessels after uncomplicated phacoemulsification; Identification of the layers with vascular parameters' change after uncomplicated phacoemulsification; Identification of the time of occurrence of changes in the blood vessels of the macula after uncomplicated phacoemulsification; Identification of the duration of macular circulatory changes after uncomplicated phacoemulsification

**Materials and methods:** Healthy aging patients with uncomplicated cataract are included in the research. Blood supply of the superficial and deep layer of the macula as well as large choroidal blood vessels and CC is monitored using optical coherence angiography. Patients are examined prior to surgery and followed 1 week, 1 month, 3 months, and 6 months after the surgery recording superficial vascular complex (SVC), formed of nerve fiber layer vascular plexus (NFLVP) and superior vascular plexus (SVP), deep vascular complex (DVC), formed of intermediate capillary plexus (ICP) and deep capillary plexus (DCP), as well as large choroidal blood vessels and CC. 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), mean E lacunarity (MEL), and foveal avascular zone (FAZ) area throughout NFLVP, SVP, SVC, ICP, DCP, DVC, CC and choroid will be analysed. A comparison of pre-operative values with values obtained 1 week, 1 month, 3 months, and 6 months after surgery will be made using Friedman ANOVA test. The significance level is set to  $P < 0.05$ .

**Expected scientific contribution:** The study will contribute to the understanding of the impact of uncomplicated cataract surgery by phacoemulsification not only on retinal blood vessels but also on large choroidal blood vessels and CC and their relationship. It will set the basis for future research (progression of diabetic retinopathy and senile macular degeneration after cataract surgery, development of Irvine-Gass syndrome) by defining changes in vascular parameters in healthy individuals needed to investigate pathological conditions.

**MeSH/Keywords:** macula; angiography; retinal vessels; blood supply; phacoemulsification; cataract;

**Poster code:** T-02-18-022

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

**PhD candidate:** Dina Lešin Gaćina

**Part of the thesis:** Personality traits, adherence and visual impairment in patients with primary open-angle glaucoma

**Mentor(s):** Assoc. Prof. Darko Marčinko, MD PhD

**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 consequent progressive optic neuropathy. Peripheral vision loss occurs first, but if glaucoma left untreated, central vision impairment and complete visual loss can occur. The most common type is primary open-angle glaucoma (POAG). Increased intraocular pressure is considered the major risk factor for development of POAG. In order to achieve positive therapeutic outcomes, the adherence of patients to the use of topical hypotensive therapy in the form of drops is necessary. Since issues of non-adherence with prescribed therapy challenges clinicians on a daily basis, there is an interest for revealing possible factors and methods for increase adherence among glaucoma patients. Psychological characteristics of patients are cited as a possible reason for such neglectful behavior. Robert Cloninger developed psychobiological model for personality traits analysis which consist of four temperament dimensions (Novelty Seeking, Harm Avoidance, Reward Dependence, Persistence) and three dimensions of character (Self-Directedness, Cooperativeness, Self-Transcendence). Results of clinical researches have shown that high temperament dimension Harm Avoidance and low character dimension Self-Directedness are mutually connected and predictive for anxiety behavior patterns. We propose that certain personality traits could therefore be the reason why glaucoma patient neglect their health and have low adherence to prescribed therapy leading to disease progression.

**Hypothesis:** POAG patients with higher temperament dimension Harm Avoidance and lower character dimension Self-Directedness are non-adherent to treatment and have greater retinal nerve fiber layer atrophy, as well as visual field defect.

**Aims:** The aim of this study is to investigate the correlation between personality traits, adherence to medication and structural and functional visual impairment in patients with POAG.

**Materials and methods:** This cross-sectional research will be conducted at the Department of Ophthalmology, Clinical Hospital Center Zagreb. The study will include at least 110 patients fulfilling the criteria for POAG diagnosis. Patients with other eye disease, psychiatric disorders, history of taking psychoactive medicines and patients with neurological diseases which have impact on visual function will be excluded. All participants eligible for entry will underwent comprehensive ophthalmological examinations. The main demographic and clinical data will be obtained from all participants. The Temperament and Character Inventory (Cloninger et al,1993) will be used in order to determine personality traits. Adherence pattern to the prescribed medication will be assessed simultaneously by validated questionnaire for adherence in chronic diseases. Structural impairment will be defined by retinal nerve fiber layer (RNFL) thickness measured by optical coherence tomography. Static automated perimetry (Octopus 900/G) will be used for quantifying visual field impairment, defined by parameter mean defect (MD).

**Expected scientific contribution:** The study will clarify weather personality traits should be considered during the assessment of adherence and treatment outcomes in glaucoma patients. We expect that the results of this research may enable the improvement of adherence to the prescribed therapy and thereby decrease disease progression.

**MeSH/Keywords:** primary open-angle glaucoma, personality traits, adherence, visual impairment, optical coherence tomography, visual field

**Poster code:** T-02-18-061

**Poster Title:** Predictability of the corneal remodeling after corneal cross linking procedure

**PhD candidate:** Fanka Gilevska

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

**Mentor(s):** Assoc. Prof. Smiljka Popović-Suić, MD PhD, Maja Boháč, PhD, research associate

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Corneal Cross-linking (CXL) procedure is an effective photodynamic treatment that halts or slows the progression of keratoconus (KC). The procedure consists of an application of Riboflavin solution on the cornea, followed by the UVA radiation that activates the photosensitive riboflavin. This results in an increased number of covalent bonds between the collagen strands and proteoglycans. The main effect of the cross-linking is a biomechanical stiffening of the cornea which leads to corneal remodeling. The change in the ultrastructure of the cornea and even the molecular organization resulting from CXL is understood, but there is no single study that has shown any link between some of the early postoperative changes following cross-linking with the final outcome of the corneal shape after the procedure.

**Hypothesis:** The postoperative optical density of the cornea during the postoperative period after CXL is a good predictability factor in relation to the shape of the corneal surfaces.

**Aims:** To analyze the optical density of the cornea (ODC) over a period of one year after the corneal CXL procedure and its impact on the corneal curvature, and explore the possibility of developing mathematical models that bring together clinical measures of the cornea following CXL

**Materials and methods:** Prospective research that includes 100 keratoconic eyes, 50 age, and gender-matched control healthy eyes without Keratoconus and 50 keratoconic eyes of patients that opted to not receive cross-linking treatment. Inclusion criteria: patients diagnosed with keratoconus and progression of the steepest meridian of 1 diopter (D) or more within a year, but not more than 60 D, a subjective decrease of visual acuity (VA), corrected distant visual acuity (CDVA) of 0.8 or less, age frame of 15 to 40 years, and pachymetry of 400 microns or more. Exclusion criteria: prior corneal surgeries, pachymetry less than 400 microns, scars or cloudings of the cornea, chemical injuries, severe dry eye, delayed epithelial healing, and any inflammatory eye surface process before CXL procedure. A therapeutical protocol will be standard Dresden protocol with given patients informed consent. Before the procedure and on every visit during the follow-up period of one year, all the eyes will be checked for corrected distant visual acuity and subjective refraction measurement, corneal tomography, and slit-lamp examination. The research plan is to analyze the corneal keratometry values, anterior and posterior curvature, diameter, central corneal thickness, and corneal densitometry preoperatively and regular intervals during the first year after CXL. Also, to develop models that could predict the amount of corneal flattening based on the preoperative values and early corneal density postoperatively.

**Expected scientific contribution:** The predictability of the corneal flattening and mathematical calculation of the keratometry changes after the CXL procedure. Mathematical models that we plan to develop will help us to plan the refractive outcome of the patient and in the future to influence it by changing the postoperative treatment protocols.

**MeSH/Keywords:** Cross-linking, Keratoconus, Corneal density

**Poster code:** T-02-18-080

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

**PhD candidate:** Marija Križić

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

**Mentor(s):** Assist. Prof. Natalija Dedić Plavetić, MD PhD, Neven Žarković, PhD, research advisor

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Breast cancer is the most frequently occurring cancer in women, and it is a highly heterogeneous disease in its pathological characteristics. The human epidermal growth factor receptor 2 (HER2) is overexpressed in 15-20% of primary breast cancers. For patients with HER2-positive breast cancer, preoperative systemic therapy (PST) with dual anti-HER2 therapy has become standard for the majority of patients. Since the pathological complete response (pCR) correlates with a better long-term treatment outcome, many studies are currently directed at identifying the predictors of response to PST. Metabolomics is a field of research concerned with the analysis of small molecules (metabolites), and its approach has been used to determine possible biomarkers and key metabolic pathways in various types of cancer.

**Hypothesis:** There are differences in the metabolic profile of patients with HER2-positive breast cancer before the beginning of PST, when comparing the group of patients with achieved pCR and the group of patients with residual disease.

**Aims:** The main goal of the study is to detect differences in the metabolic profile before PST between the group of patients with pCR and the group with the residual disease, in order to determine the predictive biomarkers of the PST response. Secondary goals are: 1. comparison of metabolic profiles of blood samples taken before and after PST, between the group with achieved pCR and the group with residual disease, in order to detect metabolites involved in the development of drug resistance; 2. radiomic approach comparison of MR findings recorded before the beginning of PST, between a group of patients with achieved pCR and a group of patients with residual disease; 3. examination of the association of the response to PST and the clinical characteristics of the patients (age, menopausal status, parity, the involvement of the axillary lymph nodes before treatment); 4. examination of the association of the response to PST and the pathohistological characteristics of the tumor (tumor stage, hormone receptor status, proliferation index, tumor grade).

**Materials and methods:** This prospective study will use the metabolomic approach on plasma samples of 30 patients with HER2-positive breast cancer treated with dual anti-HER2 therapy. An untargeted metabolomic analysis will be made using liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) methods. Multivariate statistical analyses will be performed, including building up Principal Component Analysis (PCA) models and Partial Least-Squares Discriminant Analysis (PLS-DA) and Orthogonal PLS-DA (OPLS-DA) models. Depending on the distribution of the data, for univariate statistical comparisons between groups Student's t-test or Mann-Whitney U test will be performed, followed by Bonferroni post hoc correction for multiple comparisons ( $p \leq 0.050$ ). Shapiro-Wilk test will be performed to check for normal distribution in the data set for each compound separately.

**Expected scientific contribution:** This is the first metabolomic research to analyze the response to preoperative systemic dual antiHER2 therapy with pertuzumab and trastuzumab. We expect differences in the metabolic profile before PST between the group of patients with achieved pCR and the group of patients with residual disease. These statistically significant metabolites could be used as new predictive biomarkers of PST response with the aim of researching new drugs and new treatment modalities in patients with residual disease.

