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Sveučilište u Zagrebu
Medicinski fakultet

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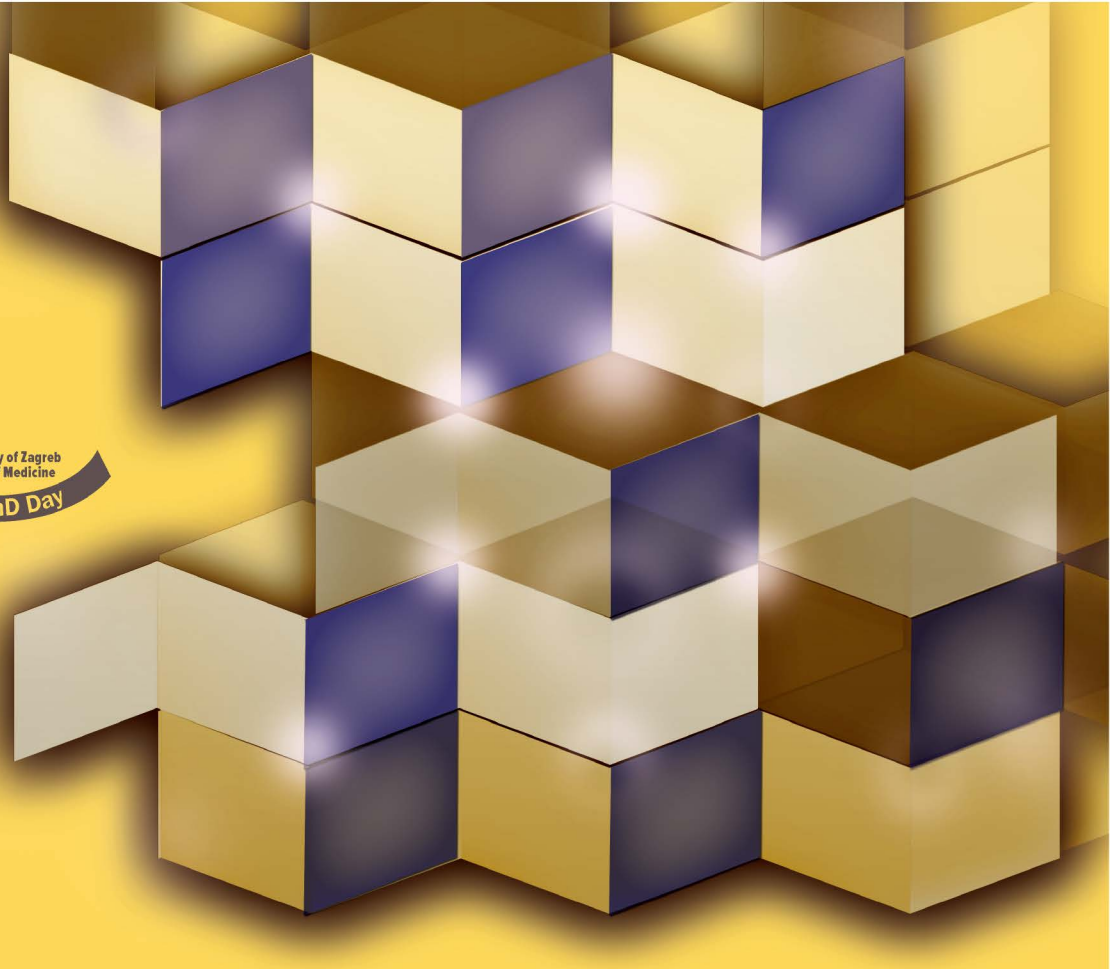
School of Medicine
PhD Day



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Abstract Book

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Research Excellence Lectures

Parkinson's disease: a puzzling widespread distribution of pathological changes

Estifanos Ghebremedhin, MD

Institute of Clinical Neuroanatomy, Goethe-University Frankfurt, Theodor-Stern-Kai 7, D-60590 Frankfurt/Main, Germany

Parkinson's disease (PD) is the most common motor disorder and the second most common neurodegenerative disease after Alzheimer's disease. The major neuropathological features of Parkinson's disease are abnormally misfolded intracellular protein aggregates, referred to as Lewy bodies and Lewy neurites, which consist largely of insoluble deposits of the alpha-synuclein protein. Currently, the etiology and pathogenesis of the neurodegenerative process in Parkinson's disease are only marginally known and, as of yet, no single diagnostic or prognostic biomarker has been identified.

Unlike many other neurodegenerative diseases, PD-associated pathology occurs not only in the central, but also in the peripheral nervous system. It is currently presumed that the mechanisms by which a pathological alpha-synuclein spreads throughout the nervous system and triggers neurodegeneration are very similar to those of prion diseases. This means that the pathologically altered or misfolded alpha synuclein may be transmitted through a chain of interconnected neurons in a prion-like manner and induces misfolding of the native alpha synuclein of the newly affected neurons. Whether the propagation of the pathological process is bi-directional or uni-directional from the central to the peripheral nervous system or vice versa is currently unknown. This talk will discuss the spectrum of pathological changes in PD and its clinical implications**.**

The complex genetics of Familial Hypercholesterolaemia (FH): from monogenic to polygenic disease

Marta Futema, MD, research associate

Centre for Cardiology in the Young, University College London, United Kingdom

Familial Hypercholesterolaemia (FH) is the most commonly inherited disease of high LDL-cholesterol (LDL-C) that leads to premature coronary heart disease (CHD). In its classical monogenic form FH is caused by mutations in LDLR, APOB or PCSK9 genes. Mutations in these genes are found in ~75-80% of definite FH cases. Further ~13% have polygenic disease due high burden of common LDL-C-rising alleles. The cause of FH in the remaining ~12% remains unknown.

Genetic testing for FH can provide crucial information for cost-effective family screening and enables early intervention. Polygenic risk score can differentiate between the monogenic and polygenic hypercholesterolaemia. Genetic diagnosis leads to a different care pathway for monogenic and polygenic FH patients, a paradigm example of the use of an individual's genotype in Precision Medicine.

Basic medical sciences - Preliminary research results

Poster Title: The effect of oral galactose on glucose homeostasis and memory in a transgenic mice model of familial Alzheimer's disease

PhD candidate: Ana Babić Perhoč

Part of the thesis: Therapeutic potential of orally administered galactose on cognitive and metabolic changes in two experimental models of Alzheimer's disease

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

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

Introduction: Decreased glucose metabolism and energy in the brain accompanies sporadic Alzheimer's disease (sAD). Our recent research revealed that oral galactose, an alternative source of energy, successfully prevented the development/normalized early-developed cognitive deficits in a non-transgenic sAD rat model. We aimed to explore whether oral galactose has therapeutic effects on cognitive deficits in a transgenic mice model of familial AD.

Materials and methods: 80 male transgenic Tg2576 (WT/TG) and wild-type (WT/WT) mice aged 5 and 10 months underwent 2-month treatment of oral galactose solution (+GAL) or tap water. Morris Water Maze (MWM), intraperitoneal glucose tolerance test, and fluorodeoxyglucose Positron-Emission-Tomography (PET) scanning were performed before sacrifice.

Results: MWM showed no deficits in 7-month but significant ($p=0.029$) cognitive decline in 12-month aged WT/TG mice compared to their respective WT/WT controls (-75%/probe trial) associated with markedly decreased movement pattern which was worsened by galactose treatment in WT/WT and WT/TG groups. PET scans demonstrated increased (+19.65%) and decreased (-17.70%) glucose uptake/metabolism in the whole brain of 7- and 12-month aged WT/TG mice, which were normalized by galactose (decreased -13.90%/7-month and increased +32.09%/12-month aged WT/TG+GAL mice) respectively. Compared to WT/WT, WT/TG group showed impaired glucose tolerance only at 12-months (glucose level increased +16 to +72%) further worsened by galactose treatment (additional +21 to +51% increment, $p<0.05$). Galactose treatment had no effects in 12-month aged WT/WT group but lowered glucose levels in 7-month old group (-32 to -45%).

Discussion: Oral galactose worsens peripheral but improves central glucose homeostasis without affecting memory in cognitively impaired transgenic AD mice.

Acknowledgments: This abstract was presented at the 11th FENS Forum of Neuroscience 7-11 July, 2018 Berlin, Germany. Supported by the Croatian Science Foundation; project no. HRZZ IP-09-2014-4639 and HRZZ DOK-10-2015.

MeSH/Keywords: Alzheimer's disease; Cognition; Galactose; Metabolism; Positron-emission tomography; Transgenic mice

Poster code: R-01-03-100

Poster Title: Use of infrared thermography and contact temperature analysis to determine human skin surface temperature after magnesium chloride solution application

PhD candidate: Stjepan Ćurić

Part of the thesis: The rate of transdermal activity of ionized magnesium chloride solution on peripheral vasoconstriction - randomized thermographic and thermocontact trial

Mentor(s): Assist. Prof. Mario Zovak, MD PhD, Assist. Prof. Srećko Sabalić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: This study presents method for determining human skin surface temperature by infrared thermography and contact temperature analysis after transdermal magnesium chloride solution and saline solution transdermal application. Comparative measurements of temperature using infrared thermography and contact temperature measurement, after applications of both solutions on the examinees both gastrocnemius muscle skin, were made.

Materials and methods: The study was conducted on 10 healthy female subjects, aged between 20-25 years. Prior to the beginning of the study, all examinees have signed an informed consent. All examinees were engaged in recreational sports. The examinees were immersed in 13^{o}C cold water up to the upper half of the both patellas for 7 minutes to achieve the peripheral vasoconstriction effect. After leaving the water tank, excess water from the surface of the body was removed with paper towels. After that, magnesium chloride solution in spray was applied to the non-dominant leg, and saline solution in spray was applied to dominant leg, in the same amounts. The examinees did not have any insight which solution was placed on which leg. Four thermocouples of T-Type then were placed in the gastrocnemius area of both lower legs. After installing the thermocouples, transparent adhesive foil has been placed on both examined areas. Recording with a thermographic camera took 10 minutes. Through the whole process, examinees were still. FLIR infrared thermography camera ThermaCam SC2000 PAL was used for testing purposes. Computer and software for detailed analysis thermogravure ThermaCam Researcher 2002 were also part of the system.

Results: In the study, the faster recovery of the skin surface temperature of the leg that was treated with a solution of magnesium chloride in spray is detected compared to the leg that was treated with saline solution. The difference recorded on the thermographic camera is visible after just two minutes of recording. The difference on the skin surface temperature of both lower legs is on average greater than 0.7 Celsius degrees after ten minutes of measurement.

Discussion: Peripheral vasoconstriction is an autonomous response to cold exposure, thus preventing the transfer of heat to the environment through the skin. Peripheral vasoconstriction is more dependent on inner body temperature than on skin temperature. Skin vasoconstriction is mainly controlled through the sympathetic part of the autonomic nervous system. During cold exposure, norepinephrine is released from the endpoints of the sympathetic nerve and induces vasoconstriction via α-receptor or vasodilatation by the β-receptor. The skin of the extremity mainly contains α2 receptors and thus exhibits strong vasoconstriction. Magnesium inhibits the release of norepinephrine by blocking N-type calcium channels on peripheral sympathetic ends. In this study, thermographic and contact temperature analysis have detected the transdermal activity of the magnesium chloride solution on the sympathetic response of the organism after exposure to cold. Finally, the proposal of the common framework for further investigations is provided.

MeSH/Keywords: magnesium chloride, infrared thermography, skin surface temperature, peripheral vasoconstriction

Poster code: R-01-03-149

Poster Title: PTCH1 protein expression in high-grade serous ovarian carcinoma cell lines

PhD candidate: Valentina Karin Kujundžić

Part of the thesis: Epigenetic changes and expression of PTCH1, SHh and IHh proteins in serous ovarian carcinomas

Mentor(s): Professor Ljiljana Šerman, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Patched-1 (PTCH1) is a transmembrane protein involved in Hedgehog (Hh) signaling pathway, which plays a significant role in the normal embryonic development of invertebrates and vertebrates. PTCH1 functions as a pathway inhibitor which constitutively represses Hh signaling in the absence of Hh ligands. Since Hh signaling promotes cell proliferation, invasion, and migration, its increased activation is associated with tumor development, while in adult organism, this signaling pathway is mostly inactive or poorly active. In order to better understand the behavior of PTCH1 in ovarian carcinoma, we decided to investigate its localization and protein expression in high-grade serous ovarian carcinoma cell lines.

Materials and methods: High grade serous ovarian carcinoma cell lines OVCAR5, OVCAR8 and OVSAHO were used for this study. Expression and localization of PTCH1 protein were examined by immunofluorescence using two different polyclonal antibodies for N-terminal region of PTCH1 and, after extraction of proteins from different cellular compartments, by western blotting, using one monoclonal and three different polyclonal antibodies for N-terminal region of PTCH1.

Results: Immunofluorescence staining using two different polyclonal antibodies showed that PTCH1 was expressed in cytoplasm and nucleus in all three high-grade serous ovarian carcinoma cell lines. Western blot results showed that PTCH1 was present in cytoplasmic, membrane, soluble nuclear and chromatin-bound nuclear protein extracts in each cell line, when polyclonal antibodies were used. In the case of monoclonal antibody, PTCH1 was present in: cytoplasmic, membrane, soluble nuclear and chromatin-bound nuclear protein extracts in OVCAR5 cell line; cytoplasmic, membrane and soluble nuclear protein extracts in OVCAR8 cell line; and soluble nuclear and chromatin-bound nuclear protein extracts in OVSAHO cell line. PTCH1 protein fragments of different molecular weight were detected in the same protein extracts.

Discussion: OVCAR5, OVCAR8 and OVSAHO cell lines exhibit aberrant (nuclear) PTCH1 protein expression. The fragments of different molecular weight indicate a possible proteolytic cleavage of PTCH1 protein, so it may be possible that some of the fragments translocate to the nucleus after proteolytic cleavage. Therefore, PTCH1 may play an active transcriptional role in these high-grade serous ovarian carcinoma cell lines. Further studies should confirm the relevance of the observed alterations.

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.

MeSH/Keywords: Ovarian carcinoma, PTCH1, OVCAR5, OVCAR8, OVSAHO

Poster code: R-01-05-024

Poster Title: Cell-free DNA methylation of RASSF1A and PRSS21 in blood and ejaculate of patients with testicular non-seminomas

PhD candidate: Jure Krasić

Part of the thesis: Cell-free DNA methylation of RASSF1A and PRSS21 in blood and ejaculate of patients with testicular non-seminomas

Mentor(s): Assist. Prof. Nino Sinčić, MD PhD, Tomislav Kuliš, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Testicular germ cell tumors (TGCT) are the most common tumors of younger male populations with an increasing incidence rate and high mortality in the Croatian population. Non-seminoma is a type of TGCT that appears at an earlier age with aggressive progression. Methylation of DNA is a prominent tumor biomarker due to its high frequency and stability and is associated with tumor growth. Due to tumor heterogeneity and the invasive nature of classic biopsies, modern science is oriented towards research of liquid biopsies as a non-invasive and precise way of cancer detection. Within liquid biopsies cell-free DNA methylation has shown itself as a prominent biomarker. Research on cell-free DNA in non-seminoma are scarce and have been done mostly on blood samples, with little to no work being done on ejaculate.

Materials and methods: Blood and ejaculate samples has been collected from 24 healthy volunteers for use as the control group. Cell-free DNA has been isolated from the samples and quantified by spectrophotometry. Non-seminoma patients blood and ejaculate samples pre and post-operation have been taken from 15 patients. The patients have been diagnosed by resident pathologists with emphasis on various non-seminoma components present in the tumor. Archived blocks have been taken from 69 non-seminoma tumors and nine healthy testes from the pathology departments of KBC Zagreb and KBC Sestre Milosrdnice for analysis of RASFF1A and PRSS21 protein expression in healthy testis tissue vs various non-seminoma components. Protein localization and expression was analyzed by immunohistochemistry semi quantitatively by morphometric analysis.

Results: Cell-free DNA yields are higher in ejaculate samples than blood samples of healthy volunteers, with the average ejaculate sample totaling 237 ng of cell-free DNA vs 95 ng of blood samples. Among the 14 non-seminoma patients there are three pure forms tumors (two embryonal carcinomas and one teratoma) and 11 mixed germ cell tumors among which there are eight embryonal carcinoma, seven teratoma, six yolk sacs, five seminoma and one choriocarcinoma components. Protein localization and expression analysis on tissue slides of archived tissue has shown a difference in both expression and localization of RASSF1A and PRSS21 in healthy testis vs non-seminoma components.

Discussion: Higher cell-free DNA yield in ejaculate samples could be explained by the reproductive system being a smaller system than the vascular circulatory system and by the generation/breakdown of sperm cells. The component distribution among our patients corresponds to ratios listed by the World Health Organization. Protein localization and expression differences in archived tissue slides suggests that absence of RASSF1A and PRSS21 could serve as a biomarker of all non-seminoma component development and presence.

MeSH/Keywords: Testicular tumors, nonseminoma, biomarker, epigenetics, cell-free DNA

Poster code: R-01-05-109

Poster Title: Ubiquitin E3 ligase Mid1 mediates subchondral bone resorption in a mouse model of rheumatoid arthritis

PhD candidate: Nina Lukač

Part of the thesis: Cellular and molecular mediators of subchondral bone destruction in arthritis

Mentor(s): Assoc. Prof. Nataša Kovačić, MD PhD

Affiliation: Department of Anatomy and Laboratory for Molecular Immunology, Croatian Institute for Brain Research, University of Zagreb School of Medicine

Introduction: Subchondral bone resorption often accompanies synovial joint inflammation in rheumatoid arthritis (RA) and causes disability which is not reversible by currently available therapeutics. Using antigen-induced arthritis (AIA), a murine model of RA, we have shown that mice deficient for Fas gene (Fas -/-) develop an ameliorated form of AIA characterized by the absence of subchondral bone resorption. This non-resorptive arthritis is marked by a lower frequency of synovial myeloid cells, which down-regulate Mid1 gene. The objective of the study was to evaluate the role of Mid1 in bone resorption in AIA.

Materials and methods: C57BL6 wild-type (WT) and Fas-/- mice were immunized with methylated bovine serum albumin (mBSA) emulsified in complete Freund's adjuvant, and arthritis was induced by subsequent intra-articular injection of mBSA. Bone resorption was assessed by micro-CT. Synovial myeloid (CD11b+Gr-1+) cells from Fas -/- and WT mice were sorted using BD FACSAria and their transcriptome was further analyzed by Affymetrix ST 2.0 arrays. Bioinformatics analysis was performed using Bioconductor and differences in gene expression were confirmed by qRT-PCR. WT mice with arthritis were treated in vivo with metformin, which inhibits proinflammatory effect of Mid1, at daily dose 1g/kg, to assess its effect on the development of arthritis.

Results: Mid1 gene was up-regulated in sorted myeloid cells (logFC=2.01, p(BH-adjusted)=0.0003, limma+BH-adjustment) and bulk joint tissue (logFC=8.74, p=0.02, Welch-test) of WT mice in comparison to Fas-/- mice with ameliorated arthritis. Despite its position on X chromosome Mid1 expression in joints was not sexually dimorphic and was up-regulated in both male (logFC=1.92, p=0.006, T-test) and female WT mice (logFC=8.74, p=0.02, Welch-test) with arthritis (WT-AIA). Furthermore, expression was associated with an increase in knee diameter (ρ=0.68, p=0.03, Spearman's rank correlation) as well as with gene expression levels of pro-inflammatory cytokines in joints of mice with arthritis (IL-1: ρ=0.78, p=0.008; IL-6: ρ=0.70, p=0.025; TNF: ρ= 0.78, p=0.008, Spearman's rank correlation). Metformin treatment of WT-AIA mice ameliorated the severity of arthritis assessed by knee diameter (3.68±0.22mm WT-AIA vs. 3.30±0.17mm WT-AIA+metformin, p=0.01, T-test) and visual score (2.33±0.88 WT-AIA vs. 0.5±0.5 WT-AIA+metformin, 0-4 scale, p=0.003, T-test). Arthritis-induced epiphyseal trabecular bone volume loss was not significantly different in metformin treated group in comparison to non-immunized (NI) control group (40.08±4.07% WT-NI vs. 33.44±4.37% WT-AIA+metformin, p=0.056, ANOVA+Tukey-Kramer), whereas it was significantly reduced in non-treated mice with arthritis (40.08±4.07% WT-NI vs. 30.38±4.43% WT-AIA, p=0.004, ANOVA+Tukey-Kramer).

Discussion: Increased expression of Mid1 gene has already been reported to promote allergic airway inflammation, which is dependent on death receptor TRAIL. Results suggest the role of Mid1 as a novel mediator joint inflammation and subchondral bone destruction in arthritis, and a potential therapeutic target for inflammation-mediated joint destruction.

Acknowledgments: This work was supported by the Croatian Science Foundation project number 7406.

MeSH/Keywords: Mid1, bone resorption, arthritis

Poster code: R-01-06-079

Poster Title: Developmental reorganization of human amygdaloid nucleus

PhD candidate: Damir Mulc

Part of the thesis: Developmental reorganization of human amygdaloid nucleus

Mentor(s): Professor Mario Vukšić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Structural and functional reorganization of neural circuits is one of the key features of the human brain during development. The amygdala (lat. corpus amygdaloideum) is a basal ganglion situated in the temporal telencephalic lobe which functionally belongs to the limbic system. Connected to various cortical and subcortical structures it plays a basic role in processing complex patterns of emotional influence. Nevertheless, studies on its prenatal development in humans are very scarce. It has been observed that the amygdala is very early recognizable in the human brain. The two main subdivisions, basolateral and corticomedial, have a diverse developmental origin and functional roles. It has previously been demonstrated a human specific transient modular organization of this structure during the midfetal period (Nikolić & Kostović, Anat Embryol 1986).

Materials and methods: To analyze the expression patterns of different developmentally regulated proteins in relation to synaptic, neuronal, glial and extracellular matrix development, histological analysis will be performed on postmortem human brains, ranging from 10 postconceptional weeks (PCW) to 6.5 years of age. The obtained results will be compared with in vitro MRI brain data to corresponding developmental age. In order to investigate the relationship between structural changes and spatio-temporal expression of specific candidate genes, we will also analyze publicly available gene expression database (Kang et al. 2011). In-situ hybridization and immunohistochemical methods will be performed to elucidate in which cells these genes are expressed.

Results: By reviewing the histological and immunohistochemical criteria in previous literature we chose to divide amygdala into nuclei that could be successfully traced in the fetal brain. We also selected several genes which are highly expressed prenatally in the human amygdala. Expression of distal-less homeobox 6 (DLX6) was visible in the amygdaloid primordiums in the prospective central nucleus. Double immunofluorescence and confocal analysis revealed that NeuN-positive cells contained DLX6. On MRI scans, reliable identification of the amygdala was seen at around 16 PCW. The measurements of amygdala volume, determined using manual segmentation, showed an increase which correlates with overall brain growth, with periods of acceletation before around 20 PCW and after 30 PCW.

Discussion: New morphometric data on the development od the human amygdala will be used for setting the baseline for studying developmental abnormalities present in various neurological, psychiatric and cognitive diseases.

MeSH/Keywords: fetal brain development, fetal amygdala MRI segmentation, fetal brain gene expression

Poster code: R-01-08-029

Poster Title: The distribution of demyelinating lesions within the cerebral white matter segments and their correlation with clinical status in patients with multiple sclerosis

PhD candidate: Sarah Stančić - Rokotov

Part of the thesis: The distribution of demyelinating lesions within the cerebral white matter segments and their correlation with clinical status in patients with multiple sclerosis

Mentor(s): Assoc. Prof. Milan Radoš, MD PhD, Anton Vladić, PhD, senior research associate

Affiliation: University of Zagreb School of Medicine

Introduction: The development of human cerebral white matter (WM) represents a complex set of developmental processes characterized by precisely regulated timetable of axonal growth and their spatial arrangement within cerebral wall. When reading radiological report, WM is roughly divided into periventricular, deep and subcortical WM. Since this division does not involve developmental and functional complexity of WM, no significant correlation can be made between radiological and clinical findings. Clinical symptoms identified in patients with MS and their neurological outcome often do not correlate with the extent of demyelination of MS lesions on MRI. In same severe demyelinated lesions induce only discrete neurological symptoms while in other patients' discrete lesions can cause severe clinical impairments. This study was based on the hypothesis that correlation between the neuroradiological and clinical findings could be improved if distribution of demyelinated lesions would be analyzed more precisely using segmentation according to Von Monakow (V) which appreciate developmental and functional complexity of WM. Each segment of WM (I-V), as classically described by von Monakow, can be visualized by using magnetic resonance imaging (MRI).

Materials and methods: This study has prospective character of MR brain examination involving 100 patients with MS. The inclusion factors are based on the McDonald Criteria for MS 2017. The excluding factor is presence of demyelinated cervical lesions. The age of the patient will be greater than 18 and less than 60 years. Patients with MR scans done according to the 3D demyelinating disease protocol, including FLAIR intercepts, will be analyzed from which the volume distribution of MS lesions can be calculated within the white matter segments. Lesions will be distributed according to its spatial distribution into one of the five segments of white matter. For manual segmentation and volumetric lesion analysis, the ITK-SNAP 3.2. program will be used (University of Pennsylvania, USA, open code program). A particularly well-trained and experienced neurologist will examine each patient prior to the MR examination of the brain to a clinical neurostatus according to Expanded Disability Status Scale and Multiple Sclerosis Functional Composite.

Results: Four patients were thoroughly processed for this analysis. First, they were examined by an experienced neurologist, who determined their neurological status, MSFC and EDSS scale. The same day an MRI scan was done with 3D FLAIR sequence and volumetric analysis of demyelinating lesions by segments. Mean volume of lesions in all patient is 3950,9 mm^3 ±520,5, thereof in 1st segment lesion volume is 5417mm^3 ± 1074, in 2nd segment 3247, 1 mm^3 ± 1106, 6, in 3rd segment is 5172,1 mm^3 ± 1319,5, in 4th segment is 1887,9 mm^3 ± 326,3. and in the 5th segment is 79,65 ± 0,5. The 5th segment which represents intracortical white matter lesion is not possible to quantify in a reliable manner. In this small sample of patient's statistical analysis of lesion volumes and neurological status is inadequate. The study is still in progress and the results are still not complete. The study should be continued and date subjected to more detailed statistical analysis to have definitive conclusion.

Discussion: Gathering the patients for the study was slower than expected due to the excluding factor, absence of cervical demyelinated lesions, with the intent of better correlation of neurological disability and presence of demyelinating lesions in segments of white matter. Our research will contribute to a better correlation of neuroradiological and clinical findings in patients with multiple sclerosis. We hope that the analysis of the distribution of demyelinating lesions in the white matter segments to Von Monakow will show which segments of white matter have the greatest significance for the development of disability.

MeSH/Keywords: Multipla sclerosis, Magnetic Resonance Imaging, EDSS, MSFC, white matter segments, demyelinating lesions

Poster code: R-01-08-061

Poster Title: Analysis of the developmental transcriptome of the human anterior cingulate cortex

PhD candidate: Mihaela Bobić Rasonja

Part of the thesis: Reorganizacijski procesi u fetalnoj prednjoj i srednjoj regiji cingularne vijuge čovjeka

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

Affiliation: University of Zagreb School of Medicine

Introduction: The developmental and differentiation processes of prenatal development of the anterior cingulate cortex (ACC), a region involved in autonomic, behavioral and emotional functions, hasn't been yet elucidated although researched to some extent. Since this region has proven roles in neurodevelopmental disorders, such as autism spectrum disorder, obsessive-compulsive disorder, and other psychiatric disorders, our aim is to time developmental and glial and neuron differentiation points in it.

Materials and methods: Using the publicly available gene expression database (Kang et al., 2011) and Partek Genomic Suite 6.0, we looked for upregulated genes (fold change>2) from 19 PCW (postconception weeks) to 6 postnatal months in medial prefrontal cortex (anterior cingulate cortex), in comparison to other limbic regions, neocortical regions and all brain regions. Using PubMed, RefSeq and GeneCards databases, we chose upregulated genes that could have or have proven a role in neuronal migration, neuronal and glial differentiation, the establishment of cell-cell connections, synapse formation, synthesis of extracellular matrix components, axon guidance, dendrite growth, apoptosis, and neurodevelopmental disorders. The selection was narrowed to genes that are protein coding in brains of other mammals, ones that have a commercially available antibody proven to be efficient in immunohistochemistry-paraffin. Immunohistochemistry on 8 human fetal postmortem brain samples aged 16 postconception weeks (PCW) to newborn age (part of the Zagreb Neuroembryological Collection) was done for proteins MEF2C (Myocyte Enhancer Factor 2C), PCDH17 (Protocadherin 17) and SEMA3A (Semaphorin3A).

Results: The greatest differences in gene expression between the anterior cingulate region and other brain regions were found during midfetal period (19 PCW-24 PCW). In this timeframe, a total of 857 upregulated genes was found in the ACC and the selection was narrowed to 26 genes using the above-mentioned criteria. Immunohistochemical detection of MEF2C, PCDH17, and SEMA3A was performed on fetal brain tissue samples. Although transcripts for these genes show high expression in ACC, contrary to our expectations, regionally specific tissue staining for these proteins in the ACC was not found.

Discussion: Many developmental processes occur and overlap in the ACC during midfetal period, hence there are surely many genes that contribute to particularities of the ACC both in development and in adult age. Thus, the midfetal period is expected to have the biggest difference in gene expression in comparison to other brain regions and our research has confirmed that. Since there is a variable time gap between mRNA expression and protein synthesis, more brain samples of different age should be stained in order to find regionally specific expression of these proteins. Also, other gene-candidates and brain samples should be researched with the purpose of explaining the specific features of anterior cingulate cortex development.

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

MeSH/Keywords: limbic system, development, axon guidance.

Poster code: R-01-08-062

Poster Title: RONNA robotic system in brain biopsies: A prospective study of 23 consecutive cases

PhD candidate: Domagoj Dlaka

Part of the thesis: Accuracy of the RONNA Robotic System in Stereotactic Neurosurgery

Mentor(s): Assist. Prof. Darko Chudy, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Robotic neuronavigation is becoming an important tool for neurosurgeons. We present a prospective study of 23 consecutive frameless stereotactic biopsies performed by the RONNA robotic neuronavigation system. The purpose of this study is to determine its accuracy, safety and diagnostic yield.

Materials and methods: Between February 2018 and March 2019 brain biopsies with RONNA robotic system were performed on 23 patients. There were 16 males and 7 females, average age 59.3 years (range 28 – 82). Trajectories were planned on preoperative MSCT. The distance between the planned and the real trajectories were computed on postoperative MSCT and MR images for detailed EPE (entry point error) and TPE (target point error) measurement together with significant clinical data.

Results: Diagnostic yield was 95.6 % (22/23). Mean trajectory length was 51.1 mm (range 15.5-86.1). The mean EPE was 1.8 mm (range 0.4-3.4) and mean TPE was 2.1 mm (range 0.4-4.7). There was no postoperative mortality or morbidity and there were no infections, one patient had significant postoperative intratumoral haemathoma (3 cm) without neurological worsening.

Discussion: This pilot study proved RONNA robotic system to be safe and precise tool for brain biopsy. Further investigation is required in a larger patient sample and for wider application of the robotic system.

MeSH/Keywords: RONNA, robotic neurosurgery, minimal invasive surgery, brain biopsy, stereotaxy

Poster code: R-01-08-083

Poster Title: Role of lactoferrin in short-term neurological outcome of very preterm newborns

PhD candidate: Damir Lončarević

Part of the thesis: Role of lactoferrin in short-term neurological outcome of very preterm newborns

Mentor(s): Assoc. Prof. Emilja Juretić, MD PhD, Professor Vlatka Mejaški-Bošnjak, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Encephalopathy of prematurity relates to cognitive, behavioural and motor deficits (eg, cerebral palsy - CP) in VPI (very preterm infants) defined as < 32 weeks of postmenstrual age (PMA). Inflammation is a major cause of developmental brain injury. Processes following perinatal brain injury include impaired axonal growth, reduced plasticity, and altered cell number. Activated microglia seems to perpetuate these effects. Late-onset sepsis (LOS) and necrotizing enterocolitis (NEC) are the most common causes of neonatal mortality and morbidities in VPI. High rate of associated comorbidities (bronchopulmonary dysplasia, BPD; and retinopathy of prematurity, ROP) increase with lower PMA. Adverse neurodevelopmental outcomes after LOS or NEC may be due to inflammatory injury. Lactoferrin (Lf), a key component of mammalian milk exerts many actions including antimicrobial, immunomodulatory and anti-inflammatory. Lf reduces microglial inflammatory activity, it enhances brain volume and it has a potential therapeutic effect in BPD and ROP. Hypothesis of this study is that oral bovine Lf (bLF) supplementation will result in significant difference in short-term neurological outcome in VPI. Secondary outcomes are differences in the incidence of LOS and NEC, as well as BPD and severe ROP.

Materials and methods: In this prospective, randomized, double blind, placebo-controlled study, bLF will be substituted to enteral feeds to 50 and sucrose (placebo) to 50 control preterm infants of ≤ 32 PMA. Intervention starts within 3 days after birth, after initial stabilisation and informed consent form parents obtained. BLf is added to expressed breast-milk or formula. Treatment is given twice daily at a dose of 200 mg of bLf or the same aliquot of sucrose and continued until 35 weeks PMA. Infants are stratified into two groups according to PMA at birth: 24-28 and 28-32 weeks. To test the difference for the primary outcome, neurologic assessment (Premie-Neuro) and electroencephalography (EEG) recordings will be carried out in regular intervals. Findings will be scored as normal, mildly, moderately and severely abnormal. LOS will be defined as detection of clinical symptoms, presence of laboratory findings with or without isolation of a causative organism. Bell's stage II or III of NEC will be reported. Severe ROP will be graded as treated medically or surgically and BPD if the infant is still receiving ventilator support or supplemental oxygen at 36 weeks' PMA. All relevant data and risk factors will be collected. Exclusion criteria are congenital malformations and recognized neurogenetic syndromes. Statistical analysis will be conducted in R programme system using "mixed effect models" analysis.

Results: In one year period 36 infants have been enrolled and 6 excluded. As expected, fewer infants in lower PMA groups have been evaluated with higher percenatge of exclusion. A trend toward higher scores on Premie-Neuro in bLf compared to placebo group has been observed. Differences seem to be more marked between lower PMA groups. EEG classifier emphasizes the difference in primary outcome with more normal findings in the immature bLf group approaching term-corrected age. There is also a trend of reduction of NEC and LOS (all subgroups). No differences have been noted between other secondary outcomes (BPD, ROP).

Discussion: A recent systematic review suggests that bLf supplementation to enteral feeds decreases LOS and NEC in preterm infants without adverse effects. Neurological outcome however hasn't been reported. Dysbiosis of gut microbiota in preterm infants could affect shaping of neural networks. Lactoferrin has been shown to play a pivotal role in establishing a healthy gut microbiome. Lf substitution could prove to be relevant neuroprotective strategy in developing brain. Preliminary results of this study are supportive in that regard. The observed differential effect in more immature infants could be due to higher amounts of bLf per body weight and longer exposure.

MeSH/Keywords: preterm newborn, lactoferrin, neurological outcome

Poster code: R-01-08-114

Poster Title: Prediction of periventricular leukomalacia and intracranial haemorrhage in preterm infants using cerebral near-infrared spectroscopy

PhD candidate: Tomislav Ćaleta

Part of the thesis: Near-infrared spectroscopy in prediction of periventricular leukomalacia and intracranial haemorrhage in preterm infants

Mentor(s): Assist. Prof. Ruža Grizelj, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: The mortality of prematurely born infants reaches 20%, and among the survivors around 25% have some form of neurodevelopmental disorders. Due to hemodynamic instability in the first days of life premature babies are at high risk for brain damage. The noninvasive measurement of cerebral oxygenation by near-infrared spectroscopy (NIRS) is a method for estimating cerebral hemodynamics by measuring regional saturation (rScO2) which correlates with cerebral blood flow and it can be considered a surrogate for brain perfusion. NIRS can be used to evaluate the autoregulation abnormalities of blood vessels. Our hypothesis is that rScO2 during the first three days of life differ in prematurely born babies that develop intracranial bleeding and/or periventricular leukomalacia in relation to those in which the above-mentioned brain damages do not occur. The aim is to analyze the impact of rScO2 pattern on the formation of brain damage. Investigate whether there are different trend patterns of rScO2 between patients with different type of brain damage.

Materials and methods: The study will include infants born ≤32 weeks gestation and was admitted at our unite on the first day of life. Patients with congenital anomalies, hemodynamically significant ductus arteriosus and IVH at the time of admission, will be excluded. Various perinatal and neonatal variables will be recorded. Serial head ultrasounds are going to be performed. Brain MRI will be performed on two occasions (before 32 weeks of postmenstrual age and at term equivalent age). According to the findings, we will classify the patients into three groups: normal, white matter damage and intracranial bleeding.

Results: So far we included 34 patients. 8 were excluded from our study (1 due to associated congenital comorbidities and 7 because they died). 8 patients are still admitted in our unit and we are still collecting their data. 7 patients haven't still reached term corrected age. The demographic findings from the patients that we have so far collected data are as follows: 54% male, 5 patients have been born from a twin pregnancy, average gestational age at birth was 30,3 weeks, average birth weight 1427g (min 720g, max 2250g), average Apgar score in the 5th minute 7,7, only 6 patients received full dose of prophylactic corticosteroid therapy for lung maturation, average score for neonatal acute physiology and perinatal extension: SNAP II was 23,7 (+/- 25,5), SNAPPE II 28,4 (+/- 32,3). Regarding complications, 6 patients developed bronchopulmonary dysplasia, 4 had necrotizing enterocolitis, 3 patients developed mild retinopathy of prematurity and didn't need any therapy for it. Most of the head ultrasound and MRI findings are still being evaluated.

Discussion: Based on the preliminary results, our patient group, according to demographic indicators, is an average group of prematurely born infants. The mortality and development of complications of our patient group are within the expected values according to existing epidemiological studies.Considering that a part of our patients is still hospitalized and that a part has not yet reached the term equivalent age, we can not derive definitive statistical conclusions about the effect of rScO2 values measured with a NIRS device and the consequent brain damage observed on the head ultrasound and MRI. However, some statistical parameters indicate that patients who had a continuous lower rscO2 had a higher mortality rate. There are also some statistical data which (for the time being still statistically not significant) indicate that the lack of brain flow autoregulation (which is manifested in the form of stabilization and absent fluctuations of the rScO2 values) is a good prognostic factor for the development of brain damage primarily in the form of intraventricular hemorrhage and/or periventricular leukomalacia.

MeSH/Keywords: premature infants, brain damage, near-infrared spectroscopy

Poster code: R-01-08-118

Poster Title: The possibility for development of non-transgenic rat tauopathy model by application of tau oligomers into the entorhinal cortex

PhD candidate: Lea Langer Horvat

Part of the thesis: The possibility for development of non-transgenic rat tauopathy model by application of tau oligomers into the entorhinal cortex

Mentor(s): Professor Goran Šimić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Sporadic Alzheimer's disease (AD) is the most common secondary tauopathy characterized by progressive loss of cognitive functions and behavioral impairment. The accumulation of hyperphosphorylated tau and the disruption of microtubules positively correlate with neuropathological changes, which progress in a stereotypical manner with the first lesions in the locus coeruleus and the entorhinal cortex from where they spread to the hippocampus and high-order neocortical regions. With this research we aimed to explore if intracerebral injection of tau oligomers and tau fibrils will induce aggregation and trans-synaptic spread of pathological tau proteins from the site of injection and will those changes be associated with expected cognitive impairment.

Materials and methods: Three to four months old male Wistar rats (n = 96) randomly divided into three groups were stereotaxically injected into the entorhinal cortex with tau oligomers (4 μg), preformed tau fibrils (4 μg) or phosphate-buffered saline. Animals were analyzed 3 days, 4 months, 6 months and 9 monthspost-injection (10 rats per group). Cognitive performance was tested using open field, T-maze rewarded alternation task, novel object recognition (NORT) and object-location test (OLT). To specifically detect tau protein changes and perform staging of tau protein pathology in the rat brain, we used anti-tau antibodies T22, AT8, HT7, and PHF1. Tentative amyloid changes were assessed using anti-amyloid antibody 4G8. Proteins isolated from the entorhinal cortex and hippocampus were analyzed by immunoblotting using anti-tau antibodies HT7, AT8, PHF-1, and Tau5.

Results: We present preliminary results obtained on 3 groups of animals sacrificed 4 months after intracerebral injection. Percentage of total investigation time of novel object as well as discrimination index showed no significant differences between groups ($p = 0.60$ for novel object investigation time). Long-term (24 h) object recognition memory was intact in both TO and TF animals at 3 months of age. In the object location test comparison of percent total investigation time of the moved object during the training trial versus the testing time showed no significant increase in investigation of the object after it is moved thus suggesting that rats didn't remember where the object was located during training ($p = 0.25$). Data obtained from T-maze rewarded alternation tasks was used to assess the working memory of rats. Tau fibril group of animals displayed impaired performance compared to their respective control in T-maze (-25%, $p < 0.05$). Immunohistochemistry revealed positive HT7 signal in the brainstem and transentorhinal region only in the group injected with tau fibrils, but not in the group injected with tau oligomers or PBS. Oligomeric tau was present both ipsilaterally and contralaterally to the injection site, but not in the control rats. The Ser202/Thr205 phosphorylated tau epitope visualized by using AT8 antibody showed weak immunoreactivity in both tau fibril and tau oligomer group of rats. PHF and HT7 immunoreactivity were much higher in animals injected with tau oligomers in comparison to control groups.

Discussion: Our preliminary results indicate that stereotaxic injection of tau oligomers or tau fibrils induces phosporylation of AT8 epitope of tau protein and tau oligomers in rat brain 4 month post-injection. Using antibody HT7, which recognizes human (and not murine) tau, revealed a signal present in the brainstem 3 days and 4 months after intracerebral injection of tau fibrils. Evaluation of hippocampal function as well as function of other cortical regions involved in object recognition showed no obvious deficit in all 3 groups of animals tested. T-maze under a food reward alteration, used to assess the working memory, showed that there was much slower learning curve with more incorrect choices in rats injected with tau fibrils in comparison to the control and the tau oligomer

group. Understanding the role of tau oligomers and tau fibrils in neurodegeneration has a great importance for revealing mechanisms underlying development and progression of AD and other tauopathies.

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MeSH/Keywords: Alzheimer disease, rats, tau proteins, neurofibrillary tangles

Poster code: R-01-08-133

Clinical medical sciences - Preliminary research results

Poster Title: Comparison of sublingual sufentanil tablet release system and local infiltration analgesia for post-operative pain management in total knee arthroplasty

PhD candidate: Burhan Hadri

Part of the thesis: Comparison of Sublingual sufentanil tablet release system and local infiltration analgesia for postoperative pain management in total knee arthropasty

Mentor(s): Assist. Prof. Daniela Bandić Pavlović, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Total knee arthroplasty (TKA) as a procedure is very common in population. Postoperative pain after total arthroplasty is severe. Postoperative pain release is very important in the earlier mobilization of the patient. Local infiltration analgesia (LIA) technique is a simple surgeon administration technique, which provides efficient analgesia in early postoperative stage in TKA. Sufentanil sublingual tablet system, is a preprogrammed, noninvasive, patient activated bedside system that enables patients to manage moderate to severe acute pain in a hospital setting. Study will compare efficacy of sufentanil sublingual tablet release system and local infiltration analgesia in postoperative pain management in patients after total knee arthroplasty.

Materials and methods: This prospective intervention clinical study will include 100 total patients undergoing elective surgery for total knee arthroplasty in a randomized clinical trial that will be conducted in the University Clinical Center of Kosovo, in Prishtina. Age, gender, and also diagnosis, duration of surgery and anesthesia, perioperative vital signs (noninvasive monitoring), length of hospitalization, will be recorded for all patients Patients will be randomized before the surgery (after indication and eligibility for inclusion). Pain intensity will be measured by 11 – point numerical rating scale (NRS) where 0- is no pain and 10 – is worst possible pain, after 15, 30, 60, 90, 120 minutes and then every 3 hours. Total pain relief scores will be recorded at 6, 12,24 hours, and 5 – point rating scale questionnaire for patient satisfaction where will be completed at 24 hours after surgery. All adverse effects during surgery and first 24h after surgery will be recorded. Clinical study consists of two groups of patient undergoing total knee arthroplasty. Spinal anesthesia will be used as type of anesthesia for all patients included in the study: Group of 50 patients will use SSTR system for postoperative analgesia and other group of 50 patients will use LIA for postoperative analgesia. Data about the NRS pain scores, total pain relief score, duration of hospitalization, patient satisfaction, complication and adverse effects, and also all other medication use for management of pain will be recorded in first 24h. Post-operative analgesia SSTR system is to be administered in a hospital setting only. It is for sublingual use only. The Sufentanil sublingual tablets are to be self-administered using the administration device. Local infiltration of knee joint using 40ml of bupivacaine 0.25% with adrenaline 1:200 000, diluted to 150ml with saline 0.9%. Rescue medicine Morphine: 3 mg bolus dosage until pain in rest is less than 3.

Results: The study is currently in the phase of data collection.

Discussion: Epidural analgesia with different mixture of drugs is efficacious, but it has side effects and major complications. Peripheral nerve blocks can lead to the nerve injury and these techniques can be technically demanding. Because of the side effects of the traditional techniques for management of pain, lately the interest has grown in local infiltration analgesia. Postoperative pain release is very important in the earlier mobilization of the patient. Research studies were directed to improve analgesia, reduce surgical stress and organ response, reducing nausea or vomiting and start early oral nutrition. Use of opioids, administered parenteral or by patient-controlled analgesia devices and epidural analgesia are very effective in pain management, but because of their systemic side effects, multimodal analgesia is developed. No previous clinical trials exist comparing opioid patient control analgesia by oral route and local infiltration analgesia for postoperative pain. Results will contribute to an improvement efficacy and safety of postoperative analgesia after total knee arthroplasty.

MeSH/Keywords: pain, postoperative,; administration, sublingual,; sufentanil,; anesthesia, infiltration

Poster code: R-02-01-056

Poster Title: Intraoperative comparison between standardized vs protective lung ventilation in controlling postoperative respiratory complications

PhD candidate: Maša Kontić

Part of the thesis: The effect of protective anesthesiological management procedures on prevention of respiratory complications

Mentor(s): Assoc. Prof. Dinko Tonković, MD PhD

Affiliation: University of Zagreb School of Medicine; General Hospital "Dr Ivo Pedišić", Department of anaesthesiology, reanimatology and intensive treatment, Sisak, Croatia

Introduction: Protective lung ventilation during general anaesthesia diminishes postoperative respiratory complications regarding standard lung ventilation. The aim of this study and its effect shall be weighed against postoperative respiratory complications and hemodynamic changes. Intensive care treatment and its measures are compared in terms of duration and outcome.

Materials and methods: The study enclosed two-year period of time between April 2015 and December 2016. Fifty three participants were divided into two groups, with written consent to participate in this study. The median age for participants was 66,4 compared between groups. It ranges from 30 to 86, including elective urological and abdominal laparotomic procedures, cardiopulmonary comorbidities, American Society of Anaesthesiologists physical status II to III. 23 subjects were analysed using standard lung ventilation, which includes higher inspiratory oxygen concentration during pre-oxygenation and maintenance of anaesthesia. 30 subjects were analysed using protective lung ventilation, lung recruitment, application of higher positive end-expiratory pressure (PEEP), optimal oxygenation by maintaining peripheral arterial saturation above 94%, lower inspired oxygen concentration and extubation using continuous positive airway pressure immediately prior to extubation. In our current study, we observe respiratory, hemodynamic and inflammatory parameters focused on data collection period.

Results: There were no statistically significant differences between the two groups in descriptive data (Mann Whitney U test ; $p>0,1$). There was no statistically significant difference when comparing the two groups in hemodynamic (systolic, diastolic and median pressure, pulse rate), respiratory (end-expiratory C02, maximal airway pressure, tidal, volume, compliance, resistance, acidobase parameters, respiratory rate), and inflammatory parameters (C-reactive protein, blood count, lactate, temperature). In addition, electronic record was reviewed for any analysis before the operation, during anaesthesia, in the early postoperative period, and 5th postoperative day when limited statistically significant difference determined systolic and diastolic arterial pressure value($0,05<p<0,10$).

Discussion: We do acknowledge that a protective lung ventilation has led to precise guidelines that take account of the acute respiratory failure, hypoventilation, and hypoxaemia. Preliminary test results suggest that in case of treating a bigger sample of the entire population, remarkable statistically relevant differences between groups in some of the measured postoperative parameters could be found.

MeSH/Keywords: general anaesthesia, respiratory complications, recruitment procedure, positive end-expiratory pressure

Poster code: R-02-01-097

Poster Title: Photoplethysmographic evaluation of sympathetic activity during epidural analgesia for vaginal delivery

PhD candidate: Krešimir Reiner

Part of the thesis: Photoplethysmographic evaluation of sympathetic activity during epidural analgesia for vaginal delivery

Mentor(s): Assoc. Prof. Slobodan Mihaljević, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: By using finger photoplethysmography as a surrogate method for evaluation of sympathetic nervous system activity, it is possible to quantify the difference in the degree of sympathectomy between 0.125% ropivacaine and 0.125% levobupivacaine. When considering that ropivacine has a lower potential for cardiovascular toxicity compared to levobupivacaine, we hypothesised that ropivacaine, during epidural analgesia for vaginal delivery, causes a lower level of sympathectomy than levobupivacaine.

Materials and methods: 60 healthy parturients, in whom epidural analgesia for vaginal delivery was indicated, were included in the study. Patients were randomised into two groups by the type of local anaesthetic solution used for epidural analgesia: 10 ml of 0.125% ropivacaine + 100 mcg of fentanyl (group 1) and 10 ml of 0.125% levobupivacaine + 100 mcg of fentanyl (group 2). Visual analogue scale for pain, non invasive blood pressure and finger photoplethysmography signal on the first finger of the left leg and first finger of the left arm, were recorded in the four timepoints: before administration of epidural analgesia (baseline values) and 5, 10 and 20 minutes after administration of epidural analgesia. Subsequently, three parameters (area under the curve, amplitude and pulse transit time) were derived from every single finger photoplethysmography recording. General data of the patients were also recorded (age, parity and body mass index), as well as the complications of the epidural block during the time of recording (maternal hypotension, fetal bradicardia, emergent ceasarean section).

Results: Pulse transit time significantly increased over time in both groups ($P<0,001$, Wilcoxon test for paired samples). Difference between the groups in the change in the mentioned parameter over time suggests a certain difference in the degree of sympathectomy between the groups ($P=0,024$, Mann-Whitney test for independent samples).

Discussion: Pulse transit time increased over time in both groups which is explained by pharmacological sympathectomy and subsequent arterioral vasodilation. Difference between the groups in the change of the mentioned parameter leads to conclusion that there might be a difference in the degree of sympathectomy between 0.125% ropivacaine and 0.125% levobupivacaine. Analysis of the changes in the other parameters derived from finger photoplethysmography are needed for final conclusions.

MeSH/Keywords: photoplethysmography, sympathectomy, epidural analgesia

Poster code: R-02-01-108

Poster Title: Dermoscopy in prediction of basal cell carcinoma margins

PhD candidate: Ivana Manola

Part of the thesis: Effectiveness of dermoscopy in preoperative discriminating superficial from other subtypes of basal cell carcinoma and in prediction of tumor margins

Mentor(s): Professor Božo Krušlin, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Basal cell carcinoma (BCC) is a slow growing epithelial skin tumor with low metastatic potential and is the most common malignancy in the fair skin population. Standard treatment of BCC includes surgical removal, and the application of imiquimod, 5-fluorouracil or photodynamic therapy should be restricted to low-risk superficial tumors. Since the recurrent BCC lesion become more aggressive, the lesion should be surgically removed in total. In addition, the appropriate margins for complete removal of recurrent BCC should be twice as big as those for complete removal of primary BCC. The main factors that can influence recurrence rates are hystological BCC subtype and surgical margins. Recurrence rates vary from 2-8% at 5 years after surgery in complete excision to 26-41% after incomplete excised BCC. Accordingly, a valid and easily applied preoperative method to define tumor margins might be useful for the clinicians. Dermoscopy, by providing a more accurate assessment of the true extension of the tumor, could allow a more precise estimation of the required surgical margins helping to minimize the BCC recurrence rate. While the diagnostic significance of pigmented structures, such as blue-gray ovoid nests, blue-gray globules and dots or maple leaf-like areas is unquestionable, the usefulness of vascular structures in defining the surgical margins of BCC remains to be further elucidated. Preoperative use of dermoscopy may improve recognition of the BCC margins and complete excison rate.

Materials and methods: We conducted a single-centre, retrospective study in the period between January 1st, 2016 and December 31st, 2018. The dermoscopic criteria applied to diagnose BCC were the following: arborizing vessels, ulceration, leaf-like areas, spoke wheel areas, large blue-gray ovoid nests and multiple blue-gray globules. After BCC diagnosis was appointed, perilesional skin was evaluated using dermoscopy. Photodocumentation of tumor appearance and dermoscopic images were performed using FotoFinder ATBM system, which was adequately stored in the database. Evaluation of dermoscopic images was based on the combined dermoscopic criteria. The BCC margins were marked with tissue staining and surgical sutures (in lateral part one suture, in medial part two sutures). After surgical excision, histopathological analysis of tumor and peritumoral tissue was performed by an experienced pathologist. Dermoscopic definition of the the BCC surgical margins was based on: vessel evaluation, detection of satelit BCC clues in perilesional tissue including whitish to erythematous translucent to opaque areas free of vascular structures, satelit pigmented dots and small, superficial ulcerations. Discrimination between tumoral vessels and teleangiectasia of the healthy skin was based on fact that BCC vessels are bright red, appear sharply in focus and exhibit evident ramifications to finer capillaries. Instead, the telangiectatic vessels of surrounding sun damaged skin was more blurred, unfocused and show few, if any, branches.

Results: Twenty lesions from 18 subjects (65% women) were included in the study. The mean age was 71,2 years. Seven lesions were superficial BCC and thirteen were nodular BCC type. The most commonly affected skin site was back (35%) followed by the temple (25%), nose (20%), leg (10%), neck (5%) and zigomatic area (5%). The most common dermoscopic features in the perilesional skin were superficial bright-red fine telangiectasia and focused vessels in 17 out of 20 lesions (85%), followed by the satelit blue-grey dots in 2 out of 20 lesions (10%) and opaque area free of vascular structures in 1 out of 20 lesions (5%).

Discussion: Subclinical extensions of tumor are unpredictable and, although dermoscopy can guide excision of lateral margins, preoperative assessment of deep margins is impossible. In this study, we evaluated the efficacy of dermatoscopy in predicting tumor margins. The data suggest that dermatoscopy could be useful in perioperative assessment of BCC margins. Randomized controlled

studies comparing dermatoscopic findings, as well as a histopathological evaluation of complete surgical excision, are required to confirm the real usefulness of dermatoscopy in defining the tumor margins. According to the findings, dermoscopic evaluation of the borders should be included in preoperative assessment of the required surgical margins in BCC.

MeSH/Keywords: BCC, margins, dermoscopy

Poster code: R-02-02-010

Poster Title: Tumour free myometrial thickness as prognostic indicator in endometrial carcinoma

PhD candidate: Ivan Babić

Part of the thesis: Tumour free myometrial thickness as prognostic indicator in endometrial carcinoma

Mentor(s): Professor Ante Ćorušić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: In cancer of the uterine body, the myometrial invasion proved to be a significant prognostic indicator associated with the recurrence of the disease, overall survival and involvement of the lymph nodes. The depth of the myometrial invasion (DIM) is defined as the distance from the endomyometrial junction to the deepest invasion of the tumor into the myometry. Myometrial invasion (MI) by tumor expressed as a percentage (more or less than 50%) is used in FIGO classification and calculated by determining DIM and total thickness of myometry. DIM measurement is difficult due to the irregularities of the endomyometrial junction etc. Therefore, there is variability in the assessment of DIM between different and the same examiners. In contrast to DIM, the outer part of myometrium which is tumor free (tumor free disease - TFD) is defined as the distance from the point of the deepest invasion to the surface of the uterine serosa. TFD is a method of assessing the invasion of myometry by a tumor that is independent of the thickness of the myometry and has a low variability between different examiners. TFD was proposed in several studies as a prognostic factor. In the literature so far there are disadvantages such as small groups of patients, inclusion in analysis of other uterine pathohistological diagnosis, exclusion of patients who did not have pelvic lymphadenectomy, lack of studies comparing TFD, DIM and percentage of myometry invasion to predict survival, recurent disease and the spread of tumor into the lymph nodes.

Materials and methods: This restrospective study involve 200 patients with diagnosed uterine cancer who were hospitalized and treated at the Department of Gynecological Oncology and the Department of Gynecological Surgery of University Hospital Center Zagreb, dated 01.01.2011. until 30.06.2014.g. The analysis include patients diagnosed with endometrioid cancer of the uterine body, grade I and II (Type 1) and by whom minimally hysterectomy with bilateral salpingooforectomy was performed during surgery. The operative material of patients with endometrial carcinoma was submitted to the Department of Gynecological and Perinatal Pathology, Clinical Institute of Pathology and Cytology KBC Zagreb. All the material was then analyzed by 1-2 pathologists, and a final histopathological diagnose was made. In this study all the slides with the deepest invasion are examined again, and morphometric analysis is made. The result is compared with a pathohistological finding in which the thickness of the myometry and the depth of the largest invasion in the myometry is recorded. An additional analysis is performed by a PhD candidate under a pathologist supervision. From the medical history records and histological diagnosis data is collected about the type of surgery, patient age, FIGO tumor stage, myometrial thickness, depth of tumor invasion in myometry, lympho-vascular space invasion.

Results: Until now 60 patients is processed, their medical history records and their pathological slides are analysed. The median age at diagnosis was 62 years (range 30– 89). Most cancers were grade 1 (37 patients, 62%) and stage IB (21 patients, 35%). Positive lymph nodes were identified in 9 patients (15%) Lymphovascular space involvement was found in 20 patients. The median DIM was 0.5 cm (range 0–3.4 cm). The median TFD was 1.1 cm (range 0–3.7 cm). 4 patients (6.5%) have recurred, with 2 recurrences confined to the pelvis and 2 with a distant component. A receiver–operator characteristic (ROC) curve generated using various cut points for TFD demonstrated that a TFD of 8 mm maximized the sensitivity and specificity of this variable in predicting disease recurrence. TFD was more predictive than DIM for outcomes that included, cervical involvement, lower uterine segment involvement, lymphovascular space involvement, lymph node metastases and tumor grade.

Discussion: Until now in our analysis we found that TFD was more accurate in predicting all surgicopathologic variables compared with DIM. A TFD value of 8 mm has shown the most effective

balance between sensitivity and specificity in predicting disease recurrence, and could be used to counsel patients regarding recurrence risk and assignment of adjuvant therapy. Since all specimens will have a different myometrial thickness, TFD as a single measurement could provide a better prognostic measurement than DIM.

MeSH/Keywords: endometrial cancer, TFD, DIM

Poster code: R-02-05-144

Poster Title: Two dimensional transvaginal ultrasound with transrectal water application in the assessment of the rectosygmoid colon wall infiltration in patients with endometriosis

PhD candidate: Marija Gregov

Part of the thesis: Two dimensional transvaginal ultrasound with transrectal water application in the assessment of the rectosygmoid colon wall infiltration in patients with endometriosis

Mentor(s): Assist. Prof. Mario Ćorić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Endometriosis is a disease defined by the presence of the uterine mucosa outside the matrix. The frequency of endometriosis in women of reproductive age is about 10% .Endometriosis is described in many sites, even in the lungs, but the most common place for endometriosis is a small pelvis, most commonly affected by ovarian and pelvic peritoneum..We can find it in the rectovaginal septum (RVS), the rectosigmoid colon, the uterosacral ligaments, the vagina vaults or the bladder wall.The presence of deep pelvic endometriosis in a patient with endometriosis is 20%.

Materials and methods: This prospective study included patients with deep pelvic endometriosis and suspected intestinal infiltration who were treated in the Department of Obstetric and Gynecology University of Zagreb, School of Medicine.In our study we included DIE patients with suspicion of endometriosis intestinal wall infiltration diagnosed by transvaginal ultrasonography(TV-US) with transrectal water administration and to whom segmental resection of the bowell was performed. After the patient's preparation the patient underwent transrectally applied to 300 ml of aquae via a single catheter number 18 under the control of transvaginal ultrasound in the presence of medical staff. This ultrasound scan determinated the depth of bowel infiltration by layers and the size of endometrial node. Patohistologically, the depth of endometriosis infiltration was measured in relation to the intestinal wall layers and the size of the node. The final pathohistological finding was compared with the preoperative transvaginal UZV finding with transrectal water application. Data were analyzed using IBM SPSS Statistics version 25.0.

Results: In 23 patients with DIE who underwent laparoscopic treatment for deep infiltrating endometriosis from March 1st 2018 until April 1st 2019, in 11 of them segmental resection of the bowel was performed. After complete patohistologycal evaluation in 9 patients of 11 (81.8 %) to whom segmental bowel resection was performed, patohistologycal examination coincided with ultrasound scan. In two patients (18.1 %) there was no infiltration of muscular layer of the bowel but the size coincided with the ultrasound examination.

Discussion: Many studies compared the sensitivity and specificity of the various diagnostic methods in the precise determination of the place of the disease.The frequency of endometriosis of the intestine in patients with deep pelvic endometriosis is 8-12% (>90% is a rectosigmoid colon). TV-US plays a very important role in detecting intestinal endometriosis because it is sufficiently sensitive, available and not expensive, compared to other radiological methods.The appearance of endometriosis in the intestine results in hyperplasia of smooth muscle cells resulting in thickening of the muscular lining.If the endometriosis is limited to the muscular lamina, then a hyperehogenic continuous line representing submucosal mucosa is shown on it. If the endometriosis infiltrates the entire thickness of the intestinal wall then the hyperehogenic line is disrupted or is not shown at all.The depth of penetration of the endometriosis is an important prognostic indication of occupation of the intestine circumference with the endometrial nodule. Infiltration of submucose means that it is very likely that more than 40% of the lumen of the intestine is occupied by the node. Transvaginal ultrasound showed lower sensitivity for the detection of endometriosis in the submucosal and mucosal layers compared to the high sensitivity to the muscular lamina. The transrectal application of water during ultrasound examination increases the sensitivity and specificity of the diagnostic method. Using two dimensional transvaginal ultrasound with transrectally applied aqua before the operation will help us to determine the depth and extent of bowel wall infiltration, the size of the endometrial node and the distance from the anocutaneous boundary

MeSH/Keywords: endometriosis, rectosigmoid colon, transvaginal ultrasound with aqua
Poster code: R-02-05-147

Poster Title: First case of transmitted drug resistance to HIV integrase-strand inhibitors in Croatia

PhD candidate: Maja Oroz

Part of the thesis: Molecular diversity of human immunodeficiency virus type 1 and the role of transmission clusters 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

Introduction: Croatia has a centralized system of HIV clinical care and all patients are treated at University Hospital for Infectious Diseases "Dr. Fran Mihaljevic", Zagreb (UHID). Previous national study showed a high prevalence of transmitted drug resistance (TDR) of 21.6% (in the period 2006 to 2008). The study analyzed primary resistance to reverse transcriptase (RTI) and protease inhibitors (PI). Our recent study confirmed that TDR is still very high in Croatia (23.4%, analysed for the time period 2014-2015). Analysis of primary resistance to integrase strand transfer inhibitors (INSTI) has not yet been examined in Croatia, however during 2016-2017 there were 3 clinical cases of INSTI resistance among INSTI-treated HIV-1 patients. With the appearance of first clinical cases that confer resistance to INSTI, there is a possibility for circulation of INSTI-resistant HIV-1 among newly diagnosed HIV-1 patients. Therefore, in this study the prevalence of TDR to INSTI was examined in a cohort of newly diagnosed HIV-1 patients from Croatia.

Materials and methods: During 2017 a total of 108 newly diagnosed HIV-infected persons entered clinical care at UHID. This study included 100 patients, providing a coverage of 92.6%. The entire protease HIV-1 gene (PR, codons 1-99), part of the reverse transcriptase HIV-1 gene (RT, codons 1-250) and the entire integrase HIV-1 gene (INI, 1-288) were sequenced by using a validated in-house method. Mutations were determined by using Stanford Drug Resistance Database. HIV subtype was determined with Rega HIV-1 subtyping tool 3.0, jumping profile Hidden Markov Model (jphmm) and COntext-based Modeling for Expeditious Typing (COMET HIV-1). Sequences subtyped B were selected for phylogenetic inference. For each of the sequences in dataset 10 closest sequences were determined with BLAST search. Phylogenetic tree was constructed with PhyML 3.0, while the Figtree version 1.4.3 was used for tree visualisation. Transmission cluster were defined as sequences ≥3 patients from Croatian cohort with the approximate likelihood ratio test value (aLRT) > 0.90.

Results: We report findings for 100 HIV patients, 97 men (97%) and 3 women (3%). TDR were found in 17 patients (17/100, 17%). TDR to INSTI was detected in one patient, G140A (1/100, 1%). TDR to RTI were detected in 15 patients (15/100, 15%) and TDR to PI were detected in 2 patients (2/100, 2%). All patients with TDR were men who have sex with men (MSM). Phylogenetice inference analysis of sequences subtype B (97/100) identified 10 distinctive transmission clusters. Totally, 77 patients diagnosed with HIV-1 during 2017 were forming local transmission clusters.

Discussion: We found a high prevalence of TDR in the cohort of newly diagnosed, treatment-naive patients with HIV-1 infection (17%) and a first case of transmitted drug resistance to HIV integrase-strand inhibitors. Ongoing national surveillance is necessary to monitor the dynamics of HIV infection and transmission networks in Croatia.

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

MeSH/Keywords: Molecular epidemiology, HIV-1 transmission clusters, HIV-1 primary resistance

Poster code: R-02-07-003

Poster Title: Immunoglobulin G glycosylation profiles in patients with influenza and bacterial pneumonia

PhD candidate: Marina Kljaković - Gašpić Batinjan

Part of the thesis: The comparison of immunoglobulin G glycosylation profiles in patients with influenza to those of patients with bacterial pneumonia

Mentor(s): Assist. Prof. Rok Čivljak, MD PhD

Affiliation: University of Zagreb School of Medicine, Zagreb; Dr. Fran Mihaljević University Hospital for Infectious Diseases, Zagreb; Genos Glycoscience Research Laboratory, Zagreb, Croatia

Introduction: Acute respiratory tract infections, especially influenza and bacterial pneumonia, are among the leading causes of morbidity and mortality. Immunoglobulin G (IgG) plays an important role in protecting the human organism from pathogens and is an example of glycoprotein whose function is regulated by glycosylation. There are two covalently linked N-glycans in the Fc region of IgG that are important for the stability of the antibody and modulating IgG effector functions. For example, the addition of sialic-acid to the N-glycan of the Fc region dramatically changes the physiological role of IgGs, by converting them from pro-inflammatory into anti-inflammatory agents. Conversely, if only one fucose molecule is removed from the N-glycan of the Fc region, IgG antibodies may become up to fifty times more effective in its proinflammatory activity. The aim of the study was to compare glycosylation profiles of IgG in patients with influenza and bacterial pneumonia with those in healthy controls.

Materials and methods: A survey was conducted in adult patients hospitalized at the Dr. Fran Mihaljević University Hospital for Infectious Diseases, Zagreb, due to acute respiratory tract infection. On three occasions (1st, 7th and 28th day after admission), the Hydrophilic Interaction Liquid Chromatography-Ultra High Performance Liquid Chromatography (HILIC-UHPLC) method was used to determine the glycosylation profile of IgG in the serum of all subjects. The results were compared with the glycosylation profile of 35 controls, which were matched by age and sex. We used a linear mixed model to analyze N-glycoprotein changes in time. We analyzed each of the 3 groups individually. P values were corrected for multiple assays using the Benjamini-Hochberg method.

Results: The study included 36 adult patients with acute respiratory tract infection, of whom 13 had laboratory-confirmed influenza, and 23 bacterial pneumonia. Regarding galactose, in patients with influenza the IgG glycome is changed toward more anti-inflammatory IgG activity; there are a statistically significant decrease of agalactosylated structures ($p < 0,05$) which are repeatedly associated with inflammation and an increase of sialylated moieties ($p < 0,01$) associated with IgG anti-inflammation activity. Contrary, in patients with pneumonia digalactosylated structures decrease ($p < 0,02$) thus promoting IgG pro-inflammatory activity and acting contrary to the observed changes in patients with influenza. Regarding bisecting N-acetylglucosamine (GlcNAc), a statistically significant decrease ($p < 0.001$) is observed in patients with pneumonia which leads to enhanced anti-inflammatory activity of IgG and is in opposite to decreased digalactosylation, while bisecting GlcNAc remains unchanged in influenza patients.

Discussion: Differences in IgG glycome isolated from patients with influenza and pneumonia clearly indicate that IgG glycosylation is affected by distinct molecular pathways underlying the diseases. IgG glycosylation in influenza and bacterial pneumonia patients differs from the glycosylation profile of healthy controls. Determination of the glycosylation profile of IgG could have a diagnostic and prognostic value and requires further investigation.

MeSH/Keywords: influenza, bacterial pneumonia, glycosylation, immunoglobulin G

Poster code: R-02-07-078

Poster Title: Distribution of Rotavirus Genotypes in Three Croatian Regions Among Children <5 Years of Age

PhD candidate: Maja Vrdoljak

Part of the thesis: Distribution of rotavirus genotypes in three regions in Republic of Croatia

Mentor(s): Assoc. Prof. Goran Tešović, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Rotavirus (RV) is the major cause of severe diarrhea in young children worldwide, with more than 140 million episodes a year. There are numerous genotypes of RV which distribution changes depending on geographic area, season or age of the patient. Two RV vaccines are available and highly effective in reducing the burden of RV gastroenteritis - RotaTeq® (Merck, Whitehouse Station, NJ), a pentavalent (G1, G2, G3, G4, P[8]) bovine-human reassortant vaccine, and Rotarix® (GSK Biologicals, Rixensart, Belgium), a monovalent (G1P[8]) human strain vaccine. However, they are not implemented in the Croatian national immunisation programme. Prior to the introduction of RV vaccine in the national immunization program, local epidemiological data on the burden of RV disease, including age distribution, seasonal trends and assessment of the genotypes that circulate in population, are important for creating effective strategies to control RV disease. Data on circulating RV genotypes in Croatia (Zagreb metropolitan area) are limited to one study. The aim of this study was to assess prevalence of RV genotypes in three different Croatian regions (North, Central and South), determine possible differences in geographic distribution of RV genotypes and monitor possible emergence of novel RV strains in the selected regions. Data can be used to direct policy decision regarding introduction of RV vaccine in the national immunization program.

Materials and methods: Children aged less than 5 years with acute gastroenteritis, treated in three Croatian hospitals (University Hospital for Infectious Diseases, Zagreb, University Hospital of Split and County Hospital Cakovec) were included in the study during the two years period. Demographic, clinical and laboratory data were collected and immunocromatographic analysis of the stool samples for RV and Adenovirus was performed. RV positive samples were further analysed (polymerase chain reaction and DNA sequencing) to identify genotype.

Results: Genotyping of 822 rotaviruses showed that the predominating circulating strain was G1P[8] (61.9%), followed by G2P[4] (19.5%), G1P[4] (3.9%) and G3P[8] (2.9%). G1 was by far the predominant G-type (67.5%), followed by G2 (three-fold lower prevalence), while G3, G9 and G4 were sporadic (□3%). Of the P-types, P[8] by far prevailed (75.6%), followed by P[4] (three-fold less) and 1 case of P[6] (“uncommon” type). G6 type was detected for the first time in Croatia.

Discussion: This is the first Croatian national study describing molecular epidemiology of RV strains among children < 5 years of age in three different Croatian regions – North, Central and South. High prevalence of “common” RV strains detected in this study (overall 88.8% and by region – in Central Croatia 88.8%, in South 95.3% and in North Croatia 80.8%) may ensure RV vaccine effectiveness but further surveillance is necessary to monitor diversity of RV strains and possible emergence of novel genotypes not included in current vaccines.

MeSH/Keywords: rotavirus, diarrhea, acute gastroenteritis, children, genotypes

Poster code: R-02-07-094

Poster Title: Comparison of predictive value of qSOFA, SOFA score and SIRS criteria for in-hospital mortality of different age-group adult patients with community-acquired bacteremia

PhD candidate: Marija Kusulja

Part of the thesis: Comparison of predictive value of qSOFA, SOFA score and SIRS criteria for in-hospital mortality of different age-group adult patients with community-acquired bacteremia

Mentor(s): Assist. Prof. Marija Santini, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Sepsis is a life-threatening organ dysfunction caused by host response to infection. Sepsis represents a common problem, with high mortality (around 26% on global scale) despite the advance of antimicrobial treatment and intensive medical care. In order to improve time to recognition and treatment of sepsis, new guidelines for sepsis were published in 2016, with new criteria for recognition of sepsis. It is unknown the new SOFA and qSOFA scores are better predictors of sepsis outcome in comparison to the old SIRS criteria.

Materials and methods: The goal of this study was to compare qSOFA, SOFA score and SIRS criteria as predictors of mortality during hospital stay and 28-day mortality. The subjects of this study were adult patients hospitalized in the University Hospital for Infectious Diseases „Dr. Fran Mihaljević“, Zagreb, with community-acquired bacteremia, during one year period (2016). The data on patients was acquired retrospectively from medical documentation. The main observed outcome was mortality during hospital stay.

Results: A total of 238 patients were included in the study. The median age was 73 years (min 20 – max 95), with 52.1% female patients. The Charlson Comorbity Index (CCI) was a median of 4 (min 0 – max 11). The most common cause of bacteremia was E. coli (n 133, 55.9%), followed by S. aureus (n 29, 12.2%). Most patients had a low-grade fever (below 38 C, 69.1%), and 71.9% reported shivering. The median heart rate (HR) was 92 /min (min 54 - max 160 /min), median respiratory frequency (RF) was 18 (min 12 - max 48) /min. Median systolic blood pressure (SBP) was 120 (min 60 – max 185) mmHg, while mean arterial pressure (MAP) was 85 (min 36 – max 128) mmHg. The median Glasgow coma score (GCS) was 15 (min 3 – max 15). The median white blood cell count (WBC) of 13.1 (min 2.4 – max 6) x 109/L with a percentage of neutrophils of 87% (min 22 – max 98%) and thrombocytes of 173 (min 19 – max 608) x 109/L. Median bilirubin value was 17 (min 5 – max 199) µmol/L and creatinine was 114 (min 60 – max 734) µmol/L. The average value of SOFA score was 2.3 (SD 2.2), qSOFA score 0.5 (SD 0.7), and SIRS 1.9 (SD 1), with percentage of patients fitting >= 2 criteria 57.7%, 12.2%, 66.4%, respectively. The median time of hospitalization duration was 13 (min 1 – max 119) days. Total mortality was 8.4% (n 20). We performed univariant statistical analysis using Exact test for categorical variables and Mann Whitney test for continuous variables and as a result identified several variables as predictors of mortality: CCI (3.5 ± 2.3 vs 4.9 ± 2.4, p value <0.01), RF (19.3 ± 4.8 vs 26.2 ± 8.2, p value <0.01) , GCS (14.9 ± 0.6 vs 12.2 ± 4.1, p value <0.01), bilirubin (21.2 ± 21 vs 39.9 ± 47.4, p value <0.01) and creatinine (143 ± 95.2 vs 198.7 ± 94.4, p value <0.01). Cut-off values od 2 or more showed strong statistical significance in connection to mortality only for SOFA and qSOFA scores and not for SIRS criteria (SOFA 2 ± 1.9 vs 5.3 ± 3.5, p value < 0.01, 52.8% survivors and 100% non-survivors met the criteria of SOFA score >= 2; 8.3% of survivors and 55% of non-survivors met the criteria of qSOFA score >= 2; 66.5% of survivors and 65% non-survivors met the >= 2 SIRS criteria).

Discussion: Previous studies have evaluated SIRS criteria as highly predictive for sepsis caused mortality in intensive care units, and new SOFA and qSOFA scores have been criticized to be more specific, but less sensitive. The new scores have still not been universally accepted, and more studies are needed for their evaluation. Our study included all patients hospitalized with community acquired bacteremia who were admitted to University Hospital for Infectious Diseases in Zagreb during a one-year period. The disadvantages of this study include retrospective analysis, population limited to one hospital, and limited availability of data. The advantages of the study include the

specific population, including all patients hospitalized with community-acquired bacteremia confirmed by blood cultures. Our preliminary results indicate that new SOFA and qSOFA scores are more reliable in predicting mortality in patients with bacteremia in comparison to SIRS criteria. Out of predictor variables, the strongest predictive value for mortality, independently of other variables, was shown for following variables: RF, GCS, bilirubin and creatinine. Further data collection and analysis is needed for more results, which is the final goal of this PhD dissertation.

MeSH/Keywords: sepsis, bacteremia, SIRS, SOFA, qSOFA

Poster code: R-02-07-096

Poster Title: Vitamin C, Vitamin B1 and Hydrocortisone in Septic Shock

PhD candidate: Gorazd Pilčić

Part of the thesis: The effect of vitamin C, vitamin B1 and hydrocortisone on clinical course and outcome in patients with septic shock

Mentor(s): Assoc. Prof. Slobodan Mihaljević, MD PhD, Assoc. Prof. Robert Likić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Septic shock is a subset of sepsis in which particularly profound circulatory, cellular, and metabolic abnormalities are associated with a greater risk of mortality than with sepsis alone. For clinical operationalization, organ dysfunction can be represented by an increase in the Sequential Organ Failure Assessment (SOFA) score of 2 or more points. Patients with septic shock require vasopressors to maintain a mean arterial pressure of 65 mm Hg or greater, and a serum lactate level greater than 2 mmol/L (>18 mg/dL) in the absence of hypovolemia. Numerous studies have been conducted in research of pharmacological agents to improve patient outcomes, but most of them did not yield satisfactory results. One of the possible treatment strategies is the parenteral administration of vitamin C, vitamin B1 and hydrocortisone.

Materials and methods: In this prospective, randomized single-blind study we plan to inroll 60 patients. The patients are divided in two groups: 30 patients in the experimental group and 30 in the control group. The experimental group patients receive standard therapy and vitamin C (1,5 g every 6 hours), vitamin B1 (200 mg every 12 hours), and hydrocortisone (50 mg every 6 hours). The control group receive only standard therapy. The drugs are administered from the start of the diagnosis for up to 4 days and ptients are followed-up until the final discharge from the ICU. In order to calculate the SOFA score, the following parameters we use for each subgroup: lactate, procaltitonin, PaO2/FiO2 ratio, platelets, mean arterial pressure, bilirubin, creatinine, Glasgow coma score, need for vasopressor therapy and diuresis. After ICU discharge, we calculate the duration of vasopressor therapy, mechanical ventilation, treatment in the intensive care unit and finally the survival and mortality of patients.

Results: The study is still in progres. Up to now we colected data for 20 patients: 10 patients in each group. Our data show that patients who recived vitamin C, vitamin B and hydrocortisone have a faster improvement in laboratory findings, less days needed for vasoactive and resiratory therapy. Patients who recived standard therapy and therapy by protocol did not differ in the mortality.

Discussion: Data collected from study participants are still not sufficient for proper statistical analysis. It appears that combination of vitamin C, vitamin B1 and hydrocortisone could be a good adjuvant therapy to standard treatment of septic shock.