**MeSH/Keywords:** HER2-positive, pathological complete response, metabolomics, preoperative systemic therapy

**Poster code:** T-02-19-085

**Poster Title:** Prognostic and predictive significance of systemic inflammation response index in treatment of patients with metastatic colorectal cancer

**PhD candidate:** Nikša Librenjak

**Part of the thesis:** Prognostic and predictive significance of systemic inflammation response index in treatment of patients with metastatic colorectal cancer

**Mentor(s):** Professor Stjepko Pleština, MD PhD

**Affiliation:** University of Zagreb School of Medicine, University Hospital Center Zagreb, Department of Oncology

**Introduction:** Systemic inflammation plays a key role in pathophysiology of many conditions including cancer so it is important to identify prognostic biomarkers. Tumor growth and progression develops as a result of interactions between the tumor, host-derived stromal tissues and host inflammatory cells. Systemic inflammation response indices include neutrophil to lymphocyte ratio (NLR) and thrombocyte-neutrophil to lymphocyte ratio (PNLR) and have been associated with worse prognosis in colorectal cancer stage II and III. However, there is still few data on exploring these indices in metastatic colorectal cancer, 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.

**Hypothesis:** An association exists between the inflammatory indices in the clinical assessment of treatment choice and prognosis of patients with metastatic colorectal cancer using modern recommended protocols and treatment guidelines.

**Aims:** To investigate the relationship between the inflammatory indices in assessing the effect of treatment according to modern guidelines and prognosis of patients with metastatic colorectal cancer, and identifying risk groups of patients for the treatment decision.

**Materials and methods:** In this observational retrospective-prospective study, relevant clinical and laboratory data of patients treated for metastatic disease will be collected at the Department of Oncology, University Hospital Center Zagreb. Study will include patients who started first-line chemotherapy in period from 1st January 2016 to 31st December 2018 and follow-up will continue up to 31st December 2021. In aforementioned period at the Department of Oncology 340 patients started first-line chemotherapy. Patients who received chemotherapy for other malignant disease, or with chronic inflammatory disease, or receiving glucocorticoids will be excluded from the study. NLR and PNLN will be correlated with overall survival (OS), progression free survival (PFS) of each treatment line, response rate (defined by RECIST 1.1 criteria, evaluated every 12 weeks using CT scan) and ECOG performance status. Also, the association between the inflammatory indices and BRAF and RAS mutational status will be explored. Statistical analysis according to distribution of variables will include  $\chi^2$  test, analysis of variance, t-test or Mann-Whitney U test and Spearman's and Pearson's bivariate (two-tailed) correlation. For the population included in the study, divided according to NLR and PNLN, Kaplan-Meier analysis will be used to estimate the probability of OS and PFS. The Cox proportional hazard model with a 95% confidence interval will be used to estimate NLR and PNLN predictions for OS and PFS.

**Expected scientific contribution:** Examination of the inflammatory indices in patients with metastatic colorectal cancer contributes to the understanding and knowledge of the effect of systemic inflammation in identification of risk groups in the overall clinical assessment of treatment protocol selection and better prognosis.

**MeSH/Keywords:** metastatic colorectal cancer, prognosis, cancer associated inflammation, neutrophil to lymphocyte ratio, platelet-neutrophil to lymphocyte ratio

**Poster code:** T-02-19-086

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

**PhD candidate:** Jure Serdar

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

**Mentor(s):** Assoc. Prof. Tomislav Smoljanović, MD PhD, Assist. Prof. Ana Pilipović, MD PhD

**Affiliation:** University of Zagreb School of Medicine

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

**Hypothesis:** Twisting of tendons by more than 180 degrees is positively associated with an increase in the tendons tensile strength during tensile load compared to the strength of non-twisted tendons.

**Aims:** The main aim of this study is to determine how tendon twisting affects the tensile strength of tendons compared to the strength of non-twisted tendons. The specific aims are: to determine the twisting angle at which the tensile strength of the tendon is increased; to determine how the thickness of the tendon graft changes after twisting in relation to the thickness before twisting, and considering the different angles of twisting; to determine how the graft length changes after twisting considering the different angles of twisting; to determine the relationship between tendon twisting angle, tendon thickness change and tendon tensile strength.

**Materials and methods:** In this study 56 matched pairs of hamstrings (semitendinosus and gracilis) cadaveric tendons will be tested. In the examined group tendons will be twisted along the entire length and in the control group plain tendons will be tested. There will be four examined groups with 14 pairs of hamstring tendons in each group. Tendons will be divided in groups according to various angles of twisting. In the first group tendons will be twisted by 180 degrees, in the second group by 360 degrees, in the third group by 540 degrees and in the fourth group by 720 degrees. Matched tendon pairs will undergo tensile testing with the purpose of determination of maximal tensile force and tensile force which brings to tendons rupture. The tensile testing will be performed on a universal testing machine (Shimadzu AGS-X, Shimadzu Corporation, Japan) with a maximum force of 10 kN. After the testing statistical data analysis will be performed.

**Expected scientific contribution:** The proposed study will contribute to a better understanding of the influence of twisting on the tensile properties of tendons. These findings will lead to the improvement of the existing method of surgical treatment of anterior cruciate ligament rupture.

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

**Poster code:** T-02-20-020

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

**PhD candidate:** Filip Vuletić

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

**Mentor(s):** Assist. Prof. Alan Ivković, MD PhD

**Affiliation:** University of Zagreb School of Medicine

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

**Hypothesis:** Treatment of focal articular cartilage lesions of the knee using nasal chondrocyte-based tissue (N-TEC) will improve the clinical efficacy for the patient after 24 months as compared to the group receiving the nasal chondrocyte-based cell therapy (N-CAM).

**Aims:** The proposed trial will evaluate whether implantation of a mature cartilage graft (N-TEC) improves the clinical efficacy, leading to an increase in the main primary outcome, Knee injury and Osteoarthritis Outcome Score (KOOS), measured 24 months after surgery. A further questionnaire (EQ-5d) at 12 and 24 month and an additional time point (12 month) for KOOS will allow the more detailed analysis of the clinical development of the patient's recovery and elucidate changes in the perceived quality of life before and after treatment. Retrospective analysis of the primary and secondary endpoint data with regard to the onset of symptoms to identify a possible selection of treatment of acute (onset <2 years) or chronic (onset >2 years) lesions.

**Materials and methods:** The study is designed as a prospective, randomized and unblinded phase II study for the comparison of a therapy with a mature (N-TEC) versus a therapy with an immature graft (N-CAM). 30 patients between 18 - 65 years old with symptomatic full-thickness cartilage lesions (2-8 cm<sup>2</sup>) of the knee treated in University hospital "Sveti Duh" will be enrolled in this trial. The clinical follow-up will be done at 6 weeks as well as at 3, 12 and 24 months to assess recovery. 12 and 24 months after treatment questionnaires will be filled out by the patient to collect the clinical data.

**Expected scientific contribution:** Novel, tissue base therapy, engineered from autologous nasal chondrocytes, can be used clinically for repair of articular cartilage defects of the knee with expected superior clinical efficacy compared with cellular based therapy.

**MeSH/Keywords:** Tissue-engineered cartilage, Autologous nasal chondrocytes, Cartilage injury, Clinical outcome, Randomized controlled

**Poster code:** T-02-20-055



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

**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 or C 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.

**Hypothesis:** Cortical thickness and length of good contact between cortex and stem influence on significant vertical subsidence of modular fluted tapered stem implanted using the transfemoral approach by Wagner in revision hip arthroplasty.

**Aims:** To determine clinical and radiographic risk factors for the significant vertical subsidence of modular fluted tapered stems implanted using the transfemoral approach by Wagner in a cohort of revision hip arthroplasties.

**Materials and methods:** A retrospective review of a single-center surgical registry will be 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 will be identified. Patient's demographic data, clinical and radiographic outcomes, and postoperative complications will be assessed and analyzed. In this study, the significant subsidence was defined as more than 5 millimeters (mm), as suggested by many authors. Therefore, two groups will be formed and analyzed, one with subsidence of 5 mm and less, and the other with subsidence of more than 5 mm.

**Expected scientific contribution:** This study will identify clinical and radiographic risk factors for significant vertical subsidence of the modular fluted tapered stems implanted using the transfemoral approach by Wagner in revision hip arthroplasty.

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

**Poster code:** T-02-20-158

**Poster Title:** The influence of mitochondrial energy metabolism and high glucose concentration on expression of pluripotency genes SOX2 and NANOG and the viability of mesothelioma cells in vitro

**PhD candidate:** Sunčana Sikirić

**Part of the thesis:** The influence of mitochondrial energy metabolism and high glucose concentration on expression of pluripotency genes SOX2 and NANOG and the viability of mesothelioma cells in vitro

**Mentor(s):** Professor Sven Seiwerth, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Mesothelioma is a very aggressive type of tumour with a long latency period after asbestos exposure. It can form in the mesothelial surfaces of the pleura, but it can also be present in the peritoneum, pericard and the tunica vaginalis testis. Since mesothelioma is a rare tumour, genomic studies are limited and usually include a small sample size. However, several genes involved in the development of this tumour have been identified, including: CDKN2A, NF2 i BAP1, TP53, DDX3X, SETD2, SF3B1 i TRAF7. Generally, the expression of pluripotency genes (e.g. SOX2, NANOG, OCT4) in different tumours is known to cause poor cell differentiation and their expression in tumours can suggest a more malignant phenotype. An important factor in the progression of mesothelioma are also the mitochondria and the metabolic changes that occur in the tumour cells. The tumour cells partly switch from oxidative phosphorylation to glycolysis and use these two processes simultaneously to maximise the production of energy needed for cell proliferation. Today several different compounds, which can take part in the modification of the mitochondrial energetic metabolism, are known. While some can affect the traffic of pyruvate, others can act as antioxidants and lower the levels of reactive oxygen species created in the electron transport chain. These modifications can cause the tumour cells to modulate their response to apoptotic signals and consequently change their viability and response to antineoplastic agents.

**Hypothesis:** High glucose concentrations stimulate the expression of SOX2 and NANOG genes in the mesothelioma cell line and increase their viability while inhibitors of the mitochondrial energetic metabolism and mitochondrial antioxidants revert this effect.

**Aims:** The aim of this research is to investigate (in mesothelial cell line Mero-14) how high glucose concentrations and inhibitors of mitochondrial energy metabolism influence the expression of pluripotency genes SOX2 and NANOG and whether these treatments change the resistance to antineoplastic agents.

**Materials and methods:** Mero-14 cells will be treated with compounds modulating the mitochondrial energy metabolism separately or together with a high glucose concentration. Immunocytochemical staining will be used to quantify SOX2 and NANOG protein expression while quantitative PCR will be used to analyse the relative expression of SOX2 and NANOG genes. To investigate the resistance of mesothelioma cells to antineoplastic agents, after the aforementioned treatment, we will test and compare cell death in response to different concentrations of the antineoplastic agents pemetrexed and cisplatin.

**Expected scientific contribution:** The results will provide new information on the role of mitochondria and glucose in cytoprotection, their role in cell viability modification and chemoresistance of mesothelioma cells.