MeSH/Keywords: septic shock, vitamin C, vitamin B1, hydrocortisone

Poster code: R-02-08-034

Poster Title: The influence of early statin administration on in-hospital and 1-year mortality after acute coronary syndrome: experience from the Croatian branch of the ISACS-CT registry

PhD candidate: Saša Pavasović

Part of the thesis: Impact of high-dose statins administered within 24 hours of an acute coronary syndrome on clinical outcomes and platelet reactivity.

Mentor(s): Academician Davor Miličić

Affiliation: University of Zagreb School of Medicine

Introduction: The relevance of statin therapy in acute coronary syndrome (ACS) is well-established, but little is known about the optimal timing of statin administration, particularly within the first 24 hours following ACS. The aim of the study was to gather data on early and late outcomes of ACS patients (pts) through the Croatian branch of the ISACS-CT (International Registry of Acute Coronary Syndromes in Transitional Countries) registry.

Materials and methods: The data was gathered retrospectively from January 2012 to October 2017. The study population included 1898 ACS pts: 46.4% (n=881) with ST-segment elevation myocardial infarction (STEMI), 36.1% (n=685) with non-ST-segment elevation myocardial infarction (NSTEMI) and 17.5% (n=332) with unstable angina pectoris (UA). Follow-up was performed on 33% (n=630) of the cohort, 43.7% (n=275) with STEMI, 34.4% (n=217) NSTEMI and 21.9% (n=138) with UA.

Results: In the first 24 hours following ACS, statins were administered in 1734 (93%) pts (for 24 pts the data is missing), while the pts who received them later or not at all were the control group. The two groups did not differ regarding age, gender, body mass index, left ventricular ejection fraction (LVEF) during initial hospitalization, smoking status, history of diabetes, chronic heart failure or arterial hypertension at initial hospitalization nor at 1-year follow-up. The overall in-hospital mortality rate was 4%, similar to that in pts treated with statins within the first 24 hours (3%), while in pts without early statin treatment, in-hospital mortality rate was 18% ($p<0.001$). The risk of in-hospital death was significantly higher in pts without early statin therapy (odds ratio [OR] 7.32, 95% confidence interval [CI] 4.371-12.27, $p<0.001$), while the risk of primary outcome at 1-year follow-up was not significantly different between groups in univariate regression analysis. Older age (OR 1.1, 95% CI 1.06-1.20, $p< 0.001$), higher creatinine level (OR 1.0, 95% CI 1.005-1.012, $p<0.001$), lower LVEF (OR0.90, 95%CI 0.86-0.94, $p<0.001$) and lack of early statin treatment (OR 3.6, 95% CI 1.0-13.10, $p=0.05$) were positively associated with increased odds for early primary outcome in multivariable regression model.

Discussion: Initiation of statin therapy within the first 24 hours following ACS is associated with a significant reduction in in-hospital mortality, although the positive effect of early statin therapy did not reach statistical significance at 1-year follow-up.

MeSH/Keywords: acute coronary syndrome, statins, mortality

Poster code: R-02-09-002

Poster Title: Association of endothelial lipase serum levels with functional characteristics of HDL particles and endothelial dysfunction in patients with metabolic syndrome: an overview of patient clinical data

PhD candidate: Iva Klobučar

Part of the thesis: Association of endothelial lipase serum levels with functional characteristics of HDL particles and endothelial dysfunction in patients with metabolic syndrome

Mentor(s): Professor Vesna Degoricija, MD PhD

Affiliation: University Hospital Centre Sisters of Charity, Department of Cardiology, Zagreb, Croatia; University Hospital Centre Sisters of Charity, Department of Medicine, Zagreb, Croatia

Introduction: HDL particles maintain normal endothelial function by inducing NO synthesis, inhibiting LDL particles oxidation and reducing adhesion molecules expression on vascular endothelial cells. Endothelial dysfunction is the first, still reversible, step to atherosclerosis and it is present in patients with metabolic syndrome (MS). Serum levels of endothelial lipase, an enzyme which degrades HDL particles, are also elevated in this condition. Hypothesis of this study is that endothelial lipase, through its impact on HDL particle structure and function, induces occurrence of endothelial dysfunction in patients with MS. The aim is to determine association between serum levels of endothelial lipase, serum levels and function of HDL particles and the endothelial dysfunction in patients with MS.

Materials and methods: This is an observational, cross-sectional study, recruiting a total of 130 participants (65 healthy volunteers (HV) and 65 patients with MS), held in the University Hospital Centre Sisters of Charity, Zagreb, Croatia, during one year. Patient history and physical examination data are recorded for each participant. A total of 24 ml of venous blood is taken from each participant, centrifuged and serum stored at the temperature of -60°C until the laboratory analysis in the Institute of Molecular Biology and Biochemistry of the Medical University of Graz, Austria. Endothelial dysfunction is quantified with flow- and nitroglycerin-mediated dilation (FMD, NMD) using brachial artery ultrasound (software FloWave.US v. 0.2.0; Coolbaugh CL, Vanderbilt University Institute of Imaging Science, Nashville, Tennessee, USA).

Results: A total of 80 participants (61.5% of the planned number) were enrolled in the study between July 2018 and March 2019, 42 HV and 38 patients with MS. 54.8% of patients in HV group and 44.7% in MS group were female, with no statistically significant difference in sex distribution between the groups (P=0.37). Median age was 57.0 (45-71) for HV group and 57.5 (33-66) years for MS group patients (P=0.78). Compared to HV, patients with MS had significantly higher median systolic blood pressure [125.0 (100-150) mmHg vs. 132.5 (115-160) mmHg; P=0.001], waist circumference [87.0 (72-118) cm vs. 110.5 (84-146) cm; P<0.001], body weight [73.5 (61-125) kg vs. 98.0 (64-146) kg; P<0.001] and body mass index [25.3 (19.4-33.5) kg/m2 vs. 31.4 (22.7-55.6) kg/m2; P<0.001]. In the MS group 86.8% of patients suffered from arterial hypertension, 76.3% had previously known dyslipidaemia and 34.2% of patients had glucose intolerance or diabetes mellitus. Blood sample analyses are ongoing, as well as the analyses of recorded ultrasound materials using software for continuous measurement of brachial artery diameter changes. Exact values for these variables are not available at the moment.

Discussion: Presented classification of study participants in HV or MS group is currently based on patient history data about previously known dyslipidaemia and glucose intolerance. The final classification will be done after laboratory blood test analyses completion. There is a possibility that the final data would differ from the currently presented.

Acknowledgments: Research is conducted in cooperation with the Medical University of Graz, Austria (Agreement on cooperation between the Univ. of Zagreb and Medical Univ. of Graz, 2005. Principal investigators: Prof. Saša Frank (Graz) and Prof. Vesna Degoricija (Zagreb)).

MeSH/Keywords: endothelial lipase, HDL, endothelial dysfunction

Poster code: R-02-09-014

Poster Title: YOGA IN RHEUMATOID ARTHRITIS

PhD candidate: Silva Pukšić

Part of the thesis: Utjecaj vježbanja joge na kvalitetu života i pokazatelje upale u bolesnika s reumatoidnim artritisom

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

Affiliation: University of Zagreb School of Medicine

Introduction: Rheumatoid arthritis (RA) is a chronic disabling inflammatory disease that substantially impacts health-related quality of life (HRQOL) of patients through increased pain, fatigue, physical disability and impaired psychological health. Yoga, a mind-body therapy, integrates physical exercises with relaxation and meditation. Published data suggest its beneficial effects on both physical and mental health in various chronic conditions. This study will determine the impact of a 12-week yoga program on measures of psychological distress, HRQOL, fatigue, pain, disease activity, levels of circulatory inflammatory markers and proinflammatory gene expression changes in RA patients.

Materials and methods: From 02/2018 43 RA patients, aged >18 years, on stable standard pharmacological treatment were randomly assigned to a 12-week yoga intervention or arthritis-education control. Yoga classes are conducted 2xweekly/ 90 minutes and consist of physical exercises (asanas), breathing exercises (pranayama), relaxation and self-enquiry meditation based on Yoga in daily life system. Arthritis-education classes are conducted 1xweekly/ 120 minutes and consist of lectures on arthritis and related issues followed by group discussion. Self-administered questionnaires are used to assess quality of life (SF-36), depressive symptoms and anxiety (HADS), fatigue (FACIT-F), RA impact of the disease (RAID), perceived stress (PSS), and pain (VAS). Blood samples are collected for CRP and hsCRP and RT-PCR analysis of expression of 5 proinflammatory genes (IL-1, IL-6, TNF, NFKB1, RELA). Disease activity is assessed by DAS28CRP score. Baseline, post-treatment and 3 month follow-up assessment include questionnaires, DAS28CRP and hsCRP measurement and baseline and post-treatment only for genomic analysis.

Results: The study is still ongoing, we are planing to include a total of 50 patients. Included patients so far are predominantly females (93%), with a mean age of 55.8 years, and 6.6 years of disease duration. Most of them are seropositive (78.6%). Both groups have similar baseline disease activity (DAS28CRP yoga group 2.55 vs. education group 2.74). Attrition rate is small as expected (14.2% yoga vs. 9% education).

Discussion: This research will provide information on potential efficacy of yoga program in improving physical and psychological outcomes in persons with RA and explore its influence on immunological system. If proved effective yoga program could be used as complementary non-pharmacological method in management of RA patients aimed at promoting their physical and psychological health.

MeSH/Keywords: rheumatoid arthritis, yoga, gene expression, inflammation, health-related quality of life

Poster code: R-02-09-015

Poster Title: ASPIRIN AND PREVALENCE OF LEFT ATRIAL THROMBOSIS IN PATIENTS WITH ATRIAL FIBRILLATION

PhD candidate: Petra Angebrandt

Part of the thesis: Povezanost genskoga polimorfizma - 455 G/A beta-fibrinogena i pojavnosti tromba u aurikuli lijevog atrija u bolesnika s atrijskom fibrilacijom

Mentor(s): Professor Anton Šmalcelj, MD PhD

Affiliation: University of Zagreb School of Medicine; Department of cardiovascular disease, University Hospital Centre Zagreb

Introduction: Atrial fibrillation is the most commonly encountered rhythm problem in adult population. It is associated with increased long-term risk of stroke as well as heart failure and death. There are many external and genetic risk factors for tromboembolic events. Anticoagulants appear to be more effective than aspirin in preventing embolisms in patients with atrial fibrillation (AF), with no increase in the risk of bleeding. Despite the evidence for the limited efficacy and poor safety of aspirin, as well as the diminished role for aspirin in recent guidelines, it is still commonly used in AF patients. Our aim was to assess the association of aspirin therapy with the prevalence of left atrial thrombosis (LAT) in patients with AF.

Materials and methods: During the performing main research (PhD thesis) we analyzed our database of patients with atrial fibrillation who were treated in our hospital department and we performed an observational study involving 131 patients with AF who underwent precardioversion transesophageal echocardiography in our institution during the period from January 2011 until January 2015, and who was not on any form of anticoagulation therapy. According to history of aspirin therapy, we divided them in two groups, Group 1 (patients on aspirin) and Group 2 (patients without aspirin). The presence of LAT was evaluated in both groups. We also analyzed thrombotic risk according to the CHA2DS2-Vasc score system.

Results: Out of 131patients, 51 (38.9%) were on aspirin. The prevalence of LAT in the Group 1 as compered to the Group 2 was 6/51 vs. 5/80 respectively, p=0.43. The two groups did not differ in the proportion of intermediate to high thromboembolic risk patients according to the CHA2DS2-Vasc score system 44/51 vs. 67/80, respectively p=0.59.

Discussion: Our study shows that therapy with aspirin is not associated with less prevalence of LAT in patients with AF in comparisons to patients who were not on any anti coagulation/anti platelet therapy. Therefore, we believe that aspirin should not be the drug of choice to prevent thromboembolic events in patients with AF. Due to the small number of patients, our study was underpowered, but our results correlate with larger studies and recent guidelines recommendations.

MeSH/Keywords: atrial fibrillation, aspirin, thrombus, transesophageal echocardiography

Poster code: R-02-09-017

Poster Title: Efficacy and safety of individualized P2Y12 receptor antagonists treatment based on agregometry versus fixed dose regimen in patients after acute myocardial infarction with ST-segment elevation

PhD candidate: Hrvoje Jurin

Part of the thesis: Učinkovitost i sigurnost individualizirane primjene antagonista P2Y12 receptora temeljem agregometrije u odnosu na fiksne doze u bolesnika nakon akutnoga infarkta miokarda s elevacijom ST-segmenta

Mentor(s): Academician Davor Miličić

Affiliation: University of Zagreb School of Medicine

Introduction: Several studies have reported high residual platelet activity (measured using platelet aggregation tests) in patients treated with clopidogrel depending on the grade of drug biotransformation in liver via cytochrome P450 system. It is also known that this high on-treatment activity as well as clinical outcomes may be improved by increasing clopidogrel dose. We report the results of group of patients who have completed 1-year follow-up and who were included in a randomized clinical trial investigating efficacy and safety of individualized P2Y12 receptor antagonists treatment based on agregometry tests versus fixed dose ticagrelor regimen in patients after acute myocardial infarction with ST-segment elevation (STEMI).

Materials and methods: We analyzed tha data of 51 consecutive patients (9 female, mean age 58.9 years) treated for STEMI. During the first month after STEMI all the patients were treated using fixed dose ticagrelor with aspirin. Aferwards the patients were randomized into two groups. First group continued with the aforementioned therapy during one year period. Patients in the second group were switched from ticagrelor to clopidogrel and treated during the one year period by indivudalizing clopidogrel dose based on aggregometry tests using Multiplate® analyzer. The optimal platelet reactivity level was determined by consensus (19-46 U). Depending on the agregometric ADP test result patients will be treated as follows: 1) patients with normal (19-46 U) as well as those with reduced platelet activity (<19 U) will be treated with a standard dose of clopidogrel until the next agregometric finding, 2) patients with measured elevated platelet reactivity (> 46 U) will continue treatment with a double dose of clopidogrel, and 3) patients with elevated residual platelet activity (> 46 U) on a double dose of clopidogrel will continue treatment with ticagrelor at standard doses. During the 12-month follow-up, a total of 7 measurements of platelet activity will be performed for each subject as follows: 1st and 30th day and 2, 3, 6, 9 and 12 months after acute coronary event. Primary outcome was the incidence of major cardiovascular events (cardiovascular death, myocardial infarction, stroke and repeat revascularization). Secondary (safety) outcome was the incidence of minor and major bleeding defined using BARC classification.

Results: During one year follow-up we haven't observed any primary outcome or major bleeding event. We found statistically significantly lower incidence of minor bleeding in the group of patients treated with individualized P2Y12 antagonists dose tailoring (p=0.027) which transfers to 4.8 times higher chance of having minor bleeding while treated with fixed dose ticagrelor in comparison to patients treated with individualized approach (OR 4.8; 95% CI 1.118 to 20.611; p=0.035).

Discussion: These results show that individualized P2Y12 antagonists treatment using aggregometry tests may represent equally effective but safer treatment approach in patients after acute coronary syndrome. Although weakened by a small number of participants included so far in the study and by the absence of primary outcomes, these preliminary research results represent, to the best of our knowledge, first study data on long-term safety and efficacy of individualized clopidogrel dose administration, based on aggregometry tests, in comparison to fixed dose ticagrelor regimen in treating patients with STEMI.

MeSH/Keywords: antiaggregational therapy, acute coronary syndrome, clopidogrel, individualized medicine

Poster code: R-02-09-023

Poster Title: Longitudinal Health-Related Quality of Life (HRQoL) in Hodgkin Lymphoma (HL) Patients and Survivors

PhD candidate: Ida Hude Dragičević

Part of the thesis: Overall quality of life among Hodgkin Lymphoma patients and survivors

Mentor(s): Professor Igor Aurer, MD PhD, Professor Peter Borchmann, MD PhD

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

Introduction: One of the most commonly used HRQoL questionnaires in oncology is the European Organization for Research and Cancer (EORTC) Quality of Life Questionnaire Core 30 (QLQ-C30). In a recent publication by EORTC Quality of Life Group, HRQoL summary score, a single higher order measurement model for QLQ-C30, was found to be robust and is recommended to supplement the traditional 15-outcome profile. Aim of this study is to evaluate longitudinal overall HRQoL from diagnosis to year 5 after end of treatment (y5EOT) in German Hodgkin Study Group (GHSG) clinical trials for early-stage favorable (HD13), early-stage unfavorable (HD14), and advanced-stage (HD15) HL using the proposed summary score (hereinafter referred to as oHRQoL).

Materials and methods: 5,306 qualified patients of the HD13-HD15 trials (ISRCTN63474366, ISRCTN04761296, ISRCTN32443041) ≤60 years at study entry were eligible for evaluation; 4,110 provided HRQoL data. Patients were treated according to different risk-adapted therapies. EORTC QLQ-C30 was used to evaluate oHRQoL from diagnosis to y5EOT with means and 95% confidence intervals. Treatment effects on oHRQoL in y2EOT and y5EOT are tested with multiple linear regression analyses adjusting for age, gender and baseline-oHRQoL. German reference data of Schwarz&Hinz (2001) are used for interpretation of results. OHRQoL is compared with QLQ-C30 item 30 (general quality of life).

Results: Analysis showed significantly worse baseline-oHRQoL with higher stage (means [95%-CI]: HD13 80.6 [79.5-81.8], HD14 76.1 [75.2-77.0], HD15 71.0 [70.0-71.9]). During chemotherapy, oHRQoL worsened in all stages (means [95%-CI]: HD13 63.5 [62.2-64.8], HD14 62.8 [61.9-63.8], HD15 62.7 [61.9-63.6]), rapidly improving thereafter. Compared to baseline, oHRQoL in the early-stage group recovered and remained stable from y1EOT, whereas in higher stages it improved significantly above baseline value, also remaining stable during follow up (y5EOT means [95%-CI]: HD13 82.6 [80.8-84.4], HD14 83.9 [82.6]85.1, HD15 82.5 [81.4-83.7]). Age- and gender-adjusted German reference values of oHRQoL (93.5-93.9 in HD13-HD15) were not reached at any time point. Comparison of standard vs. successful experimental treatments showed that treatment intensity had no significant influence on long-term oHRQoL. Age, physical and cognitive functioning at baseline were prognostic QLQ-C30-parameters for oHRQoL. Compared to item 30 of the QLQ-C30, the new summary score shows a higher differential validity (= higher differences to reference data), a higher precision (= smaller confidence intervals) and less response bias (responses of survivors less increased compared to baseline).

Discussion: There are multiple scientific contributions of this study: 1) this is a comprehensive overall HRQL analysis on a largest HL sample size published thus far; 2) the feasibility of oHRQoL QLQ-C30 summary score in hematologic cancer is investigated for the first time. Results show that oHRQoL is largely impaired among HL patients. Despite significant baseline differences, oHRQoL after therapy seems independent of initial stage or treatment regimen and does not recover to normal values, warranting further investigation which should lead to improvements in timely interventions. As expected, older age results in lower oHRQoL of survivors, most probably due to less tolerance to chemotherapy; these negative consequences of older age increase with higher disease burden and more intensive treatments. Physical and cognitive functioning at baseline are the best prognostic QLQ-C30-parameters for oHRQoL of survivors. The new oHRQoL summary score is more sensitive, more precise and less biased than item 30 of the QLQ-C30. Therefore, it seems like a promising tool for HRQoL evaluation, encouraging further testing.

Acknowledgments: Special thanks to Mr. Horst Mueller, PhD, for valuable input in study design and statistical analysis.

MeSH/Keywords: Hodgkin lymphoma, Combined Modality Therapy, Quality of life, Survivorship

Poster code: R-02-09-033

Poster Title: Serum biomarkers of degradation and formation of type III, IV and V collagen are associated with disease activity in patients with Crohn's disease

PhD candidate: Viktor Domislović

Part of the thesis: Serum biomarkers of degradation and formation of type III, IV and V collagen are associated with disease activity in patients with inflammatory bowel disease

Mentor(s): Professor Željko Krznarić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Crohn's disease (CD) is characterized by episodes of relapse and remission and therefore requires continuous evaluation of disease activity. Extra Cellular Matrix (ECM) consists of basement membrane (BM) and interstitial matrix (IM). BM) is positioned directly underneath the epithelial cells and consists mainly of type IV collagen, while IM consists mainly of type I, III and V collagen, and is produced by fibroblasts. Pathological environment, such as inflammation and fibrosis, leads to impaired remodeling, structure, quality and function of the collagen in the ECM. We investigated biomarkers of collagen degradation and formation and their association with disease activity and in patients with CD.

Materials and methods: In this cross-sectional study we measured five biomarkers of ECM remodeling in 75 patients with CD (60% males, age 35(IQR 26.5-43.5)), and 29 healthy controls matched by age and gender. Biomarkers of type III collagen degradation (C3M) and formation (PRO-C3), type IV collagen degradation (C4M) and formation (PRO-C4), type V collagen formation (PRO-C5) and C-reactive protein (CRP) were measured in serum by ELISA. Inflammatory activity was defined as combination of clinical or biochemical disease activity (CDAI≥150 or CRP>5). Disease behavior was assessed by Montreal classification. One-way ANOVA (Tukey's multiple comparisons test), and ROC analysis was applied in statistical analysis.

Results: Biomarkers of interstitial matrix remodelling showed that C3M was significantly elevated in active CD compared to inactive CD ($P<0.05$) and HD ($P<0.05$), whereas PRO-C3 and PRO-C5 were significantly elevated in active CD and inactive CD compared to HD ($P<0.001$, $P<0.05$). (Figure 1) Turnover type III collagen showed highest diagnostic accuracy for active disease (AUC=0.74). Area under curve was for C3M 0.63, PRO-C3 0.36 and PRO-C5 0.52. Biomarkers of basement membrane remodelling showed significantly higher C4M in active CD compared inactive ($P<0.05$) and HD ($P<0.001$), whereas PRO-C4 was significantly elevated in active and inactive CD compared to HD ($P<0.01$). Area under curve was for C4M 0.64, C4M/PRO-C4 ratio 0.57 and PRO-C4 0.56.

Discussion: Both biomarkers of interstitial matrix (C3M) and basement membrane (C4M) were associated with disease activity. PRO-C3, PRO-C5 and PRO-C4 were associated with CD regardless of disease activity. Interstitial matrix biomarkers of turnover type III collagen C3M/PRO-C3 showed highest diagnostic accuracy for disease activity. In conclusion, these biomarkers may be used in monitoring and prediction of disease activity and in differentiation between patients with CD and healthy individuals.

MeSH/Keywords: Crohn's disease, collagen, inflammation

Poster code: R-02-09-041

Poster Title: Autophagy and the role of osteogenic macrophages in chronic Philadelphia-negative myeloproliferative neoplasm

PhD candidate: Marin Međugorac

Part of the thesis: Autophagy and the role of osteogenic macrophages in chronic Philadelphia-negative myeloproliferative neoplasm

Mentor(s): Professor Rajko Kušec, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Homeostasis of hematopoietic stem cells is under influence of specific anatomic-functional unit, so-called hematopoietic niche. Osteogenic machrophages by excretion of Oncostatin M influence on Nestin-positive mesenchymal stem cells, which then produce cytokines essential for quiescence of stem cells (CXCL12, ANGPT1, KITL). Autophagy is a self-degradation mechanism of cellular molecules and organelles which helps cell survival in conditions of increased stress (hypoxia, nutrient deficiency, tumor growth). LC3B-II is the best marker which can be used to quantify autophagy. Chronic Philadelpia-negative myeloproliferative neoplasms are group of clonal disorders of hematopoietic stem cells that comprise the following entities: polycythemia rubra vera, essential thrombocythemia, primary and secondary myelofibrosis.

Materials and methods: The examined group is composed of patients with chronic Philadelphia-negative myeloproliferative neoplasms. The study is prospective and will include newly diagnosed patients. Control group is comprised of patients whose blood and bone marrow were sampled in a diagnostic procedure of agressive lymphoma in limited stage. Expected cohort of respondents is 30 in examined and 10 in control group. The material that will be examined in this study is bone marrow obtained at bone marrow biopsy and venous blood. All respondents will be examined for clonal nature of the disease by determining mutations JAK2, CALR and MPL. Bone marrow tissue will be examined by using immunohistochemistry based on binding of antibodies to specific macrophage antigens (CD169, F4/80) to determine the number of macrophages as percentage of all cells with the exception of adipocytes. Bone marrow tissue will be tested based on immunohistochemistry and molecular testing (q-PCR) to determine the expression of Oncostatin M, Osteonectin, LC3B-II and Beclin1.

Results: There are 10 respondents included in examined group for now. They all suffer from myelofibrosis. Average age at diagnosis is 61. The ratio between men and women is 6:4. Nine examinees suffer from primary myelofibrosis, and one patient has secondary myelofibrosis that has arised from essential thrombocythemia. Mutation in JAK2V617F gene is detected in 3 of 10 respondents. One patient has positive mutation CALR. In one respondent is detected abnormal karyotype in bone marrow cells (del13q14, del20q13). The mean hemoglobin value is 120 g/L at the time of diagnosis. The mean leucocyte count at the time of diagnosis is 17 x 109/L, and the average number of thrombocytes at the time of diagnosis was 755 x 109/L. The mean craniocaudal diameter of spleen at the time of diagnosis was 17,5 cm. Immunohistochemical staining of macrophage antigens (CD169, F4/80) in bone marrow is currently beeing performed.

Discussion: Epidemiologic data concerning age at the diagnosis of myelofibrosis are consistent with the literature data. Based on hematologic parameters, at the time of diagnosis, myelofibrosis was mostly manifested with leucocytosis, thrombocytosis and mild secondary anemia. The JAKV617F gene mutation status is positive at a slightly lower percentage in regards to the known results so far. Immunohistochemical staining to specific macrophage antigens and markers of autophagy is currently beeing performed.

MeSH/Keywords: osteogenic macrophages, autophagy, Oncostatin M, osteonectin, chronic Philadelphia-negative myeloproliferative neoplasm

Poster code: R-02-09-042

Poster Title: Gender and clinical parameters in the first year follow up of treatment outcomes in patients with acute coronary syndrome

PhD candidate: Igor Tagasovski

Part of the thesis: Gender and clinical parameters in the first year follow up of treatment outcomes in patients with acute coronary syndrome

Mentor(s): Academician Davor Miličić

Affiliation: University of Zagreb School of Medicine

Introduction: Despite the marked improvement of in-hospital outcome of patients with Acute Coronary Syndrome (ACS), long-term outcome remains poor. Therefore, studies on a role of specific factors that could be causative associated with worse clinical outcomes are still on-going. In this study we analyse concurrently non-variable risk factors with specific predictors for worse outcome within first year in ACS patients. We use data collected throughout of 6 years on patients included in the ISACS-TC Registry– Croatian Branch, such as non-variable factors -age and gender; as well as variable ones-glomerular filtration rate, bundle branch block in the electrocardiogram (ECG), and left anterior descending artery (LAD) as the infarction-related artery in persons who undergo primary percutaneous coronary intervention. So far separate investigations have shown association between each of these specific factors with worst clinical outcome whiten first year in patients who suffer acute coronary syndrome. Up to the present there have been no investigations which links these specific predictors in one unify study.

Materials and methods: In our case control study patients are divided into two groups survived/deceased, stratified by their non-variable risk factors (gender/age), and compared on the basis of predictors (selected according to their significance, i.e. low p-value) through log odds ratio. 3.000 patients are included from the ISACS-TC Registry-Croatian Branch. Eligible patients were those aged >18 years, who had been hospitalized for ACS between January 1, 2013, and December 31, 2017 and have been followed up for 1 year after their hospital discharge. ISACS-TC is one of the world's largest registries of patients who suffered acute coronary syndromes, with a headquarter at the University of Bologna. Department of Cardiovascular Diseases, University Hospital Centre Zagreb, has been participating within the Registry since January 2013. The data analysis is consisted of descriptive and inferential statistical methods. Patients will be split into two groups survived and deceased, stratified by their non-variable risk factors (gender and age). As part of descriptive analysis, the data will be represented through absolute frequencies, percentages and mean tendency. In order to summarize each observed variable we will report the mean, standard deviation, minimum and maximum value, variance or interquartile range. Furthermore, to better understand the relationships between the observed variables and categories, we will calculate Pearson or Spearmans' correlation coefficient in the closed range [-1, 1]. All exploratory analysis will be done for the complete dataset, as well as per stratified group. To test for differences in clinical findings between patients who died or survived, we will use odds ratio. However, before applying any test, we will need to determine the type of the data distribution (a known parametric family/nonparamertric) so that a suitable test method can be implemented. In determining the distribution of the data, suitable parametric or nonparametric methods will be used. For all tests the level of significance will apriory be set to 5%, which will provide 95% confidence interval for accepting or rejecting our hypothesis by observing p-values from two-sided tests.

Results: The study is currently in the phase of statistical processing and preliminary results are expected to be finalized at the end of April 2019.

Discussion: This study will enable detection of parameters which are possible marker of bad outcomes (reinfarction or lethal outcome) in first year after the primary percutaneous coronary interventions in ACS patients. According to this results we can detect possible high risk subgroups, and for the patients in this groups we will suggest more frequent cardiological follow ups and tests (accordingly to the predictors for bad outcomes).

MeSH/Keywords: acute coronary syndrome, predictors, high risk subgroup

Poster code: R-02-09-049

Poster Title: Gene RAD50, STARD3, ORMDL3 and IL6R polymorphisms in asthmatic patients

PhD candidate: Anamarija Štajduhar

Part of the thesis: Gene RAD50, STARD3, ORMDL3 and IL6R polymorphisms in asthmatic patients

Mentor(s): Assoc. Prof. Sanja Popović-Grle, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Asthma is a common inflammatory respiratory disease, resulting from the interaction of genes and environment. Thus, previous researches have shown that IL-6 gene is responsible for the immunomodulatory and proinflammatory effect. RAD50 affects the expression of Th2 cytokines. ORMDL3 gene is particularly expressed in cells involved in inflammatory response and associated with more frequent asthma exacerbations. STARD3 gene is associated with allergic asthma. IL6R, RAD50, STARD3, and ORMDL3 gene single nucleotide polymorphisms (SNP) are associated with earlier disease onset, a severe form of asthma, and atopic asthma phenotype. The aim of this study is to investigate the frequency of aforementioned SNPs, to examine how they affect inflammatory component and severity of the disease, the time of asthma occurrence, and how they interact with environmental factors.

Materials and methods: This case-control study will be carried out at the Clinic for Pulmonary Disease Jordanovac at the Clinical Hospital Center Zagreb in collaboration with the University of Manchester. The study will include 540 adult subjects of both sexes, 240 asthma patients and 300 healthy controls who signed the informed consent. Asthma will be determined based on anamnesis, clinical status, specific tests and validated ECRSH II and ECRSH II Smoking questionnaires. Anamnestic data will be obtained from medical documentation dating from 2003 to 2018. The allergic status will be established with a skin prick test. Spirometry with bronchodilator test will be used to assess pulmonary function, the fractional exhaled nitric oxide will be determined and lung diffusion test conducted. The DNA from leukocytes will be amplified by chain reaction polymerization, and genotyped using the MALDI-TOF MS. Additional venous blood will be taken for the determination of total and specific IgE, eosinophilic cationic protein, CRP and complete blood count. In selecting SNPs of interest, data from previous SNP researches and linkage disequilibrium (LD) data will be used. The relationship between relevant SNPs and traits of interest will be analyzed using $\chi 2$-test, multivariate logistic regression, and ANOVA.

Results: Insofar a total of 204 participants have been enrolled in the study. Among them, 79 (38.72%) were men, and 124 (61.08%) were woman. The mean age was 44.32±17.07 years. Most of the participants suffered from mild to moderate asthma - 105 (52%) and 72 (35%) respectively, whilst only 15 (7%) were in the severe asthma group. The mean BMI was 25.7164±4.62 kg/m2. In a total of 190 patients, it was possible to determine the age of onset of the disease, and most of them had adult type disease (142; 75%). Most of the patients had concomitant asthma-rhinitis phenotype (190.00; 93%). At the moment of inclusion into the study, the mean asthma duration of the population of interest was 12.889±10.53 years. Most of the included subjects were never-smokers (121; 62%), and the rest of the group were predominantly ex-smokers (44; 23%). In the group of people who were current smokers or ex-smokers, the mean pack-years were 4.718±10.92 years. Lung function tests showed that the mean FEV1 was 2.67±0.94 L. Finally, the duration of asthma negatively correlated with FEV1 (Pearson Correlation -0.027, $p<0.001$).

Discussion: This study is aimed to describe the adult population of patients suffering from asthma and as accurately as possible show current population trends in Croatia pertaining to the disease burden and risks and preventable traits. So far, it was shown that the most of the patients in Clinical Hospital Center Zagreb had mild or moderate disease which is in correlation with the current world situation. Also, most of them were adults at the moment of asthma diagnosis, which can be under the influence of confounders, mostly insufficient data. Asthma in Croatia is predominantly allergic, and most of this population were smokers. It shows that the unfavorable smoking trends are still present in this population, which is one of the major problems in asthma patients, and continues to

burden this population group despite current anti-smoking efforts. The lung function worsens with the asthma duration time. In conclusion, although these are just a fraction of intended study results, the situation in Croatia is similar to that present in other world population studies.

MeSH/Keywords: asthma, atopy, genes, SNP, phenotype, pulmonary function

Poster code: R-02-09-059

Poster Title: Knowledge and self-efficacy as predictive factors of regulation in type 2 diabetes mellitus

PhD candidate: Miroslav Ćaćić

Part of the thesis: Knowledge and self-efficacy as predictive factors of regulation in type 2 diabetes mellitus

Mentor(s): Professor Milan Vrkljan, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Diabetes mellitus (DM) is one of the most common chronic diseases whose treatment depends largely on compliance. DM is the world's leading cause of blindness, kidney failure, heart attacks, stroke and lower extremity amputation. Assessment of the patient's level of knowledge of diabetes is an important step in adapting group education programs to better achieve treatment outcomes. Self-efficacy refers to an individual's belief that he can successfully achieve the behavior necessary to achieve a specific goal. Patient's ability to correctly measure blood glucose levels, adhere to the recommended diet, exercise with physical activity, and adjust therapies as needed, results in better control of DM and greater patient satisfaction.

Materials and methods: The study was carried out in the health centers of Zagreb-Centar, Zagreb-West, Zagreb-East, Health centers of the Zagreb County and the Institute for Endocrinology, Diabetes and Metabolic Disorders "Mladen Sekso", Clinic for Internal Diseases of Clinical Hospital Center Sestre milosrdnice (CMC SM). The study was conducted as a cross-sectional study of patients with type 2 diabetes, with 70% of examined patients being treated by family physicians (health centers of examined patients being treated by family physicians (health centers of the City of Zagreb and Zagreb County), while 30% of patients are treated by diabetologists (KBC SM).

Results: The first translation was made by a professional translator, familiar with the vocabulary and semantics of the terms used in the diabetes environment. In order to be able to translate some specific expressions, a diabetologist with excellent English knowledge was involved. The translation version was then revised by four diabetologists with excellent English language knowledge. The translators worked independently and all four versions are merged into one final version. This final version of the questionnaire was translated into English by a physician who is an native speaker and English and Croatian. Such backward reward was additionally evaluated by 2 general practictioners and 1 diabetologist, all with excellent English language skills. Since there was no difference between the text versions of the question, the translation was considered adequate. In the second part of the study, a psychometric test was performed to assess the reliability (Cronbach alpha - internal consistency (homogeneity of the questionnaire) and test-retest reliability), validity (constructive, convergent and divergent validity) and suitability of the questionnaire - good psychometric properties for all questionnaires were confirmed.

Discussion: Research has shown that knowledge should be a central result in the control of diabetes. Investigations reveal that knowledge about medications, diet, physical activity, blood glucose monitoring at home, care for the feet and on the necessary modifications is essential to manage diabetes.

MeSH/Keywords: diabetes mellitus type 2, DKQ-24, DMSES, psychometric properties, validation

Poster code: R-02-09-064

Poster Title: Characteristics of prehypertension

PhD candidate: Margareta Fištrek Prlić

Part of the thesis: The importance of serum concentrations of hepatocyte growth factors in prehypertension

Mentor(s): Professor Bojan Jelaković, MD PhD

Affiliation: University of Zagreb School of Medicine, Department of Nephrology, University Hospital Center Zagreb

Introduction: Prehypertensives have an increased cardiovascular risk, and are twice more likely to develop hypertension than subjects with optimal blood pressure (BP). Presence of additional risk factors (i.e. dyslipidemia, hyperglycemia) and/or target organ damages is important for therapeutic plan. Our aim was to analyze the prevalence and characteristics of prehypertensives in our cohort of subjects enrolled in an epidemiological survey performed in continental rural part of Croatia.

Materials and methods: Data obtained from 913 subjects (333 men, 580 women; average age 55.0 ± 16.6 vs. 56.3. ± 17.2; $p > 0.05$) were analyzed. There were no differences between genders except in weight ($p < 0.0001$) and triglycerides (TC) ($p = 0.04$). BP and heart rate were measured using OMRON devices recommended by the ESH, performing it in the sitting position after 5 minutes of rest, three times in two visits, and mean values were calculated.

Results: Prevalence of prehypertension in the whole group was 36% (men vs. women 39% vs. 33%; $p < 0.001$). Significant difference between men and women was observed in frequency of prehypertension (74% vs. 50%) and optimal BP (26% vs.50%) values ($p < 0.0001$). Prehypertensives were older ($p = 0.0001$) and had higher values of BMI ($p = 0.029$) and fasting blood glucose (FBG) ($p = 0.004$) than subjects with optimal BP. We failed to find differences between prehypertensives and stage 1 hypertensives in heart rate, BMI, waist circumference, FBG and TC. Significantly higher values of age ($p < 0.0001$), heart rate ($p = 0.03$), waist circumference ($p = 0.03$), FBG ($p = 0.0006$) and TC ($p = 0.00016$) were observed in stage 2 and 3 hypertension than in prehypertensives.

Discussion: Prevalence of prehypertension is high, significantly higher in men than in women. Obtained higher values of FBG in prehypertensives than in subjects with optimal BP might point on insulin resistance. Prehypertensives do not differ in cardio-metabolic risk factors from hypertensives in stage 1, and that could explain their higher cardiovascular and renal risk.

MeSH/Keywords: Prehypertension, Cardiovascular Risk, Metabolic Syndrome

Poster code: R-02-09-076

Poster Title: Cross Sectional Study On Serum Ghrelin, Leptin And Proinflammatory Cytokines Levels In Inflammatory Bowel Disease

PhD candidate: Ana Barišić

Part of the thesis: Cross sectional study on serum ghrelin, leptin and proinflammatory cytokines levels in inflammatory bowel disease

Mentor(s): Professor Željko Krznarić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Crohn's disease and ulcerative colitis are chronic, idiopathic, and relapsing forms of inflammatory bowel disease (IBD). The pathogenesis of IBD likely involves complex interactions between genetic, environmental or microbial factors and immune responses. Leptin and ghrelin participate in a broad spectrum of biological pathways, which in turn modulate metabolism as well as immunity. Available data suggest leptin and ghrelin may have role in intestinal inflammation. In addition, it is well known that IBD is frequently associated with loss of appetite and weight loss, where ghrelin and leptin may also play a role. The aim of this study is to evaluate serum ghrelin, leptin, IL-6 and TNFα levels and their association with disease activity and body composition.

Materials and methods: 180-200 patients diagnosed with IBD, based on clinical, histological and endoscopic criteria and 50 healthy controls will be included in this research. Plasma ghrelin, leptin, IL6 and TNFα levels will be determined using an enzyme immunoassay (EIA) technique. All participants will undergo anthropometric measurements (including muscle strength) as well as analysis of body composition measured by bioelectrical impedance analysis (TANITA body composition analyser, BC-420MA). In order to evaluate disease activity, we will use laboratory parameters (faecal calprotectin, CRP) and clinical indices: Crohn's Disease Activity Index (CDAI) for Crohn's disease patients and Partial Mayo Score for ulcerative colitis patients.

Results: From March 2018 till April 2019, a total of 199 participants were enrolled (CD=121; UC=60; healty controls 18; among which 57 % were male and 43% female). The median age was 67 years (range 18-70). Epidemiological and clinical data, including clinical activity by appropriate scales, date of diagnosis, initial and current treatment, phenotype, disease extension, extraintestinal manifestations and laboratory parameters, including CRP and faecal calprotectin levels were obtained. All participants underwent anthropometric measurements and bioelectrical impedance analysis. Serums for plasma ghrelin, leptin, IL6 and TNFα analysis were was collected, aliquoted and stored at −70°C and will be analysed when target control group number will be reached. In order to assess association of body composition and muscle strength with disease activity we analysed data for 75 patient (CD=58, UC=17; 50.7% male, 49.3% female). Clinically active disease (defined as CDAI > 150 or Partial Mayo Score≥3) was present in 25 patients (16.9% CD and 42.9% UC). There were no statistically significant differences among patients with active and inactive disease in fat-free mass index -FFMI [kg/m2] 17.7 (14.9-20.1) vs. 18.2 (15.3-26.2), skeletal muscle index-SMI [kg/m2] 8.9 (7.1-10.7) vs. 9.3 (7.8-10.8), lean mass - LM [kg] 50.4 (43-72.4) vs. 55 (43.3-90.5), and fat mass [%] 19.7 (14.9-45) vs. 23 (17.5-45), $p>0.05$. Muscle strength was significantly lower in patients with active disease (26.6±10.9 kg) comparing to inactive group (32±11.5 kg), $p=0.048$. Underweight patients, defined as BMI<18.5 kg/m2, were significantly more prevalent in active group comparing to inactive (27.8% vs 8.8%, $p=0.04$, $\chi 2=4.219$).

Discussion: Results of the analysis haven't shown consistent association between body composition and disease activity. However, muscle strength was lower in group of patients with clinically active disease. Further analysis of serum biomarkers (ghrelin, leptin, IL-6 and TNFα levels) in study participants will help to determine possible association of ghrelin, leptin, IL-6 and TNFα levels with disease activity and body composition.

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MeSH/Keywords: inflammatory bowel disease, ghrelin, leptin, proinflammatory cytokines
Poster code: R-02-09-082

Poster Title: Influence of nutritional status and educational level on the outcomes in chronic hemodialysis patients

PhD candidate: Lea Katalinić

Part of the thesis: Influence of nutritional status and educational level on the outcomes in chronic hemodialysis patients

Mentor(s): Assist. Prof. Nikolina Bašić Jukić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Problem of protein-energy wasting (PEW) is complex, and its prevalence in the population of chronic kidney disease (CKD) patients is high. Although largely associated with the presence of inflammation, uremia and metabolic acidosis, its progression is most certainly influenced by the restrictions our patients are constantly faced. Although dealing with maintaining fluid and electrolyte balance is an important first step to successful treatment, research show that 19-99% hemodialysis (HD) patients fail to comply with the given instructions. However, research focused on the impact of education and sociodemographic characteristics is scarce, and the results questionable. The main aim of our study is to show that higher educational level as well as continuous nutritional education could change nutritional habits of HD patients, prevent malnutrition and improve long-term treatment outcomes.

Materials and methods: 134 patients undergoing HD were included in this prospective study. Clinical data were obtained from the medical records and charts. Dietary habits and socio-demographic characteristics were evaluated using a self-administered questionnaire. Laboratory parameters were measured at the beginning of the study and controlled in 6-months intervals. Anthropometric measurements were performed prior to HD session. Malnutrition-inflammation score (MIS) was used as a scoring system representing the severity of PEW. First educational visit was conducted by a nephrologist and a dietitian during which every patient received individual nutritional recommendations. Six months later, a follow-up questionnaire was carried out in order to identify if any changes in food intake were present.

Results: There were 58.32% male and 41.8% female patients, mean age 60.8+/-16.15 years. The leading cause of CKD was chronic glomerulonephritis (27.72%). The mean HD vintage was 96.03+/-102.521 months. When dividing the patients by MIS, 44.7% patients were well nourished (MIS 0–2) or slightly malnourished (MIS 3–7), and 55.3% patients were malnourished (MIS≥8). Malnourished patients were significantly older ($p<0.05$). They had significantly lower residual diuresis, body mass index (BMI), serum proteins and albumins and lean tissue index (LTI) when compared to non-malnourished patients (all $p<0.05$). When observing dietary habits only 69% of patients had breakfast every day and 22% didn't eat until lunch. 83% of patients received a meal during HD and 17% refused it. Most patients undergoing HD had three to four meals daily. There was a strong correlation of meat intake with age ($p<0.001$). Greater number of household members and higher educational level correlated with higher creatinine, protein and albumin leves as well as BMI and LTI (all $p<0.05$). More educated patients had lesser interdialytic weight gain ($p=0.03$) and lower phospate levels ($p=0.04$). 66% of patients answered positively when asked whether they attempted to change their eating habits after nutritional education. 34% of them did not even try. 23 patients died during the study. Malnourished patients survived significantly shorter than non-malnourished patients. Only malnourishment and age were associated with higher overall mortality in all groups of patients.

Discussion: Despite great efforts put into treating well known and highly prevalent traditional risk factors, and continuous improvements in HD techniques, mortality rates of CKD patients remained inexplicably high. Results of our study demonstrate that survival of HD patients is strongly associated with the parameters regarding nutritional status. When estimating nutritional status through MIS, it is evident that by improving serum albumin and protein levels, and therefore LTI and MIS, we could be able to achieve better nutritional and survival outcomes. However, this is neither simple nor easy when bearing in mind poor nutritional habits of HD patients as demostrated by our study. Specific

educational activities along with continuous evaluation and therapy adjustments are needed to improve patient compliance in order to achieve better outcomes.

MeSH/Keywords: kidney disease, hemodialysis, protein-energy wasting, education

Poster code: R-02-09-090

Poster Title: Associated risk factors for hepatitis E seroprevalence among liver transplant recipients

PhD candidate: Petra Dinjar Kujundžić

Part of the thesis: Hepatitis E viral infection after liver transplant

Mentor(s): Assist. Prof. Anna Mrzljak, MD PhD, Professor Adriana Vince, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Hepatitis E virus (HEV) is an emerging disease in Europe, especially important among solid organ transplant (SOT) recipients who are at greater risk of developing acute and chronic graft hepatitis with progression to cirrhosis. It is proposed that foodborne transmission is the main route of HEV infection in developed countries. However, risk factors for the acquisition of HEV infection among SOT recipients are incompletely understood. This study aimed to determine exposure of HEV infection and associated socio-demographic risk factors.

Materials and methods: In this cross-sectional study, 242 Croatian SOT recipients were screened during routine post-transplant outpatient visits. All participants completed a risk factor assessment questionnaire. Blood samples were tested for anti-HEV IgG using an enzyme immune assay (Mikrogen, Germany) and nucleic acid extracts of whole blood were tested by an in-house real-time reverse-transcriptase polymerase chain reaction assay for HEV RNA.

Results: Anti-HEV IgG seroprevalence in Croatian SOT recipients was 24.38%. No SOT patients had HEV viraemia at time of testing. The median time after transplant was 5 years (range 19 years). The majority of the recipients were male (69.0%) and major indication for SOT was alcoholic liver disease (50.4%). Older age (OR=1.05; 95%CI=1.02-1.09), female gender (OR=2.61; 95%CI=1.42-4.81), rural area of residence (AOR=2.17; 95%CI=1.10-4.27), and specific factors within a household: a farm (AOR=2.79; 95% CI=1.31-5.92), a water-well (AOR=3.09; 95%CI=1.11-8.57) and a sewage system connected to a septic tank (AOR=3.38; 95%CI=1.64-6.95) were detected as potential risk factors, while highest level of education (AOR=0.05; 95%CI=0.01-0.43) and a recent travelling experience (AOR=0.39; 95%CI=0.17-0.88) as protective factors. Contrary to initial assumptions, production and/or consummation of cured meat and occupational exposure had no statistically significant strength of association with anti-HEV IgG seropositivity.

Discussion: Prevalence of anti-HEV IgG in SOT recipients in Croatia is 24.38%. Identified socio-demographic factors associated with the seropositivity set up a platform for further research directions to evaluate sources/routes of transmission and clinical impact of HEV infection after SOT.

MeSH/Keywords: Hepatitis E virus infection, seroprevalence, risk factors, liver transplantation

Poster code: R-02-09-112

Poster Title: The impact of CRT therapy on valvular regurgitation differs between men and women

PhD candidate: Marija Mance

Part of the thesis: The impact of CRT therapy on valvular regurgitation differs between men and women

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

Affiliation: University of Zagreb School of Medicine

Introduction: This study was conducted on patients with symptomatic chronic heart failure (CHF) and implanted cardiac resynchronization therapy device (CRTD) according to guidlines in order to evaluate possible differences in mitral (MR) and tricuspid (TR) valvular regurgitation grades and left ventricle ejection fraction (LVEF) between male and female CRTD patients during follow up of 6 months after CRTD implantation.

Materials and methods: We analyzed 135 patients (89 M, 46 F) with CHF due to any cause, implanted CRTD, optimal medical therapy with atrial fibrillation or in sinus rhythm. Clinical (NYHA class) and echocardiographic data (valve pathology, LVEF) were assesed before and 6 months after CRTD implantation. Using color and continuous flow Doppler, PISA, regurgitation volume and vena contracta measurments, MR and TR were stratified in 3 grades.

Results: Mean age of patients was 60 (± 10) years.The results showed improvement in LVEF from 28% up to 37% ($p < 0.001$) in all patients regardles of sex and age, as well as worsening of TR ($p < 0.001$) and no difference in MR ($p = 0.195$). In male patients (N = 89), significant worsening in MR and TR was present, $p < 0.05$. while in female patients (N = 46) there was no significant worsening in TR ($p = 0.06$) nor MR ($p = 0.42$). In patients older than 60 years (N = 82, female 27 (33%), male 55 (67%)), as well as younger than 60 years (N = 53, female 19 (36%), male 34 (64%)) significant worsening of TR grade was observed ($p < 0.05$) while the grade of MR remained the same ($p = 0.255$ and $p=0.534$).

Discussion: Six months after CRTD implantation the grade of MR and TR remained the same in female patients while significant wosening in MR and TR grade was found in male patients. TR worsening was probably due to implanted electrodes. The changes in LVEF, TR and MR did not differ in pts younger and older than 60 years of age. Despite improvement in NYHA class and LVEF, the changes of MR did not meet our expectations.

Acknowledgments: 1. Randolph TC, Hellkamp AS, Zeitler EP, Fonarow GC, Hernandez AF, Thomas KL i sur. Utilization of cardiac resynchronization therapy in eligible patients hospitalized for heart failure and its association with patient outcomes. Am Heart J. 2017 Jul;189:48

MeSH/Keywords: CRTD, gender differences in CRT patients, valvular regurgitation in CRT

Poster code: R-02-09-145

Poster Title: The influence of decontamination protocols on structural characteristics of heart valve allografts

PhD candidate: Ivica Šafradin

Part of the thesis: The influence of decontamination protocols on structural characteristics of heart valve allografts

Mentor(s): Assist. Prof. Bojan Biočina, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Heart valve allografts are well established substitutes used for heart valve replacement. Microbiological decontamination is a standard procedure in heart valve allografts preparation. Although an increase in temperature of microbiological decontamination could increase its efficacy, there is no published data about its influence on structural characteristics of heart valve allografts. Functional durability of heart valve allografts depends on remodeling capacity of extracellular matrix. Remodeling capacity of extracellular matrix depends on the balance between biosynthetic and proteolytic activity. The aim of proposed study is to analyze influence of different protocols of microbiological decontamination on remodeling capacity of extracellular matrix of heart valve allografts.

Materials and methods: Heart valve allografts used in this study do not meet functional and morphological criteria for the storage in Cardiovascular tissue bank at UHC Zagreb. Heart valve allografts will be incubated in antibiotic solution at 4°C and 37°C. Tissue samples will be taken at beginning of decontamination and after 6 and 24 hours. Tissue samples will be routinely processed in pathohistological laboratory. Immunohistochemical analysis of expression of matrix metalloproteinase 2 and procollagen type I will be performed and tissue samples for fibroblast culture will be taken.

Results: Preliminary results include measurements for 7 heart valve allografts (targeted to collect 24 heart valve allografts). Results for fibroblast culture and microbiological analysis are available. Levels of MMP-2 and procollagen type I expression, due to high cost, are planned to be measured when all samples are collected. Preliminary results are expected although number of collected heart valve allografts and measurements is still not enough for statistical data analysis.

Discussion: Preliminary results are in accordance with our hypothesis although we need further measurements to achieve enough data for adequate statistical analysis. We hope that results of future measurements will help us to increase efficacy of decontamination protocol without influencing original characteristics of heart valve allografts.

Acknowledgments: I would like thank to my mentor Professor B. Biocina and to the staff of Cardiovascular Tissue Bank and Department of Pathology at University Hospital Center Zagreb for their unconditional support.

MeSH/Keywords: Heart valve, allograft, decontamination, extracellular matrix

Poster code: R-02-10-032

Poster Title: Effect of resveratrol on oxidative stress parameters and systemic inflammatory response in patients undergoing cardiopulmonary bypass during cardiac surgery

PhD candidate: Mislav Planinc

Part of the thesis: Effect of resveratrol on oxidative stress parameters and systemic inflammatory response in patients undergoing cardiopulmonary bypass during cardiac surgery

Mentor(s): Professor Željko Sutlić, MD PhD, Ivana Novak Jovanović, PhD, research associate

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

Introduction: There is a well-established connection between cardiopulmonary bypass (CPB), oxidative stress and systemic inflammatory response syndrome (SIRS) in cardiac surgery patients. Reactive oxygen species thought to be responsible for such effects are produced during surgical trauma, contact of blood with non-biological surfaces and due to ischemic – reperfusion injury which all lead to SIRS. Previous researches have shown that intake of antioxidants in preoperative period can markedly reduce the incidence of SIRS and lower the negative effects of CPB. Scientific interest for resveratrol, a member of stilben family, started with observation of "French paradox" – lower incidence of cardiovascular diseases in people who regularly ingest moderate amounts of red wine. There has not been a research yet about a resveratrol as an antioxidant in cardiac surgery.

Materials and methods: In this placebo-controlled double blinded randomized clinical trial 30 elective valve patients that meet inclusion criteria will be included and administrated with resveratrol (RES group - 2 x 400 mg 2 days before and 3 days after index day of operation) or placebo (CON group). Inclusion criteria are elective aortic valve replacement for aortic stenosis, age 18 – 85 and signed informed consent. In five time points during and after the procedure the blood samples from venous system will be drawn to measure levels of biomarkers of oxidative stress as well as clinical and laboratory signs of inflammation. Those time points are V1 – admission to hospital, V2 – after induction to anesthesia, V3 – after coming to intensive care unit after the surgery, V4 – 24 hours after the surgery and V5 – 72 hours after the surgery. We will analyze each of the following biomarkers – superoxide dismutase, malondialdehyde and total antioxidative plasma capacity (TAPC) using spectrophotometry and liquid chromatography. Regarding clinical signs of SIRS body temperature, respiration and pulse are measured. Laboratory markers of inflammation that are prospectively measured are leukocytes (L), C-reactive protein (CRP) and erythrocyte sedimentation rate (SE).

Results: We present preliminary results done for first 10 patients (4 in RES group and 6 in CON group) enrolled to this randomized controlled trial. Mean age in both groups is 67 years, with male prevalence (60%). Ejection fraction was 60 +/- 8 % and 59 +/-9% respectively and EuroSCORE II was 2.1 +/- 0.2 in both groups. Two groups did not differ in body temperature (C˚; 36.8 +/- 0.3 vs 36.9 +/- 0.5, $p>0.5$), heart rate (80 +/- 8 vs 80 +/-9 beats/min; $p>0.5$) nor in number of respirations (respirations/min; 12 +/-2.3 vs. 13 +/- 2.6; $p>0.5$) in all 5 time points. Comparing RES group with CON group there has been a difference in leukocytes (109/L; 11 +/-3 vs. 16 +/-7; $p<0.5$), CRP levels (mg/L; 60 +/-40 vs. 170 +/- 114, $p<0.05$) bot not in SE (mm/h; 85 +/-47 vs 86 +/-55, $p>0.5$). Regarding markers of oxidative stress when comparing RES and CON – MDA (pM/mL; 145 +/-67 vs. 190 +/-102; $p<0.05$) and TAPC (mM/L; 1.2 +/- 0.15 vs. 0.6 +/- 0.15; $p<0.05$) were significantly different but total SOD (U/mg; 45 +/- 15 vs. 40 +/- 10; $p>0.5$) was not shown to have the difference.

Discussion: General characteristics and clinical parameters of inflammation of patients in RES and CON group do not differ. However, when comparing laboratory signs of inflammation such as L and CRP group that received resveratrol showed less high levels of both markers, but SE rate did not follow. When investigating oxidative stress markers, MDA and TAPC both showed significantly higher levels in group given resveratrol, implying that less MDA is been activated in blood after blood exposition to CPB machine is due to possible resveratrol effect. Also, TAPC is higher in RES group meaning that resveratrol is promoting total antioxidative capacity of plasma antioxidants. However,

SOD levels did not follow the same results. We still have a small number of patients enrolled as only third of patients have been evaluated, so we may expect to have changes in SOD results. Other possible explanation is that resveratrol in not having effects of SOD levels. In conclusion, we can say that upon first 10 patients enrolled and analyzed resveratrol, which is potent antioxidant, is showing promising and positive results on levels of oxidative stress markers in plasma in this group of patients.

MeSH/Keywords: Cardiac surgery, resveratrol, oxidative stress, systemic inflammatory response

Poster code: R-02-10-048

Poster Title: Interdependence of intracranial and lumbar cerebrospinal fluid pressure in patients with ruptured intracranial aneurysms - preliminary results

PhD candidate: Ivan Pašalić

Part of the thesis: Interdependence of intracranial intradural and lumbar cerebrospinal fluid pressure in patients with ruptured intracranial aneurysms

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

Affiliation: University of Zagreb School of Medicine

Introduction: According to the ''classical hypothesis'' of cerebrospinal fluid (CSF) hydrodynamics, its production takes place at the choroid plexuses located in the brain ventricular system. From the lateral ventricles CSF circulates towards the third ventricle through the foramen Monroi and towards the fourth ventricle through the Sylvian aqueduct. Hereupon, CSF reaches the subarachnoid cisterns through the Luschka and Magendie openings. Finally, CSF resorption takes place at the level of the dural venous system. On the other hand, the „new“ hypothesis states that the CSF hydrodynamics is based on the hydrostatic and osmotic pressures at every single point along the ventricular system. Thus, CSF cicrulation, as well as its secretion and resorption, are a result of net pressure gradient along the ventricular system.

Materials and methods: The research is being carried out at the Zagreb University Hospital Centre's Department of Neurosurgery. Twenty patients are expected to be enrolled by the end of a two year period. Patients older than eighteen years and diagnosed with a ruptured intracranial aneurysm are included in the study. Diagnostic evaluation consists of a detailed clinical examination and extensive neuroradiological findings (MSCT of brain, MSCT angography and, if necessary, cerebral DSA). Each patient undergoes external ventricular and external lumbar drainage, followed by aneurysm „clipping“. Following the neurosurgical procedure patients' intracranial and intraspinal pressures are continuously monitored in the intensive care unit during a four-day period. Standard measurements are performed continuously, in a lying position (with a thirty-degree headboard tilt), while non-standard measurements are performed intermittently, in a horizontal position (with a zero-degree headboard tilt). As a standard of care, biochemical analysis of CSF samples is performed on a daily basis. Decision whether the external ventricular drainage will be removed or converted to a ventriculoperitoneal shunt depends on the findings of the CT-scan follow-up and the detection of hydrocephalus.

Results: Preliminary data are based on the first three included patients (two males and one female, with an average age 49.7±14.2 years), none of which developed postoperative hydrocephalus. Median GCS was 14, while the mean ventricular opening pressure was 10 mmHg. No significant difference was detected between the average intracranial and intraspinal CSF pressures during a four-day follow-up. Preliminary results also shows no differences in changes of both intracranial and intraspinal CSF pressures in a four-day follow-up. Interestingly, we found a strong trend towards lower values of intraspinal CSF pressures measured in the standard position when compared to the non-standard position during the first postoperative day. This trend was lost in the following days. No significant changes in CSF laboratory findings were detected in the follow-up period.

Discussion: Although based on a small sample, presented preliminary results are in accordance with the „new“ hypothesis of CSF hydrodynamics. No changes among the values of intracranial and intraspinal CSF pressure gradients were found, which was expected as no patients developed hydrocephalus following the „clipping“ procedure. A trend towards lower intraspinal pressures in the standard position can be explained by an increased resorption of CSF fluid due to initially increased hydrostatic pressures when the patient's head is tilted upwards. Finally, finishing the research is warranted in order to confirm the „new“ hypothesis of CSF hydrodynamics and to check for an impact of invasive CSF pressure monitoring on outcomes in patients with a ruptured intracranial aneurysm.

MeSH/Keywords: Ruptured intracranial aneurysms, cerebrospinal fluid hydrodynamics, intracranial cerebrospinal fluid pressure, lumbar cerebrospinal fluid pressure, pressure gradient.

Poster code: R-02-10-110

Poster Title: Influence of external lumbar drainage on pressure and volume of cerebrospinal fluid, CSF biomarker concentrations and clinical status of patients with idiopathic normotensive hydrocephalus

PhD candidate: Klara Brgić

Part of the thesis: Influence of external lumbar drainage on pressure and volume of cerebrospinal fluid, amyloid beta and tau protein concentrations and clinical status of patients with idiopathic normotensive hydrocephalus

Mentor(s): Professor Marijan Klarica, MD PhD, Assist. Prof. Goran Mrak, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Normotensive hydrocephalus is a clinical condition that includes symptoms such as dementia, urinary incontinence and gait disturbance and is characterized by an increased amount of cerebrospinal fluid intracranially. The pathophysiology of normotensive hydrocephalus is unclear. Although there are numerous studies on the intracranial volume of cerebrospinal fluid (CSF) and changes in intracranial volume of fluid and brain tissue after ventriculoperitoneal drainage, there are few studies that describe the changes in volume in spinal compartment. New neuroradiological research argue against one-way circulation of cerebrospinal fluid and prove that CSF is constantly pulsing in both directions. In the last decade, interest has increased for biomarker research in CSF, especially in diseases such as Alzheimer's dementia, but also hydrocephalus, which is one of the few reversible causes of dementia. Previous research has shown heterogeneous results of biomarker concentrations such as β-amyloid protein 42 (Aβ42) total tau (t-tau) and phosphorylated tau (p-tau) in patients with idiopathic normotensive hydrocephalus.

Materials and methods: Patients with clinical presentation and neuroradiological signs of idiopathic normotensive hydrocephalus were subjected to testing by evacuation of CSF through external lumbar drainage over a 72 h period, during which the values of intracranial pressure, corresponding to lumbar pressure in lying position, were monitored. Prior to external lumbar drainage placement, the subjects were evaluated (MMSE, Japanese NPH scale) to determine the severity of the clinical symptoms. Each patient was thoroughly informed about the procedure. MRI volumetric analysis of CSF spaces of the neuroaxis using standard high-resolution sagittal T1 sequences for the haed (MPRAGE), and standard high-resolution sagittal T2 sequences for the cervical, thoracal and lumbosacral segment of the spine (1x1x1 mm voxel dimensions) , prior to the insertion of external lumbar drainage and after its removal, will be coupled with changes in clinical parameters. Biomarkers from CSF (amyloid β - c (Aβ1-42) / pg/ml, c (Aβ1-40) pg/ml, total and phosphorylated tau proteins (c (Total tau) (pg/ml), c Tau [pS199] / pg/ml, c Tau [pT181] / pg/ml) and c (NF-L)/pg/ml) were quantified in three time points (0, 36 and 72 h)

Results: Preliminary results were calulated on a total number of ten tested patients. Distribution by gender was equal and the mean age was 74 ± 5,8. Mini mental scores ranged from 3-29, with the mean of 23.2. Mean opening pressure was 6,8 mmHg. CSF concentration of all analyzed biomarkers were significantly higher in the CSF samples in the last time point (72 h after ELD) then those from the initial samples (0h –ELD placement). Three patient had significant improvement after ELD testing and underwent surgery.

Discussion: MRI images obtained prior to ELD placement and 72 hours after drainage, support our hypothesis that there is no significant change in the volume of intracranial cerebrospinal fluid, suggesting that spinal fluid space is the dominant compensatory space and intracranial pressure depends on the intraspinal volume. Since biomarker values are significantly higher in the last sample, it is possible that this is a protective mechanism of the brain to remove harmful substances via CSF.

MeSH/Keywords: Cerebrospinal fluid, normotensive hydrocephalus, intracranial and spinal cerebrospinal fluid volume, intracranial pressure, external lumbar drainage, CSF biomarkers

Poster code: R-02-10-115

Poster Title: Protein expression of SCUBE2 in aortic valve stenosis - preliminary data

PhD candidate: Josip Varvodić

Part of the thesis: The role of Hedgehog-Gli signaling pathway in development of calcific aortic valve stenosis

Mentor(s): Assist. Prof. Igor Rudež, MD PhD, Assist. Prof. Frane Paić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Calcific aortic stenosis is the main heart valve disease in the elderly, leading to massive focal calcification and thickening of the valve cusps. Signal peptide CUB EGF-like domain-containing protein 2 (SCUBE2), a member of the SCUBE family of proteins, was recently found to play an important role in atherosclerotic plaque progression via Hedgehog signal transduction. However, little is known regarding its biological function in aortic valve stenosis. The aim of this study was to investigate SCUBE2 expression and localization in normal and stenotic aortic valve tissue.

Materials and methods: The study comprised 15 tricuspid aortic valves obtained from patients with severe aortic valve stenosis (n=10) and aortic valve insufficiency (n=5) as well as normal, pathologically unaltered aortic valve tissue (n=5) obtained from patients undergoing heart transplantation. Upon surgical resection the aortic valve tissue tissues were fixed in formalin and embedded in paraffin. The paraffin-embedded tissue blocks were cut into 5µm sections using a microtome. The sections were incubated for 1 hour in 10% normal goat serum/PBS solution, then incubated overnight with SCUBE2 primary antibody (Abcam, ab117625 at concentration 5 µg/ml), in 0.1% BSA/PBS solution in humid chambers at 4°C. Secondary goat anti-Rabbit IgG H&L (HRP) (Abcam, ab205718 at concentration 5 µg/ml) were applied following incubation (10 min at room temperature) in peroxidase blocking solution according to manufacturer protocol. Immunostaining was visualized by 1x diaminobenzidine (DAB) solution (DAKO; Hamburg, Germany), subsequently counterstained with hematoxylin, and mounted with Permount (Sigma). Immunostaining without primary antibody was carried out as negative control. The protein expression of SCUBE2 was evaluated on a semi-quantitative scale [0 - without staining, 1 - rare, focal staining (<25% positive cells), 3 - high staining (>25% positive cells)]. Statistical analysis was performed by the Pearson chi-square or Fisher exact test as appropriate.

Results: There was significantly higher SCUBE2 protein expression in calcified aortic valve tissue ($P<0.05$) compared to AVI and control valve tissue samples. The SCUBE2 expression was mainly localized in cytoplasmatic regions of valvular interstitial cells localized in subendothelial space and fibrotic regions surrounding the calcific nodules. There was no statistically significant difference in SCUBE2 expression between AVI and control aortic valve tissue.

Discussion: The findings of increased expression of SCUBE2 in diseased aortic valves suggest its potential role in the pathogenesis of human aortic valve calcification.

Acknowledgments: I want to thank my mentor assistant professor Igor Rudez, and especially my co mentor assistant professor Frane Paic from the Department of medical biology for his devoted work in helping me make this thesis and work easy and enjoyable.

MeSH/Keywords: Aortic valve stenosis, SCUBE2, immunohistochemistry

Poster code: R-02-10-129

Poster Title: The impact of the preoperative nutritional preparation in patients with colorectal cancer on incidence of anastomotic leak

PhD candidate: Dubravka Mužina Mišić

Part of the thesis: The impact of the preoperative nutritional preparation in patients with colorectal cancer on incidence of anastomotic leak

Mentor(s): Assist. Prof. Mario Zovak, MD, PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Treatment of colorectal cancer depends on the stage of the disease at the time of diagnosis, but for the majority, surgery is a fundamental part of colorectal cancer treatment. The reality of colorectal surgery is a risk of anastomotic leak, a complication that leads to higher morbidity and mortality, permanent stoma rates and increased local recurrence rates. Every surgery induces stress response, inducing a wide range of metabolic and physiological processes. After colorectal surgery, patient's body has to overcome consequences of stress response and to be able to heal colonic anastomosis and wound. In those cases, nutritional demands for amino acids are increased. Majority of patients with cancer, already have preoperatively a certain degree of malnutrition, which also affects healing and recovery after surgery.

Materials and methods: Patients with histologically confirmed colorectal cancer, who underwent elective surgery, between January 2018. and January 2019. at a single centre were included. Patients had routine preoperative blood sampling including serum C reactive protein (CRP) and were divided in two groups, based on their preoperative nutritional status, measured by the Nutritional Risk Screening 2002. Patients with higher risk for malnutrition were given preoperatively oral nutrition supplements. Exclusion criteria included patients under 18 years of age, emergency operations and current steroid use. Surgery involved resection of the involved part of colon and anastomosis with mechanical stapling device. Intraabominal drainage was placed near the anastomosis. Postoperatively, every 24 hours for the first 4 postoperative days, CRP and cytokine levels (TNF-alpha, IL-6) were measured from intraabdominal fluid. At the same time, serum CRP levels were measured. In patients with suspected anastomotic leak MSCT of abdomen and pelvis was done, or surgical exploration. To compare difference between two groups repeated- measures ANOVA, ROC analysis and chi-square test were used.

Results: In total, 59 patients were included in the study. 30 patients had their normal diet until the day of surgery, and 29 patients had additional oral nutritional supplements with higher protein content 14 days prior to surgery. The majority were male (32, 54,2%), over 61 years old (42, 71,1%),and had BMI higher than 25(42, 71,2%). Comparing two groups of patients, with and without preoperative enteral nutrition, there was a statistically significant difference between those two groups, ($\chi 2=4.975$, df=1, $p=0.026$): higher proportion of anastomotic leak was in the group of patients without enteral nutrition. Repeated measures ANOVA showed statistically significant change in serum CRP in four postoperative days ($F(1.6,55)=6.107$, $p=0.006$). Similar results were shown for drainage CRP ($F(1.8,53)=40.761$, $p<0.001$) , IL-6 ($F(2.4,54)=4.463$, $p=0.009$), and for TNF alpha ($F(2.2,54)=7.378$, $p=0.001$). There was statistically significant difference between two groups of patients , all being higher in group with dehiscence. ROC analysis showed that serum CRP levels had highest sensitivity for anastomotic leak on postoperative day 4 (85,7), with a cut-off value of 121. Drainage CRP levels were significant only on the 4. postoperative day, with sensitivity of 71.4. Similar findings were for TNF-alpha, with sensitivity of 71.4 on the 4. postoperative day. Levels of IL-6 were statistically significant on the 1. and 4. postoperative day, with the highest sensitivity on the first day of measurement(75).

Discussion: The nutrition of a patient with cancer is one of the basic factors which impacts on the safety of a surgery.Malnutrition has a significant impact on appearance of complications, especially infectious complications and impaired wound healing. Anastomotic leak is a combination of infectious complication and impaired healing of colonic anastomosis.Although this study enrolled

small number of patients, and further research is needed, significant difference in development of anastomotic leak was shown between groups with and without preoperative oral nutrition supplement with higher content of protein.The present study also suggests that levels of serum CRP is a usefull tool for earlier and improved diagnosis of anastomotic leak, leading to earlier intervention. This approach may result in improved postoperative morbidity and mortality following surgery for colorectal cancer.

MeSH/Keywords: colorectal cancer, surgery, anastomotic leak, enteral nutrition, malnutrition

Poster code: R-02-10-131

Poster Title: Protein expression of APEX1 in degenerative calcific aortic valve stenosis (CAVS) - preliminary data

PhD candidate: Dubravka Šušnjar

Part of the thesis: DNA demaging and demethylation pathways in development of calcific aortic valve stenosis

Mentor(s): Assist. Prof. Igor Rudež, MD PhD, Assist. Prof. Frane Paić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Degenerative calcific aortic valve stenosis (CAVS) is the most common cardiac valve disease facing the elderly population in western societies. Recent findings suggest that accumulation of reactive oxygen species (ROS) that occurs during the early phase of disease progression leads to increased oxidative DNA damage. Formation of the apurinic/apyrimidinic (AP) sites occurring spontaneously due to the attack of reactive oxygen species (ROS) or by the action of DNA glycosylase (DG) are central to oxidative DNA damage in both the cell nucleus and mitochondria. They are repair by the action of AP endonuclease 1 (APEX1), a multifunctional enzyme component of the base excision DNA repair (BER) pathway. However, there is no available data regarding the extent of AP site formation or the expression and biological function of APEX1 enzyme in pathological settings of aortic valve stenosis. The aim of this part of study was to investigate APEX1 gene expression and localization in normal and stenotic aortic valve tissue.

Materials and methods: This preliminary study comprised 15 tricuspid aortic valves obtained from patients with severe aortic valve stenosis (n=10) and aortic valve insufficiency (n=5) as well as normal, pathologically unaltered aortic valve tissue (n=5) obtained from patients undergoing heart transplantation. Upon surgical resection the aortic valve tissues were fixed in formalin and embedded in paraffin. The paraffin-embedded tissue blocks were cut into 5µm sections using a microtome.

Results: The CAVS tissue sections were incubated for 1 hour in 10% normal goat serum/PBS solution, then incubated overnight with Anti-APE1 antibody primary antibody (Abcam, ab2717 at concentration 6 µg/ml), in 0.1% BSA/PBS solution in humid chambers at 4°C. Secondary Donkey Anti-Goat IgG H&L (HRP) (Abcam, ab205723at concentration 5 µg/ml) were applied following incubation (10 min at room temperature) in peroxidase blocking solution according to manufacturer protocol. Immunostaining was visualized by 1x diaminobenzidine (DAB) solution (DAKO; Hamburg, Germany), subsequently counterstained with hematoxylin, and mounted with Permount (Sigma). Immunostaining without primary antibody was carried out as negative control. We are currently finishing immunohistochemical staining of AVI and control aortic valve tissue samples obtained from patients subjected to heart transplantation.

Discussion: The protein expression of APEX1 will be evaluated on a semi-quantitative scale [0 - without staining, 1 - rare, focal staining (<25% positive cells), 3 - high staining (>25% positive cells)]. Statistical analysis of final data will be performed by the Pearson chi-square or Fisher exact test as appropriate. The final results of this preliminary study will be presented on poster section.

MeSH/Keywords: Aortic valve stenosis, APEX1, immunohistochemistry

Poster code: R-02-10-136

Poster Title: The role of polymorphic genes ABCG2, ABCB1 and SLCO1B1in predicting adverse drug reactions of rosuvastatin

PhD candidate: Ivana Radman

Part of the thesis: The role of polymorphic genes ABCG2, ABCB1 and SLCO1B1in predicting adverse drug reactions of rosuvastatin

Mentor(s): Assoc. Prof. Iveta Merćep, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: The 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) are very efficient drugs for treatment of hypercholesterolemia but can cause side effects like myopathy, rhabdomyolysis and hepatotoxicity. Interactions of rosuvastatin with many different drugs are well documented. Rosuvastatin does not undergo extensive biotransformation by P450 (CYP) enzymes, thus pharmacokinetic variability and significant drug interactions depend on variable drug transport by transporter proteins ABC and SLC. Recent studies showed a strong association between myotoxicity of rosuvastatin and variants in the SLCO1B1 gene which encodes OATP1B11-3, one of the key hepatocellular uptake transporters providing extraction of statins from portal venous blood into the liver. However, data about genetic risk factors for developing rosuvastatin induced hepatotoxicity and myotoxicity are missing. The aim of this study was to examine the role of polymorphic genes ABCG2, ABCB1 and SLCO1B1 as possible predictors of the damaging effects of rosuvastatin.

Materials and methods: We analyzed blood of patients who developed adverse drug reactions by statins for SLCO1B1 388 A>G and 521T>C, ABCB1 2677G>T/A and 3435C>T. Genotyping was performed by real-time PCR method with ready- made kits of TaqMan®Drug Metabolism Genotyping Assays (Applied Biosystems, Ca, USA): ABCG2 421C> A (rs2231142) test ID C_15854163_70; SLCO1B1 388A> G (rs2306283) test ID C_1901697_20), SLCO1B1 521T>C (rs4149056) test ID C_30633906_10); ABCB1 1236C> T (rs1128503) testom ID: C_7586662_10. Genotyping will be performed on Applied Biosystems 7500 Real Time PCR, according to the manufacturer's instructions (Applied Biosystems, Ca, USA).

Results: We analyzed 30 patients (14 male and 16 female, range 33-77 years) who developed hepatotoxicity or myotoxicity or both caused by rosuvastin (10-20-40 mg/day), Nine patients developed hepatoxicity and 4 among them were the homozygote carrier for both low activity SLCO1B1 genotypes (521C/C and 388G/G) had worst clinical presentation. All patients were carriers of at least one polymorphism in SLCO1B1 gene. Five patients with hepatotoxicity had also increased creatin phosphokinase levels. Sixteen patients developed myotoxicity and among them 2 patients developed rhabdomyolisis

Discussion: Our preliminary data indicate associations between miotoxicity and hepatotoxicity caused by rosuvastatin and SLCO1B1 polymorphysms. ABCB1 and ABCG2 allele variants increases susceptibility to rosuvastatin toxicity.

MeSH/Keywords: rosuvastatin, side effects, gene polymorphism, ABCG2, ABCB1, SLCO1B1

Poster code: R-02-13-148

Poster Title: Professional and ethical attitudes of medical professionals on treatment of end-of-life patients in intensive care units in Republic of Croatia

PhD candidate: Diana Špoljar

Part of the thesis: Professional and ethical attitudes of medical professionals on treatment of end-of-life patients in intensive care units in Republic of Croatia

Mentor(s): Assoc. Prof. Ana Borovečki, MD PhD, Assoc. Prof. Dinko Tonković, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Medical professionals working in intensive care units (ICUs) face numerous ethical dilemmas when treating and caring for patients who are at the end of their lives. Studies conducted throughout Europe have demonstrated that end-of-life practices, namely limitation of life- sustaining treatment, are widely practiced. However, considerable international differences are reported. The Ethicus study has shown that the limitation of life-sustaining treatment is more common in northern than southern European countries, and that end-of-life decision-making is more paternalistic in southern countries. There were no national or international studies conducted in Croatia concerning the experiences and attitudes of medical professionals on end-of-life decisions.

Materials and methods: A cross-sectional study involving physicians and nurses is currently being conducted in intensive care units of several university and general hospitals using a questionnaire constructed by Grosek et al. It is designed to assess the attitudes and experiences of the participants regarding the treatment of end-of-life patients in ICUs, focusing on limitation of life-sustaining treatment. The questionnaire is handed out to all nurses and specialist doctors working in adult intensive care units pertaining to departments of anaesthesiology, internal medicine and neurology.

Results: Preliminary results from questionnaires collected in ICUs pertaining to departments of anaesthesiology and neurology in 2 clinical hospitals in Zagreb are shown. Results from several questions are singled out and presented here. Due to the sensitive nature of the research and in order to retain participants' anonymity, only the combined results of all doctors and all nurses included thus far are shown. The response rate was equal or above 50% in all ICUs. The results show that both doctors and nurses use written or oral instructions on limiting life-sustaining treatments (81% and 65%, respectively). Seventy-three percent of doctors state they are included in the decision-making process about limiting life sustaining treatments, while only 14% of nurses state the same. Limiting life-sustaining treatments in end-of-life patients is found morally acceptable by 78% of doctors and 51% of nurses, while 22% of doctors and 41% of nurses do not have a clear opinion on the matter. Forty percent of doctors and 22% of nurses do not think there are any moral differences between withholding and withdrawing of treatments. Most doctors and nurses think that patient's will regarding limitation of life sustaining treatments should be respected (88% and 86%, respectively). However, only 52% of doctors find that patient's (or patient's legal representative's) religious and cultural values should be respect, while 64 % of nurses share that opinion.Forty-eight percent of doctors and 26% of nurses state they are often aware of patient's (or patient's family's) wishes regarding limitation of life sustaining treatments.