**MeSH/Keywords:** mitochondria, SOX2, NANOG, mesothelioma, Mero-14

**Poster code:** T-02-23-073

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

**PhD candidate:** Matija Horaček

**Part of the thesis:** Characteristics of patients with thin basement membrane nephropathy combined with focal segmental glomerulosclerosis

**Mentor(s):** Assoc. Prof. 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/or COL4A5 genes, which encode chains of collagen IV, 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 researches 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.

**Hypothesis:** Patients with TBMN associated with FSGS have a more severe clinical course compared to patients with isolated TBMN, and this is partly due to the type and severity of the underlying collagen IV mutation.

**Aims:** The aim of this study is to determine clinical, pathohistological and genetic characteristics of patients with TBMN associated with FSGS and to compare them to patients with isolated TBMN. We plan to test included patients for COL4A3, COL4A4 and COL4A5 mutations to determine which mutations are present in our population and how they affect the clinical course of the disease.

**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 chemicals (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.

**Expected scientific contribution:** This study will provide better insight into the genetic background and histological characteristics of patients with TBMN associated with FSGS with the potential detection of new mutations that cause AS spectrum disorders. Understanding the genetic etiology, severity and progressiveness of these disorders is 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:** T-02-23-100

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

**Mentor(s):** Assoc. Prof. Slavko Gašparov, MD PhD, Assoc. Prof. 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). Genes GMNN, PCNA and EZH2 are some of decisive in process of replication and chromatin reassembly, and their mutations allow accumulation of errors and represent one of the main steps in tumorigenesis.

**Hypothesis:** Changes of expression of genes and proteins GMNN, PCNA and EZH2 are important step in development of DLBCL-NOS.

**Aims:** 1. Determining methylation levels of promoter regions of GMNN, PCNA and EZH2 genes by bisulfide conversion of DNA and pyrosequencing of selected promoter regions. 2. Determining level of expression of genes GMNN, PCNA and EZH2 using real time polymerase chain reaction. 3. Determining level of expression of proteins GMNN, PCNA and EZH2 using immunohistochemical staining. 4. Comparing methylation levels of promoter regions of GMNN, PCNA and EZH2 genes with their level of expression and presence of proteins they code. 5. Determining significance of detected changes in studied genes and proteins in pathogenesis of subgroups of DLBCL-NOS.

**Materials and methods:** Study population group will include 102 patients with newly diagnosed DLBCL-NOS (51 of GCB subtype and 51 of non-GCB subtype). Archived paraffin blocks will be used (formaldehyde fixated, paraffin imbedded biopsy tissues) on which diagnose of DLBCL-NOS was signed out in Department of pathology and cytology, University hospital Merkur from year 2010 to 2015. Only patients with newly diagnosed DLBCL-NOS without any previous treatment will be included. Standard immunohistochemical staining will be used for determining level of expression of proteins CD20, CD3, CD10, BCL6, MUM1, BCL2, Ki67, GMNN, PCNA i EZH. Control group will include 51 patient reactive tonsillectomy tissue. Standard immunohistochemical staining will be used for determining level of expression of proteins GMNN, PCNA and EZH2 in all control group specimens. I will isolate DNA and RNA form all tissue samples. Using PCR based DNA methylation analysis I will determine methylation levels of promoter regions of GMNN, PCNA and EZH2 genes. Using real time polymerase chain reaction, I will determine level of expression of genes GMNN, PCNA and EZH2 using specific hydrolysing probes. Using specific antibodies, I will determine expression of proteins GMNN, PCNA and EZH2 by immunohistochemical staining. For statistic analysis, depending on type of distribution of results I will use several statistic tests: ¥ Distribution of scores (Shapiro-Wilk test) ¥ Student t-test or Mann-Whitney U test ¥ chi-square test ¥ Pearson correlation coefficient

**Expected scientific contribution:** By analysing changes in process of replication and chromatin reassembly through changes of expression of genes and proteins GMNN, PCNA and EZH2 we will better understand their potential role in B-lymphomagenesis.

**MeSH/Keywords:** diffuse large B-cell lymphoma, lymphomagenesis, GMNN, PCNA, EZH2

**Poster code:** T-02-23-106

**Poster Title:** Involvement of cancer stem cells and PD-L1 in the aggressiveness of oral cavity squamous cell carcinoma

**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; University Hospital Dubrava

**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. Despite advances in therapy, overall survival rates have remained unchanged in the last 40 years. Combined therapy – therapy directed against CSCs and immunotherapy, is currently being intensively explored. Correlation between tumor aggressiveness 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. Extranodal extension in metastatic regional lymph nodes is considered to be the worst prognostic factor in OCSCC patients. This research will analyse the correlation between expression of CSC and PD-L1 in the primary tumors of the patients and extranodal extension in positive lymph nodes.

**Hypothesis:** The expression of cancer stem cell markers (CD44 and ALDH-1) and programmed death receptor ligand 1 (PD-L1) is stronger in the primary tumors of oral cavity squamous cell cancer patients with regional lymph node metastases and extranodal extension than in those without extranodal extension.

**Aims:** The aims of this study are to determine the correlation between the expression of cancer stem cell markers and PD-L1 in the primary tumors of oral cavity squamous cell cancer patients and regional lymph node extracapsular extension, as well as the correlation between the markers themselves and between the markers and standard clinico-pathological parameters.

**Materials and methods:** This study will involve oral cavity squamous cell cancer patients with regional lymph node metastases, who will be divided into two groups: those with and those without extranodal extension. Samples of their primary tumors will be immunohistochemically processed and the expression of cancer stem cell markers CD44 and ALDH-1 and expression of PD-L1 will be analysed and correlated with extranodal extension. Statistical analysis of the correlation between the markers themselves and between the markers and standard clinico-pathological parameters will also be performed.

**Expected scientific contribution:** This study will determine the correlation between cancer stem cells, the ability of the tumor to evade the immunological response of the organism and extranodal extension in a regional metastasis of oral cavity squamous cell cancer, which is related to the aggressiveness of the tumor and considered to be the worst prognostic factor for patients with this disease. This will enable more accurate predictions of tumor behaviour and more precise treatment planning, as well as point to a biomarker profile which will potentially represent new therapeutic targets on cancer cells.

**MeSH/Keywords:** oral cavity squamous cell carcinoma, metastasis, cancer stem cells, PD-L1, extranodal extension

**Poster code:** T-02-23-109

**Poster Title:** Expression of P53 in mantle-cell lymphomas at diagnosis and in relapse

**PhD candidate:** Adriatik Berisha

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

**Mentor(s):** Assist. Prof. Snježana Dotlić, MD PhD, Professor Igor Aurer, MD PhD

**Affiliation:** University of Zagreb School of Medicine

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

**Hypothesis:** Hypothesis 1: Aberrant immunohistochemical expression of P53 is a negative prognostic marker in mantle-cell lymphoma; Hypothesis 2: Frequency of aberrant immunohistochemical P53 expression increases in relapse.

**Aims:** Determine the frequency of aberrant P53 expression in MCL at diagnosis and in relapse. Determine the correlation of aberrant P53 expression with classic prognostic factors: MIPI, Ki67 and cytology (blastoid vs. classic MCL). Analyze the prognostic impact of aberrant P53 expression.

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

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

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

**Poster code:** T-02-23-131

**Poster Title:** The role of physical activity in the occurrence and treatment of muscle and bone mass disorders in children with inflammatory bowel disease

**PhD candidate:** Ivana Trivić

**Part of the thesis:** The role of physical activity in the occurrence and treatment of muscle and bone mass disorders in children with inflammatory bowel disease

**Mentor(s):** Professor Sanja Kolaček, MD PhD

**Affiliation:** University of Zagreb School of Medicine; Department of Pediatrics, Children's Hospital Zagreb

**Introduction:** Inflammatory bowel disease, comprising Crohn's disease, ulcerative colitis and indeterminate colitis; is chronic, relapsing-remitting inflammatory disorder of the gastrointestinal tract. Impaired digestive function leads to unfavorable changes in body composition. According to available data, more than 90% of children with Crohn's disease and almost 50% of children with ulcerative colitis have reduced lean body mass, a clear reflection of the reduced absolute and relative muscle and bone mass. Possible underlying mechanisms include constantly present systemic inflammation, negative effects of the therapeutical drugs used, but also decreased physical activity. Few studies have shown that decreased physical activity is related to decreased bone mineral density in children and have speculated that regular physical activity could have a beneficial effect on the body composition. Moreover, the potential beneficial effect of physical activity on the activity and course of the disease and children's quality of life should be investigated.

**Hypothesis:** Muscle and bone mass disorders in children suffering from inflammatory bowel disease are associated with decreased physical activity and can be corrected by a 24-week long moderate intensity exercise program.

**Aims:** Primary objective of the study is to examine the relationship between physical activity and muscle and bone mass in children suffering from inflammatory bowel disease. Secondary objectives are to examine physical activity of children in remission before a 24-week moderate intensity exercise program and determine whether it correlates with muscle and bone mass and to examine whether such exercise program could significantly increase muscle and bone mass in these children, as well as, influence disease activity, quality of life and later physical activity.

**Materials and methods:** We plan to include 38 children, aged 10 to 18 years, suffering from inflammatory bowel disease. Relevant clinical, laboratory and anthropometric data will be collected for all subjects. Nutrient intake will be assessed using a three-day dietary diary, while body composition analysis will be performed using GE Lunar iDXA. Physical activity will be assessed using the Physical Activity Questionnaire and based on the five-day long measurements during which subjects will be instructed to wear a triaxial accelerometer. In all subjects, hand grip and global muscle strength will be examined and quality of life will be assessed using the IMPACT-III questionnaire. The study will be conducted over 26 weeks, in three stages. Upon inclusion in the study, each subject will be given a triaxial accelerometer and a three-day dietary diary. After the five-day physical activity measurement, clinical, laboratory and anthropometric data of the subjects will be recorded, nutrient intake assessed, body composition determined, physical activity, muscle strength and quality of life examined. For each subject a 24-week moderate intensity exercise program will be designed. The program will include weight bearing exercises, assigned three times a week, with a progressive increase in the number of repetitions every four weeks. Upon completion of the 24-week intervention, final evaluation, identical to the initial one, will be undertaken.

**Expected scientific contribution:** The study will examine the impact of physical activity on the status of muscle and bone mass and the activity of inflammatory bowel disease in ill children. It will contribute to the resolution of uncertainty whether regular physical exercise can yield significant anti-inflammatory effects. Consequently, the results of the study could provide a scientific basis for active promotion of physical activity as a supportive therapeutic modality in children suffering from inflammatory bowel disease, with beneficial effects on the course of the disease, muscle and bone mass status and quality of life.

**MeSH/Keywords:** inflammatory bowel disease, children, muscle and bone mass, body composition, physical activity

**Poster code:** T-02-24-012

**Poster Title:** Doctoral thesis proposal: Contribution of the whole exome sequencing in the identification of genetic variants associated with childhood-onset systemic lupus erythematosus and IgA vasculitis

**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 erythematosus and IgA vasculitis

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

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** The Doctoral thesis proposal 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 or Henoch Schönlein purpura (HSP).

**Hypothesis:** Using whole exome sequencing (WES) in carefully selected patients with cSLE and HSP and in the family members of affected individuals it is possible to identify novel and rare gene variants that may be contributing to etiopathogenesis of these diseases and expand existing genetic databases, which will represent a small but important step towards understanding the complex pathophysiology of two diseases.

**Aims:** General aim of the research is to identify novel genes and variants involved in cSLE and HSP. The specific aims are as follows: to identify individuals and families with a likely monogenic cSLE and HSP according to inclusion criteria and to obtain appropriate DNA samples from patients and consented family members; to perform WES in selected individuals; to analyze exome sequencing data to identify genetic variants in all individuals sequenced; to predict the pathogenicity of putative variants by comparison with exome sequencing data and using prediction tools; to characterize and define possible mode of inheritance of the variants.

**Materials and methods:** In this multiple case study at least 15 "trios" containing proband case with cSLE or HSP 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 will undergo genomic testing using WES. For WES DNA samples will be enriched and sequenced by Illumina HiSeq 2000 (Illumina, inc). The exomes of these sequences will be analysed through bioinformatics pipeline: single nucleotide variants (SNV) and small insertions and deletions (indels) will be called from the aligned data using SAMtools. Two in silico panels will be used for performing targeted, outcome-driven analyses of WES data. The first in silico panel of genes will include 75 genes known to cause monogenic SLE, interferonopathies or found within GWAS SLE loci. The second in silico panel of genes will include 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 will narrow 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.

**Expected scientific contribution:** The research aims to contribute to elucidating the complex pathogenesis of cSLE and HSP finding new genetic variants, which would expand existing genetic databases and highlight the role of gene technology for the better diagnosis and classification of patients with cSLE and HSP and encourage further medical research toward the development of personalized and precision medicine.

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

**Poster code:** T-02-24-042



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

**PhD candidate:** Lana Žigjć Antić

**Part of the thesis:** Predictive factors of mental health in children and adolescents with juvenile idiopathic arthritis

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

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

**Introduction:** Mental health is a condition in which a person realizes their abilities, can cope with everyday stress, work productively and fruitfully, and contribute to his own community. It means much more than the mere absence of mental illness. Early study shows that there are some risk factors of mental health (mental illness of parents or children, parents' divorce, parental conflicts, unemployment, etc) and protective factors (personality, family support, positive relationships between parents and children, social support etc.). 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. The data obtained from various studies in the field of mental health of children and adolescents with chronic pain are not consistent.

**Hypothesis:** Trait anxiety, anxiety sensitivity, family support, social support, and pain components are associated with the mental health of children and adolescents with JIA, while impaired mental health is associated with the assessment of poorer general condition.

**Aims:** The main aim of this study is to examine the risk and protective factors for the mental health of children with JIA from the biopsychological perspective.

**Materials and methods:** 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, The Mental Health Continuum – 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 will be administered in 108 JIA patients aged 9-18 years, monitored at the Pediatric Clinic of the Sestre milosrdnice University Hospital Center in Zagreb. Part of the questionnaire will be completed by a parent, as well as by the attending pediatric rheumatologist. All data will be processed with appropriate statistical analyses.

**Expected scientific contribution:** To 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 help us to better understand factors influencing the assessment of the general condition, in addition to gaining a better understanding of patients and their family members. Our results will allow us to record the factors that contribute to the maintenance of chronic pain as well as planning future preventive strategies.

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

**Poster code:** T-02-24-075

**Poster Title:** Histological classification in predicting outcomes in Henoch–Schönlein purpura nephritis

**PhD candidate:** Nastasia Kifer

**Part of the thesis:** Histological classification in predicting outcomes in Henoch–Schönlein purpura nephritis

**Mentor(s):** Assoc. Prof. Marija Jelušić, MD PhD, Assoc. Prof. Marijana Ćorić, MD PhD

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

**Introduction:** Although Henoch-Schönlein's purpura (HSP) is most often self-limiting, up to 60% of children develop nephritis (HSPN), the main cause of morbidity and mortality. The confirmation of kidney diseases in children with HSP relies on invasive renal biopsy. Albeit the evidence for the association between clinical features and histological findings, there are no studies comparing the performance of the most frequently used histological classifications and it remains unknown which one has the strongest association with the severity of HSPN.

**Hypothesis:** By determining the best histological classification for HSPN it is possible to identify patients with unfavourable disease outcome and create with selected histological variables from existing classifications a model for early prediction of prognosis in children with HSPN.

**Aims:** The primary aim is to determine which histological classification for HSPN is the best for prediction of HSPN progression while constructing the best classification model. Secondary aims are to reclassify retrospective and prospective biopsy samples using four aforementioned classifications, to establish which histological variables most significantly predict renal outcomes and to explore the interdependence between biopsy renal findings, outcome, routine laboratory examinations, disease activity and damage indices.

**Materials and methods:** This research will include patients with HSPN diagnosed by EULAR/PRINTO/PRES criteria. Subjects included prospectively during two years will undergo clinical examination at baseline and follow up visits. Demographic, clinical, laboratory, and medication administration records will be maintained. Samples of venous blood, urine and stool will be collected at the disease diagnosis and at the six months follow up. Renal biopsy findings will be analysed. Retrospective part of the project will include statistical analysis of data extracted from past medical records, and the reanalysis of prior biopsy samples. Renal biopsy findings will be examined using light microscopy, immunofluorescence, and electron microscopy analyses. Four classifications will be used: ISKDC, Haas classification, Oxford classification, and SQC classification. The clinical outcome will be defined through four categories, graded according to the modified classification of Counahan, based on physical examination, haematuria, proteinuria, urine albumin-to-creatinine ratio, hypertension and estimated glomerular filtration rate. The disease activity and degree of kidney damage will be determined by Paediatric Vasculitis Activity Score and Paediatric Vasculitis Damage Index. The minimal sample size for determining correlation between outcome and each classification is 60.

**Expected scientific contribution:** The optimal histological classification could determine patients with poor prognosis and be a useful predictor of the severity of renal impairment. In the preliminary cross-sectional study including 52 pediatric patients retrospectively collected in five Croatian Centers for pediatric rheumatology, the SQC classification proved to be the best reducing the deviation (of the model-predicted outcome value from the observed value) by 8.5% ( $p = 0,003$ ), followed by the Oxford classification ( $p = 0,005$ ), then the ISKDC classification ( $p = 0,008$ ), and finally the Haas classification ( $p = 0,042$ ). Analysis of individual variables of Oxford and SQC classifications singled out interstitial fibrosis and tubular atrophy (SQC), and mesangial hypercellularity (Oxford) as variables affecting outcome. Further research with a larger cohort will enable reliable results and the creation of a model for early prediction of prognosis in children with HSPN, enabling intensive clinical care for vulnerable patients and help design future research aiming to prevent progression to end-stage kidney disease.

**Acknowledgments:** This research is part of Croatian Science Foundation project PURPURAPREDICTORS IP-2019-04-8822.

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

**Poster code:** T-02-24-104

**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):** Assist. Prof. Gordana Ivanac, MD PhD, Assist. Prof. Zrinka Biloglav, MD PhD

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

**Introduction:** 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 coronary artery disease, 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 in comparison with previously suggested CT based scores in patients with suspected coronary artery disease.

**Hypothesis:** Patients with high CT-Leaman score will have significantly shorter event-free survival time compared with patients with lower CT-Leaman score.

**Aims:** Aims of this research are to analyse ten-year prognostic value of CT Leaman score (CT-LeSc) on survival in a cohort of patients with suspected coronary artery disease (CAD). Its specific aims are (1) to assess event-free survival in patients with high and low CT-LeSc defined by cutoff  $\geq 5.52$ , and (2) to cross-compare event-free survival in patients with nonobstructive CAD and high/low CT-LeSc and obstructive CAD and high/low CT-LeSc. Segment involvement and segment stenosis score as well as Agatston calcium score will be compared in multivariate analyses with CT-LeSc. Prognostic and therapeutic implication of baseline statin and aspirin therapy will also be included in multivariate analyses.

**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 will be obtained. Causes and dates of death will be crosschecked from registry of Croatian Institute of Public Health ending with 30th June 2018. For each patient Agatston calcium score, CT-LeSc, segment involvement and segment stenosis score will be calculated. Predictors of outcome—all cause death or cardiovascular death, will be estimated based on Kaplan-Meier survival curves and proportional Cox regression models.

**Expected scientific contribution:** More precise risk stratification of CAD patients through the estimate of association of all-cause or cardiovascular death with the clinical predictors and CCTA defined extent and severity of CAD assessed with different CT based scores. Quantification of long-term prognostic value of CT based scores in conjunction with the impact of baseline medicamentous pharmacotherapy use on mortality.

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

**Poster code:** T-02-25-008

**Poster Title:** Impact of Preoperative Breast Magnetic Resonance Imaging on Surgical Procedure Decision Making and Treatment Outcome in Women with Breast Cancer

**PhD candidate:** Vanja Posavec

**Part of the thesis:** Impact of Preoperative Breast Magnetic Resonance Imaging on Surgical Procedure Decision Making and Treatment Outcome in Women with Breast Cancer

**Mentor(s):** Professor Boris Brkljačić, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Breast MRI (magnetic resonance imaging) is a proven method for diagnosing and local staging of breast cancer due to its superior sensitivity and specificity compared to breast ultrasonography and mammography. The preoperative use of breast MRI increased significantly in the last two decades, although its impact on the surgical treatment outcome is still subject of debate.

**Hypothesis:** Preoperative breast MRI has a significant impact on surgical procedure decision making and surgical treatment outcome in women with breast cancer.

**Aims:** The primary goal of this study is to examine whether preoperative breast MRI is associated with greater odds for breast conservative surgical treatment, lower rate of surgical resection margin involvement, and lower rate of reoperation. Furthermore, we will investigate the impact of preoperative breast MRI on 5-year breast cancer recurrence.

**Materials and methods:** We designed a retrospective case-control study in a tertiary clinical centre specialized in breast cancer treatment. Our non-randomized sample (N=871) includes all women with diagnostic work-up and treatment from 2009 to 2018. We stratified this sample to a control non-MRI group composed of women with preoperative mammography and breast ultrasound (N=442; 51%), and MRI group composed of women with additional preoperative breast MRI (N=429; 49%). Sample groups will be paired by age, breast tissue density, tumour histology and grade, TNM stage, operator experience, and tumour palpability. Univariate and multivariate regression analysis will be used to explore the association of preoperative breast MRI and surgical procedure type, rate of surgical resection margin involvement, and rate of reoperation. Differences in locoregional breast cancer recurrence rate and 5-year disease free survival rate will be examined.

**Expected scientific contribution:** This study will further examine the impact of preoperative breast MRI in women treated with breast conservative surgery, and skin and nipple sparing mastectomy.