Discussion: These preliminary results are from a small sample, and do not include all of the types of ICU's included in the research, therefore no clear conclusions can be drawn at this time. However, there are distinguishable differences between experiences and opinions of doctors and nurses in certain areas, which are expected to be confirmed by further research.

MeSH/Keywords: Intensive care unit, end-of-life, ethics, attitudes

Poster code: R-02-15-068

Poster Title: Biochemical phenotypes 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 Europe and worldwide, there is the presence of the uropathogenic Escherichia coli genetic line O25 ST131. Cause of the occurrence of a clonal group of E. coli O25 ST131 around the world is investigated in relation to metabolic activity, belonging to a certain phylogenetic group, the frequency of virulent factors, as well as the acquisition of resistance to antibiotics. Antibiotic resistant profile characteristic of E. coli O25 ST131 is fluoroquinolone resistance and the presence of extended-spectrum of beta-lactamases primarily CTX-15. The prevalence of E. coli O25 ST131 within fluoroquinolone-resistant UPEC is 24% in Europe, and in North America from 44% to 60%. There is no data on the prevalence of E. coli O25 ST131 in the outpatient population in Croatia, but there is published data on the presence of strain E. coli ST131 isolated from blood in hospital patients in 2005. In this study, characterization of biochemical phenotypes of uropathogenic strains of E. coli serogrous O25 is made, and will be estimated how many of these strains is going to be the genetic line E. coli O25 ST131.

Materials and methods: In this study were included uropathogenic E. coli strains isolated in outpatients of all age groups with proven urinary tract infections (significant bacteria, number of bacteria ≥105 CFU / mL in pure culture - monoculture and positive leukocyte esterase) that were resistant to fluoroquinolones (norfloxacin and ciprofloxacin) from March 2011 to January 2012 (153 strains). The study did not include catheter urine samples. The E. coli strains were serotyped by the slide agglutination method with Denka Seiken Co. Ltd. Japan, serum for serogroup O25 according to the manufacturer's instructions. Biochemcal phenotypes of E. coli O25 were determined using the automated instrument Vitek 2 and the identification card GN according to the manufacturer's instructions.

Results: Total number of 153 fluoroquinolone-resistant E. coli strains were agglutinated with serum for serogroup O25 and 47 strains belong to E. coli serogroup O25. The automated instrument Vitek 2 and the identification card GN have obtained 35 different biochemical phenotypes from the total number of 47 E. coli O25 tested. One biochemical phenotype repeated up to four times, and nine biochemical phenotypes were repeated two times. Gibreel et al. found in the genetic line E. coli O25 ST131 there are characteristic biochemical reactions for this clone, but also that there is no uniform biochemical phenotype. The correlation between the positive biochemical reactions, whose positivity was between 45% and 87%, was tested by using Fisher's exact statistical test. Biochemical reaction of alpha-galactosidase (AGAL) and L-lactate alkalinization (ILATk) reactions were not statistically significantly related ($p > 0.05$), while positive AGAL and succinate alkalinization (SUCT) reactions were statistically significantly related ($p < 0.05$) and total common positive reactions is 24. Positive reactions of ILATk and SUCT were also statistically significant ($p < 0.05$) and total common positive reactions is 17. Positive reaction of SUCT and E. coli ESBL phenotype did not show statistically significant association ($p > 0.05$).

Discussion: Positive biochemical reactions of alpha-galactosidase(AGAL), beta-glucuronidase(BGUR), L-lactate alkalinization (ILATk), ornithine decarboxylase (ODC), L-proline arylamidase (ProA) and sucrose (SAC), Gibreel et al. associated with the biochemical phenotype of genetic line E. coli O25 ST131, but our results differ from their study. In this study the AGAL reaction was positive in 87%, BGUR 45%, ILATk 51%, ODC 96%,%, ProA 19%, SAC 96% and SUCT 51%. Total number of E coli O25 ESBL was 31 in this study, but were not statistically related to the positive reaction of SUCT and this result differs from Gibreel et al. study. Based on the biochemical propriety, it can not be estimated

how much E. coli O25 ST131 will be proven in further research and biochemical phenotypes can not be used as marker for prediction.

MeSH/Keywords: Escherichia coli, fluoroquinolone, urinary tract infection

Poster code: R-02-16-071

Poster Title: Postoperative thinning of lamellar donor graft after conventional Descemet's Stripping Automated Endothelial Keratoplasty

PhD candidate: Ana Meter

Part of the thesis: Povezanost debljine lamele s vidnom oštrinom nakon stražnje slojevite transplantacije rožnice

Mentor(s): Assist. Prof. Tomislav Kuzman, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Penetrating keratoplasty (PKP) was the gold standard of corneal transplantation in the past. For the diseases of corneal endothelium which is the most inner layer of cornea, PKP was replaced with lamellar endothelial keratoplasty procedures. Currently, the most common endothelial keratoplasty procedure is Descemet Stripping Automated Endothelial Keratoplasty (DSAEK). Several authors found that the thickness of the lamellas measured in the eye bank preoperatively and after the surgery postoperatively do not correspond. It is recognized that after cutting, the graft deturgesces and becomes thinner. The purpose of this study is to find correlation between preoperative thickeness of lamellas measured in eye bank and final postoperative thickness of lamellas. Inclusion criteria were lamellae that meet the definition of conventional DSAEK. We have devoted a special interest to the conventional DSAEK because it is the most widely used method of endothelial keratoplasty and is performed in our eye bank and country.

Materials and methods: This prospective study enrolled 55 eyes of patients who undergone DSAEK corneal transplantation. Criteria for inclusion were corneal edema secondary to Fuchs endothelial dystrophy and pseudophakic bullous keratopathy. Lamellar thickness was measured by anterior OCT device (Visante OCT Carl Zeiss Meditec) which is a non-invasive, non-contact imaging method that provides a high micrometric resolution of eye tissue sections. All surgeries were performed by two surgeons under general anesthesia. After successful conventional DSAEK transplantation patients were examined postoperatively at the University Hospital Center Zagreb Ophthalmology Department and the final measurement of corneal and lamellar thickness was performed by anterior OCT six months after surgery. Lamellar thickness preoperatively and postoperatively were compared.

Results: Study included 55 eyes of patients who undergone DSAEK, mean age 70,9 ± 9,4 years, range from 48 to 87 years. There were 61,8% (N=34) female patients and 38,2% (N=21) male patients. Central graft thickness before surgery measured by ultrasound pachymetry ranged from 110 to 198 µm with average of 142 ± 27 µm. Central graft thickness six months after surgery measured by anterior OCT ranged from 100 to 187 µm with average of 124 ± 20 µm. When statistical analysis was performed on average lamellar values measured before and after surgery it can be seen that a statistically significant difference in the thickness of the lamellae before and after the surgery ($t = 5.148$, $p < 0.01$) was obtained so that the subjects had statistically significantly greater thickness of the lamellae before the surgery (M = 142) compared to thickness after surgery (M = 124). Deturgescence of lamellas six months after conventional DSAEK surgery was 12% on average.

Discussion: It is recognized that after cutting and transplanting the graft in to the eye, the graft deturgesces and becomes thinner. Di Pascuale in his study reported that mean graft thickness decreased from 243 µm first day postoperatively to 148 µm at the last visit, stabilizing approximately six months after surgery. The thinning in that study was 39%. In study conducted by Woodward et al. presented deturgescence was from 199 µm preoperatively to 165 µm postoperatively with average thinning of 17%. The above mentioned studies show that thicker grafts deturgescences more than thinner grafts. Results from our study showed deturgescence from 142 µm to 124 µm with average 12% thinning ($p<0,01$). That is little lower than other studies and can be explained by different methods of measurement with preoperative ultrasound pachymetry and more precise anterior OCT postoperatively. The similar study was conducted by Woodward et al. on 64 eyes with same measurement methods, and our results are coherent with that study group. At the end we want to conclude that after performing conventional DSAEK corneal transplantation, surgeons should expect

deturgescence of corneal graft and reduction in thickness of lamellae according to our study about 12% of initial thickness. We found that information important for better planning of surgical procedures and knowing what to expect after surgery, and for better cooperation with eye banks when ordering precut corneal tissue.

MeSH/Keywords: Corneal Transplantation, Descemet Stripping Endothelial Keratoplasty, Corneal Pachymetry, Tomography, Optical Coherence

Poster code: R-02-18-020

Poster Title: The influence of palpebral fissure width on tear film dysfunction

PhD candidate: Ana Čović

Part of the thesis: The influence of palpebral fissure width on tear film dysfunction

Mentor(s): Assist. Prof. Igor Petriček, MD PhD

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

Introduction: Palpebral fissure width in primary eye position determines the eye opening and exposure to external conditions. Since an enlarged exposed ocular surface area negatively affects the tear film stability and alters the eye desiccation, changes in palpebral fissure width among healthy individuals may have an impact on development of tear film dysfunction. Therefore, the aim of this study is to investigate the influence of palpebral fissure width in the primary eye position in healthy individuals on tear film dysfunction, in the same age and sex group.

Materials and methods: This preliminary research included 50 adult subjects, divided into 2 groups according to age: group 1 (N=25, subjects aged 18 to 50 years) and group 2 (N=25, subjects aged 51 and older). First a standardised Schein questionnaire was used in order to determine the severity of dry eye symptoms. Palpebral fissure width was measured to all subjects on both eyes in primary gaze position with a clear plastic ruler. After that, other tests were preformed that are routinely used for dry eye diagnostics (conjunctival hyperemia, LIPCOF, NIBUT, TBUT). The data were analysed with the help of Statistica ver. 13.3 (TIBCO Inc., USA).

Results: Study included 50 healthy subjects of both sex, 13 men (26%) and 37 women (74%). Average age was 51,38 ± 18,18 years. Subjects of group 2 had statistically significant narrowed palpebral fissure width compared to subjects of group 1 ($p<0,001$). Statistically significant difference in palpebral fissure width between and inside the groups was influenced by the values of width in older women group. The groups did not differ significantly according to dry eyes symptoms determined with a standardised Schein questionnaire. Subjects of group 2 had statistically significant more pronounced hyperemia (CCRLU) ($p=0,004$), shorter NIBUT ($p=0,052$), shorter TBUT ($p=0,022$) and more lid- parallel conjunctival folds (LIPCOF) ($p=0,004$) compared to subjects of group 1. Palpebral fissure width was statistically significant and negatively related to age in all subjects, specially in group 2 where is significant correlation with female sex determined. There was no statistically significant correlation between palpebral fissure width and dry eye symptoms ($p=0,985$), NIBUT ($p=0,776$), TBUT ($p=0,873$) and LIPCOF ($p=0,079$) in all subjects.

Discussion: Results of this research showed the difference in palpebral fissure width between 2 examined age groups, as well as between women and men inside the groups. Limiting factor was the imbalance between number of women and men participating the research with a overrepresentation of women. More precise and correct results of existing difference in subjective symptoms and clinical signs of tear film dysfunction between examined age groups and between women and men inside the groups, as well as a correlation between palpebral fissure width and tear film dysfunction, will be accomplished with further analysis of examined variables when required sample size will be collected.

MeSH/Keywords: Palpebral fissure width, Dry eyes, Tear film dysfunction

Poster code: R-02-18-113

Poster Title: Prognostic significance of lymph node ratio in clinically node-negative (cN0) oral cancer: a preliminary research report

PhD candidate: Iva Nikles

Part of the thesis: Prognostic significance of lymph node ratio in patients with clinically node-negative oral cancer

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

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

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 tumors and a significant cause of morbidity. Although the incidence of OSCC has decreased in most developed countries over the past decades, it remains 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. The aim of our study was to evaluate the concept of LNR as a potential prognosticator of survival in clinically node-negative oral cancer patients.

Materials and methods: Inclusion criteria were: histologically proven OSCC, no clinical evidence of regional metastases (cN0), pathologically confirmed 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. The 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 the 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. Survival analyses were performed using the log-rank test and Cox regression analysis. Overall survival (OS) was measured from the date of surgery to the date of death or last follow-up. P values <0.05 were considered to be statistically significant. Follow-up intervals were calculated in months from the date of first treatment to the date of last follow-up or death.

Results: A total of 61 patients were primary surgically treated. Among these, 26 patients were diagnosed with occult neck disease (24 males and two females). Mean age was 55.5 ± 8.8 years. A five-year OS for the entire cohort (N=61) was 39.9%. In a subgroup analysis of patients with histologically positive neck, 5-year OS was 31,7%. Three or more positive lymph nodes (HR=2.88, P=0.022) and high LNR (HR=3.73, P=0.009) were significantly associated with inferior overall survival. Association of high LNR with worse outcome remained significant in a multivariate model adjusted for age and gender (HR=3.97, P=0.034). Follow-up information was available for all patients ranging from 7 to 87 months the average being 39 months. Twelve patients had died due to disease recurrence, while five patients died from other causes. All surviving patients were followed-up for a minimum of 3.5 years.

Discussion: According to the results of this study, a higher number of node metastases (>3) and high LNR showed statistically significant association with an inferior 5-year OS. To our knowledge, this is a first report implying that LNR could be used as a new prognosticator in cN0 OSCC with possible impact on choice and intensity of adjuvant therapy.

MeSH/Keywords: oral cancer, lymph node ratio, overall survival

Poster code: R-02-19-022

Poster Title: Prognostic value of tumor-infiltrating lymphocytes and androgene receptors in patients with early triple negative breast cancer

PhD candidate: Ana Tečić Vuger

Part of the thesis: Prognostic value of tumor-infiltrating lymphocytes and androgene receptors in patients with early triple negative breast cancer

Mentor(s): Professor Damir Vrbanec, MD PhD, Professor Božena Šarčević, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Triple Negative Breast Cancer (TNBC) is the most aggressive and deadliest BC, with a representation of about 15% in the total number of cases. The common feature of all TNBC is the lack of immunohistochemical expression of estrogen, progesterone and HER2 receptors. Therefore, TNBC is not susceptible to endocrine or antiHER2 therapy. To date TNBC stayed an "orphan" cancer, with neither any usable biomarkers nor specific therapeutic target. Previous studies have shown that the expression of the androgene receptor (AR) and the presence of tumor - infiltrating lymphocytes (TIL) as an immune response in TNBC has a positive effect on the behavior of this tumor type

Materials and methods: We performed a retrospective cohort study on the consecutive sample of early TNBC patients treated at the University Hospital for Tumors 2009-2012. The inclusion criteria were: age (35-75 years), gender (women) and PHD (early TNBC). The primary outcome was OS. We assessed TIL and AR using the standard FFPE pathohistological tissue samples. We analyzed TIL in accordance with the recommendation of International Working Group for the Evaluation of TIL on H - E samples in central tumor and in invasive margin, and AR using immunohistochemistry. The study was approved by the Ethics Committee of the Sestre milosrdnice University Hospital Center and was performed in accordance with the Declaration of Helsinki. We adjusted the analysis for age, pathohistological type, tumor stage and size, number of positive lymph nodes, Ki-67, type of surgery, and treatment with adjuvant chemotherapy and radiotherapy.

Results: In this preliminary report we included 115 (68%) of the planned sample size. Median (IQR) patients' age was 58 (48-71) years. Majority of patients (84%), were diagnosed with ductal carcinoma in situ with median (IQR) size of 2.2 (1.6-3.0) cm, 46% in stage I, 23% in stage II, and 31% in stage III. Total of 28% died during the follow-up. Positive AR (≥1%) was present in 31%. AR was not significantly associated with OS (ORbivar=1.00; CI95% 0.99-1.00; p=0.589; ORadj=0.52; CI95% 0.97-1.02; p=0.515). Prevalences of intermediate or high TIL expressions defined as ≥10% were: CTs 81 (48%), CTi 37 (22%), IMs 145 (86%), and IMi 83 (49%). In bivariable analysis, significant predictors of OS were CTs (OR=0.96; CI95% 0.93-0.99; p=0.014), IMs (OR=0.96; CI95% 0.94-0.98), and IMi (OR=0.93; CI95% 0.88-0.98; p=0.007). CTi was not significant predictor of OS (OR=0.95; CI95% 0.90-1.01; p=0.078). After the adjustment, significant predictors of OS were IMs (ORadj=0.92; CI95% 0.84-0.10; p=0.044), and IMi (ORadj=0.77; CI95% 0.60-0.97; p=0.028). The best OS prognostic model included three variables: TIL IMi (HR=0.87; CI95% 0.78-0.96; p=0.008), number of positive lymph nodes (HR=1.39; CI95% 1.24-1.56; p<0.001), and treatment with adjuvant chemotherapy (HR=6.54; CI95% 1.88-22.74; p=0.003). Although AR was not significant predictor of OS, it's interaction with TIL was. In patients with AR=0, odds for death were OR=0.96; CI95% 0.90-1.01; p=0.096. In patients with AR≥1, odds for death were OR=0.80; CI95% 0.66-0.97; p=0.022.

Discussion: Positive AR prevalence in TNBC was lower than in studies on nonTNBC. As AR is generally associated with better prognosis, its relatively lower prevalence in TNBC may be associated with the higher aggressiveness of TNBC. By itself AR is not significant prognostic factor. TIL, and particularly TIL in invasive margin are. Could it be that presence of TIL in the majority of samples (at least in one measured compartment) really speaks of high immunogeneity of TNBC and further, about true protective role of immune system in this situation? Furthermore, the interaction of AR and intratumor invasive margin TIL also shows unexpected effect, and should be further studied.

MeSH/Keywords: early breast cancer, triple negative breast cancer, tumor-infiltrating lymphocytes, androgene receptors, biomarkers

Poster code: R-02-19-060

Poster Title: Correlation of hormonal receptor expression and clinical parameters in liposarcoma

PhD candidate: Milena Peitl

Part of the thesis: Correlation of hormonal receptor expression and clinical parameters in liposarcoma

Mentor(s): Assoc. Prof. Fedor Šantek, MD PhD, Assist. Prof. Lovorka Batelja Vuletić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Although our understanding of soft tissue sarcoma has significantly improved, there is still a growing need for a more efficacious therapy. Estrogen receptors alpha (ERα), progesterone (PR) and androgen receptors (AR) have been implicated in the pathophysiology of other malignant diseases. More specifically, uterine leiomyosarcomas (uLMS) and their hormonal receptor expressions have quite often been a subject of scientific research and its results led to the establishment of hormonal therapy as treatment option in these sarcomas. In light of that fact, research of hormonal receptor expressions in liposarcomas (LS) may prove as fruitful as previous sarcoma (uLMS) research.

Materials and methods: This research will be performed on 40 pathohistologicaly verified LS in the form of adequately prepared paraffin and/or formalin tissue cubes. Tissue specimens is gathered from the archive of the Department of pathology of UHC Zagreb and The University of Zagreb School of Medicine. The research is in itself a retrospective one, which will encompass the time period from 2012 to 2018. Tissue material is under immunohistochemical validation using targeted monoclonal antibodies for ERα, PR and AR, according to manufacturer instructions.Cut off values for expression of ERα, PR and AR in LS will be 1% and 10% of positively coloured cells. ERα/PR positive tumour breast tissue will be used as a positive control for ERα and PR, while AR positive tumour prostate tissue will be used as a positive control for AR. Spleen tissue bioptates will be used as negative controls. Pathohistological subgrouping of LS will be according to the grading system of French Federation Nationales des Centres de Lutte Contre le Cancer(FNCLCC) and the LS is further subdivided into 4 subtypes:well-differentiated, dedifferentiated, myxoid/round-cell and pleomorphic. Investigated clinical parameters will include:age (over 18 years of age), both genders, anatomical localisation, tumour size and the presence of metastases at the time of diagnosis.Anatomical localisation will be grouped according to the affected body area: head and neck, extremities, trunk, retroperitoneum and visceral localisation. Tumours will also be grouped according to their size into three groups:smaller than 5 cm, 5 to 10 cm and above 10 cm in size, respectively.Presence of metastases at the time of diagnosis will also be noted.Information regarding the clinical parameters of age, gender, anatomical localisation, tumour size and the presence of metastases are gathered from the existing archives.

Results: Preliminary results for baseline characteristics revealed that in research group there were 21 male and 19 female patients. Their age ranged between 30-85 years, with a mean age of 55.7. Pathohistological subgrouping was as follows: 17 well-differentiated, 14 dedifferentiated, 5 myxoid/round-cell, and 5 pleomorphic LS. Tumor anatomical localisation was: 12 extremity localised tumors, 14 retroperitoneally localised, 5 with truncal and 9 with a visceral localisation. Regarding tumour size, analysis revealed that only 1 patient had tumour <5 cm, 17 had tumors between 5-10 cm, and 22 had tumors >10 cm. Only 4 patients had metastases at the time of diagnosis.

Discussion: As this research is focused on very rare types of sarcomas (which are themselves one of the rarest types of malignancies), that have seldom been part of scientific research, gathering any new data is of significant scientific interest. Further planned research analyses are subject to immunohistochemical validation, which has not been completed at this point. Expected scientific contribution is to determine the suspected existence of certain subtypes of LS, according to their hormonal status, which may lead to development of targeted and more efficacious hormonal treatment.

MeSH/Keywords: Liposarcoma; Estrogen Receptor alpha; Progesteron; Receptors, Androgen
Poster code: R-02-19-063

Poster Title: The analysis of transcription factors SOX2, OCT-4 and NANOG and the PI3/AKT/BCL2 pathway in malignant pleural mesothelioma - preliminary results

PhD candidate: Fran Seiwerth

Part of the thesis: The analysis of transcription factors SOX2, OCT-4 and NANOG and the PI3/AKT/BCL2 pathway in malignant pleural mesothelioma - preliminary results

Mentor(s): Assoc. Prof. Marko Jakopović, MD PhD, Assist. Prof. Filip Sedlić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Malignant mesothelioma is an aggressive mesenchimal tumor, originating from the pleural or peritoneal serous mesothelial cells. The grade of cell differentiation and pluripotency-related gene expression in mesothelioma cells correlates with poorer overall survival and greater resistance to chemoterapeutic agents. Our goal is to analyse the correlation between the expression of pluripotency-related proteins, patohistological and clinical features of patients with malignant pleural mesothelioma (MPM).

Materials and methods: Tissue samples of patients with MPM from the archive of the Department of Pathology, School of medicine, University of Zagreb are being used, from patients diagnosed and treated at Jordanovac Department for respiratory diseases, UMC Zagreb. Analyses on patient tissue samples have been performed using antibodies for immunohistochemistry staining: 1) Anti-Oct4 antibody, (EPR 17929), mouse monoclonal IgG, 1/1000, 2) Anti-Nanog (EPR 2017(2)), rabbit monoclonal IgG, 1/200, 3) Anti-Sox2 atb. (EPR 3131), rabbit monoclonal IgG, 1/100, 4) Anti-Bcl2 (ab692) 5) Anti-pan-AKT (ab8805), rabbit monoclonal IgG, 1/1000, i 6) Anti-PI3 kinase p110 beta (EPR5515(2))

Results: Preliminary results showed immunohistochemical positivity for SOX-2 and NANOG, especially in solid subtypes of MPM. Staining for OCT-4 and BCL2 were negative, and Anti-Pi3 kinase showed some positivity, regardless of patohistological subtype in this stage.

Discussion: Preliminary results are showing some promise regarding different histological subtypes expressing different positivity for pluripotency-related proteins. The semiquantitative analysis and grading is still to be performed, as well as the analysis of further tissue samples. Correlation with clinical data follows, with definitive conclusions.

MeSH/Keywords: malignant pleural mesothelioma, SOX-2, NANOG, OCT-4

Poster code: R-02-19-123

Poster Title: Comparison of biochemical changes in patients with trochanteric region fracture fixation with DHS versus PFN

PhD candidate: Kushtrim Grezda

Part of the thesis: Comparison of systemic inflammatory response in patients with trochanteric region fracture fixation with Dynamic Hip Screw versus Proximal Femoral Nail

Mentor(s): Assoc. Prof. Mislav Jelić, MD PhD, Professor Cen Bytyqi, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Hip fractures are one of the most frequent fractures in older adults. There is still controversy which surgical strategy is the best option for treatment of hip fractures especially trochanteric region fractures. Surgical intervention that follows hip fracture induces biochemical, physiological and fibrinolytic changes that are so-called “second hit phenomenon” which trigger systemic inflammatory response syndrome.

Materials and methods: 52 patients with AO/OTA 31.A1–31.A2 trochanteric region fractures will be included in study divided into two groups each containing an equal number of patients which will be treated with Dynamic Hip Screw versus Proximal Femoral Nail. The blood sample will be taken 1 hour prior to surgery and 24 hours after the end of the surgery.

Results: Our study is currently in the phase of recruiting and the preliminary results we anticipate to have by July 2019.

Discussion: Major surgery can lead to systemic inflammatory response which is related to different post-operative complications. Our primary objective will be the difference of the levels of interleukin-6 in patients with hip fractures treated either with Dynamic Hip Screw or Proximal Femoral Nail. Different studies have shown that the levels of IL-6 are increased after surgery. There are only few studies that have compared the levels of IL-6 after different types of surgeries but to our knowledge there is no study in literature that has measured the difference of this marker after DHS and PFN in the same study. We hope that with our result we will help surgeons in everyday practice to choose the most suitable surgical treatment for patients with trochanteric region fracture and give scientific community more evidence which methods is better since there is still controversy.

MeSH/Keywords: hip fracture, Interleukin-6, inflammation, comparative study

Poster code: R-02-20-073

Poster Title: Association of immunohistochemical expression of thrombin, PAR-1 and tumor budding in pancreatic ductal adenocarcinoma

PhD candidate: Anteja Krištić

Part of the thesis: Association of immunohistochemical expression of thrombin, PAR-1 and tumor budding in pancreatic ductal adenocarcinoma

Mentor(s): Assist. Prof. Gorana Aralica, MD PhD

Affiliation: Clinical Hospital Dubrava

Introduction: Pancreatic ductal adenocarcinoma (PDAC) is a tumor with poor prognosis (90% patients die within 1 year). Tumor budding (TB) is a histological representation of epithelial-mesenchymal transition (EMT) which is an independent parameter of poor prognosis in pancreatic cancer. Frequently detected molecular event in EMT is the loss of membrane expression of E-cadherin and increased expression of N-cadherin. Grade of TB can be histologically evaluated at the invasive margin of the tumor. TB was found in PDAC and also a statistically significant association between the presence of a high grade-TB and an increased risk of mortality and/or recurrence of disease. Elevated thrombin production not only increases the risk of thrombosis, but also promotes tumour growth and metastasis and as a consequence, thrombin and its contributors present opportunities for treatment of cancer-associated thrombosis and the underlying cancer. The immunohistochemical (IHC) presence of prothrombin and prothrombin fragment 1+2 was investigated in pancreatic carcinoma. PAR-1 (whose agonist is thrombin) is detected in the pancreatic cancer but not detectable in the epithelium of healthy pancreas, as found in the literature. There is no found data on the combined relationship between these five: thrombin, PAR-1, TB, EMT and PDAC. The formed hypothesis of this research is that positive IHC expression of thrombin and PAR-1 in PDAC is associated with areas of TB and with positive N-cadherin IHC expression and negative E-cadherin IHC expression.

Materials and methods: Retrospective research (cross-sectional study) at the Department of Pathology and Cytology of Clinical Hospital Dubrava (CHD), which will include archive material of the department and patient data (selected data from the pathohistologic reports) of 80 patients operated in CHD due to PDAC in 2011-2017 period). TB grade will be determined on IHC (CK AE1/AE3) PDAC tissue slides. The corresponding sample of tumor tissue incorporated in the paraffin block will be processed with multiple-punch tissue microarray technique in four areas (with punch sample from the central/epithelial part of the tumor, area of TB at the invasive front, peripheral/stromal/mesenchymal part of the tumor and surrounding healthy pancreatic tissue) to produce IHC slides (thrombin, PAR -1, E- and N-cadherin). The results will be analyzed with light microscope.

Results: Previously defined patient data were collected. There are 44 men and 36 women recorded. Mean age of the patients in the time of the pathohistologic report is 64,84 years (standard deviation 9,89 years). Median tumor size in one dimension is 4,052 cm (minimum 1,5 cm, maximum 14 cm, range 12,5). Most tumors were moderately differentiated (73,7%). Tumor spread beyond the pancreatic tissue is in 93,8% of patients. Resection margin infiltrated with tumor is in 41,3% of patients. Vascular invasion is recorded in 92,5% and peri- and/or intraneural infiltration in 97,5% of patients. The existence of positive lymph nodes is recorded in 67,5% and distant metastases (in other organs/tissues) in 5% of patients.

Discussion: In the literature pancreatic cancer is described as a disease of the elderly with a median age at diagnosis ranging between 68-72 years and it seems that this group of patients is somewhat younger. The frequency of vascular invasion is higher in these tumors as opposed found in the literature. Next steps are to collect archive material of the patients and perform further research: to investigate grade of TB, IHC expression of thrombin, PAR-1, E- and N-cadherin in PDAC tissue (in four areas), association of IHC expression of thrombin, PAR-1, E- and N-cadherin with TB area and its

grade, association of IHC expression of thrombin, PAR-1, E- and N-cadherin with patient data and association of grade of TB with patient data.

MeSH/Keywords: pancreatic ductal adenocarcinoma, epithelial-mesenchymal transition, EMT, tumor budding, thrombin, PAR-1

Poster code: R-02-23-121

Poster Title: Association of weight gain acceleration and retinopathy of prematurity phase 2

PhD candidate: Ana Čolić

Part of the thesis: Association of weight gain acceleration and retinopathy of prematurity phase 2

Mentor(s): Assoc. Prof. Nenad Vukojević, MD PhD

Affiliation: Division of neonatology, University hospital for obstetrics and gynecology, University hospital center Zagreb

Introduction: Premature birth exposes undeveloped organs to extrauterine environment that interrupts regular development further altered by medical interventions. Abrupted normal retinal vascularization continues with abnormal blood vessels growth in retinopathy of prematurity (ROP) phase 2. Periodic ophthalmic examinations are scheduled by adopted protocols to detect severe cases requiring treatment. Stress and pain of diagnostic procedures have immediate and long-term consequences. Prevention, reduction and treatment of pain and stress are beholden part of contemporary neonatal practice in endevour to improve the outcome of very premature infants. Weight loss is unavoidable after very premature birth, followed by slow, then accelerated catch-up growth. Weight gain trend correlates with levels of insulin-like growth factor 1 which is necessary for normal retinal vascularization and neovascularization in ROP. Aim is to prove that weight gain acceleration is followed by emergence of ROP phase 2.

Materials and methods: In this retrospective study at least 42 premature infants of gestational age ≤28 weeks and birth weight ≤1250 grams born in our Birth Center will be included. Phases of ROP are determined based on ophthalmic examinations and graded according to the Early Treatment of Retinopathy of Prematurity (ETROP) study. Acceleration of weight gain is calculated. Relations of ROP phases and weight gain acceleration are analyzed. Cessation of oxygen therapy as a possible confounding factor for the onset of ROP phase 2 is analyzed as well.

Results: Data are collected for 30 infants. Immature avascularized retina persists within a week after weight gain acceleration. Two weeks after weight gain acceleration ROP phase 2 emerges in most infants and by the time of three weeks in all cases. Severe ROP requiring treatment is not detected during observed period. Cessation of oxygen therapy is not associated with emergence of ROP phase 2 in analyzed cases.

Discussion: Collected data indicate that weight gain acceleration is followed by emergence of ROP phase 2. Association of weight gain velocity shift and onset of neovascularization contributes to comprehension of ROP pathogenesis. Such association might also be applicable in clinical practice. The relatively latent period for emergence of ROP phase 2 enables the recognition of weight gain acceleration that might serve as an indicator to start monitoring the disease. Absence of severe disease in this period secures timely diagnosis for infants requiring treatment. Postponing first ophtalmic examination would reduce the number of stressful procedures and concomitant neurological sequelas.

Acknowledgments: I would like to thank my mentor, family and friends for their support.

MeSH/Keywords: premature infants, newborn, retinopathy of prematurity, growth, insulin-like growth factor I

Poster code: R-02-24-047

Poster Title: Fecal Calprotectin as a Biomarker of IgE-Mediated Food Allergy in Children with Atopic Dermatitis

PhD candidate: Alen Švigir

Part of the thesis: Fecal Calprotectin as a Biomarker of IgE-Mediated Food Allergy in Children with Atopic Dermatitis

Mentor(s): Assoc. Prof. Suzana Ljubojević Hadžavdić, MD PhD, Marta Navratil, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Fecal calprotectin (FCP) is a biomarker of intestinal inflammation. As intestinal inflammation plays an important role in the development of immune-mediated diseases, such as allergies, it is proposed that FCP could serve as a diagnostic biomarker of food allergy (FA) in children. The aim of this preliminar investigation was to compare FCP level in infants and children under 4 years of age suffering from atopic dermatitis (AD) with and without IgE-mediated food allergy (FA), with FCP level in healthy controls.

Materials and methods: In total, 23 infants and children (age, mean 13 months ± 12) newly diagnosed with AD were devided in two groups: G1, children with atopic AD with FA (N=15), 2) G2, children with AD without FA (N=8). Control group (G3) consisted of healthy children of the same age (N=7). In G1 and G2, a complete blood count, total immunoglobulin (Ig) E, specific IgE to nutritive allergens, immunoglobulins, FCP and SCORAD score were assessed, while in G3 only FCP was assessed.

Results: The median FCP was 67 (IQR 187) μg/g in the 23 infants and children with AD and 74.1 (IQR 666.4) μg/g in the control group (p=0.9024). There was no difference between G1 and G3 in FCP level (G1: median 70, IQR 413 μg/g vs G3: median 74.1, IQR 666.4 μg/g; p=0.8878). There was no difference between G2 and G3 in FCP level (G2: median 31, IQR 73.5 μg/g vs G3: median 74.1, IQR 666.4 μg/g; p=0.5628). The median FCP was significantly higher in G1 in comparison to G2 (G1: median 70, IQR 413 μg/g vs G2: median 31, IQR 73.5 μg/g; p=0.0259). We have found a significant correlation between the FCP level and percentage of eosinophils (r=0.6041, p=0.0287), immunoglobulin (Ig) G level (r=-0.7281, p=0.0073) and IgA level (r=-0.7250, p=0.0076). There was no significant correlation between FCP level and the SCORAD score (r=0.3657, p=01488). SCORAD score significantly correlated with white blood cells (r=-0.7275, p=0.0048), IgG (r=-0.6590, p=0.0197), IgA (r=-07263, p=0.0075) and IgM (r=-0.6836, p=0.0142).

Discussion: Our preliminary results showed a significant difference in FCP level in patients with AD without FA in comparison to patients having both, AD and FA. However, these results should be confirmed on a larger number of subjects in order to asses the role of FCP as a biomarker of IgE-madiated FA in infants and children with AD and FA.

MeSH/Keywords: Fecal calprotectin, atopic dermatitis, nutritive allergy

Poster code: R-02-24-066

Poster Title: Serum calprotectin as an early biomarker for bacterial urinary tract infections in children: preliminary research results

PhD candidate: Mirta Lamot

Part of the thesis: Serum calprotectin as an early biomarker for bacterial urinary tract infections in children

Mentor(s): Assoc. Prof. Miroslav Harjaček, MD PhD, Slaven Abdović, PhD, research associate

Affiliation: Sestre milosrdnice University Hospital Center

Introduction: Fever is one of the most common symptoms of illness in children and a reason for medical consultation in childhood. Although self-limiting viral infections are the principal cause of fever in the majority of cases, it is estimated that 5% of febrile children younger than three years of age would have a urinary tract infection (UTI). Despite the increased availability of laboratory testing and development of various clinical scores, there is still no reliable predictor of UTI in children, with urine culture remaining the reference standard. However, this is a time-consuming test that often leads to engagement of antibiotic treatment while pending the results. Recent advancements in the understanding of inflammatory process have highlighted the role of S100 proteins, with many studies suggesting their clinical value as a biomarker of inflammation. Therefore, the aim of this research is to investigate the possible role of serum calprotectin (sCAL), a heterodimer consisting of S100A8 and S100A9 proteins from S100 family, as a biomarker for bacterial urinary tract infection in children.

Materials and methods: Patients aged 0-36 months who came to Pediatric Emergency Department of Sestre milosrdnice University Hospital Center between December 2018 and March 2019 with a fever of 38C and higher, lasting less than 72h, with no history of chronic illness or ongoing use of antibiotics, were enrolled in the study after a detailed clinical examination and signing of an informed consent. In every patient, serum calprotectin was measured with Quantum Blu rapid test (Buhlman, Switzerland), along with testing of standard inflammatory markers, including complete blood count (CBC), c-reactive protein (CRP) and procalcitonin (PCT), and urinalysis with dipstick test. In patients positive for leukocyte esterase (LE), urine was sent for culture, and those with ≥ 10^5 CFU/mL were defined as having the UTI. Further investigations, including respiratory syncytial virus (RSV) and adenovirus testing, was directed by treating physician, and patients with clinical signs and symptoms and/or positive RSV and adenovirus testing were diagnosed with a viral acute respiratory infection (ARI). Statistical analysis was performed using the software GraphPad Prism version 8 for Mac OS X (La Jolla California, USA). The data did not meet the normality assumption; therefore, the results were described as median with interquartile range (IQR). Mann-Whitney test was applied for sCAL concentrations analysis in UTI and ARI patients, while Spearman correlation was applied for comparison of sCAL with PCT, CRP, white blood cell count (WBC), absolute neutrophil count (ANC) and duration of fever (DoF). The statistical significance level was set to 0,05.

Results: Among 24 patients included in the study (median age 1 year (IQR 1), median DoF 24h (IQR 37,5), M:F=6:18), 20 were diagnosed with ARI (median age 1 year (IQR 1), median DoF 27h (IQR 17), M:F=6:14) and 4 with UTI (median age 0,5 years (IQR 1), median DoF 8,5h (IQR 9,25), M:F=0:4). In patients with UTI, the median value of sCAL concentration was 5,83ug/mL (IQR 3,0), which was significantly higher (p=0,0228) than in patients with ARI, whose median value of sCAL concentration was 3,03ug/mL (IQR 1,21). There was a moderate positive correlation of sCAL with CRP (r=0,481; p=0,017), WBC (r=0,606; p=0,02) and ANC (0,569; p=0,004), while there was no significant correlation with PCT and DoF.

Discussion: Despite the small number of participants, the preliminary results of this study have shown that sCAL concentration differs significantly among patients with bacterial UTI and viral ARI during the first three days after the onset of fever. Besides, the correlation of sCAL with DuF and inflammatory markers standardly used to infer the presence of bacterial infection was only moderate or insignificant, suggesting the potential differences in their dynamics. Hence, the sCAL concentration in febrile children could add to timely and accurate diagnosis of UTI, which is essential to reduce

treatment delay and improve treatment outcomes but also to avoid unnecessary use of antibiotic treatment in children.

Acknowledgments: The author wishes to thank mentors for their insightful guidance, dr. Nora Nikolac Gabaj and Marijana Miller for their generous support, and dr. Ivana Trutin for her kind assistance.

MeSH/Keywords: calprotectin, fever of unknown origin, urinary tract infections, children

Poster code: R-02-24-093

Poster Title: Croatian national survey of urosepsis and antimicrobial resistance profile in maternity wards, neonatal intensive care units and divisions for pediatric nephrology

PhD candidate: Ana Meyra Potkonjak

Part of the thesis: Croatian national survey of urosepsis and antimicrobial resistance profile in maternity wards, neonatal intensive care units and divisions for pediatric nephrology

Mentor(s): Assoc. Prof. Boris Filipović Grčić, MD PhD, Professor Danko Milošević, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Urinary tract infections (UTI) are one of the most common causes of neonatal infections, especially in premature and low birth weight neonates. Improperly treated UTI can lead to long-term complications, renal scarring, recurrent UTI that require long-term prophylaxis, arterial hypertension and renal failure in adults. Sepsis and UTI are the most common hospital acquired infections in neonatal intensive care units. Antimicrobial resistance among uropathogens that cause UTI is increasing. However, the frequency of UTI as a source of neonatal sepsis, their antimicrobial resistance as well as the frequency of associated congenital urinary abnormalities has not been previously investigated in Croatia.

Materials and methods: The aim of the study is to determine the frequency of UTI as a source of neonatal sepsis. Analyzing the data obtained in 2005. and 2015., investigation of the susceptibility patterns of most common uropathogens and evaluation of antibiotic treatment regimens used will be performed. Data population will include all neonates treated for UTI and urosepsis in maternity wards, neonatal intensive care units and divisions for pediatric nephrology in Croatia.

Results: Preliminary data show high resistance patterns rates of Escherichia coli, Klebsiella pneumoniae and Enterococcus faecalis to empirical antibiotic treatment regimens. Further analysis evaluating susceptibility rates of other pathogens with regard to local resistance patterns is necessary. After performing complete analysis which will include 15 hospitals, risk factors for developing urosepsis in neonates will be identified.

Discussion: Our data for neonatal population is consistent with antimicrobial resistance patterns in general population published by the Interdisciplinary Section for Antimicrobial Resistance Control in Croatia. The results of this study will be important for managing further approach in treatment of neonatal urosepsis.

MeSH/Keywords: neonatal urosepsis, antimicrobial resistance, risk factors

Poster code: R-02-24-132

Poster Title: The value of sonoelastography in assessment of lesions detected on magnetic resonance imaging of the breast after the surgical treatment of breast carcinoma

PhD candidate: Eugen Divjak

Part of the thesis: The value of sonoelastography in assessment of lesions detected on magnetic resonance imaging of the breast after the surgical treatment of breast carcinoma

Mentor(s): Professor Boris Brkljačić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Breast carcinoma is a heterogeneous disease with high mortality rate and growing incidence. The importance of imaging techniques in both detecting breast carcinoma and in evaluation of neoadjuvant chemotherapy efficacy is increasing. Contrast-enhanced magnetic resonance imaging (MRI) of breast is a well-established imaging method in breast carcinoma diagnostics, offering insight in both lesion morphology and vascularisation patterns. The full potential of breast-MRI and possible indications for use are still subject to clinical research. Sonoelastography is a novel ultrasound method displaying tissue elasticity, and shear-wave elastography (SWE) can quantify the lesion stiffness by measuring multiple elasticity indicators. The results regarding differences between MRI and SWE characteristics of benign postoperative changes and cancer recurrences are presented.

Materials and methods: 587 patients that underwent postoperative breast MRI and second-look US with SWE were included in the study. Lesions were categorized using American College of Radiology Breast Imaging-Reporting and Data System (BIRADS). Majority of patients had MRI findings of BIRADS 2 (normal finding) category, and diagnosis was based on imaging findings only, since any invasive procedure would be unethical for this category. In 86 of examined patients, second-look ultrasound was indicated and SWE exam was performed using state-of-art US scanner (Aixplorer, Supersonic Imagine, Aix en Provence, France) with measurements of mean stiffness (stiffest part of the lesion, region of interest 2 mm in diameter) measured in kilopascals (kPa). In case of suspicious breast lesions, a core-biopsy was performed using a 14 gauge biopsy needle, under local anesthesia, to obtain histological findings. Patients with positive findings of carcinoma recurrence were referred to a plastic surgeon and oncologist for further treatment. An informed consent was obtained. Appropriate statistical tests were used to seek differences between benign and malignant lesions based on sonoelastographic stiffness and MRI characteristics (T2-intensity, diffusion-weighted imaging (DWI) signs of diffusion restriction, postcontrast kinetics).

Results: In 36 out of 587 examined patients (6,13%) a cancer recurrence was verified by core-biopsy. Type of surgery performed for primary tumor and incidence of cancer recurrence didn't show statistically significant association. Regarding lesion SWE stiffness, cancer recurrences tend to have higher El mean values than postoperative changes of breast tissue (145.7 kPa vs 106.3 kPa, $p<0.001$). Cut-off value of 100 kPa yields high sensitivity (78%) but low specificity (50%) for cancer recurrence detection (ROC curve analysis), probably due to stiff areas of postoperative scarring. Regarding MRI characteristics, high intensity on DWI and low signal on ADC maps show good association with cancer recurrence ($p<0.001$, Chi squared test), while positive finding of diffusion restriction has sensitivity of 94% and specificity of 76% for cancer recurrence detection. Other MRI characteristics didn't show statistically significant association with cancer recurrence.

Discussion: MRI is a powerful tool in follow up of patients who underwent surgery for breast carcinoma, especially after breast conserving surgery. However, there are many cancer mimics in postoperative breast and second-look US is often needed to exclude cancer recurrence or for biopsy guidance in suspicious lesions. Our results indicate that sonoelastography may show useful in differentiation between benign postoperative changes and cancer recurrence. Caution is needed due to overlap of stiff areas of postoperative scarring and cancer recurrence. Also, cases of soft malignant tumors are known, which can result in false-negative result on SWE (e.g. small tumors, mucinous tumors). Regarding MRI, postoperative scarring and inflammation can result in rapid contrast

enhancement which may mimic malignant lesion. T2 signal can be heightened due to edema (both postoperative and postiradiation) and is not always reliable in exclusion of malignant lesions. DWI can detect areas of hypercellularity, such as in malignant tumor tissue. Our results suggest high sensitivity and specificity of DWI sequences in cancer recurrence detection.

Acknowledgments: This work is supported by Croatian Science Foundation under the project IP-2016-06-2997 "Sonoelastography and MRI in diagnosis and treatment of breast cancer".

MeSH/Keywords: breast cancer; magnetic resonance imaging; ultrasound; sonoelastography

Poster code: R-02-25-009

Poster Title: Tibial tuberosity–tibial intercondylar midpoint distance in diagnosing patellofemoral instability on the axial CT-images of the knee

PhD candidate: Dinko Nizić

Part of the thesis: Tibial intercondylar midpoint distance in diagnosing patellofemoral instability on the axial CT-images of the knee

Mentor(s): Assoc. Prof. Mislav Jelić, MD PhD, Assist. Prof. Goran Pavliša, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Proper alignment of the leg implies that the centres of the hip, the knee and the ankle are in the straight line with leg assuming the natural neutral position. The ultimate goal of the extensor mechanism of the knee is to achieve the upright position by putting the upper and the lower leg into the same plane using the knee joint. It is believed that the malalignment of this mechanism could cause various grades of patellar shear leading to anterior knee pain, a very frequent complaint associated with the knee joint, and, likely, patellar dislocation. This malalignment is routinely demonstrated with radiological imaging, namely computerized tomography (CT), and, rarely, magnetic resonance imaging (MRI). On the axial CT images of the knee joint, TT-TG distance is measured between the tip of the tibial tubercle and the bottom of the femoral trochlea. The TT-TG value greater than 20 mm is considered advisable for translocation (mostly medialization) of the tibial tubercle, sometimes with the additional repair of the medial patellofemoral ligament. It can be reliably presumed that the bottom of the femoral trochlea reflects the centre of the knee joint. Often, the femoral trochlea is not groove-shaped, and thus the bottom cannot be defined. Seitlinger et al. propose an alternative measurement of the TT-PCL distance between the tibial tubercle and the soft-tissue landmark—the medal margin of the posterior cruciate ligament (PCL), at its tibial insertion. Yet, the TT-PCL distance can only be measured on the knee MR images, and in the case of ruptured PCL it cannot be measured at all. Therefore, the distance between the tip of the tibial tubercle and the tibial intercondylar midpoint (TT-TIM) on the axial CT images of the knee is proposed as the new measuring method for evaluation of the malalignment of the extensor mechanism of the knee.

Materials and methods: Two independent raters retrospectively performed three measuring methods for evaluation of malalignment of the extensor mechanism of the knee (as per Koëter and Nizić, along with a new, tibial tuberosity-tibial intercondylar midpoint distance) on each of the included 129 knees of 79 patients scanned by the CT-protocol for patelofemoral instability in the Clinical institute for diagnostic and interventional radiology of Clinical hospital centre Zagreb. The methods were compared regarding differences due to knee instability, the correlation with age, sex, laterality, and longitudinal rotational knee angles, and interrater agreement. Lastly, the threshold of the new method was proposed.

Results: All methods were different in objectively unstable knees (p from <0.001 to 0.002). TT-TGs were dependent on age (p from 0.002 to <0.001), TT-TIM on sex ($p=0.009$), and none on laterality (p from 0.08 to 0.78). Only TT-TIM was not correlated with knee rotation ($p=0.10$ in contrast with both $p<0.001$), and the overall knee rotation angle ($p=0.86$ in contrast with both $p<0.001$). The interrater agreement was almost perfect (ICCs from 0.84 to 0.94). The TT-TGs had sensitivity from 76.8 % to 78.6 %, and specificity from 56.2 % to 63.0 %. The threshold value for TT-TIM was >14 mm for males (sensitivity 84.6 %, specificity 60.0 %), and >12 mm for females (sensitivity 72.1 %, specificity 60.4 %). The OR for all methods was very strong (from 4 to 7).

Discussion: The TT-TIM is a reliable substitute for use in patellofemoral instability assessment, yet is independent of knee rotation. TT-TIM seems solely independent of age. Still, no table values exist to accommodate for this correlation with the TT-TGs, though the link has been known. Unlike the TT-TGs, TT-TIM is dependent on sex. Since male and female skeleton differ, we can speculate this difference transcends to the patellofemoral instability indices such as TT-TIM. The excellent interrater reliability signifies great practical value of all methods. For TT-TG as per Koeter, the

threshold value is believed to be >20 mm, with many other numbers circulating. TT-TG according to Nizić has no established threshold, thus the same value has been transposed. The thresholds should be dealt with caution due to lack of reference regarding age dependence. The TT-TIM threshold has been sex-branched. Still, this should be dealt with in a separate study designed to meet the needs for threshold approximation.

Acknowledgments: I thank the other radiologist, Marko Šimunović.

MeSH/Keywords: knee, computed tomography, TT-TG distance, extensor mechanism malalignment, sex, laterality, rotational knee angles

Poster code: R-02-25-126

Poster Title: Efficiency of ultrasound-guided radiological intervention methods in the treatment of the rotator cuff calcific tendinitis – preliminary results

PhD candidate: Andro Matković

Part of the thesis: Efficiency of ultrasound-guided radiological intervention methods in the treatment of the rotator cuff calcific tendinitis – preliminary results

Mentor(s): Assoc. Prof. Vinko Vidjak, MD PhD, Professor Božidar Šebečić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Rotator cuff calcific tendinitis (RCCT) is a disease caused by the deposition of hydroxyapatite crystals in the rotator cuff tendons. The literature describes different minimally invasive treatment methods indicated in the acute and resorptive stage of the disease. Thus, the aim of this study was to compare the efficiency of four different ultrasound-guided interventional methods in the treatment of RCCT.

Materials and methods: The total sample available until this point in the study was randomly assigned into three groups without knowledge in which group they belong to. Each group was subjected to a different treatment: ultrasound-guided percutaneous irrigation with saline heated at 37° C (IUZV), ultrasound-guided percutaneous irrigation with saline heated at 37° C augmented with corticosteroid injection (IUZVK) and ultrasound-guided percutaneous irrigation with saline heated at 37° C augmented with protein-rich plasma injection (IUZVPRP). On treatment day the pain level was measured by visual analog scale (VAS) and shoulder function with QuickDASH and SPADI questionnaires. Due to the duration of the preliminary research follow-ups were done at 3 weeks and 3 months after the treatment. Descriptive parameters were calculated and the differences between groups were tested with Whitney U test.

Results: From the total planned number of patients, until now, 29 patients have been included in the research. According to the planned groups, 7 subjects were included in the IUZV group, 21 in the IUZVK group and 1 in the IUZVPRP. Since there is only one person in the last group, this group will not be considered further. By gender, there were more women (N = 20; 71.4%) than men (N = 8, 28.6%). The mean age of patients was 49.3 years (range 33 to 69 years, median 48 years). The dominant arm was affected in most cases (N = 18; 64.3%). The mean duration of the symptoms in the subjects was 5.2 months (0.2 to 24 months, median 2 months). At the beginning of the study, there were no differences between groups in the measured parameters – VAS, SPADI and QuickDASH. At three weeks follow-up the IUZVK group shown lower values in all the measured parameters but statistically significant only for SPADI disability and QuickDASH parameters. There were no statistically significant differences between the groups at three-month follow-up although the IUZVK group still had lower values.

Discussion: The acquired preliminary results show that the treatment used in IUZVK group is superior to the treatment used in the IUZV group. It is more effective in reducing pain and improving shoulder function. Even though the results were statistically significant only in function improvement, we believe that the complete results will also show a statistically significant pain reduction. Overall ultrasound-guided percutaneous irrigation with saline heated at 37° C augmented with corticosteroid injection into the subacromial-subdeltoid bursa can be recommended as a treatment for RCCT as it is effective in lowering the pain and improving shoulder function.

MeSH/Keywords: Rotator cuff; Calcific tendinitis; Interventional musculoskeletal procedures; Ultrasound.

Poster code: R-02-25-134

Poster Title: The effect of pharmacogenetic variations of P-glycoprotein and inosine-5'-monophosphate dehydrogenase on treatment outcomes in patients with kidney transplant

PhD candidate: Luka Penezić

Part of the thesis: The effect of pharmacogenetic variations of P-glycoprotein and inosine-5'-monophosphate dehydrogenase on treatment outcomes in patients with kidney transplant

Mentor(s): Professor Željko Kaštelan, MD PhD, Assoc. Prof. Nada Božina, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Interindividual and intraindividual variability in clinical response to immunosuppressants – mycophenolic acid and calcineurin inhibitors, is well documented. Numerous studies investigated pharmacogenetic associations of P-glycoprotein and IMPDH SNPs on kidney transplantation clinical outcomes. The primary aim of this study is investigation of IMPDH2 and P-glycoprotein SNPs' influence on kidney transplantation outcomes, mainly acute rejection, graft function, graft and patient survival as well as association with adverse effects, tumors and infections incidence.

Materials and methods: The study is retrospective and includes 250 patients transplanted at the Department of Urology, Clinical Hospital Center Zagreb, from January 2011 onward. Inclusion criteria are: capability for informed consent and the availability of DNA. Exclusion criteria are: early postoperative complications that result with graft loss. Genotypization will be performed form recipients' blood samples and donors' archived materials. We have reviewed recipients' medical documentation for clinical outcomes and laboratory measurements during the initial and minimum 3 years follow up. Basic data such as sex, age, BMI, miss match, cold ischemia time, concomitant medication, etc. was collected along with relevant clinical (graft function, graft loss, death, BPAR according to Banff classification, infections, lymphocele, tumors, etc.) and laboratory (creatinine, whole blood count, electrolytes, urinalysis, immunosuppressive drug concentrations, etc.) data. DNA extraction will be done by salting-out method according to Miller and the genotypization using Real-Time PCR.

Results: We have identified 250 patients that meet inclusion and exclusion criteria during a period from from January 2011 until October 2014. There are 98 female (39,2%) and 152 (60,8%) male renal transplant recipients. The average age in the time of transplantation was 51 years (range 15-76 years). Mean follow up is 5,4 years (range from 1 month to 8 years). During follow up, 26 (10,4%) patients died and 29 lost graft function (11,6%).

Discussion: At this moment we are able to report only preliminary demographic data for our study participants. The blood samples are being collected and appropriate genotypization will be performed when enough samples have been gathered. Genotypization and clinical outcome analysis will show IMPDH2 and P-glycoprotein SNPs' influence on long term kidney transplantation outcomes in population of Croatian kidney graft recipients for the benefit of better individualization of immunosuppressive therapy.

Acknowledgments: I would like to thank The UHC Zagreb Kidney Transplantation Team for their approval of this study and my mentors, prof. Željko Kaštelan and prof. Nada Božina, for their guidance and support.

MeSH/Keywords: Kidney transplantation, immunosuppressive agents, pharmacogenetics, single nucleotide polymorphism, IMP dehydrogenase, P-glycoprotein

Poster code: R-02-28-080

Poster Title: The impact of UGT1A9 and SLCO1B1 pharmacogenetic variations on treatment outcomes in kidney transplant patients

PhD candidate: Sandra Nađ Škegro

Part of the thesis: The impact of UGT1A9 and SLCO1B1 pharmacogenetic variations on treatment outcomes in kidney transplant patients

Mentor(s): Assoc. Prof. Nada Božina, MD PhD, Assist. Prof. Tvrtko Hudolin, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Mycophenolate acid (MPA) is immunosuppressive agent widely used in kidney allograft recepients to prevent graft rejections. MPA displays variable pharmacokinetics which can be partly explained by presence of genetic polymorphisms in key enzymes and patients concomitant therapy with calcineurin inhibitors. This research analyzes the role of genetic polymorphisms UGT1A9 and SLCO 1B1 on treatment outcomes (graft function, patients survival, acute rejaction, adverse effects..) in kidney transplant patients. The hypothesis of this study is that genetic polymorphisms of UGT 1A9 (-275T>A, -2152C>T) and SLCO 1B1 (521T>C) can be used as predictors of the risk of graft rejections and the incidence of adverse drug reactions of immunosuppressive therapy in patients with kidney transplant.

Materials and methods: The study includes 250 patients who had kidney transplantation in Department of urology , Clinical Hospital Centre Zagreb, from January 2011 to October 2014. Patients DNA will be isolated from the whole blood samples by salting-out method. Genotyping of UGT1A9 (-275T>A, -2152C>T) and SLCO1B1 (521T>C) will be performed by real time PCR method. From patients medical documentation we will collect relevant basic, clinical and laboratory data. Most important clinical data include: biopsy proven acute rejection (Banff classification), patients death, graft function, graft loss, immunosuppressive adverse reactions (myeloproliferative, gastrointestinal), infections and tumors.

Results: Study is still in progress, the blood samples for genotypization are being collected so we do not have genotypization results yet. Following the inclusion and exclusion criteria we analyzed the medical records of 240 (250 in plan) kidney transplant patients, and we have preliminary demographic data. The study includes 37.92% female (N=91), and 62.08 % male (N=149) patients, with average age of 51.3 years in time of transplantation. During follow up 27 patients lost graft function and 24 patients died.

Discussion: So far the study is still in process and we do not have all data to make final conclusion. Further investigation and data will show genotype correlation with clinical outcome in kidney transplant patients.

Acknowledgments: I would like to than Prof. Nada Božina and Assist.Prof. Tvrtko Hudolin for providing guidance with this project.

MeSH/Keywords: kidney transplantation, UGT1A9, SLCO1B1, genetic polymorphism, acute graft rejection, clinical outcome, immunosuppression, adverse effect

Poster code: R-02-28-127

Poster Title: Reasons for Using Depression Internet Forums in Croatia

PhD candidate: Nikola Žaja

Part of the thesis: Razlike u razlozima korištenja internetskih foruma o shizofreniji i o depresiji u Hrvatskoj

Mentor(s): Assist. Prof. Tea Vukušić Rukavina, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: The majority of people uses Internet every day to acquire needed information. A wide range of information regarding mental health can be found on the Internet and there are various ways of communication, one of them are Internet forums. Internet forums have become the source of information for any health or psycho-social problem. Participation and communication in the forums are anonymous, which greatly contributes to honest and immediate communication, especially on sensitive topics such as mental illness. The aim of this study was to examine the motives for using depression Internet forums in Croatia.

Materials and methods: The study sample consists of 297 user-generated posts on the largest Croatian depression Internet sub-forum over a period of one year, analyzed using qualitative methodology-grounded theory.

Results: The results showed that the majority of depression Internet forums user in Croatia use Internet forums to receive emotional support from others with the same illness. Other important reasons were to exchange information about medications, symptoms and prognosis of the illness.

Discussion: Users of depression Internet forums in Croatia, mostly use those forums to receive emotional support from others with the same illness. For some of the users, Internet forums are the first place where they seek help to cope with symptoms of depression. Because of this reason, Internet forums could be used to detect untreated individuals who could then be provided earlier with psychiatric treatment.

MeSH/Keywords: Depression, Internet forums, mental health

Poster code: R-02-29-011

Poster Title: Serum concentrations of zinc, albumin, C-reactive protein and interleukin-6 in patients with major depressive disorder and depressive episode of bipolar disorder

PhD candidate: Tihana Bagarić

Part of the thesis: Serum concentrations of zinc, albumin, C-reactive protein and interleukin-6 in patients with major depressive disorder and depressive episode of bipolar disorder

Mentor(s): Professor Alma Mihaljević-Peleš, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: There is no difference in clinical features of depressive episodes in bipolar disorder compared to those in major depressive disorder. On the contrary, pharmacotherapeutic approach to the depressive episode of bipolar disorder and the major depressive disorder is significantly different. To distinguish these two disorders, it is necessary to rely on the anamnestic data. So far conducted studies suggest the existence of a biological marker that could help differentiate these two disorders, but none is sufficiently reliable.

Materials and methods: The research will include total of 128 participants of both sexes, aged 18 to 65, fulfilling the criteria for major depressive disorder and depressive episode of bipolar disorder according to ICD 10 and DSM 5. Research will be conducted individually using sociodemographic questionnaire. The severity of depressive symptoms will be measured by the Montgomery Asberg Rating Scale (MADRS) and the Hamilton Depression Scale (HAM-D-17). Clinical Global Impression Severity Scale (CGI-S) will be used to assess severity of the disorder. Blood samples will be obtained from a brachial vein from each study participant, in order to determine serum concentrations of zinc, albumin, C reactive protein and interleukin-6.

Results: The participant's sociodemographic data are collected through the structured questionnaire. The research is currently in data collecting phase, at the moment data for 40 participants are collected and partially assessed. There is expected distribution on gender, 53% (N=21) are male and 47% (N=19) of them representing female gender. Median of age is 50,72 with range from 22 to 65 years, and majority of participants are with high school education and lower socio-economic status. 27,5% (N=11) of them are diagnosed with bipolar disorder and 72,5% (N=29) of them are diagnosed with major depressive disorder which is in line with expected distribution among diagnosis. Blood samples are being analyzed for serum concentrations of zinc, albumin and C-reactive protein, while analyses of serum concentrations of interleukin-6 will be commenced when half of participants are enrolled.

Discussion: Currently available results for all required parameters in the research, nor the number of participants is sufficient to bring any conclusions.

MeSH/Keywords: major depressive disorder, bipolar disorder, zinc, albumine, C reactive protein, interleukin-6

Poster code: R-02-29-091

Poster Title: Comparison of personality traits in patients with advanced cervical cancer who did not perform routine gynecological screening with women without cervical cancer diagnosis who underwent regular gynecological screening

PhD candidate: Anastazija Aleksandrova Stanojević

Part of the thesis: Comparison of personality traits in patients with advanced cervical cancer who did not perform routine gynecological screening with women without cervical cancer diagnosis who underwent regular gynecological screening

Mentor(s): Assist. Prof. Marina Šagud, MD PhD, Assist. Prof. Joško Lešin, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: The majority of our patients with cervical cancer have advanced disease due to irregular check-ups. The reasons for such neglectful behavior may be lower psycho social circumstances and certain personality traits as is shown in many research papers. Cloninger's “unified biosocial” model of personality traits analysis several Character- Self-Directedness (SD), Cooperativeness (C), Self-Transcendence (ST) and Temperament dimensions- Novelty Seeking (NS), Harm Avoidance (HA), Reward Dependence (RD), Persistence (PS). People with high temperament dimension "Harm Avoidance" (HA) are pessimistic, fearful, shy, fatigable. People with low character dimension "Self-Directedness" (SD) are blaming, aimless, inept, vain. Clinical studies that analysed different temperament dimensions in patients, especially HA and character dimensions like SD showed that these dimensions are mutually connected and predictive of depression development, emotional trauma in childhood or adult life, and could therefore be the reason of patients neglecting their health. After years of treating many cervical cancer patients we observed many similarities in their social circumstances, personality profile and traumatic experiences. We propose that our cervical patients have higher harm avoidance, lower self-directedness and were previously exposed to higher intensity of traumatic events compared to women without cervical cancer diagnosis who regularly go to gynecologist.

Materials and methods: This research will include 124 women (62 cancer patients treated in Gynecological Cancer Center, Petrova 13, Zagreb and 62 controls without cervical cancer who regularly attend gynecological screening). They will fill out three questionnaires: 1.Temperament and Character Inventory (Cloninger i sur,1993), 2. Childhood Traumatic Events Scale (Pennebaker and Susman, 2013), and 3. General item questionnaire.

Results: Up until now five patients filled out Temperament and Character Inventory questionnaire with 140 questions. All of them readily participated in this study. By observation their answers our patients show tendency to avoid new, unknown situations, they show high level of empathy, and high work responsibility. They like to please other people, they tend to worry more then other people, and feel more insecure then others. They are not perfectionists, do not like to be group leaders or stand out from others.

Discussion: Although this is a small sample of patients without controls to compare or thorough statistical analysis, by observing answers in Temperament an d Character Inventory, there are indications that point to character and temperament similarities among this group. By adding Childhood Traumatic Events Scale and General Item Questionnaire we will be able to show other traits among cervical cancer patients.

MeSH/Keywords: perosnality traits, cervical cancer, harm avoidance, self directedness, trauma

Poster code: R-02-29-146

Poster Title: Simultaneous bilateral cerebral circulation monitoring during cognitive tasks performance

PhD candidate: Petra Črnac Žuna

Part of the thesis: The effect of aerobic exercise on cognitive functions and cerebral circulation in persons with subcortical vascular cognitive impairment, no dementia

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

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

Introduction: Subcortical vascular cognitive impairment without dementia (scVCI-ND) refers to the loss of cognitive function, particularly executive functions, due to cerebrovascular disease, with a high risk of progression to dementia. The aim of our study is to evaluate the effect of additional structured aerobic exercise on cognitive functions and cerebral circulation parameters measured by functional transcranial Doppler sonography (fTCD) compared to standard treatment protocol in subjects with scVCI-ND. So far, systematic reviews have shown improvement in cognitive tests results after aerobic exercise, but mostly non-significant, possibly due to a short intervention period or follow-up. Sensitive biomarkers are needed to detect early exercise-induced changes in brain function. Previous studies used mostly brain MRI as a biomarker. High temporal resolution and accessibility are some of the advantages of TCD in comparison to MRI. fTCD enables monitoring of blood flow velocity (BFV) changes caused by specific neural activation. A preliminary study has been conducted to find the most suitable activation tests to elicit BFV changes in the brain regions of interest and to test the suitability of fTCD as a biomarker for cognitive activation. We performed simultaneous BFV monitoring in both anterior (ACA) and middle cerebral arteries (MCA) during cognitive tasks to obtain temporal and spatial relations between each cognitive task and BFV.

Materials and methods: The preliminary study included 14 right-handed healthy subjects aged 20 to 33 years. Blood pressure, heart rate (HR), visual analogue anxiety scale (VAS) and handedness (with the Edinburgh Handedness Inventory (EHI)) have been assessed before completing the fTCD paradigm. BFV in both ACAs and MCAs, as well as HR were monitored during performance of cognitive tasks. The paradigm consisted of the breath holding test (BHT), followed by cognitive tasks designed to activate frontal lobes: Phonemic Verbal Fluency Test (pVFT), Stroop Test (ST), Trail Making Test A and B (TMT A, TMT B). Cerebral BFV were recorded with 2 MHz TCD probes attached to a headframe placed over the transtemporal window at a 65–70 mm insonation depth. Mean BFV values were averaged and analyzed for each artery and each cognitive task.

Results: A statistically significant BFV increase in both ACAs and both MCAs was found during the performance of all cognitive tasks. Statistically significant right ACA dominance was found during the performance of pVFT and TMTB. The highest blood flow velocity increase was obtained during the performance of TMTB, both in ACAs and MCAs. Statistically significant lateralization was found during performance of Stroop test with incongruent stimuli. No statistically significant differences were found in systolic and diastolic pressure or VAS before and after task performance. No statistically significant correlation between HR and BFV was found. There was no statistically significant difference in BFV between right ACA and left ACA or right MCA and left MCA during the 2-min resting phase between tasks.

Discussion: The aim of this preliminary study was to determine the cognitive tests with greatest activation potential for BFV monitoring in ACAs and MCAs, as well as the suitability of fTCD as a biomarker for cognitive activation. All tests were chosen based on known activations of medial and orbital frontal lobe shown on functional neuroimaging studies. Excellent fTCD temporal resolution enables the monitoring of rapid and transient BFV increase that is not possible to record by fMRI. Our preliminary results show good temporal relation and specific temporal pattern between BFV in the ACAs and performance of ST and TMT B, and between BFV in both ACAs and MCAs and performance of pVF. Some of the cognitive tasks have shown good potential for BFV monitoring in ACAs (TMT B,

pVFT), and MCAs (TMT B). The most significant BFV increase appeared during performance of more demanding tasks (TMT B and pVFT). Our preliminary results have shown good suitability of BFV monitoring by using fTCD as a biomarker of neural activation for the brain regions of interest and a good activation potential of pVF, Stroop test with incongruent stimuli and TMT-B on BFV increase in ACAs and MCAs, for the medial and orbital frontal lobe region.

Acknowledgments: I would like to thank my mentor for her guidance.

MeSH/Keywords: Cerebral arteries; Ultrasonography, Doppler, Transcranial; Exercise; Dementia, Subcortical Vascular; Neuropsychological Tests

Poster code: R-02-30-089

Public health and health care - Preliminary research results

Poster Title: Development of the model for predicting of interval breast cancer based on effectiveness indicators of the National Program for early detection of breast cancer in the Republic of Croatia

PhD candidate: Romana Tandara Haček

Part of the thesis: Development of the model for predicting of interval breast cancer based on effectiveness indicators of the National Program for early detection of breast cancer in the Republic of Croatia

Mentor(s): Assoc. Prof. Nataša Antoljak, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: The National Program of Breast Cancer Early Detection in the Republic of Croatia has been conducted since 2006 using mammography method as primary screening method. It covers women aged 50 to 69 years who are invited to mammography every two years. In the first three cycles of screening 890.124 women were scanned over the period from October 2006 to May 2014. The quality indicators ensure monitoring implementation and data collection quality. The quality and effectiveness of the screening program is subject of continuous evaluation. Performance indicators reflect the provision and the quality of activities constituting the screening process. Acceptable and desirable values have been set for them according to EU guidelines. The functional organisation and web-based screening registry are prerequisites for organized screening. However, some countries still do not have all needed conditions. If all minimum indicators cannot be recorded, it must be assured on other way by surrogate indicators analyse. One of surrogate quality indicators, but also indicator of possible fast-growing neoplasm is interval cancer. Interval cancer is primary breast cancer diagnosed to the woman who has made screening examination with or without further assessment that was negative to the malignancy prior to the next screening call or within a period of time equal to a screening interval if the woman reaches the upper age limit for inclusion in the screening. Second surrogate indicator is the result of data linkage with Cancer Registry and finding of greater proportion of woman detected in localised or early stage of disease in screened group, compared to non-screened or mixed non-screened with opportunistic screened woman.

Materials and methods: Material 1. Anonymised data from the screening registry of National Program for early detection of breast cancer for first three cycles of invitation. 2. Regarding stage of disease, anonymised data from the Cancer Registry for women of the age group covered by the program were used. Ethical approval was obtained from institutions. Methods After accessing the screening program registry data, without insight into the woman's identity, data linkage with cancer registry was done. The MS Office Access 2016 program was used. Data are represented as proportions and difference between groups is tested by chi-squared test.

Results: 2.7% of women who did not participate in mammography screening and 3.8% of them who participated at least in one mammography screening cycle had breast cancer in situ. 47% of women who did not participate in mammography screening and 61% of screened woman were diagnosed in localised stage, while regional metastases were detected in 42% in nonparticipant group and 33.3% in screened group. Distant metastases were detected in 8.4% of non-participants, and only in 2.4% of screened group.

Discussion: There is substantial problem of quality control in organised screening program even if robust web registration application exists. Mainly, the reason is lack of recording data in separated system during the diagnostic procedure conducted by clinicians. They are not aware that data must be recorded because they are indicators of quality and usually hospital databases do not consist structured findings and are hardly monitored. Medical findings are usually stored as pdf or similar kind of documents. Moreover, screening cancer is highly sophisticated procedure which purpose is measuring and improving quality and effects, so it differs of routine work. Even medical doctors who do exams must be the best in their field of specialty. Thus, analyse of surrogate quality indicators must be performed. In this paper we got results that there is significant difference between stage of

breast cancer in female population which did not participate in organised screening and for which we do not have proper data on opportunistic screening and group of females who were diagnosed as breast cancer within organised program. That supports other country data and confirm that screened female population was detected in earlier phase that nonparticipated females.

MeSH/Keywords: quality, assessment, breast cancer screening, interval breast cancer

Poster code: R-03-01-013

Poster Title: Googling for Suicide: Suicide Risk Screening using Internet Search Data

PhD candidate: Vanja Pajić

Part of the thesis: Epidemiological characteristics of suicide and the possibility of preventive intervention among Internet users in the Republic of Croatia

Mentor(s): Professor Stjepan Orešković, MD PhD, Kyle L. Grazier, PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Literature research demonstrated that the analysis of internet search data can be utilized as a tool in predicting the occurrence of suicide risk in a given population of internet users. Preliminary results presented here have shown that there is a relationship between the use of suicide-related keywords and the real world suicide patterns such as the general rise of suicide incidence and the annual fluctuations of committed suicides. However, conclusion has not yet been reached on the relationship between the usage of selected keywords.

Materials and methods: In this preliminary research, the Google Trends tool was to used compile suicide-related Google search data for years 2012-2018. Statistical analysis was performed for monthly search data on a month-to-month basis. Official data on suicide in Croatia were obtained from the Croatian Institute of Public Health. The keywords were selected after a literature search. The following terms were chosen for this preliminary research: a) "suicide" ("samoubojstvo"), b) "how to kill yourself" ("kako se ubiti"), c) "kill yourself" ("ubiti se") and d) "suicide crimes & accidents pages" ("samoubojstvo crna kronika"). Only the Croatian terms were used and the Google Trends analysis was selected and performed only for the internet search results from the territory of Croatia. Presented results were presented in the Google Relative Search Volume (GRSV), a standardized unit introduced by Google representing how often a word was searched using Google search in a given time and region. Based on the GRSV score, time series of suicide-related keywords used were created for the selected period. The time series were extracted into a CSV file in order to be further analyzed. Data was collected into a MS Excel spreadsheet and analyzed using the MS Excel Data Analysis tool. Multiple regression was performed for the selected period in order to establish the relationship between the keywords. The level of statistical significance was set at $p<0.05$.

Results: Similar to the data from the national gazette published by the Croatian Institute of Public Health which show a steady increase in committed suicides, the gradual increase in GRSV score for the selected keywords was observed in years 2012-2018. For the keyword "samoubojstvo" the lowest GRSV score was observed in February 2012 (GRSV = 31), whereas the lowest GRSV score for the same term in December 2018 (GRSV = 46). The February 2012 score is also the lowest overall for the 2012-2018 period, second only to November 2012 (GRSV = 25). The December 2018 score was the highest low score in the 2012-2018 period. Similar observations are true for other selected keywords as well. It is also interesting to note that the highest internet search volumes for the selected keywords were recorded in autumn and winter months. Usually, there is a peak of internet search using the selected keywords in September and October, following a steep fall in November and then peaking up again in December and January. Both occurrences follow the distribution of yearly suicide occurrences from the official statistical data. However, the relationship between the use of keywords themselves seems problematic. While it seems that the general trend of using suicide-related keywords follows the yearly occurrence of suicides, a correlation between the keywords themselves has not yet been established. Although the correlation between the selected keywords was calculated to $R=0.20$ for the 2012-2018 period, the p-value was not statistically significant and therefore the results are not conclusive.

Discussion: The results of this preliminary research seem promising as they show a pattern of using suicide-related keywords similar to the observed incidence of suicides in Croatia for the selected period. This may seem important as it could add predictive value to analyzing the keywords searched in real time. However, the connection between the keywords themselves is not yet fully clear. One step towards better understanding the relationship between suicide-related keywords could be

further explored by grouping keywords according to the manner of execution (e.g. "hanging") and according to known suicide risk factors (e.g. "divorce", "death", "loss of job", etc.).

MeSH/Keywords: mental disorders, behavioral disorders, suicide, epidemiology, digital epidemiology, public health, public health informatics

Poster code: R-03-02-005

Poster Title: Seasonal variation of benzodiazepine utilization among the Croatian population

PhD candidate: Marija Delaš Aždajić

Part of the thesis: Ocjena racionalnosti izvanbolničkoga propisivanja benzodiazepina u Republici Hrvatskoj

Mentor(s): Assoc. Prof. Danijela Štimac Grbić, MD PhD

Affiliation: University of Zagreb, School of Medicine

Introduction: Drugs acting on the central nervous system are the leading group of drugs utilized worldwide, followed by cardiovascular drugs. Within this group, benzodiazepines are medications widely used to treat anxiety and insomnia, and in managing alcohol withdrawal. However, these drugs should be only used in a short period of time, because within weeks of chronic use, dependence and withdrawal symptoms may develop. Additionally, abrupt interruption of the therapy occurs as a rebound phenomenon in the form of anxiety or insomnia.

Materials and methods: The data for this study was extracted from Croatian Health Insurance Fund database. This data includes the information about frequency of benzodiazepines prescription via outpatient utilization, retrospectively for years 2015 and 2016. For this study, only patients who utilized minimum of seven prescriptions in one year were included in further analysis, and for the purposes of our analyses, we assumed that the patients took the number of pills as prescribed. Correlations for diagnosis, sex, age and duration of therapy will be assessed in future reports, and the rationality of benzodiazepines utilization according to the gender as seasonality pattern is shown in this paper.

Results: The results have shown an increasing trend of benzodiazepine utilization in a two consecutive years (2015 and 2016), with an average number of prescriptions of 259 535 in 2015, and 270 774 in year of 2016. The highest benzodiazepine utilization was noted during summer months, the peak month in 2015 was June (212 453 prescriptions), while in 2016 the peak month was August (219 786 prescriptions). The overall increase was found with the exception of slight decline in July 2016 in a comparison to July 2015.

Discussion: Inappropriate prescribing is a known risk factor for adverse drug reactions especially in the elderly. Previous studies have shown that patients tend use benzodiazepines over longer time periods than recommended by current guidelines. The main adverse drug reactions of benzodiazepines are central nervous system depressant effects, such as drowsiness and sedation, falls, hip fracture, memory problems, muscle weakness, and respiratory depression. Whether prescribing benzodiazepines might raise a patient's risk of suicide is frequently being questioned in the past years, and recent studies reported a positive correlation between prescribed benzodiazepine and attempted or completed suicide. Additionally, the high-lethality suicide attempts group peaked in the months with a higher sunlight exposure (months such as June and July). This analysis shows that the use of benzodiazepines in Croatia is particularly high in the summer months, which may contribute to suicide risk. Further analysis should be focused on general practitioner's drug prescribing patterns; especially for the elderly and measure the adverse clinical and financial consequences of irrational pharmacotherapeutic decision making process.

MeSH/Keywords: benzodiazepine, Croatia, drug utilization, inappropriate medication, prescription

Poster code: R-03-02-135

Poster Title: Understanding environments in Croatian elementary schools as a first step in childhood obesity prevention

PhD candidate: Maja Lang Morović

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

Mentor(s): Assist. Prof. Sanja Musić Milanović, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Obesity, especially in childhood, is a growing worldwide public health problem due to its negative consequences on all aspects of lifelong health. In order to tackle this problem, interdisciplinary public health interventions are required. In joint work of public health and educational professionals, school environments in which children spend the majority of their time, can be the setting for intervention. In order to implement successful interventions, it is important to know and understand strengths and limitations of school environments. The purpose of this paper is to analyse current status of Croatian school environments and its potential for childhood obesity prevention.