**MeSH/Keywords:** breast neoplasms, diagnostic imaging, surgery, magnetic resonance imaging, reoperation, treatment outcome

**Poster code:** T-02-25-052

**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):** Assist. Prof. David Ozretić, MD PhD, Assist. Prof. Hrvoje Budinčević, MD PhD

**Affiliation:** Clinical Hospital "Sveti Duh"

**Introduction:** ASL (arterial spin labeling) is a non-invasive MR imaging technique to measure cerebral blood flow (CBF). Dynamic susceptibility contrast (DSC)-magnetic resonance imaging (MRI), computed tomography (CT) perfusion imaging, single-photon emission tomography (SPECT), and H<sub>2</sub>[15O] positron emission 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.

**Hypothesis:** ASL magnetic resonance imaging technique differentiate Alzheimer's disease from vascular dementia based on specific pattern of perfusion in Alzheimer's disease.

**Aims:** The main goal of this research is to determine the role of ASL magnetic resonance imaging technique in differentiation between Alzheimer's disease and vascular dementia. Specific goals are: Determine if there are differences in cerebral blood flow in frontal, parietal, occipital and temporal lobes in Alzheimer's disease and vascular dementia. Determine if there are differences in cerebral blood flow in posterior cingulate cortex and precuneus in Alzheimer's disease and vascular dementia. Determine if there is correlation of cerebral blood flow values with the degree of cognitive impairment. Determine if there is correlation of cerebral blood flow values with the duration of disease. Determine if there is higher hippocampal cerebral blood flow in Alzheimer's disease in comparison with vascular dementia.

**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 equipped with 64-channel head coil. After standard MRI brain protocol, pulsed 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.

**Expected scientific contribution:** The expected scientific contribution from this research would be confirmation of ASL imaging technique as new completely non-invasive diagnostic test for differentiation between Alzheimer's disease and vascular dementia.

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

**Poster code:** T-02-25-068

**Poster Title:** Sex determination based on osteometric measurements of the humeral, radial and ulnar bones of the victims of the Homeland War

**PhD candidate:** Anton Mažuranić

**Part of the thesis:** Sex determination based on osteometric measurements of the humeral, radial and ulnar bones of the victims of the Homeland War

**Mentor(s):** Assist. Prof. 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 sexual dimorphism. Sex determination of the skeletal remains can be done using morphological or osteometric method. The morphological method gives excellent results, with high percentage of accuracy in determining sex. The disadvantages of the morphological method are subjectivity as well as the fact that the person examining the bones must be experienced. The osteometric method uses discriminant equations that include certain measures of bones. The advantage of the osteometric method is its objectivity and the fact that it can be applied by unexperienced examiners. The disadvantage of the osteometric method is the fact that discriminant equations are population specific. Research has also shown that due to a positive secular trend, socioeconomic circumstances, and technological advances, discriminant equations for archaeological and contemporary populations in the same geographic region do not match. In cases where the pelvic bones, which are best for sex determination, are missing or are insufficiently preserved, the sex is determined on the basis of other skeletal remains present, namely long bones of the limbs and bones of the skull. Long bones of the arm (humerus, radius and ulna) are suitable for osteometric determination of sex because, due to their strength, they are often very well preserved, and due to the large number of measurable parameters they can be used in case of fragmented skeletal remains.

**Hypothesis:** Osteometric parameters measured on the humerus, radius and ulna show statistically significant differences between the sexes.

**Aims:** Primary aim of this study is to determine the discriminant equations for sex estimation based on measures of the humerus, radius and ulna for the contemporary Croatian population. The primary aim of this study will be achieved by measuring a number of standard and new osteometric measures of the humerus (10 measures), radius (7 measures) and ulna (6 measures) which will then be tested for sex determination quality.

**Materials and methods:** Measurements as part of the research will be carried out on the skeletal remains of victims of the Homeland War (1991-1995). As part of the identification of victims of the Homeland War, the sex on these skeletal remains was determined morphologically, as well as by DNA analysis. Measurements will be performed on eighty male and eighty female skeletal remains. Skeletal remains with obvious pathology, ante / perimortem damage and incomplete bones will be excluded from the study. Measurements will be made using a movable gauge (sliding caliper) and a flexible millimeter. To examine the repeatability of measurements between different authors, measurements on randomly selected twenty male and twenty female bone remains will be performed by another colleague and the results will be compared with the original measurements. In order to examine the repeatability of the measurements over time, after the original measurements were made, the author of the study will repeat the measurements on randomly selected twenty male and twenty female bone remains.

**Expected scientific contribution:** This research will determine the importance of measures of the humerus, radius and ulna in the procedures of sex determination of the skeletal remains. The research aims to obtain discriminant equations for sex estimation for the contemporary Croatian population. For the first time, three new parameters will be measured on the humerus, two on the radius and two on the ulna. The results of this research will have practical application in future work of forensic medicine experts.

**MeSH/Keywords:** osteometric sex determination, humerus, radius, ulna, discriminant equations, contemporary population

**Poster code:** T-02-27-043

**Poster Title:** Determination of 1,5-anhydroglucitol concentration in pericardial fluid in diagnosing diabetes mellitus postmortem

**PhD candidate:** Martina Tkalčić

**Part of the thesis:** Determination of 1,5-anhydroglucitol concentration in pericardial fluid in diagnosing diabetes mellitus postmortem

**Mentor(s):** Assoc. Prof. Davor Mayer, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Diabetes mellitus is one of the worlds leading causes of morbidity and mortality and as such represents a major public health problem. It is suspected that one third of the cases are never diagnosed. In forensic setting and from the autopsy point of view diabetes and its acute complications, such as diabetic ketoacidosis and hyperglycaemic hyperosmolar coma, present a tremendous challenge since usual diagnostic tests for diabetes (blood glucose concentrations) are not reliable due to postmortem changes. The other problem that arises from postmortem changes or environment where the body was found is the lack or inadequacy of appropriate specimens for analysis. Thus, the need for additional reliable specimens and stable quantifiable compounds with diagnostic significance. One of such compounds is recently studied 1,5-anhydroglucitol, an inert deoxy-form of glucose, regularly ingested by food. 1,5-anhydroglucitol binds competitively with glucose in kidney tubules and is indirect indication of blood glucose levels. Since 1,5-anhydroglucitol is more sensitive to daily changes in blood glucose levels it provides a better insight into metabolic status of the deceased in shorter period prior to death than glycated haemoglobin. As far as the specimens go pericardial fluid has been proven as a stable body fluid in which measured parameters are comparable to those in other specimens.

**Hypothesis:** Pericardial fluid is suitable specimen for determination of 1,5-anhydroglucitol concentration in postmortem diagnosis of diabetes mellitus.

**Aims:** To determine concentrations of 1,5-anhydroglucitol in pericardial fluid of deceased diabetics and how they correlate to vitreous, urine and femoral blood concentrations in goal of determining if pericardial fluid is a stable alternative specimen in case of inadequate or unavailable other specimens.

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

**Expected scientific contribution:** Research would prove that pericardial fluid is good alternative specimen for determining 1,5-anhydroglucitol concentrations in postmortem diagnosis of diabetes mellitus which would in turn help us understand metabolic occurrences prior to death and their contribution to it. Such understanding will lead to defining cause of death more precisely. It would be especially useful as a diagnostic tool in cases of sudden unexpected deaths of younger people as they are primary group in which diabetes mellitus type 1 occurs, with ketoacidosis as the most severe acute complication that can lead to fatal outcome.

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

**Poster code:** T-02-27-084

**Poster Title:** Urethral functional profile length before radical prostatectomy as an early postoperative continence predictor

**PhD candidate:** Mirko Bakula

**Part of the thesis:** Urethral functional profile length before radical prostatectomy as an early postoperative continence predictor

**Mentor(s):** Assist. Prof. Tvrtko Hudolin, MD PhD, research advisor

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Prostate cancer is the second most common cancer in men in the world and the fifth most common cause of death from malignant disease. Radical prostatectomy (RP) is the gold standard of treatment for patients with localized prostate cancer and can be performed by open access, laparoscopically, or robot-assisted. Urinary incontinence (UI) is one of the most common complications after surgical treatment that affects the quality of life of patients, often requires subsequent treatment and creates an additional financial burden on the health system. Shortening the period of incontinence or reducing the severity of urinary problems would greatly contribute to improving the quality of life of patients. One of the main causes of UI after RP is impaired urethral sphincter function, but the mechanism of complete or partial recovery of urinary continuity has not been fully elucidated. In order to learn more about the possible causes of UI, better and earlier recovery of urinary function and improving the quality of life of patients, this study will use the urodynamic method of profilometry (Urethral Pressure Profile - UPP). This diagnostic method provides data on the function of the urethral sphincter, ie the pressure it creates during closure (Maximal Urethral Closure Pressure - MUCP) and the length of the functional part of the urethra (Functional Profile Length - FPL). The severity of urinary incontinence and the impact on quality of life are assessed by the international validated questionnaire ICIQ-SF.

**Hypothesis:** Functional profile length and maximal urethral closure pressure are predictors of an early continence recovery after radical prostatectomy.

**Aims:** Aim of this research is to determine the correlation of profilometric values in patients with prostate cancer and early continence recovery after radical prostatectomy (RP). The impact of preoperative profilometric values (FPL, MUCP) on the quality of life of patients with postoperative UI will be assessed and the correlation between chronic diseases, demographic and morphological characteristics of patients and postoperative complications with emphasis on UI will be determined.

**Materials and methods:** This prospective study on a patient cohort with prostate cancer will be conducted at the Center for urodynamics, University Hospital Center Zagreb. Urethral pressure profile will be performed before RP which will give us values of functional profile length and maximal urethral closure pressure. Every patient will sign an informed consent as well as the validated ICIQ-SF questionnaire; before and at the specified time-points after the RP (2, 8, 16 and 24 weeks after surgery).

**Expected scientific contribution:** This research will contribute to a better understanding of the mechanism of urinary incontinence after RP. According to the available medical literature, there are no data on a research conducted by the proposed time-frame concept and perceiving the preoperative profilometric data.

**MeSH/Keywords:** profilometry, urethral pressure profile, functional profile length, maximal urethral closing pressure, early continence recovery, urinary incontinence, prostate cancer, radical prostatectomy

**Poster code:** T-02-28-066



**Poster Title:** Comparison of targeted cognitive multiparametric magnetic resonance-guided prostate biopsy with a repeat systemic biopsy guided by a transrectal ultrasound in a patient with a negative initial biopsy

**PhD candidate:** Sven Nikles

**Part of the thesis:** Comparison of targeted cognitive multiparametric magnetic resonance-guided prostate biopsy with a repeat systemic biopsy guided by a transrectal ultrasound in a patient with a negative initial 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.