Materials and methods: This paper is a part of Childhood Obesity Surveillance Initiative conducted by the World Health Organization Regional Office for Europe. The goal of this surveillance is to gain comparable, standardized data on childhood weight status. For the data collection purposes three questionnaires were used: School Form, Parental Form and Examiner's Form. The surveillance was administered in randomly selected 182 second and 182 third grades from 164 main elementary schools in Croatia. School data for physical activity opportunities for children was collected through variables outdoor playground and its availability to children, and for school nutrition through variables concerning school nutritional facilities and the food they offer.

Results: Results show inconsistency in 164 schools in relation to physical activity and nutrition environments. In relation to physical activity promotion opportunities for children, 95,7% of school have outdoor playgrounds. Children can use these playground during free time in 95,1%, and during extreme weather in 34,1% of schools. Further, 84,1% of schools have indoor gyms for physical education classes, that children can freely use during free time in 48,8%% of schools.Regarding nutrition, 93,9% of schools offer a for of nutrition education. School kitchen exists in 69,5% of schools, while 9,1% of school s have vending machines. Water is free of charge to children in 93,9% of schools, fresh fruit and vegetables in 62,8% of schools, while other foods including soft drinks and snacks can be purchased in most schools.

Discussion: Results show that schools vary in great deal in ways they support children's physical activity and nutrition. The biggest infrastructural weakness for physical activity of children are the lack of school gyms regarding physical activity and the lack of school kitchens that would offer healthy foods to children. From this starting point and with using this baseline knowledge of school environments, recommendations will be made on their possible strengthening in order to make them more protective for the development of healthy habits in children.

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

Poster code: R-03-02-150

Poster Title: Skin changes in Croatian hairdressing apprentices at the beginning of vocational education

PhD candidate: Zrinka Franić

Part of the thesis: Interaction of constitutional and occupational risk factors on the incidence of occupational contact dermatitis in hairdressing apprentices during vocational training

Mentor(s): Assoc. Prof. Suzana Ljubojević Hadžavdić, MD PhD, Jelena Macan, research advisor

Affiliation: Institute for Medical Research and Occupational Health, Zagreb

Introduction: Hairdressing apprentices are at high risk of developing occupational contact dermatitis related to various workplace exposures to hazardous skin agents. Major risk factors include exposure to skin irritants and sensitizers, particularly wet work, hair dyes, preservatives, and fragrances. Previous study on Croatian hairdressing apprentices at the end of apprenticeship showed that work-related skin symptoms were present in 40% of apprentices.

Materials and methods: In a screening phase of a prospective cohort study, from September to December 2017, we recruted 352 hairdressing apprentices attending vocational schools in 24 Croatian towns at the beginning of their education. The study protocol included: Nordic Occupational Skin Questionnaire and International Study on Asthma and Allergy in Children Questionnaire for the evaluation of self-reported skin and atopy symptoms, Osnabrueck Hand Eczema Severity Index (OHSI) for the clinical assessment of the severity of hand eczema, genotyping filaggrin (FLG) gene polymorphisms 2282del4 and R501X from buccal swabs, skin pH and transepidermal water loss (TEWL) measurements.

Results: We screened 352 hairdressing apprentices (334 females and 18 males; mean age 15 years). A history of respiratory and/or skin atopy symptoms was reported in 158 (44.89%) apprentices, hand/wrist eczema in 42 (11.93%), and a history of dry hands (without eczema) in 121 (34.38%) apprentices. At the clinical examination, one or more hand/wrist skin changes were found in 64 (18.18%) apprentices, with the OHSI score ranging from 1 to 6. Washing hands more than 20 times per day was identified in 45 (12.78%) apprentices. An FLG gene mutation (R501X) was found in only one apprentice. The median (range) for hand TEWL and pH was 13.1 (4.36-62.69) and 5.68 (4.28-7.13), respectively. OHSI score was positively correlated with hand TEWL (Spearman rho 0.16; p=0.0026), and pH (Spearman rho 0.13; p=0.0186).

Discussion: The results indicate a high prevalence of self-reported atopy (44.89%), moderate prevalence of self-reported hand/wrist eczema (11.93%) and clinically observed skin symptoms (18.18%) on the hands/wrists in hairdressing apprentices already at the beginning of education, without FLG mutations as a risk factors. This emphasizes the need to ameliorate preventive examinations of children before enrolling to schools for professions with high risk of exposure to hazardous skin agents.

MeSH/Keywords: occupational contact dermatitis, self-reported skin and atopy symptoms, filaggrin gene polymorphism, TEWL

Poster code: R-03-03-085

Poster Title: Mortality and causes of death in male Croatian Olympic medalists

PhD candidate: Vedran Radonić

Part of the thesis: Mortality and causes of death in male Croatian Olympic medalists

Mentor(s): Assist. Prof. Tomislav Letilović, MD PhD, Mario Šekerija, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: To compare the overall and disease-specific mortality of Croatian male athletes who won one or more Olympic medals representing Yugoslavia from 1948 to 1988 or Croatia from 1992 to 2016, and the general Croatian male population standardized by age and time period.

Materials and methods: All 233 Croatian male Olympic medalists were included in the study. Information about life duration and the cause of death for the Olympic medalists who died before January 1, 2017, was acquired from their families and acquaintances. We asked the families and acquaintances to present medical documentation for the deceased. Data about the overall and disease-specific mortality of the Croatian male population standardized by age and time period were obtained from the Croatian Bureau of Statistics (CBS). Overall and disease-specific standard mortality ratios (SMR) with 95% confidence intervals (CI) were calculated to compare the mortality rates of athletes and the general population.

Results: Among 233 Olympic medalists, 57 died before the study's endpoint. The main causes of death were cardiovascular diseases (33.3%), neoplasms (26.3%) and external causes (17.6%). Overall mortality of the Olympic medalists was found to be significantly lower than that of the general population (SMR=0.73, CI=0.56-0.94, p=0.013). Regarding specific causes of death, athletes' mortality from cardiovascular diseases was significantly reduced (SMR=0.61, CI=0.38-0.93, p=0.021).

Discussion: Croatian male Olympic medalists benefit from lower overall and cardiovascular mortality rates in comparison to the general Croatian male population. This paper was published in Croatian Medical Journal and presented at the conference Cardiology Highlights Dubrovnik 2017. In my disertation, we included all Croatian male and female Olympic participants.

MeSH/Keywords: Mortality, Athletes, Causes of death

Poster code: R-03-03-137

Basic medical sciences - PhD thesis proposals

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 (C40H52O4) 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.

Hypothesis: Astaxanthin, administered intraperitoneally, has a protective effect on testicular torsion-detorsion injury of 720° in rats.

Aims: General aim of this study is to investigate the potential protective effect of astaxanthin on testicular torsion-detorsion injury. Specific aims are: determine the values of malondialdehyde (MDA); determine the enzymatic antioxidant values; superoxide dismutase (SOD) and glutathione peroxidase (GPx); calculated the mean diameter of the seminiferous tubules (MSTD); calculate the mean value of Johnsen's score; determine the number of apoptotic caspase-3 positive cells per 100 tubules (apoptotic index).

Materials and methods: Thirty-two Fisher rat will be divided into 4 groups of 8 individuals. At the first (sham) group, a cut will be made in the right inguinoscrotal region by which the ipsilateral testis will be take out and immediately returned to its natural position. In the second group, a 720° testicle torsion will be performed, which will be detorsion after 90 minutes. In the third group, at the time of detorsion, astaxanthin will be given intraperitoneally as a single dose of 75 mg / kg, while in the fourth group, astaxanthin will be given intraperitoneally 45 minutes from the moment of detorsion at an equal dose. After 90 minutes of reperfusion the testis will be removed and will be divided into two halves. On one half of the testicle will be conducted biochemical research while in the second half will be performed histological and immunohistochemical research. Lipid peroxidation will be determined by measuring malondialdehyde (MDA) in testis homogenates by the thiobarbituric acid. The activity of superoxide dismutase (SOD) and glutathione peroxidase (GPx) will be measured using ready-made kits and absorbance measurement. For the purpose of histological research the preparations will be dyed with haematoxylin and eosin (HE). Using a microscope we will measure the diameter of the tubule from which the mean diameter will be obtained – MSTD for each group. For each group randomly selected 20 sections of tubules. The diameter of the tubule will be measured at a smaller and larger axis from which the mean diameter will be obtained. Also, for each group, the Johnsen value will be determined depending on the maturity of the germ cell. The number of apoptotic caspase-3 positive cells per 100 tubules (apoptotic index) will be 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®) will be used.

Expected scientific contribution: Testicular torsion through several mechanisms leads to testicular dysfunction, spermatogenesis loss, permanent testicular damage and ultimately infertility of men. If multimodal (biochemical, histological, immunohistochemical) approaches prove the protective effect

of astaxanthin, the dissertation 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: T-01-02-053

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, Assoc. Prof. Ivica Lukšić, MD PhD

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

Introduction: Sexual dimorphism represents differences in structural, physical, chemical and behavioral characteristics between two sexes of the same species and 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 stucture between male and female lacrimal glands (tertiary sexual characteristics). Observed differences in the structure of human lacrimal glands between sexes have not been studied adequatly. Prevalence of dry eye syndrome raises 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 development of lacrimal gland disfunction.

Hypothesis: Qualitative histological analysis, quantiative (stereological) histological analysis and immunohistochemistry for androgen and estrogen receptors expression of human lacrimal glands will show sexual dimorphism in researched parameters (volume density of acini (Vva), volume density of ducts (Vvd), volume density of connective tissue (Vvct), length density of intralobular channels (Lvi), length density of excretory channels (Lve), numerical density of androgen receptors (Nva) and numerical density of estrogen receptors (NvE)).

Aims: General aim of this study is thorough research of sexual dimorphism of human lacrimal gland. Specific aims include: 1. Qualitative analysis of structural differences between acini, excretory ducts and interstitium of male and female subjects using light and electrone microscope, 2. Quantitative analysis- comparison of volume density of acini (Vva), volume density of ducts (Vvd), volume density of connective tissue (Vvct), length density of intralobular channels (Lvi) and length density of excretory channels (Lve) between male and female group using Weibels 32 point multipurpose test system, 3. Immunohistochemical analysis- comparison of numeric density of estrogen (Nva) and androgen receptors (Nve) expression in male and female group and corelation of results with level of dimorphism and age of subjects.

Materials and methods: Samples will be human lacrimal glands collected during 60 autopsies. Both lacrimal glands of each donor will be explanted. Donors will be deceased patients from University Hospital Dubrava also eligible for eyeball donation and with signed informed consents. 30 donors will be male and 30 female. Qualitative histological analysis will be performed using binocular light microscope and transmission electron microscope. Results of qualitative analysis will be descriptive. Quantitative (stereological) analysis will be performed using Weibels 32 point multipurpose test system intergrated into light microscope. Immunohistochemical analysis will be performed using primary antibodies 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 18 months.

Expected scientific contribution: This research is the first detailed stereological analysis of human lacrimal gland. Results of this study should broaden our knowledege of sexual dimorphism in histological features of human lacrimal gland and also contribute to better understanding of mechanisms responsible for lacrimal gland dysfunction and dry eye syndrome.

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

Poster code: T-01-02-067

Poster Title: Prevalence and molecular background of RHD allele with decreased or altered antigenic expression:epidemiological and clinical relevance.

PhD candidate: Hana Safić Stanić

Part of the thesis: Prevalence and molecular background of RHD allele with decreased or altered antigenic expression:epidemiological and clinical relevance.

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 blood group antigens in the Rh system, the RhD antigen is the most immunogenic as it can strongly stimulate the immune response and formation of anti-D antibodies, and lead to strong transfusion haemolytic reactions and haemolytic disease of the newborn. Rh antigens are encoded by a pair of highly homologous genes (RHCE and RHD), which lie in tandem on chromosome 1. Presence or absence of the RhD protein differs the D+ from D- phenotype, contributing to the the high immunogenicity of D. Around 85 % of Caucasians carry a normal D antigen on the erythrocyte membrane, and 14% have no D antigen. Although most people are either D+ or D−, there is a plethora of D variants. Around 0.2-1% Europeans are carriers of aberrant RHD allele-that encode different variants of D antigen, categorized as weak D, partial D, and DEL. Individuals carrying these variants may test antigen negative, weakly positive, or positive by serology. Identification of the D variant allele is important because anti-D alloimmunization can occur in some variants. Weak D types 1, 2, and 3 are seldom, if ever, associated with alloanti-D production, so these patients can be treated as D+, to preserve D− stocks, whereas patients with all other D variants would be treated as D−. All donors with the D variants should be treated as D+. The RHD genotyping implementation in routine screening can improve transfusion therapy strategy, rationalize use of D - blood units and RhIG.

Hypothesis: Molecular characterization of the D variant allele could prevent D-alloimmunization, reduce unnecessary administration of Rh immune globulin in women with a weak D, and decrease transfusion of Rh-negative RBC units in most recipients with a serological weak D phenotype.

Aims: Determine type and prevalence of D variant allele in the blood donor population, compare our results with the published data, compare genotyping results with serological typing, estimate the rate of unnecessary transfusion of RhD-negative RBC and unnecessary RhIG Injections and propose new serological-molecular algorithm for D antigen typing

Materials and methods: The study will be carried out at Croatian Institute of transfusion medicine, Zagreb. The blood specimen-6ml will be collected from voluntary blood donors (n-6000) all typed as D negative on standard serologic testing including indirect antiglobulin test. The age range of respondents will be between 18 and 65 years (the blood donor age limit in Croatia) with no excluding criteria. The study was approved by the Ethics Committees of CITM . 1.Serological RhD typing; 2.DNA extraction and RHD screening- pooled sample using QIAamp DNA Blood Mini kit (Qiaqen, Germany) on QIAcube station (Qiaqen, Germany). RHD screening will be done by real-time PCR (qPCR) method (RT-PCR System 7500, Applied Biosystems, USA) with TaqMan chemistry used for exon 7 and 10 amplification; 3.RHD genotyping-by use of the polymerase chain reaction with sequence specific priming (PCR-SSP), using commercial genotyping kits: (Inno-Train, Germany); 4.Sequencing of RHD gene-In cases where RHD allele could not be classified by routine diagnostic PCR-SSP method, genomic DNA sequencing will be performed for all ten exons of the RHD gene.

Expected scientific contribution: Determination of type and prevalence of aberrant RHD alleles in the studied population, potential discovery of new RHD alleles and Rhesus Base update.

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

Poster code: T-01-05-035

Poster Title: Analysis of the human placentas with intrauterine growth restriction using Infrared spectroscopy with Fourier's transformation

PhD candidate: Petra Kejla

Part of the thesis: Analysis of the human placentas with intrauterine growth restriction using Infrared spectroscopy with Fourier's transformation

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. It may be assumed that those changes can be detected by specific physical methods. There is no data on potential differences between the structure of the tissue and the DNA isolated from normal and IUGR placentas.We will isolate tissue and DNA from IUGR and control nonIUGR placentas to measure the vibration characteristics of the solutions using Infrared spectroscopy with Fourier's transformation (FTIR). Elaborated FTIR spectra will be analyzed by the principal components analysis and regression analysis in order to determine the potential difference in the secondary DNA structure.

Hypothesis: The wave numbers and intensities of FTIR bands will differ between tissue isolated from control and IUGR placentas. The wave numbers, intensities of FTIR bands and DNA conformation A and B markers will differ between the DNA isolated from control and IUGR placentas.

Aims: General aim: To define the difference in structure of tissue and DNA isolated from IUGR and control placentas using Infrared spectroscopy with Fourier's transformation. Specific aims: 1. To define the optimal protocol for preparation of tissues and isolated DNA and to determine the optimal parameters for FTIR spectroscopy; 2. To determine characteristic bands in FTIR spectra of the tissues isolated from control placentas and to determine the wave number mistakes; 3. To determine characteristic bands in FTIR spectra of the tissues isolated from IUGR placentas and to determine the wave number mistakes; 4. To compare vibration characteristics of control and IUGR tissues; 5. To compare vibration characteristics of the DNA isolated from control and IUGR placentas.

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

Expected scientific contribution: Determination and testing of FTIR protocols for tissue and DNA analysis as well as wave number shift and intensity changes will set the basis for future studies. The results of this study will show potential differences in tissues and secondary and tertiary DNA structure between IUGR and placentas from normal pregnancies.

MeSH/Keywords: DNA, IUGR, placenta, FTIR spectroscopy

Poster code: T-01-05-039

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 "third mission" of the university, in addition to teaching and research, includes various other forms of action known as technology or knowledge transfer (TT, KT), translation or more accurately, exchange of knowledge between public and the private sector. The success of the TT of some institution, apart from the organizational structure and strategy, is largely influenced by the innovation environment and the entrepreneurial university, and particularly the motivation of the researchers, as shown in all areas of research in many countries. Technology transfer is even more important in the field of biomedicine where the end users are patients in need for innovative treatments. 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. Biomedical scientists from academic institutions are very often main source of new ideas for inventing new medical interventions, but only if their research results are successfully transferred to patients.

Hypothesis: Croatian scientists in the field of biomedicine are poorly involved in technology transfer and commercialization of their research results due to insufficient knowledge of the process and inadequate institutional support.

Aims: Aims of these studies are to identify the status of knowledge and technology transfer activities among Croatian scientists in the field of biomedicine, determine their motivation for technology transfer and attitude towards commercial exploitation of the research results. Finally, the objective is to propose measures to increase the impact of Croatian scientists in the field of biomedicine on society and make better use of their innovativeness.

Materials and methods: For the study of participation of biomedical scientists in technology transfer processes in the Republic of Croatia and their motivation, we will perform the survey of the targeted population (sample) listed in the Register of Biomedicine and Health Sciences Officers in the Republic of Croatia, led by the Ministry of Science and Education. Prior to main survey, we will perform qualitative exploratory research aiming at identifying problems, obstacles and motivations of scientists. It will consist of two components: Depth, semi-structured interviews with scientists / representatives of different groups and case studies with Technology Transfer Offices. 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 technology transfer 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. We will use STATISTICA support available at MEF.

Expected scientific contribution: The results of this study will bring new insight into the activities of transferring knowledge and technology in the Republic of Croatia. The results and proposed measured could help technology transfer offices, research institutions and decision makers in developing strategies for improvements in this filed and strengthening impact of research results in biomedical field in Croatia and beyond.

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: T-01-05-040

Poster Title: De novo DNA methylation dynamics and cell cycle regulators Rb and p53 expression in testicular development of human, mouse and rat

PhD candidate: Marta Himelreich Perić

Part of the thesis: Comparative analysis of de novo DNA methylation dynamics i ekspresije regulatora staničnog ciklusa Rb i p53 u razvoju testisa čovjeka, miša i štakora

Mentor(s): Assist. Prof. Ana Katušić Bojanac, MD PhD

Affiliation: University of Zagreb School of Medicine and Centre of Excellence for Reproductive and Regenerative Medicine

Introduction: Disturbances of the complex prenatal testicular development are a possible cause of male reproductive system disorders, diagnosed however later in adult life, such as infertility and testicular tumors. It is known that critical epigenetic events like DNA de novo methylation (sex-specific imprinting and retrotransposonal sequences) in male germ cells take place in the fetal period, suggesting its importance for germ cell homeostasis in adult testis. Importantly, de novo DNA methylation takes place in quiescent germ cells, arrested in G1 phase of the cell cycle, which is thought to be caused by p53 and/or Rb signalling pathways.

Hypothesis: De novo DNA methylation in the fetal testes is concomitant with the expression of negative cell cycle regulators pRb and p53 during late gestation in human, mouse and rat.

Aims: The aim of this study is to systematically analyse and compare the cell cycle, global methylation and imprinting dynamics of male germ cells in developmental stages of human, mouse and rat testis. For the first time, time correlation of an expression of Rb and p53 to DNA methylation will be analysed in human samples. 1. Determine global DNA, H19 and Rasgrf1 gene methylation level in different developmental stages of fetal testicular tissue samples in human and complementary mouse and rat developmental stages. 2. Determine gene expression dynamics and epigenetic modifications ofcell cycle regulators pRb and p53, with their phosphorylated forms on protein and mRNA level in the same samples. 3. Investigate whether silencing of Rb and p53 gene via siRNA affects germ cell quiescence period that starts in the late pregnancy of mice and rat in in vitro culture.

Materials and methods: The study will be conducted on formalin-fixed, paraffin-embedded archive human fetal samples, 14.-35. week of pregnancy, a prepubertal sample; mouse and rat fetal testes samples isolated from pregnant animals in a timeline of the second trimester to the juvenile postnatal period. An in vitro testis organoid culture will be established to investigate siRNA-Rb effect on the germ cell fate analysing the proliferation , apoptosis, de novo DNA methylation). Formalin-fixed, paraffin-embedded (FFPE) samples will be accessed by histological, immunohistochemical/immunofluorescence and sterological methods. Frozen samples from in vitro culture will be stored on -80 °C and together with FFPE, used for molecular diagnostics – DNA methylation, pyrosequencing, DNA isolation and gene expression by ddPCR method.

Expected scientific contribution: The results of the study will contribute in validating the animal model in investigation of factors that affect the development and function of testis and possibly in clarifying causes of male infertility and/or germ cell neoplasms.

Acknowledgments: This work is supported by the Scientific Center of Excellence for Reproductive and Regenerative Medicine, Republic of Croatia, and by the European Union through the European Regional Development Fund, under grant agreement No. KK.01.1.1.01.0008, project R

MeSH/Keywords: testis, development, de novo DNA methylation, quiescence, human, rat, mouse

Poster code: T-01-05-106

Poster Title: Model for impact evaluation of biomedical scientific projects of Republic of Croatia

PhD candidate: Olja Ulični Nikšić

Part of the thesis: Model for impact evaluation of biomedical scientific projects of 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. As a consequence, question of how to measure and assess investment returns in research remain highly relevant. The researchers still mostly use bibliometric indexes instead of measuring more long-term and significant research impact. In recent years there was a growing number of frameworks which 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. Still review shows that majority of the proposed indicators have different criteria to fulfil these goals.

Hypothesis: Impact evaluation model tailored to Croatian biomedical scientific projects will better contribute to the evaluation of targeted research area compared to the current existing models.

Aims: To design the Croatian biomedical-specific RIAF and to compare it with already existing RIAFs and bibliometric evaluation.

Materials and methods: The bibliometric data describing the results of Croatian biomedical projects relevant for the study will be collected from the open repositories and data available on the internet. The criteria to be used for the Croatian biomedical-specific RIAF would be defined by qualitative study based on the interviews with stakeholders representatives. Finally, the newly designed RIAF would be compared to the existing RIAFs for the Croatian-based research projects. 1. Collect and compare bibliometric data for Croatian biomedical projects Identification of Croatian biomedical projects in period from 1.1.2007. to 31.12.2017. will be carried out by searching online registers such CORDIS, ESIF, HRZZ, MZO, Uni pages and private foundations. Publications resulting from listed projects will be identified through databases such as CROSBI, Scopus, PubMed, WoS and Google Scholar (published up to 5 years after project ending). Project impact will be measured by impact factor of publication research area, citations number, degree of co-authorship, author's h-index, publication timing in relation to project ending, number of publications vs. project duration, and number of publications vs. granted funds. 2. Evaluation of existing models of evaluation of successful scientific projects Identification of existing RIAFs will be performed through databases such as CROSBI, Scopus, PubMed, WoS and Google Schoolar. Key impact factors of Croatian biomedical projects will be identified by interviewing stakeholders (researchers, librarians, editors, end-users and management bodies). After defining key impact factors for each stakeholder group, an evaluation frame for identified RIAFs will be created according to factors chosen by stakeholders. 3. Creating a evaluation model for Croatian biomedical projects impact Aim is to create RIAF whose parameters correspond to consensus of impact factors defined by stakeholder groups and by key international RIAFs. Representative projects identified by bibliometric indexes will be evaluated by newly created RIAF and results of both evaluations will be compared.

Expected scientific contribution: The new RIAF would be in particular developed to serve emerging "Croatia-like" research communities. The parameters of impacts and excellence would contribute to the societal definition of the financial priorities, and to the academic approaches oriented toward new knowledge production.

Acknowledgments: OUN was supported by FP7 project GlowBrain, H2020 project BioChip and Scientific Center of Excellence for Reproductive and Regenerative Medicine. No ethical approval was required.

MeSH/Keywords: Research impact assessment framework, Research impact

Poster code: T-01-07-075

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 hence the aim of the study is to see how the Tlr2 gene expression and the ischemic lesions are modified depending on the prior splenectomy.

Hypothesis: Splenectomy, due to the decrease in the number of monocytes in the area of ischemic lesion caused by MCAO, decreases the size of the lesion, hence acts neuroprotective.

Aims: The aims of this study are to elucidate the effect of splenectomy on the size of ischemic lesion and intensity of the mediated Tlr2 inflammation, to determine the size of ischemic lesion using MR imaging in different groups of mice depending on intervention, to prove that splenectomy reduces inflammatory response using bioluminescence imaging of transgenic animals in which luciferase expression is determined by Tlr2 promoter and to determine critical parameters of brain recovery visible on MRI.

Materials and methods: Transgenic mice B6-Tyrc-Brd-Tg (Tlr2-luc/gfp)/Gaj provide by luciferase reporter and green fluorescence protein insight into TLr2 gene expression. Groups of animals with or without ischemic damage will be compared previously subjected to splenectomy or sham operated. To determine the lesion size, MR imaging, histological and immunohistochemical analysis will be done.

Expected scientific contribution: Clarifying effects of splenectomy after stroke, the path of establishing new therapeutic approaches in the treatment of stroke, directed at peripheral inflammatory cells, would be opened.

MeSH/Keywords: stroke, ischemia, inflammation, microglia, splenectomy

Poster code: T-01-08-025

Poster Title: Developmental origin of the subthalamic nucleus

PhD candidate: Ema Bokulić

Part of the thesis: Developmental origin of different subtypes of subthalamic nucleus

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 dorsally. Most STN neurons are glutamatergic projection neurons, but small populations of GABAergic neurons have also been described. This nucleus is one of the key structures regulating basal ganglia motor circuits. Its major afferent inputs come from the globus pallidus pars externa, the cortex, the brainstem, and the thalamic nuclei. The STN projects to the globus pallidus pars interna, thus regulating the output structures of the basal ganglia circuit. Although the STN has gained new clinical relevance with the development of deep brain stimulation as a treatment for movement disorders, little is known about its developmental origin. To this day, two theories have been postulated about the developmental origin of the STN. One theory proposes that the STN originates from the separate longitudinal subthalamic zone between the ventral thalamus and the hypothalamus, while the other suggests that the nucleus originates from the germinative zone lying caudally from the mammillary recess. Moreover, information about the molecular factors important for the establishing of the STN are scarce. Five different transcription factors have been identified in regulating the STN development: Pitx2, Lmx1b, Foxa1, Foxp1, and Foxp2. These factors are important for the development of post-mitotic neurons and their migration, but progenitors and their exact place of origin still remain unknown.

Hypothesis: The hypothesis of this research is that different subtypes of STN neurons have specific developmental origin and separate molecular factors influencing their development.

Aims: This doctoral thesis aims to define the developmental zone and the progenitors of STN neurons in mouse and human, as well as establish a transcriptomic profile of the STN neurons and affirm specific molecular markers involved in the development of different subtypes of the STN neurons.

Materials and methods: The research will be conducted on human and mouse tissue with the approval of the University of Zagreb, School of Medicine Ethics Committee and in accordance with the Ethical Codex of Croatian Society for Laboratory Animal Science.We will obtain mouse embryonic tissue from timed pregnancies with day 0.5 designated as the morning the plug was identified. Pregnant female mice will be anesthetized and the embryos will be harvested at E9,5-10,5, E12,5-14,5, E17,5-19. Mice at the age of P14 will be anesthetized and transcardially perfused with buffer solution 1xPBS followed by 4% paraformaldehyde. All human tissue will be from the Zagreb collection of human brains, age range spanning from 12-15 PCW, 20-24 PCW, 30-35 PCW to 0-2 years. Both mouse and human tissue will be processed for RNA analysis, immunohistochemistry for paraffin-embedded and frozen tissue, and in-situ hybridization. We will analyze the transcriptome of the STN using RNAseq or microarray method. For histological methods we will use parvalbumin, calbindin, calretinin, and vGLUT2 as markers of different subtypes of STN neurons, while PITX2, LMX1B, FOXP1, FOXP2, and FOXA1 will be used as markers of progenitors and early post-mitotic neurons. We will conduct statistical analysis of transcriptome data using the program R.

Expected scientific contribution: This research will help elucidate the developmental origin of the STN and the molecular origin of its neurons, setting the ground for future research of anatomo-functional subdivisions in the STN.

MeSH/Keywords: Diencephalon, Human brain development, Subthalamic nucleus, Transcription factors, Transcriptome

Poster code: T-01-08-101

Poster Title: Morphological analysis and intracellular filling of dendritic spines in hippocampal granule cells of TNF-alpha KO mice.

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: The microanatomy of neurons can provide ample information concerning structure and function. In the dentate gyrus of the hippocampus are granule cells that have a strictly defined structure. Dendritic spines on these granule cells, which protrude from dendrites, establish synaptic connections and are the recipients of most excitatory inputs. Spines are considered to be key elements in learning, memory, and cognition. The morphology of spines changes in an activity-dependent manner and their structure has functional correlates. Intracellular filling with fluorescence dyes in combination with high-resolution confocal imaging allows the filling of individual cells completely, including all of the spines of the neuron. This method can be easily combined with immunofluorescence, enabling precise identification of intracellular, intraspinal proteins or receptors. This study aims to characterize dendritic and spine morphology of hippocampal granule cells in mouse mutants lacking the pro-inflammatory cytokine Tumor Necrosis Factor-alpha (TNF-alpha), which is known to be an important factor involved in the regulation of synaptic plasticity. In addition, immunohistochemistry for synaptopodin, a specific marker for intracellular calcium stores, will be applied to analyze the relation of synaptopodin presence to spine morphology. Obtained results will provide new insights into the role of this important pro-inflammatory protein on neuronal morphology and will represent a baseline for future studies on neural plasticity using this mouse mutant.

Hypothesis: Gyrus dentatus granule cells in mice that do not express TNF-alpha have smaller spine sizes and synaptopodin cluster levels when compared to wildtype controls.

Aims: The aim of this research is to investigate the influence of TNF-alpha on neuronal morphology in mice, and the influence that synaptopodin presence in spines has on the spine morphology.

Materials and methods: A gene-targeted C57BL/6 mouse strain deficient for TNF-alpha will be used. After an overdose of 100mg/kg pentobarbital intraperitoneally, intracardial perfusion will be applied with 0,1M PBS for 1-3 minutes and then switched to 4% PFA for 4 minutes. Postfixation of the complete brain in 4% PFA. Brains will be sliced on vibratome to prepare fixed hippocampal at 250 µm thickness. Micropipettes will be backfilled with an Alexa Fluor 568 hydrazide. Cells will be impaled under visual control. The dye will be iontophoretically injected for 10 minutes. Sections with filled cells will be resliced on a vibratome at 40 µm thick frontal reslices. Sections will be incubated for 30 min with 10% bovine serum antigen (BSA) in 0.5% Triton X-100 containing Tris-buffered saline and subsequently incubated for 72h at RT in guinea pig polyclonal anti-SP antibody [1:2000 in TBS + 0.01% NaN3, 0.5% Triton X-100; Synaptic Systems]. Sections will be washed and then incubated for 4 h with Alexa 488 labeled anti-guinea pig antibody [1:2000 in TBS + 0.01% NaN3, 0.5% Triton X-100; Dianova]. Sections will be transferred onto glass microscope slides and mounted with anti-fading mounting medium DAKO. Confocal images will be acquired using a Nikon Eclipse C1si laser-scanning microscope and Zeiss LSM META 510 with a 60x objective lens. All high-resolution images will be acquired from 5-20 µm depth. Immuno-labeled SP-clusters and dendritic segments will be analyzed in the outer molecular layer of the dentate gyrus using the Image-J software package. Three granule cells of each mouse will be analyzed and three segments per granule cell will be quantified. Statistics will be done with GraphPad Prism 7 software. Statistical comparisons will be made using t-tests, ANOVA or Wilcoxon-Mann-Whitney test as appropriate. P-values of less than 0.05 will be considered statistically significant.

Expected scientific contribution: The study of spine morphology in vivo in TNF-alpha deficient mice will provide new insights into the effect of this important pro-inflammatory protein on spine morphology and the distribution of synaptopodin will explain how TNF-alpha affects excitatory synaptic structures.

MeSH/Keywords: TNF-alpha, synaptopodin, morphology, spines, granule cells, hippocampus

Poster code: T-01-08-151

Clinical medical sciences - PhD thesis proposals

Poster Title: Impact of anaesthesia on patient care

PhD candidate: Tea Fabijanić

Part of the thesis: Health related quality of life after hip fracture surgery in regional versus general anaesthesia

Mentor(s): Assist. Prof. Daniela Bandić Pavlović, MD PhD

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

Introduction: Hip fractures are one of the most common traumas in general population and as such represent significant public health care problem regarding short-term and long-term medical management. Each step in the process of medical therapy is vital to final outcome and as such should be treated accordingly. Hip fractures usually require surgical treatment under general or regional anaesthesia. Studies investigating the quality of life after the hip fracture surgery show that hip fractures and the treatment process have a significant effect on the quality of life of the patient and that there are significant cognitive changes associated with poor mood and significant physical limitations, chronic pain and other socio-emotional components despite the good outcomes of operations. Since hip fractures have a profound impact on the quality of life in women and men regardless of age, it points to the need for special and individualised care in perioperative and postoperative recovery. There are still undefined areas regarding the procedure which will represent best possible care for the patient. It is essential to define parameters that will represent the pivoting point to centre patient care on.

Hypothesis: Patients undergoing regional anaesthesia during hip fracture surgery have better quality of life after the surgery.

Aims: Assessing the impact of two most commonly used anaesthesia treatment modalities in hip fracture surgery on the health related quality of life in patients undergoing hip fracture surgery.

Materials and methods: Patients selected for hip fracture surgery and meeting the eligibility criteria will be divided into two groups, those undergoing hip fracture surgery under regional anaesthesia and those operated on under general anaesthesia, with a follow up accordingly. All subjects will be timely and thoroughly informed and made familiar with the research, research protocol, research goals and potential benefits and will be able to ask questions and get clear and easy-to-understand answers. After full information, the subject will sign informed consent and consent to participate in the research. Subjects will be allowed to withdraw from the study at any time during the research regardless of the reasons. Patients will fill out easy to understand standardised, validated and population adapted health related quality of life questionnaires that are applicable to these specific groups of patients. Personal, demographical and medical data will be collected. Data will be extrapolated and compared. The collected and obtained data will be described by numeric and graphical descriptive statistical methods, in the form of tables and graphs, and the conclusions will be clearly explained in text form.

Expected scientific contribution: Comparing effects of regional and general anaesthesia on health related quality of life may have significant future benefits in terms of enhancing patient care.

MeSH/Keywords: hip fracture, quality of life, regional anaesthesia, general anaesthesia

Poster code: T-02-01-057

Poster Title: Determination of the effectivnes of biofeedback and kinesiotherapy in relation to electrical stimulation and kinesiotherapy in patients with knee osteoarthritis.

PhD candidate: Silvija Mahnik

Part of the thesis: Determination of the effectivnes of biofeedback and kinesiotherapy in relation to electrical stimulation and kinesiotherapy 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. The disease primarily affects the joint cartilage but also all the other joint structures. Treatment of osteoarthritis may be nonpharmacological and pharmacological. Nonpharmacological conservative treatment includes changing life style and physical therapy. Physical therapy uses kinesiotherapy and passive physical methods to reduce the pain and improve the function. Biofeedback is a method that displays muscular contraction as a sound or visual signal for the purpose of increasing or decreasing voluntary muscle activity. Electrical stimulation refers to the application of electrical current in order to achieve muscle contraction.

Hypothesis: Patients treated with biofeedback will have better knee function, increased muscle strength and reduced pain compared to patients treated with electrical stimulation.

Aims: The aim of this study is to examine whether there is a difference in reducing pain and improving knee function in patients with knee osteoarthritis treated with kinesiotherapy and biofeedback therapy compared to those who received electrical stimulation and kinesiotherapy.

Materials and methods: The research will be conducted at the Department of Orthopaedic surgery, Clinical Hospital Center Zagreb. Every patient will be familiar with the participation in the research through informed consent. The study will include patients 55 years old and older who have been diagnosed with knee osteoarthritis after clinical and radiological examination. We will establish diagnosis of knee osteoarthritis according to the guidelines of the American College of Rheumatology (ACR) classification for knee osteoarthritis and by radiological images according to Kellgren-Lawrence classification grades 1 and 2 of joint cartilage degradation. Patients with following conditions will not be included in the study: previous surgery on that knee, thrombophlebitis, skin infection, malignant disease, bleeding disorders, neurological diseases, inflammatory rheumatic disease, secondary knee osteoarthritis, hip and ankle contracture deformity, grades 3 and 4 knee osteoarthritis according to Kellgren-Lawerence classification, who have received intaarticular injection into the knee during the last 3 months, patients with pacemaker or metallic foreign body. Patients will be randomly assigned to three groups of 30 subjects. he 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. We will collect information about gender, age, height and weight, leg length, use of orthopeadic aids and about patient's occupation. Patients will fill in a 36 Item Short Form Health Survay (SF 36). For pain assessment we will use a visual analogue scale (VAS) and an analgesic journal. To evaluate function we will use Oxford Knee Score (OKS) and International Classification of Functioning, Disability and Health (ICF) for osteoarthritis. Patients will also fill in questionnaire Falls Efficacy Scale Inernational (FES-I) to assess fear of falling. The strength of quadriceps muscle will be determined by EMG biofeedback. The data will be gathered on the first day of physical therapy, the last day of physical therapy, after 3 months and 6 months after physical therapy.

Expected scientific contribution: Our goal is to examine the effectiveness of passive physical methods (biofeedback and electrical stimulation) in treating osteoarthritis of the knee. We expect that biofeedback will have a better effect on the pain and knee function then electrical stimulation in patients with knee osteoarthritis.

MeSH/Keywords: kinesiotherapy, biofeedback, electrical stimulation, knee osteoarthritis

Poster code: T-02-03-007

Poster Title: Diagnostic ultrasound in monitoring patients with rheumatoid arthritis

PhD candidate: Valentina Delimar

Part of the thesis: The use of diagnostic ultrasound in monitoring patients with rheumatoid arthritis

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

Affiliation: University of Zagreb School of Medicine

Introduction: Rheumatoid arthritis (RA) is a chronic inflammatory disease which 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 heterogenicity of existing US scoring systems. Therefore, US is still not incorporated into disease activity indices or classification criteria for RA.

Hypothesis: Diagnostic US provides additional value in monitoring disease activity, response to therapy and remission in RA patients in comparison with standard methods of monitoring.

Aims: To determine the added value of sonographic parameters of disease activity (synovitis, tenosynovitis, erosions) in monitoring RA patients in relation to standard methods DAS 28, SDAI, CDAI. To investigate if there is a difference between sonographic parameters of disease activity and DAS index regarding the number of examined joints (DAS 28 and DAS 44). To investigate the relationship between US findings and functional scores based on Health Assessment Questionnaire (HAQ) and Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F). To propose and validate a new monitoring method for RA patients – a composite scoring system based on the US detected synovitis, HAQ and DAS 28 scores. To propose a model of Croatian US joint count (CroUS).