**Hypothesis:** Targeted cognitive guided multiparametric magnetic resonance imaging prostate biopsy has a higher detection rate of prostate cancer than repeated systemic biopsy guided by transrectal ultrasound in patients with previously negative systemic biopsy

**Aims:** The aim of the study is to determine the usefulness of targeted cognitive multiparametric magnetic resonance-guided prostate biopsy to detect lesions that cannot be diagnosed in any other way.

**Materials and methods:** This prospective study will be conducted in the period from 1 October 2016 until 1 October 2020 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  $\leq$  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.

**Expected scientific contribution:** The obtained research results will offer additional understanding of the role of 1.5 T magnetic resonance imaging in the detection of prostate cancer and determine the optimal number of targeted cores.

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

**Poster code:** T-02-28-114

**Poster Title:** Psychopathological characteristics and personality traits of adolescents prone to cannabinoid use

**PhD candidate:** Anita Alegić Karin

**Part of the thesis:** Psychopathological characteristics and personality traits of adolescents prone to cannabinoid use

**Mentor(s):** Assist. Prof. Zrnka Kovačić Petrović, MD PhD

**Affiliation:** Andrija Stampar Teaching Institute of Public Health, Department of Mental Health and Addiction Prevention

**Introduction:** Adolescence is a period marked by significant physical, cognitive, emotional and social changes. Certain first symptoms of more serious mental disorders show rapid development: suicide and suicide attempts, alcoholism, psychoactive substance abuse, schizophrenia, anorexia nervosa and depression. Nevertheless, most adolescents undergo this transition without significant emotional problems. According to the developmental model proposed by Jessor and Jessor, adolescence is a period in which deviation from accepted rules and engaging in problematic behavior is not only expected, but can also be a sign of a healthy development. Therefore, our aim is to identify psychopathological characteristics and personality traits in adolescents who are prone to cannabinoid use, as one of the components of the problematic behavior.

**Hypothesis:** Adolescents prone to cannabinoid consumption differ significantly from adolescents who are not prone to cannabinoid use in terms of psychopathological deviations and personality traits.

**Aims:** To identify psychopathological deviations and personality traits in adolescents prone to more regular cannabinoid use and to compare their psychopathological deviations and personality traits in relation to the other participants – those who tend to experiment with cannabinoids and those who do not consume psychoactive substances.

**Materials and methods:** Approximately 320 participants, included in the treatment at the Andrija Stampar Teaching Institute of Public Health in the period from 2016. to 2019., will be included in the study. On their first arrival, all of the participants underwent urine samples test for the presence of psychoactive substances, a clinical interview was conducted, and the participants filled out psychological measuring instruments PAI-A (Personality Assessment Inventory- Adolescent; for the assessment of psychopathological deviation) and PIE (Plutchik Emotional Profile Index), the personality questionnaire that measures the personality traits like incorporation, self-protection, deprivation, oppositionality, aggressiveness, reproduction, exploration, uncontrollability. At each subsequent visit, participants underwent urine samples tests for the presence of psychoactive substances. On average, each participant was 6 months in the treatment. According to the results of urine samples tests, participants will be divided into two groups: those who are prone to the use of cannabinoids and those who are not prone to the use of any psychoactive substances. Furthermore, the group of participants prone to the use of cannabinoids and / or other psychoactive substances will be divided into two groups; one group will consist of participants who use cannabis exclusively for experimental purposes (experimentators), and the other group will consist of those who consume more frequently (consumers) and who have developed an addiction disorder. The results will be analyzed and processed using a statistical package.

**Expected scientific contribution:** By using psychological measurement instruments in the assessment of psychopathological deviation (PAI-A) and the assessment of personality traits (PIE), together with urine testing for the presence of psychoactive substances in adolescents, we expect a more objective assessment of psychopathological deviations and more accurate diagnosis. This would contribute to a more objective, timely, and adequate treatment of the disorder and the creation of more specific public health actions as part of prevention programs. Contribution to the theoretical postulates of the developmental conceptual theory by Jessor R and Jessor SL is also expected, in which "problematic behavior" is considered part of the normal development of adolescents.

**MeSH/Keywords:** cannabinoid, adolescents, psychopathological characteristics, PAI-A, PIE

**Poster code:** T-02-29-070

**Poster Title:** Effect Of Acute Response To Stress On Cardiovascular Pathology

**PhD candidate:** Sonja Udovičić

**Part of the thesis:** Arterial Stiffness, Concentration of Cortisol and Alpha Amylase in Saliva nad Serum Highly Sensitive Troponin T in Patients with Acute Stress Disorder

**Mentor(s):** Assoc. Prof. Marina Šagud, MD PhD, Assoc. Prof. Ingrid Prkačin, MD PhD

**Affiliation:** University of Zagreb School of Medicine; Department of Psychiatry, University Hospital Center Zagreb; Department of Internal Medicine, University Hospital Merkur

**Introduction:** Cardiovascular diseases are the most common cause of morbidity and mortality in the world and are responsible for half of the causes of death in people over 65 years of age. The traumatic event causes a strong psychophysiological reaction that can accelerate the development of cardiovascular disease by activating the hypothalamic pituitary axis (an indicator is increased cortisol) and sympathetic system (an indicator is increased concentration of alpha amylase and highly sensitive troponin T and increased arterial stiffness). Acute stress has an adverse effect on the cardiovascular system in the laboratory, but its effect on these indicators in patients with acute stress disorder (ASD) is largely unknown.

**Hypothesis:** Patients with ASD have increased arterial stiffness (expressed as pulse wave velocity - PWV), increased concentration of cortisol and alpha amylase in saliva and elevated levels of serum highly sensitive troponin I compared to the control group, and these parameters are improved after a month of treatment.

**Aims:** Aim of this research is to compare the concentration of cortisol and alpha-amylase in saliva (sAA), serum highly-sensitivity troponin T (hsTnT) and arterial stiffness expressed as PWV in patients with ASD and healthy persons, and within a group of patients after 4 weeks.

**Materials and methods:** At least 85 people with ASD and 60 healthy persons will be included. Within 5 days of acute stress, PWV will be measured with a Mobil-O-Graph, and saliva samples taken to determine cortisol and sAA and serum to determine hsTnT, and compared with the values of healthy persons. Measurements will be repeated after four weeks In patients with ASD.

**Expected scientific contribution:** We expect that the results of this research will help us understand the effect of acute stress on the cardiovascular system.

**MeSH/Keywords:** acute stress disorder, arterial stiffness, cortisol, alpha amylase, highly sensitive troponin T

**Poster code:** T-02-29-095

**Poster Title:** Correlation between brain neurotrophic factor and depression in patients after percutaneous coronary intervention

**PhD candidate:** Sara Medved

**Part of the thesis:** Correlation between brain neurotrophic factor and depression in patients after percutaneous coronary intervention

**Mentor(s):** Professor Alma Mihaljević-Peleš, MD PhD, Assist. Prof. Joško Bulum, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Brain-derived neurotrophic factor (BDNF) has a potential to serve as a common biomarker in cardiovascular diseases (CVD) and depression due to the ability to regulate vascular growth and repair and to promote the survival, differentiation and preservation of neurons. A majority of CVD patients undergo percutaneous coronary intervention (PCI). Changes in BDNF levels upon PCI could affect both cardiac and depression status.

**Hypothesis:** An increase in serum BDNF concentration in patients after PCI with stent placement is associated with a decrease in depressive symptoms.

**Aims:** The aim of the study is to determine the correlation of serum BDNF concentration changes with symptoms of depression in patients six months after PCI with stent placement.

**Materials and methods:** This is a cohort study with six months follow-up. Participant will be enrolled after PCI with stent placement due to angina pectoris or myocardial infarction. Exclusion criteria include left ventricular ejection fraction (LVEF) less than 40%, presence of other cardiac diseases, recent PCI or psychopharmacological treatment, acute inflammatory condition and cognitive impairment. The study obtains medical data collected using a survey, provides structured psychiatric interview, applies cardiologic, psychiatric and quality of life (QoL) questionnaires, and collects blood samples. Medical data include medical history, socioeconomic data and data about risk habits. Cardiology questionnaires (Seattle Angina Questionnaires, Duke Activity Status Index) will assess the cardiac status. Depression will be detected using structured psychiatric interview and psychiatric questionnaires (Beck's Depression Inventory II, Hamilton Rating Scale for Depression and Montgomery-Asberg Depression Rating Scale). QoL will be assessed with EQ-5D-3L questionnaire. Blood samples will be collected for analyzing BDNF serum concentrations using enzyme-linked immuno-sorbant assay (ELISA). Upon participant enrollment, medical data, psychiatric and cardiologic status, QoL and BDNF levels will be obtained using a survey, structured psychiatric interview, questionnaires and blood samples. Subjects who will meet the diagnostic criteria for a depressive episode will be provided with psychiatric treatment. The treatment will be evaluated using Clinical Global Impression scale. If psychopharmacological treatment is indicated, escitalopram or sertraline will be prescribed. Subjects who require different psychiatric treatments will be excluded from the study since the changes in BDNF levels in other interventions are still unclear. Six months after enrollment same data as baseline will be collected.

**Expected scientific contribution:** The research has the potential to deepen the knowledge about the BDNF as a possible biomarker in both cardiovascular and psychiatric disease.

**MeSH/Keywords:** brain derived neurotrophic factor (BDNF), depression, cardiovascular disease, percutaneous coronary intervention (PCI), psychocardiology

**Poster code:** T-02-29-107

**Poster Title:** Association of symptoms of anxiety, depression and stress with symptoms of autonomic nervous system disorders

**PhD candidate:** Anamari Junaković

**Part of the thesis:** Association of symptoms of anxiety, depression and stress with symptoms of autonomic nervous system disorders

**Mentor(s):** Assoc. Prof. Mario Habek, MD PhD, Assist. Prof. Milena Skočić Hanžek, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** The autonomic nervous system acts largely unconsciously and regulates key involuntary body functions with a major role in maintaining homeostasis. Anxiety and depression are known to be common disorders, and have been shown to have symptoms that overlap with those of the autonomic nervous system (ANS). On the other hand, stress caused by unpleasant neurological symptoms can trigger and worsen the symptoms of anxiety and depression. Symptoms of ANS disorders can be measured using the Composite Autonomic Symptoms Score (COMPASS-31) questionnaire and with various neurophysiological tests for ANS. COMPASS-31 is the only questionnaire to detect ANS symptoms, so the primary goal of this study is to limit the values of the COMPASS-31 questionnaire, which indicated the existence of ANS disorders depending on the presence of anxiety, depression, and stress symptoms. All subjects will be subjected to a neurophysiological examination of ANS and the estimated emotional state of depression, anxiety and stress with the Depression, Anxiety and Stress Scale (DASS-21) questionnaire. The results will be compared to a healthy control group. This research will have scientific and clinical value. Scientific, as it will contribute to a better understanding of the interactions of autonomic dysfunction and the presence of symptoms of anxiety, depression and stress, which is clinical as it will help to diagnose faster and more accurately, and with time start treatment earlier.

**Hypothesis:** The presence of symptoms of anxiety, depression, and stress is associated with the results of the COMPASS-31 questionnaire

**Aims:** Determine the limit values of the COMPASS-31 questionnaire which indicates the existence of ANS disorders depending on the presence of symptoms of anxiety, depression and stress  
**Specific objectives:** 1. Determine the limit values of the COMPASS-31 questionnaire which indicates the existence of a functional disorder of the ANS depending on the presence of symptoms of anxiety, depression and stress; 2. Determine the limit values of the COMPASS-31 questionnaire that indicate the existence of a structural disorder of the ANS depending on the presence of symptoms of anxiety, depression and stress; 3. Determine which questions in the COMPASS-31 questionnaire depend on the presence of symptoms of anxiety, depression and stress.

**Materials and methods:** 1000 consecutive patients  $\geq 18$  years of age referred by a specialist doctor for a tilt table test or an ANS testing. Healthy control group of 500 subjects  $\geq 18$  years. The following data will be collected: detailed anamnesis, detailed clinical data on diseases and drugs, socioeconomic data and anthropometric measures. An objective and subjective assessment of the ANS function will be performed. Objective assessment will be performed using cardiovascular tests, heart rate response to deep breathing test and Valsalva maneuver, and sympathetic adrenergic function tests using blood pressure response to Valsalva maneuver and tilt test. A subjective assessment of ANS will be made using the COMPASS-31 questionnaire. An assessment of the emotional state will be made using the DASS-21 questionnaire. Statistical processing will be performed by the Smirnov-Kolmogorov test to examine whether the data have a normal distribution. Differences in the distribution of qualitative variables will be determined by the  $\chi^2$  test, while for differences in quantitative variables with respect to the distribution, a parametric t-test or a nonparametric Mann-Whitney test will be used. Correlations between individual variables will be examined using the Pearson or Spearman correlation coefficient. The characteristic curve of the receiver operator (ROC) will be used to examine the sensitivity and specificity of certain values of the variables of interest to us.

**Expected scientific contribution:** Improving the COMPASS-31 questionnaire into a questionnaire with greater reliability and validity, as a screening tool for detecting ANS disorders thus saving resources and shortening the time required to make an accurate diagnosis.

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

**Poster code:** T-02-30-039

**Poster Title:** Epidemiologic and Clinical Characteristics of the Patients Treated with Epilepsy in the Republic of Croatia

**PhD candidate:** Filip Đerke

**Part of the thesis:** Epidemiološka i klinička obilježja pacijenata liječenih od epilepsije u Republici Hrvatskoj

**Mentor(s):** academic Vida Demarin

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Epilepsy is a common neurological disease affecting about 1% of the world's population. Due to its clinical picture, epilepsy is a serious medical and social problem. To date, few epidemiological studies have been conducted on patients treated for epilepsy, mostly based on a smaller sample, geographically limited, on the local and country level. The proposed research is designed to cover a larger sample, at the level of the whole of Croatia, treated from epilepsy.

**Hypothesis:** Epidemiological and clinical characteristics and pharmacotherapeutic approach to patients treated for epilepsy in Croatia do not differ from the European population of patients treated for epilepsy. There is a negative association between the level of physical activity and the clinical manifestation of the disease in the population treated for epilepsy, of at least 25%. There is a negative association between eating habits and clinical manifestations of the disease in the population treated for epilepsy, of at least 25%.

**Aims:** The primary aim of the proposed study is to determine the epidemiological and clinical characteristics of patients treated for epilepsy in the Republic of Croatia. Secondary aims are (1) to present a descriptive age, gender and geographical distribution of patients with epilepsy in the Republic of Croatia, (2) determine the pharmacological approach to treatment, (3) to determine the connection between the level of physical activity and diet concerning the clinical manifestations of the disease, (4) identify possible differences in the clinical manifestations of epilepsy in urban and rural patient populations, and (5) compare the obtained results with the data available for European countries.

**Materials and methods:** Cross-sectional research has been proposed, designed to show the epidemiological and clinical characteristics of patients treated for epilepsy in Croatia. According to the assumption of the approximate number of patients in Croatia, the required sample size is 1385. Inclusion criteria are: (1) the patient suffers from epilepsy, (2) the diagnosis of epilepsy was made by a neurology specialist with subspecialization in epileptology or the diagnosis of epilepsy was confirmed by epileptologist; (3) the respondent is older than 18 years; (4) the respondent has a permanent residence in Croatia. The collected data will not be linked to a person. The research is planned to conduct according to a validated questionnaire consisting of (1) modified versions of the questionnaire by Željka Josipović-Jelić MD PhD used in the dissertation entitled: Incidence of epilepsy in Šibenik-Knin County in the period from 1995 to 2005.; (2) dietary analysis: questionnaire by the author Assist. Prof. Jozica Šikić used in the dissertation entitled: Psychosocial characteristics as risk factors in hospitalized coronary patients in Croatia; (3) determining the level of physical activity. Data collection will take place from August 2020 to February 2021 at the Dubrava University Hospital, Department of Neurology at the Preoperative Assessment of Patients with Pharmacoresistant epilepsy. Statistical significance will be determined at the level of  $\alpha = 0.05$ . The measured variables will be presented in tables and graphs.

**Expected scientific contribution:** The proposed research provides a detailed insight into the epidemiological and clinical characteristics of patients treated for epilepsy in Croatia. The research will use scientific methodology to determine the relationship between the level of physical activity and diet to the clinical manifestation of epilepsy. The research will determine whether there are differences in clinical approach and disease manifestation between urban and rural population.

**MeSH/Keywords:** Epilepsy, Croatia, epidemiology, clinical characteristics

**Poster code:** T-02-30-060

**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):** Assoc. Prof. Porin Perić, MD PhD, Assist. Prof. Marina Boban, MD PhD

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

**Introduction:** Psoriatic arthritis (PsA) is a spondyloarthritis associated with psoriasis. Frequent comorbidities include cardiovascular disease, metabolic syndrome, obesity, diabetes, inflammatory bowel disease and depression. Systemic inflammation, increased cardiovascular risk, chronic pain, associated depressive symptomatology and some 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 only on Montreal Cognitive Assessment (MoCA) test results.

**Hypothesis:** PsA patients will have significantly lower scores on cognitive function tests (MoCA and Trail Making Test) compared to the healthy control group.

**Aims:** General aim is to assess cognitive functions in PsA patients in relation to control group of healthy individuals. Specific aims are: – to examine relationship between sociodemographic as well as disease related factors, and cognitive function testing results in PsA patients – to examine relationship and possible mediator effect of anxiodepressive symptomatology and cognitive function testing results.

**Materials and methods:** Approximately 65 subjects diagnosed with PsA according to the Classification criteria of Psoriatic Arthritis (CASPAR) will be consecutively included in the test group, and same number of healthy controls without inflammatory rheumatic disease or PsO in the control group. Data will be collected through clinical examination, standard laboratory testing, cognitive function testing, and patient reported outcome measures. Subjects' medical history, anthropometric and sociodemographic data will be taken. Standard clinical examination will be performed. Inflammatory markers (ESR, CRP), thyroid stimulating hormone, vitamin B12, folic acid and lipid profile will be determined. In a PsA patients' group, additional data on articular disease treatment, and tender and swollen joint count according to the scoring system of 68 joints will be collected. PsA patients will determine pain level, general health and disease activity on visual analogue scale (VAS), and complete Croatian version of Health Assessment Questionnaire (HAQ), Bath Ankylosing Spondylitis Functional Index (BASFI) and Functional Assessment Chronic Illness Therapy-Fatigue (FACIT-F) questionnaire. Standard disease activity indices like Disease Activity Score (DAS) 28, Disease Activity in Psoriatic Arthritis (DAPSA), Ankylosing Spondylitis Disease Activity Score (ASDAS) and Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) will be calculated. The severity of PsO will be determined by Physician Global Assessment, Body Surface Area and Dermatological Quality of Life Index. Cognitive status of all subjects will be assessed by independent investigator using MoCA test and Trail Making Test (TMT) A and B. MoCA is a mental status scale with moderate assessment time, sensitive to early cognitive changes. Neuropsychiatric status will be evaluated through The Beck Depression Inventory-II (BDI-II) and The State-Trait Anxiety Inventory (STAI) scale.

**Expected scientific contribution:** Results of this research will demonstrate whether PsA patients differ from healthy controls in assessed cognitive functions. According to our present knowledge, this is the first research of its kind and represents original scientific contribution. Additionally, research results will contribute to rheumatology clinical practice improving patient monitoring and identification of potential cognitive impairment.

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

**Poster code:** T-02-30-111

Public health and healthcare - thesis proposals



**Poster Title:** Relationship of stress and depression in pregnant woman with pregnancy outcomes and anthropometry of newborn

**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, Assist. Prof. Natalija Novokmet, MD PhD

**Affiliation:** University of Zagreb School of Medicine, Institute for anthropological research

**Introduction:** In the fields of health psychology, behavioral medicine and social epidemiology, the importance of the impact of adverse psychological factors such as stress and depression during pregnancy on the health of the pregnant woman, as well as the possible adverse pregnancy outcomes, is increasingly emphasized. The proposed study gives us insights from Croatian Island's Birth Cohort Study (CRIBS) approved by the Croatian Science Foundation from November 2015 to October 2018.

**Hypothesis:** The severity of stress and depression in pregnant women are associated with pregnancy outcomes and anthropometric measures of the newborn.

**Aims:** GENERAL AIM: To examine the relationship between stress and depression in pregnant women with pregnancy outcomes and anthropometric measures of the newborn. SPECIFIC AIMS: 1. To examine the association of stress and depression with biomedical, socio-demographic, socio-economic variables and lifestyle in pregnant women. 2. To examine the relationship between pregnancy outcomes and anthropometric measures of the newborn with biomedical, socio-demographic, socio-economic variables and lifestyle in pregnant women. 3. To examine the relationship of biomedical, socio-demographic, socio-economic variables and lifestyle in pregnant women on association between stress and depression in pregnant women with pregnancy outcomes and anthropometric measures of the newborn.

**Materials and methods:** The research will be conducted on a representative sample of participants from the continental area (town of Split and its surroundings) and the related islands (islands of Brač and Hvar) of the Split-Dalmatia County. About 300 pairs of pregnant women and children will participate from 2016 onwards. The inclusion of pregnant women in the study and follow-up was carried out at the doctor's offices in which the pregnancy was monitored and at the Clinic for Women's Diseases and Obstetrics at the Clinical Hospital Center Split. During the period from 22. to 26. weeks of pregnancy, blood samples of pregnant women (plasma and serum) were collected at KBC Split, on the basis of which extended biochemical analyzes were made in the laboratory of KB Dubrava. The biochemical parameters from the blood used for research purposes are glucose and lipids (triglycerides, HDL, LDL). Blood pressure values were collected from pregnancy booklets in the second trimester of pregnancy. Psychological characteristics of pregnant women were examined in the period from 18. to 28. weeks of pregnancy using The Social Readjustment Rating Scale (SRRS) and Edinburgh Postnatal Depression Scale (EPDS). Data which was collected from questionnaires were age, level of education, working status and consumption of cigarettes in pregnant women. The children were included in the study at birth. Anthropometric measures of an infant collected from a discharge letter: length of the child, weight of the child on the basis of which the categories were made in SGA (small for gestational age), AGA (appropriate for gestational age) and LGA (large for gestational age). The outcome of pregnancy was divided in categories preterm delivery and birth at the term.

**Expected scientific contribution:** The findings of this study will highlight the importance of psychological risk factors of stress and depression in pregnant women for adverse pregnancy outcomes and changes in neonatal anthropometric measures. It opens the possibility to design psychological preventative education based on screening of psychological status of potential and existing pregnant women. This is a significant contribution to the development of guidelines for public health interventions.

**Acknowledgments:** This research was funded by grant of the Croatian Scientific Foundation (HRZZ UIP-2014-09-6598). We thank all the participants, researchers and health professionals who have made this study possible.

**MeSH/Keywords:** pregnancy, cohort study, stress, depression, birth outcomes, anthropometry of new born

**Poster code:** T-03-02-011

**Poster Title:** Determinants of the long-term health care integration process in the Republic of Croatia

**PhD candidate:** Dorja Vočanec

**Part of the thesis:** Determinants of the long-term health care integration process in the Republic of Croatia

**Mentor(s):** Assoc. Prof. Aleksandar Džakula, MD PhD, Assoc. Prof. Ana Borovečki, MD PhD

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Medical and technological advancements have posed new challenges for healthcare systems. Life expectancy has been extended, and the leading causes of mortality and global disease burden are chronic noncommunicable diseases. That has increased the complexity of health care delivery, resulting with the fragmentation of care. Therefore, one of the challenges of today's health care systems is the response to fragmented delivery of care, which leads to poorer health outcomes and increased costs. This is especially important in the long-term care of people with chronic and terminal illnesses. The organization and strengthening of the long-term care system are highlighted in the strategic documents and are part of the health care reforms in Croatia. Nevertheless, the long-term care system is one of the least developed. It is characterized by fragmentation of services, without mutual coordination. Integrated care as a set of measures and models in organization, care, clinical work, administration, which aim to improve quality of care and life, increase patients' satisfaction and the efficiency of the healthcare system, is the answer to fragmentation. One of the forms of long-term care is palliative care. Palliative care is active, holistic care of patients whose disease is not responsive to curative treatment. In Croatia, palliative care has been implemented since 2014. Although progress has been made to date in the areas of organized resource development, regulation and professionalization, as key elements of the paradigm shift in the care of terminally ill and chronic patients, the new concept is still not fully accepted. In this research, determinants of its implementation as an integrated care model will be analyzed.

**Hypothesis:** The integration of long-term care can be improved by developing a new governance model that will include factors of the existing health care governance system and be guided by specific indicators of integration and quality of long-term care processes and outcomes.

**Aims:** To determine the factors influencing the success of integration of long-term care and indicators of the effects of integrated care on users and based on them to propose improvements in the health system in the Republic of Croatia.

**Materials and methods:** In the first phase, comparison of structures and processes of the governing and organizational model of long-term care in the Republic of Croatia with the determinants of the concept of integrated care will be performed. Next, based on the mapping of sources of information in the field of long-term care, the indicators of the integration will be determined, and based on them, the progress in achieving the integration of long-term care will be analyzed. In the second phase, qualitative statistical methods will be used. Data will be collected through semi-structured interviews and focus groups. The aim of focus groups and interviews will be to identify factors that affect the integration of long-term care and to identify general and specific indicators of the quality of long-term care integration. The indicators will be grouped according to Donabedian's quality model into structure, process and outcome indicators.

**Expected scientific contribution:** Defining determinants in the process of integration of long-term health care is a complex and important research task in all health systems. This research will point out general and specific critical points for the implementation and quality improvement of the integrated care model. The findings of this research will be useful for countries with a similar organizational and cultural-social profile in the field of implementation, specifically in the segments of response to individual health needs.

**MeSH/Keywords:** integrated care, long-term care, implementation science, quality improvement

**Poster code:** T-03-02-153

**Poster Title:** The Effect of Noise on Salivary Cortisone Concentration and Auditory Brainstem Response Characteristics

**PhD candidate:** Roko Žaja

**Part of the thesis:** The Effect of Noise on Salivary Cortisone Concentration and Auditory Brainstem Response Characteristics

**Mentor(s):** Assist. Prof. Mihael Ries, MD PhD, Assist. Prof. Milan Milošević, MD PhD, senior research associate

**Affiliation:** University of Zagreb School of Medicine

**Introduction:** Exposure to noise levels of intensity  $\geq 85$  dB(A) is associated with an increase in the salivary cortisol concentration, and a decrease in the amplitude ratio of the first and the fifth wave (A1/A5) of the auditory brainstem response (ABR). Activation of the adrenal glands by noise causes an increased cortisol secretion. In the salivary glands, cortisol is converted to the cortisone that is secreted into the saliva. Salivary cortisone concentration (SCC) correlates with the concentration of unbound blood cortisol. The occupational noise-induced hearing damage is common and initially manifested by difficult hearing in noise, tinnitus and hyperacusis. It is associated with a decrease in the number of cochlear synapses and dysfunction of outer hair cells. When there is no permanent threshold shift registered on the audiogram, further evaluation of the early hearing impairment can be directed toward analysis of the otoacoustic emission and the ABR.

**Hypothesis:** There is a negative correlation between the A1/A5 of the ABR and the SCC in persons who have a normal audiogram finding and are exposed to noise in the workplace between 1 and 2 years.

**Aims:** The aim of the study is to examine the correlation between the A1/A5 of the ABR and the SCC in workers exposed to occupational noise. Specific objectives of the research are: risk assessment of noise exposure and the analysis of perceived stress and psychosocial risk factors in the workplace.

**Materials and methods:** Materials: Audiometer AC40 with earphones DD45, tympanometer Interacoustics AZ26, otoacoustic emission measuring instrument Audera GSI with earphones, ABR Interacoustics Eclipse, EPA Preamplifier, 3M E-A-RTONE Insert Earphone ABR, cotton swabs Cortisol-Salivette, high performance liquid chromatography instrument, validated Health and Safety Executive's (HSE) questionnaire for the assessment of occupational psychosocial risk factors, validated Perceived Stress Scale (PSS) and Megerson's noise exposure questionnaire (NEQ). The non-concurrent cohort study will be conducted on a convenient sample of participants aged 19 to 30 with occupational noise exposure duration of 1 to 2 years. Assuming the association of ABR characteristics with SCC with a correlation coefficient of 0.4, a power of 80% and a significance level of 0.05, at least 46 subjects should be included in the study. Power analysis was performed using MedCalc Statistical Software, version 19.1. Data distribution will be analysed by Kolmogorov-Smirnov test and appropriate parametric and/or non-parametric tests will be applied. Differences in the values of numerical variables between the examined group and the control group will be analysed by the Student's t-test for independent samples, and the Mann-Whitney U test for independent samples. Differences in categorical variables will be analysed by chi-square test, or Fisher's exact test when the number of participants in the group is less than 5. Pearson's or Spearman's correlation coefficients will be calculated between clinical variables. The variables that in previous statistical analysis have  $P \leq 0.20$  will be included in predictive regression model for the group at risk for the development of noise-induced hearing loss. In all statistical tests, P values will be considered significant when less than 0.05. Licenced IBM SPSS Statistics software, version 25.0 will be used in the statistical data analysis. A linguistic validation of the NEQ will be done as well as the calculation of the Cronbach's alpha for the PSS and HSE questionnaire.

**Expected scientific contribution:** In contrast to the previous studies, we will examine both auditory and non-auditory noise effects non-invasively and interpret their occurrence and association according to the reported noise exposure. Therefore, we expect to determine the relevance of the SCC in the risk assessment of noise-induced hearing damage during early occupational exposure, which includes the analysis of the ABR, insight into the Workplace risk assessment and self-assessment of noise exposure.

**MeSH/Keywords:** occupational noise; cortisone; auditory brainstem response

**Poster code:** T-03-03-134

**Poster Title:** Knowledge assessment on modifiable cardiovascular risk factors in patients with arterial hypertension

**PhD candidate:** Sonja Frančula-Zaninović

**Part of the thesis:** Knowledge assessment on modifiable cardiovascular risk factors in patients with arterial hypertension

**Mentor(s):** Assoc. Prof. Iskra Alexandra Nola, MD PhD, Assist. Prof. Matias Trbušić, MD PhD

**Affiliation:** University of Zagreb School of Medicine, Health Center Zagreb-Centar

**Introduction:** CV prevention is based on population and high-risk strategy. The population strategy is based on health promotion, health education, lifestyle habits, facilitated access to health activities, availability of medical diagnostics and therapy. Assessing patients' knowledge of CV risk factors can help in the clinical and population-based CV prevention. Arterial hypertension (AH) is the most important independent CV factor. Patients' knowledge and attitudes about CV risk factors improve compliance, treatment outcomes, control of AH, reduce morbidity and mortality. Education should be an integral part of CV prevention. It is necessary to monitor the performance of educational programs, assess knowledge about CV risks and opportunities to reduce CV risks.

**Hypothesis:** Hypertensive patients do not have adequate knowledge about modifiable CV factors

**Aims:** To assess knowledge of modifiable CV factors with the Heart Disease Fact Questionnaire (HDFQ) in hypertensive patients in relation to sociodemographic and anthropometric characteristics, the presence/absence of coronary heart disease (CHD). To identify the variables of respondents to whom specific secondary CV education should be directed.

**Materials and methods:** A cross-sectional survey will include around 800 hypertensive respondents, mostly urban population, older than 18 years, both genders at the cardiology outpatient clinic. The exclusion criteria is the history of diabetes mellitus. The validation of HDFQ was performed on the first 107 respondents (the Cronbach  $\alpha$  0.68); CV risk factors, anthropometric and sociodemographic characteristics were included. Statistical analysis will be performed using TIBCO Software Inc. (2018), Statistica, version 13. Descriptive values will be presented by contingency tables. The normality of the distribution of continuous variables will be examined by the Shapiro-Wilkov test. Sociodemographic and anthropometric data will be compared with the answers to individual questions and with the total score. Appropriate statistical tests will be used: hi square test, Man Whitney U test, Pearson's correlation. The regression methods will be used to examine the influence of individual sociodemographic and anthropometric predictors on the overall score.

**Expected scientific contribution:** The results would have clinical applicability, especially at the secondary CV prevention in identifying patients to whom additional educational programs should be directed to increase knowledge and awareness of CV risk factors and proper lifestyle habits.

**MeSH/Keywords:** arterial hypertension, cardiovascular risk, heart disease, questionnaire, health education

**Poster code:** T-03-06-062