Materials and methods: A minimum of 100 patients with diagnosed RA according to the American College of Rheumatology/European Leauge Against Rheumatism (ACR/EULAR) 2010 criteria, treated at the Clinic for Rheumatic Diseases and Rehabilitation, University Hospital Centre Zagreb, will be included in the study. A minimum of one control examination is planned after 3 months. On study entrance, patient history and demographic data will be collected, inflammatory markers ESR and CRP determined, and plain radiographs of hand and feet obtained. Clinical examination will include tender and swollen joint count according to the scoring system of 28 and 44 joints. Patients will determine the pain level, general health and disease activity on visual analogue scale and fill the HAQ and FACIT-F questionnaires. DAS 28, SDAI and CDAI indices will be calculated. US examination will be done according to the standardized EULAR protocol. The examined joints for synovitis and erosions will include the combination of published US12 and US7 joint scores: elbows, radiocarpal joints, second and third metacarpophalangeal and proximal interphalangeal joints, knees, ankles, second and fifth metatarsophalangeal joints on both sides. The examined tendons for tenosynovitis according to the Outcomes Measures in Rheumatology (OMERACT) proposition for RA will include 2nd, 4th and 6th wrist extensor compartment, finger flexor digitorum superficialis and profundus tendons 3 and 4, peroneal tendons and tibialis posterior tendon. Additionally, every clinically swollen or painful joint or tendon outside the initial set will be examined. Synovitis will be scored according to the latest EULAR/OMERACT scoring system, erosions according to the binary scoring system and tenosynovitis according to the OMERACT 2012 scoring system. The proposed composite scoring system based on the US detected synovitis, HAQ and DAS 28 scores will be calculated. The CroUS joint count will be determined using the applicable regression model.

Expected scientific contribution: This research will determine the value of diagnostic US in monitoring RA patients and the relation of US findings with the patient's functional status. We will propose a new monitoring method for RA patients, a composite scoring system based on US detected synovitis, HAQ and DAS 28 scores and a model of CroUS joint count.

MeSH/Keywords: rheumatoid arthritis, ultrasound

Poster code: T-02-03-052

Poster Title: Fixed versus Flexible GnRH antagonist protocol 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: Various protocols for growth and maturation of follicles during ovarian stimulation in assisted reproductive technology are being used. Although gonadotropin agonists have been used world-wide, based on research findings over the past years, GnRH antagonists take over. It has been showed that GnRH antagonists have less side effects and fewer amounts of drugs used with the same efficacy. The standard protocol that determines when to start antagonist treatment is not known. Two main principles are: fixed protocol, when antagonist is started on the day 6 of cycle, or flexible protocol when antagonist is started depending on follicle size (13-14mm). Based on Bologna criteria, proposed by ESHRE, patients who comply two out of three following criteria are defined as poor responders: age >/= 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). Gonadotropin-releasing hormone antagonists inhibit the premature LH surge and reduce suppression in early follicular phase and therefore are considered possibly to be beneficial in poor responders. However the answer to the question when to start GnRH antagonist stimulation is not known. The optimal protocol for routine clinical use in poor responders has not yet been identified.

Hypothesis: Timing of GnRH antagonist protocol administration for ovarian stimulation affects the outcome of medical assisted reproduction techniques in patients with poor ovarian response.

Aims: Main aim of this study is to compare IVF outcome in fixed and flexible antagonist protocol in controlled ovarian stimulation among patients with poor ovarian response. Specific aims are: 1. To determine whether levels of endogenous LH, estradiol and progesterone during early follicular faze in both stimulation protocols affect number and quality of oocytes and IVF outcome; 2. To establish if the levels of LH, estradiol and progesterone in early follicular phase of menstrual cycle can help choose GnRH antagonist protocol in patients with poor ovarian response; 3. To explore which is the optimal protocol in patients with poor ovarian response; 4. To point that the administration of optimal GnRH antagonist protocol will decrease dose of medicine being used, decrease withdrawal and increase the number of pregnancies.

Materials and methods: This prospective randomized trial includes patients being treated for infertility with poor ovarian response based on Bologna criteria. Subjects are divided in two groups, one in which fixed and the other in which flexible GnRH antagonist protocol is used. LH, estrogen and progesterone levels are measured on 2nd cycle day, on the day of GnRH antagonist administration and on the day of hCG administration. Each patient receives recombinant FSH starting on day 2 of menstrual cycle (CD). In patients with fixed protocol, GnRH antagonist Cetrorelix is administrated from 6 CD (0,25 mg/day) up to ultrasound follicle measure of 17-18 mm. In patients with flexible protocol GnRH antagonist Cetrorelix is administrated when follicle is 13-14 mm (0,25mg/day) up to follicle measure of 17-18 mm. When at least 3 follicles measure 17-18 mm, 10000 IU of hCG is administrated. Thirty-six hours after HCG administration, ultrasound-guided transvaginal aspiration is performed. Three to five days after oocyte aspiration, embryo transfer of 1-2 embryos follows. The outcome measures are: oocyte quality, number of oocytes retrieved, number of embryos, confirmation of pregnancy (positive BhCG 2 weeks after embryo transfer), clinical pregnancy at 12 weeks of gestation, the number of withdrawal and the total dose of used medicine. For data analysis appropriate statistical analysis will be used.

Expected scientific contribution: Determining optimal start of GnRH antagonist protocol in poor responders is of great importance in enhancing IVF outcome and pregnancy rates, reducing amount of used drugs and diminishing cancellation.

MeSH/Keywords: Fixed and flexible GnRH antagonist protocol, poor responder, ovarian stimulation, female infertility

Poster code: T-02-05-072

Poster Title: Working the graveyard shift - is it stiffening our arteries and ruining our work ability?

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

Affiliation: University of Zagreb School of Medicine

Introduction: It has been proven that shiftwork has adverse effects on the cardiovascular system, beginning after 5 to 10 years of shift work (1), which is why early diagnosis is important. Research on the link between shiftwork and cardiovascular risk is demanding as long monitoring time is required, which is why other parameters for assessing cardiovascular risk are being explored, such as arterial stiffness. A recent extensive study of over ten thousand subjects showed a connection between shiftwork and a significant increase in arterial stiffness (2). However, there are no studies on the correlation of arterial stiffness and shiftwork among healthcare workers. Measuring pulse wave velocity is a new method of measuring central aortic pressure and stiffness of the arteries, thus measuring the pulse wave velocity (aortic level) is considered a measure of arterial stiffness (3,4). Shift work can also affect the work ability of workers (5,6).

Hypothesis: Following a nightshift, there is a significant increase in arterial stiffness and a decline in the work ability index in hospital healthcare professionals.

Aims: The aim of this study is to examine the impact of shiftwork on arterial stiffness and the work ability index in hospital healthcare workers. This study will try to assess the work ability index and its connection to shiftwork, the impact of socio-demographic characteristics, the impact of the work environment on the parameters of arterial stiffness and evaluate the influence of physical activity on the parameters of atherosclerotic risk and work ability in hospital healthcare workers.

Materials and methods: A prospective survey will be carried out on all on-call physicians and nurses who work in the Emergency Department at the University Hospital Dubrava with their informed consent. Respondents will be divided into two groups: nurses who work 12-hour night shifts and resident physicians who work in night shifts of either 16 or 24 hours. Each group will include at least 46 participants according to the power analysis. This research will include an analysis of sociodemographic and clinical data collected by a questionnaire and two measurements using an arteriograph. The questionnaire is comprised of sociodemographic data (age and sex), information on habits, the presence of 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 (7). The Physical Activity Questionnaire - Short Form will be used to assess physical activity (8,9). The first measurement using an arteriograph will be carried out in the morning while the subjects rest in basal conditions and the second measurement will take place after their nightshift. The arteriograph is an electronic device (ArteriographTM, TensioMed, Hungary) which calculates the mean arterial pressure, aortic pulse wave velocity, central systolic arterial pressure and central pulse pressures by means of non-invasive pulse, systolic and diastolic pressure measurements taken on the upper arm. The method is fast, simple, operator-independent, validated and with good reproducibility (10–13). Statistical analysis will be conducted using the IBM SPSS Statistics version 25.0.

Expected scientific contribution: According to currently available literature and data, no similar study has been completed to date. With this research, we will try to determine in a non-invasive, repeatable way which parameters affect the atherosclerotic risk and diminish work ability index in hospital healthcare workers. The results of this research will assist in the adoption of health preservation measures, disease prevention and in preserving the working ability of healthcare workers.

MeSH/Keywords: Vascular Stiffness, Health Personnel, Atherosclerosis, Shift Work Schedule, Blood pressure, Hypertension.

Poster code: T-02-06-004

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. Previous studies have demonstrated a high NT-proBNP sensitivity in the diagnosis of acute heart failure. Routine use of chest X-ray in the diagnosis of acute heart failure has 70% sensitivity and 100% specificity. Unfortunately, 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.

Hypothesis: Lung ultrasound in patients with acute dyspnoea is at least as sensitive as chest X-ray and NT-proBNP. Lung ultrasound is monitoring the effect of heart failure therapy and accelerates the treatment of dyspnoeic patients in the emergency department.

Aims: General aim is to establish the diagnostic value of lung ultrasound in the differential diagnosis of acute dyspnoea in the emergency department.

Materials and methods: This study will start at the beginning of 2019 in the Emergency department of Clinical Hospital Centre Zagreb and will include 120 patients both sexes 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.

Expected scientific contribution: The most important contribution is the result of testing the diagnostic values of the lung ultrasound in the population of urgent patients. Confirmation of the hypothesis of the diagnostic non-inferiority of lung ultrasound to the chest X-ray and laboratory tests would be a significant contribution in the creation of the diagnostic algorithms for patients with acute dyspnoea in emergency department

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

Poster code: T-02-06-044

Poster Title: Diagnostic value of non-contact thermography in the detection of lower-extremity deep vein thrombosis in the emergency medicine

PhD candidate: Ivan Jurić

Part of the thesis: Diagnostic value of non-contact thermography in the detection of lower-extremity deep vein thrombosis in the emergency medicine

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

Affiliation: University of Zagreb School of Medicine

Introduction: Despite improvements made in diagnostics, prophylaxis and treatment, deep vein thrombosis (DVT) is a significant public health problem and challenge. Venous ultrasound is the standard imaging test for patients suspected of having acute DVT. Because VTE diagnosis is frequently suspected but confirmed in less than 20% of suspected cases it is not ideal to perform imaging in every suspected case. Diagnostic strategies for VTE are based on assessment of the pretest probability for individual patients, which provides an estimate of the expected prevalence of VTE at a population level, followed by D-dimer testing and imaging as appropriate. The signs, diagnostic algorithms including pretest probability assessment and D-dimer testing alone have failed to consistently or adequately diagnose DVT and have diagnostic accuracies around 29%. Infrared thermography (IRT) is a non-invasive, non-contact method of plane visualization of infrared radiation emitted on the surface of the body. As a homotherm, a person can maintain his body at a constant temperature, with simmetrical signal distribution, regardless of environmental temperature. The body temperature is closely connected with the local blood circulation and methabolism which can change during various diseases. However, the published results of the effectiveness of infrared thermography for DVT in the last 40 years are contradictory, based on technologically limited devices, mainly on a small number of subjects, mostly based on the use of direct thermography methods.

Hypothesis: Non-contact infrared thermography is a sufficient method of exclusion of acute deep venous thrombosis.

Aims: The aim of the study is to determine the effectiveness of an indirect method of infrared thermography in excluding and verifying the localization of lower extremity DVT, compared to the compressive Color-Doppler ultrasound results.

Materials and methods: The diagnostic accuracy study will be conducted on 150 patients, range of age 18-90, with clinical suspicion for DVT admitted through the emergency department. Thermal imaging of legs will be performed in all subjects, and the results will be compared to the findings of the compressive Color-Doppler ultrasound (CDUS) by applying statistical methods. For each subject, the region of interest (ROI) will be chosen, expressed as the mean value of the temperature with the standard deviation. If the heat distribution is not homogeneous and there is an abnormality, the ROI will be determined, otherwise a homogeneous distribution will yield itself as ROI. Temperature values of ROI will be statisticaly compared with anatomically equal region of contralateral legs. Any difference in temperature between ROI DVT and contralateral legs higher than 0,5°C will be considered as a positive finding. An appreciation of the anatomy of the major muscle groups and their draining veins possible will allouwnce the recognition of predictable thermographic images when different muscle groups and veins are involved by DVT. The data obtained from the clinical evaluation, laboratory results (CRP, D-dimer) and Wells score system used in this study will be compared with the CDUS. The estimation of the accuracy will be described with sensitivity, specificity, positive and negative likelihood ratios, diagnostic odds ratio and agreement analyses (kappa statistics) with 95% confidence intervals. Using the calculated sigma values for all subjects, a general sigma value will be set to provide the maximum diagnostic accuracy and the Youden's index for diagnosis (PATIENT-NORMAL; DVT-PATIENT).

Expected scientific contribution: The results obtained by this research could provide new insights into the use of infrared thermography in distinguishing between thromboembolic and inflammatory as well as other changes in lower extremities, which would be not only a scientific, but also a clinical contribution.

MeSH/Keywords: infrared thermal imaging (IRTI); deep vein thrombosis (DVT); Color Doppler Ultrasonography, lower limb; diagnostic accuracy study

Poster code: T-02-06-055

Poster Title: The effect of fascia iliaca compartment block on stress response and acute confusional state in preoperative pain management for hip fractures: Randomized controlled trial

PhD candidate: Anđela Simić

Part of the thesis: The effect of fascia iliaca compartment block on stress response and acute confusional state in preoperative pain management for hip fractures

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

Affiliation: University of Zagreb School of Medicine

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.

Hypothesis: Preoperative FICB in patients with a hip fracture 65 years aged and older decreases the stress response and the frequency of acute confusional state.

Aims: To evaluate the effectiveness of the FICB in preoperative pain management in the emergency department, especially the effect on stress response and the effect on the acute confusional state. Secondary objectives are pain assessment at rest and pain assessment on passive movement, vital signs, the need for rescue analgesics, patient satisfaction with analgesia, and the outcome of hospitalization.

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" will be conducted. As standard care, all participants will receive parenteral paracetamol 1 gram every 6 hours. If needed, rescue analgesic will be given, intravenous tramadol, 100 mg to maximum 400 mg a day. Inclusion criteria will be a hip fracture after minor intensity trauma, age 65 and older, and signed informed consent. Exclusion criteria will be 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 will be 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, patient satisfaction with analgesia (Likert's scale) 24 hours after therapeutic protocol, and the outcome of hospitalization classified as discharge to rehabilitation /patients home or death.

Expected scientific contribution: The effect of preoperative FICB on the intensity of pain in hip fracture patients is well documented, but there are no sufficient data concerning the effect on the stress response and the frequency of acute confusional state among hip fracture patients 65 years of age and older. New evidence obtained from this research could significantly improve the standard and quality of pain management in this fragile group of patients.

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

Poster code: T-02-06-098

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 Hospital for Infectious Diseases "Dr. Fran Mihaljević"; 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.

Hypothesis: The degree of liver steatosis may be used in prediction of the outcome of sepsis.

Aims: The aims of this research are: a) to analyse the relationship between the degree of liver disease and severity of sepsis; (b) to analyse the relationship between certain parameters and their role in predicting sepsis outcome; (c) to analyse the relationship of liver steatosis and sepsis etiology; (d) to analyse the relationship between liver steatosis serum markers and other laboratory parameters in sepsis; (e) to analyse the relationship of liver steatosis and sepsis complications; (f) to determine the frequency of liver steatosis in patients treated for sepsis; (g) to determine the frequency of hepatic dysfunction in patients with different stages of liver steatosis.

Materials and methods: A prospective observational cohort study lasting three years involving 150 patients. The diagnosis of sepsis will be made using the Sepsis-3 consensus guidelines while liver status will be evaluated using abdominal ultrasound and transient elastography (liver stiffness measurement – LSM, controlled attenuation parameter - CAP). Routine biochemical and microbiological patient evaluation will be performed upon admission. Biochemical markers of liver fibrosis (cytokeratine 18 and adiponectine) will be sampled upon admission. The levels of cytokeratine 18 and adiponectine will be measured using ELISA. Parameters specific to the intensive care unit will be taken into account. The data obtained will be analysed with the appropriate statistical tests in the program SAS v. 9.4.

Expected scientific contribution: Comprehension of the role of different stages of liver steatosis in the course and outcome of sepsis along with potential clinical use of liver steatosis evaluation in the prognosis of the outcome of sepsis.

MeSH/Keywords: sepsis, chronic liver disease, liver steatosis, ultrasound, elastography, serum markers, adiponectine, CK-18

Poster code: T-02-07-028

Poster Title: Comparison of vancomycin application via continuous infusion and intermittent dosing on pharmacokinetic variables and pharmacodynamic outcomes

PhD candidate: Vedran Lokošek

Part of the thesis: Effect of vancomycin in continuous intravenous infusion on drug serum levels and treatment outcomes in patients in intensive care unit

Mentor(s): Assoc. Prof. Slobodan Mihaljević, MD PhD, Assoc. Prof. Robert Likić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Vancomycin is drug of choice in infections caused by various gram-positive bacteria resistant to ß-lactam antibiotics including methicillin-resistant Staphylococcus aureus, methicillin resistant S. epidermidis and ampicillin resistant enterococci and as such is important in intensive care unit patients. Current guidelines suggest vancomycin dosing by intermittent infusions two to three times a day according to current body weight and disease severity. Several studies showed that insufficient serum drug concentrations are achieved when drug is administered this way. Therefore, continuous vancomycin infusion is better administration method considering drug serum concentration and adverse effects such as nephrotoxicity. This study is aimed at testing vancomycin infusion algorithm to achieve optimal serum drug concentration and to minimize undesired side effects.

Hypothesis: Vancomycin application via continuous infusion results in 35% increase in number of patients with plasmatic drug concentration within therapeutic range in comparison to intermittent drug application.

Aims: General aim of this research is to target therapeutic drug concentration achievement proportional difference among two groups of patients at the time of first serum concentration measurement. Specific aims are to determine 1) AUC/MIC > 400 achievement proportional difference, 2) total daily doses difference, 3) total daily costs difference, 4) difference in total number of days spent on therapy difference in total number of days spent in ICU, and 5) nephrotoxicity and other side effects incidence difference.

Materials and methods: Inclusion criteria: patients older than 18 years of age; Exclusion criteria: patients younger than 18 years of age, vancomycin therapy in previous 48 hours, renal replacement therapy, patients treated with extracorporeal membrane oxigenation therapy, pregnant women, patients with burn injuries; Dosing: Continuous infusion: initial loading dose of 25mg/kg (of TBW; maximum of 2g) given during 3 hours, followed by continuous infusion with daily doses based on creatinine clearance, aiming target serum concentration of 20 to 30mg/l. Intermittent infusion: 2g per day divided in two doses, or 3g per day for CNS infections. Measurement: Continuous infusion: 24 hours after start of infusion (48 hours for patients with CrCl 20-50mg/l), three more measurements every 24 hours up to four samples to the end of fourth day of therapy. Intermittent infusion: steady state trough concentration measurement just before fourth dose following three more measurements every 24 hours. Statistical analysis: An a priori power analysis determined that to obtain an alfa of 0.05 and 80% power, a sample size of 60 patients was needed to detect a 35% absolute difference in target plasma concentration attainment. A Fischer exact or X2 test was used for all categorical data, and Student t test was used for all continuous data comparisons. A $P<0.05$ was considered significant.

Expected scientific contribution: Acquired comprehension will contribute to better dosing and methodology understanding with final purpose of side effects reduction and better patient outcomes.

MeSH/Keywords: Vancomycin, continuous infusion, serum concentration, nephrotoxicity

Poster code: T-02-08-036

Poster Title: Nucelotide polymorphism rs531564 of pri-miR-124 gene in patients with primary sclerosing cholangitis and inflammatory bowel disease

PhD candidate: Mislav Jelaković

Part of the thesis: Nucelotide 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, 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 unlcear pathogenesis. MicroRNAs (miRs) are small, non-coding RNAs that play a role in post-transcriptional gene regulation and may have a tumor-suppressor role which has been demonstrated in multiple studies. Polymorphism of a single nucleotide (SNP) of the pri-miR-124 gene may significantly alter its expression, depending on the present allel/genotype. For example, the G/C genotype shows a higher expression compared to CC.

Hypothesis: Frequency of G/C genotype of single nucleotide polymorphism rs531564 of the pri-miR-124 gene, is higher in inflammatory bowel disease patients compared to patients diagnosed with inflammatory bowel disease and primary sclerosing cholagitis

Aims: The general aim is to analyse the frequency of genotypes of single nucleotide polymorphism rs531564 of the pri-mir-124 gene, among patients diagnosed with primary scleroisng cholangitis and/or inflammatory bowel disease, and to compare it between groups. Furthermore, we will test for possible associations between SNP rs531564 and clinical characteristics of IBD patients (clinical course of the disease, initial extension) and PSC/IBD patients.

Materials and methods: In this cross-sectional, observational study, we are planning to include a random sample of adult (older than 18 years) female and male participants diagnosed with either inflammatory bowel disease or primary sclerosing cholangitis according to international guidelines. Patients will be enrolled after written informed consent was signed, with all inclusion criteria and non of the exclusion criteria present. Our plan is to enrol a total of 120 subjects which includes 60 with IBD (30 UC and 30 CD) and 60 with PSC/IBD (20 PSC, 20 PSC/UC, 20 PSC/CD). The sample size was calculated using minor allele frequency (alpha=0.05, beta 0.20). Relevant clinical data will be obtained from electronic medical chart of each patient. Single nucelotide polymorphism rs531564 of the pri-miR-124 gene will be analysed using real-time PCR method (Applied Biosystems 7300 Real Time PCR System). After all of the relevant data is available, statistical analysis will be conducted in collaboration with a statistician.

Expected scientific contribution: In this study, we will analyse the frequency of genotypes of single nucleotide polymorphism rs531564 of pri-mir-124 gene in patients diagnosed with primary scleorising cholangitis, ulcerative colitis and Crohns disease; and for the first time, in patients with diagnosed with both primary sclerosing cholangitis and inflammatory bowel disease

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

Poster code: T-02-09-021

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 a rare hereditary disorder of clotting, and most commonly occurs as haemophilia A and haemophilia B. 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 ethiology has not yet been clarified but it could possibly be due to lower arterial stiffness in patients with severe haemophilia.

Hypothesis: Lower arterial stiffness could be responsible for lower cardiovascular mortality in patients with severe haemophilia despite higher cardiovascular risks.

Aims: The aim of the study is to determine arterial stiffness in patients with severe haemophilia comparing it with cardiovascular risk factors (arterial hypertension, age, diabetes mellitus, hyperlipidemia, smoking, positive family history, BMI index, etc.).

Materials and methods: We plan to enrol 100 patients suffering of haemophilia A and haemophilia B. The data of subjects will be collected using standard laboratory methods. The blood pressure will be measured 3x. The arterial stiffness will be measured by non invasive Arteriograph. Anamnestic data and clinical status will also be performed.

Expected scientific contribution: The 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.

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

Poster code: T-02-09-046

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'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. Pituitary adenomas and adrenocortical tumors are the most common endogen causes of CS and that is why we differentiate between ACTH-dependent and ACTH-independent CS. The purpose of this retrospective study is 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 evaluate quality of life, time of postoperative recovery of the hypothalamus-pituitary-adrenal axis (HPA) and differences between ACTH-dependent and ACTH-independent CS on this changes.

Hypothesis: Reduction of waist circumference, improvement of lipid parameters as well as better regulation of blood pressure and glycaemia will develop six months after remission of CS.

Aims: The main aim of this study is to investigate the influence of remission of CS on individual components of metabolic syndrome (including waist circumference, glycaemia, blood pressure and lipid profile). Specific goals of this study will investigate differences between ACTH-dependent and ACTH-independent CS on recovery of HPA axis, quality of life, cardiovascular and thromboembolic events as well as cardiovascular risk before and after remission.

Materials and methods: A retrospective study will be conducted at the Department of Endocrinology, Clinic of Internal Medicine, University Hospital Zagreb. All patients with CS who are treated at the Department of Endocrinology in the period 2011-2017 will be included in this study. Data will be retrieved from available medical documentation. CS is defined as elevated free cortisol levels in 24hour 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 distinguishes ACTH-dependent from ACTH-independent CS. Other investigations to define the etiology of CS include magnetic resonance imaging of the pituitary gland and inferior petrosal sinus sampling (IPPS) if necessary as well as CT scan of adrenal glands. This study will include only patients with either ACTH-dependent (pituitary) CS or ACTH-independent CS (adrenal). Patients with ectopic CS will not be included in this study as well as patients with adrenocortical carcinomas. The treatment of pituitary CS includes transphenoidal ablation of the tumor as for adrenal CS it involves laparascopic adrenalectomy. Remission of CS is defined as normalization of cortisol levels in 24h urine sample as well as adequate suppression of cortisol after overnight dexamethasone suppression test (< 80 nmol/L). Improvement of metabolic parameters after remission of CS is 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 anti-hypertensive drugs.

Expected scientific contribution: Results of former published studies investigating differences in metabolic parameters in patients with CS before and after remission show contradictory results. This study will help in better understanding of the role of cortisol in metabolic syndrome respectively on blood pressure as well as glucose and lipid metabolism and investigating the difference in clinical outcomes depending on the etiology of CS.

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

Poster code: T-02-09-074

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): Andrea Vukić Dugac, MD PhD, research associate, Professor Lada Rumora, PhD

Affiliation: University of Zagreb School of Medicine, University of Zagreb Faculty of Pharmacy and Biochemistry, University Hospital Centre Zagreb, Clinic of pulmonary diseases

Introduction: Chronic Obstructive Pulmonary Disease (COPD) is characterised by permanent progressive reduction of air flow through the respiratory tract. Numerous studies have already identified inflammation, both localised and systemic, as a key factor in COPD etiopathogenesis. COPD is currently the fourth leading cause of death worldwide but is projected to be the third leading cause of death by 2020 due to tenacious exposure to COPD risk factors as well as ageing of the population. It represents an important public health challenge. Pathological changes characteristic that include chronic inflammation with increased numbers of specific inflammatory cell types are found in the airways, lung parenchyma and pulmonary vasculature in patients suffering from COPD. In general, the inflammatory and structural changes in the airways increase with disease severity and persist even upon smoking cessation. The wide variety of inflammatory mediators that have been shown to be increased in COPD patients attract inflammatory cells from the circulation, amplify the inflammatory process, and induce structural changes. The mechanisms for such amplified inflammation are not yet understood.

Hypothesis: Expression of NLRP3 mRNA and concentration of IL-1 beta are increased in patients with COPD compared to the control group of healthy smokers and non-smokers.

Aims: The overall aim of this study is to investigate the role of inflammasome NLRP3 in systemic inflammatory response in patients with stable COPD and to further investigate the association of inflammasome NLRP3 and cytokine IL-1beta with lung function disorders and biomarkers of systemic inflammation.

Materials and methods: The proposed study is a prospective monitoring of systemic inflammatory biomarkers in groups of patients in stable phase of COPD and healthy subjects (smokers and non-smokers) during a three year period. The research will not be anonymous due to the need to monitor each individual patient. In the selection of the patients, a pulmonologist will participate during regular outpatient control. All COPD patients involved in the study will receive optimal medical therapy according to current guidelines and good clinical practice. A total of three visits of the patients are planned during 3 years of monitoring at one-year intervals and for the control group only one visit is scheduled during the first year of the study. Each time all relevant demographic data, therapy data, smoking status and comorbidities are to be collected. Pulmonary function will be evaluated in all groups of subjects by determining spirometry, CO diffusion, and plasma arterial blood flow analysis. Patients with COPD will complete the CAT questionnaire, SGRQ-C questionnaire and mMRC scale. During the first and second visit, blood samples will be taken.

Expected scientific contribution: The results of this study should contribute to a better understanding of systemic inflammation in COPD etiopathogenesis as well as phenotyping of patients in COPD, hopefully resulting in improved treatment modalities.

MeSH/Keywords: COPD, NLRP3 inflammasome, cytokine IL-1beta, phenotype

Poster code: T-02-09-095

Poster Title: Prevalence of asthma among adolescents in the City of Zagreb

PhD candidate: Kristina Lalić

Part of the thesis: Prevalence of asthma among adolescents in the City of Zagreb

Mentor(s): Professor Neven Tudorić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Asthma is a severe chronic disease representing significant burden on patients, caregivers and health care systems. The prevalence of asthma in developed countries increased, mostly due to changes in lifestyle and environmental conditions. However, little is known about trends of asthma prevalence in Croatia.

Hypothesis: The prevalence of asthma among adolescents in City of Zagreb increased significantly in the last two decades.

Aims: Firstly, to determine the prevalence of asthma among adolescents in City of Zagreb using standardized ISAAC (International Study of Allergy and Asthma in Childhood) methodology. Secondly, to estimate changes of asthma prevalence by comparing the obtained findings with existing ones, collected in the same way 20 years ago. In addition, we will also investigate the possible impact of multiple risk factors on expected change in asthma prevalence.

Materials and methods: A randomly chosen sample of 3,000 school children aged 15-16 will be included in this study. All participants will complete the standardized ISAAC questionnaire designed to detect the risk for asthma. Participants suspected for being at risk of asthma will be invited to Allergy Department of Clinical Hospital Dubrava for further tests: thorough disease history, skin prick test with standard inhaled allergens and peak expiratory flow rate measurement.

Expected scientific contribution: The proposed study will give us data about trends of asthma prevalence in adolescents in City of Zagreb. The analysis of documented risk factors might explain the causes of observed changes. We believe that the obtained results could be used in planning different interventions targeted toward better asthma management based on earlier diagnosis and diminishing potentially harmful environmental risk factors.

MeSH/Keywords: asthma, adolescents, prevalence, ISAAC

Poster code: T-02-09-099

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

PhD candidate: Jelena Bielen

Part of the thesis: The connection between inflammatory markers, mean platelet volume and red blood cell volume distribution width in occurrence of post-thrombotic syndrome after proximal deep venous thrombosis

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

Affiliation: University of Zagreb School of Medicine

Introduction: Post-thrombotic Syndrome (PTS) is a late chronic complication of deep vein thrombosis (DVT). Inflammation 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), mean platelet volume (MPV) and red cell volume distribution width (RDW) and PTS formation after proximal DVT and evaluation of quality in PTS patients.

Hypothesis: Inflammation markers (C- reactive protein, total leukocytes, neutrophil and lymphocyte ratio), platelet count, mean platelet volume and red cell volume distribution width are independent risk factors for post-thrombotic syndrome formation after proximal deep venous thrombosis.

Aims: To investigate the connection of inflammation parameters (C reactive protein, total leukocytes, neutrophil and lymphocyte ratio), mean platelet volume (MPV) and red cell volume distribution width (RDW) and PTS development after proximal DVT.

Materials and methods: This observational cohort study will include 110 patients hospitalized due to the first proximal DVT. In addition to clinical examination, diagnosis of proximal DVT will be confirmed by duplex ultrasound examination the lower extremity veins. The data on medical history, physical examination of the patient, the findings of the diagnostic and therapeutic procedures and laboratory findings will be collected from medical documentation and hospital information system. 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-etiologic-anatomic-pathophysiologic 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).

Expected scientific contribution: The study would provide further insight in the role of inflammation, platelet count, mean platelet platelet volume and erythrocyte volume distribution in post-thrombotic syndrome development. This would allow an earlier screening of a risk group of patients after proximal deep vein thrombosis for the development of post-thrombotic syndrome.

Acknowledgments: I would like to thank my mentor associate professor Mislav Vrsalović, MD, PhD for his continuous support.

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

Poster code: T-02-09-103

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 osteoporosis in liver transplant candidates

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

Affiliation: Merkur University Hospital, Zagreb

Introduction: Orthotopic liver transplantation (OLT) is a standard treatment for acute or chronic liver failure. Decompensated liver cirrhosis is the most frequent indication for OLT. Among cirrhotic patients, bone loss (osteopenia and osteoporosis) is the most common bone disease. Well known risk factors for bone loss in cirrhosis are endocrine disorders (hypogonadism, hyperthyroidism, hyperparathyroidism, diabetes, metabolic syndrome), malnutrition, alcoholism, hyperbilirubinemia, corticosteroid usage, low level of fibronectin and increased levels of proinflammatory cytokines. Bone cells produce fibroblast growth factor 23 (FGF 23) which interferes with bone mineralization by inducing phosphaturia, lowering vitamin D, suppressing tissue-nonspecific alkaline phosphatase and increasing pyrophosphate. FGF 23 is elevated in cirrhotic patients and correlates with the stage of cirrhosis, increased pre-OLT mortality and depletion of osteoprogenitor cells.

Hypothesis: Bone loss (osteopenia and osteoporosis) in OLT candidates is associated with an elevated level of FGF 23, with chronic inflammation and insulin resistance as contributing factors.

Aims: The aims of this research are to determine the prevalence of bone loss (osteopenia and osteoporosis) and osteoporotic fractures in OLT candidates, to determine osteoporotic fracture risk in OLT candidates using Fracture Risk Assessment Tool, to determine the serum level of FGF 23 and its association to bone loss (osteopenia and osteoporosis) in OLT candidates and to assess the association of the serum level of proinflammatory markers (hsCRP, IL-6, sialic acid) and insulin resistance with FGF23 and bone loss (osteopenia and osteoporosis) in OLT candidates.

Materials and methods: Patients who are candidates for OLT at the Merkur University Hospital will be included in the study. Patients with an acute inflammatory condition or with chronic renal insufficiency, diabetes, malignant disease or those on corticosteroids and/or bone antiresorptive bone drugs will be excluded. Before enrolling in the study, a signed informed consent will be obtained from the participants. Patient's history and demographic data will be collected and known risk factors for bone loss in cirrhosis recorded at the study entrance. In addition to standard pretransplant blood tests, level of FGF23, hsCRP, IL-6 and sialic acid will be measured. Plain radiographs of thoracic, lumbar spine and pelvis will be obtained and signs of bone loss and osteoporotic fractures will be recorded. Bone densitometry of lumbar spine and hips will be obtained. According to statistical analysis, a minimum of 80 patients (40 patients with recorded bone loss and 40 patients without signs of bone loss) need to be included in the study. Homeostatic Model Assessment 2 is going to be used for the evaluation of insulin resistance. The risk of osteoporotic fracture will be measured using Fracture Risk Assessment Tool.

Expected scientific contribution: This study will determine the prevalence of bone loss (osteopenia and osteoporosis) and osteoporotic fractures in OLT candidates and establish their association to the serum level of FGF23 and proinflammatory markers, as well as insulin resistance. These findings will increase knowledge and could lead to a better understanding of the metabolic background of bone loss, which could enable more efficient prevention of fractures and improve quality of life and life expectancy in OLT candidates.

MeSH/Keywords: Cirrhosis, Osteoporosis, Fibroblast Growth Factor 23

Poster code: T-02-09-124

Poster Title: Transient elastography in assessment of fibrosis and steatosis of donor livers

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: Non-alcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases and is presumed to become the most common indication for liver transplantation (LT). NAFLD has a negative impact on liver donors because it is known that steatosis of allografts is associated with high risk of ischemic-reperfusion injury development, primary non-function and lower overall survival of allografts. In the study, we will analyse the efficiency of transient elastography as a non-invasive method in the detection of fibrosis and steatosis of liver donors in context of LT.

Hypothesis: Transient elastography is an effective non-invasive method for assessment of fibrosis and steatosis of donor livers.

Aims: Primary aim is to determine effectiveness of transient elastography in detection of fibrosis and steatosis in donor livers after LT in comparison with pathohistological analysis of liver biopsy Secondary aims are to determine presence of steatosis and fibrosis in pathohistological analysis before and after LT, to determine presence of metabolic syndrome components (MetS) in the liver recipient, to determine relation of MetS components and type of immunosuppressive therapy with the presence and course of steatosis and fibrosis after LT, determined by liver biopsy, to determine relation between MetS components and graft fibrosis and steatosis assessed by transient elastography (LSM (liver stiffness measurements) and CAP (controlled attenuation parameter)) after LT, and to determine relation between MetS components and transient elastography findings with recipient and graft survival after LT.

Materials and methods: In this prospective study at Department of Gastroenterology, University Hospital Merkur, we plan to include at least 150 patients, liver transplant recipients from the time of transplantation. Each subject participating in the prospective study will sign a current Institutional Review Board approved consent form. For all donor livers, biopsy and transient elastography by FibroScan®-CAP will be done before implantation. For all liver transplant recipients, liver biopsy and FibroScan®-CAP will be done at 6 months and 12 months after LT. We will evaluate the presence of MS and its components in liver transplant recipients in the follow up period of 12 months. Metabolic syndrome (MS) will be defined according to the International Diabetes Federation criteria. Anthropometric measurements will be performed for all patients at the beginning of the study and during the follow up period as well as blood pressure measurement with digital blood pressure monitor. Statistical analysis of data will be performed using descriptive statistics (mean and standard deviation). Categorical variables will be tested by chi-square test or Fisher's exact test. Testing the importance of the difference of two independent groups will be performed using t-test or ANOVA. The Pearson or Sperman correlation coefficient will be used to express correlations between variables. Univariable and multivariable regression analyses will be performed using the logistic regression analysis (and the results will be express as odds ratio [OR] and 95% confidence intervals [CI]). Covariates that will be include in the multivariable regression models will be chosen on the basis of their statistical significance in univariable regression analyses. P-value <0.05 will be considered to be statistically significant. Statistical analysis will be made using MedCalc statistical software package, version 10 (MedCalc, Mariakerke, Belgium).

Expected scientific contribution: This will contribute to a better evaluation of the quality of liver donors before and after LT using non-invasive methods, stratification of patients at risk of fibrosis and steatosis progress and the development of follow-up strategy with non-invasive methods.

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

Poster code: T-02-09-125

Poster Title: Effects of inhaled corticosteroids on bone turnover markers in patients with asthma

PhD candidate: Sanda Dokoza Terešak

Part of the thesis: Effects of inhaled corticosteroids on bone turnover markers in patients with asthma

Mentor(s): Marija Gomerčić Palčić, PhD, research associate

Affiliation: University Hospital Centre Sisters of Charity, Zagreb; University of Zagreb School of Medicine

Introduction: Asthma is a chronic respiratory disease with incidence characteristic of younger age. Several studies link long-term use of high dose of inhaled corticosteroids with faster development of osteoporosis which is characterized by decreased bone density. Currently, the golden standard for detection of osteoporosis is central dual-energy x-ray absorptiometry (DXA) of the hip and lumbar spine. Bone turnover markers are useful tools in assessment of bone metabolism. There are two groups of bone turnover markers: markers of bone formation and bone resorption. Osteocalcin, one of the major non-collagenous proteins in bone matrix, is synthesized by osteoblasts and is a specific marker of bone formation. On the other side, beta-crosslaps, specific product of Type I collagen degradation, is used as marker of bone resorption. Additional bone turnover marker is cathepsin K, 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.

Hypothesis: The use of inhaled corticosteroids in patients with asthma increases the level of cathepsin K, osteocalcin and beta-cross laps and has influence on development of osteoporosis.

Aims: The aim of this study are as follows: 1. to determine the levels of cathepsin K, osteocalcin and beta cross laps in a group of patients with asthma treated with inhaled corticosteroids; 2. to explore whether treatment with inhaled corticosteroids in patients with asthma, independently to dose and type of inhaled corticosteroid, has influence on biochemical bone turnover markers (chatepsin K, osteocalcin and beta-crosslaps). 3. to explore the relationship between cathepsin K and other bone turnover markers (osteocalcin and beta-crosslaps), and densitometry; 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: This present study will include 250 adult patient with asthma divided into three groups depending on the inhaled corticosteroids 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 participants without significant comorbidity or history of corticosteroid use, who had done densitometry that showed no pathologic findings. All data will be shown using tables and graphs. A $P<0.05$ will be considered significant. IBM SPSS Statistics Version 25.0 will be used for the analysis

Expected scientific contribution: Cathepsin K level in a serum as well as its dynamic 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, bone densitometry

Poster code: T-02-09-130

Poster Title: The role of TLR7 gene in the formation and development of lung carcinoma

PhD candidate: Fedža Džubur

Part of the thesis: The role of TLR7 gene in the formation and development of 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-kB, enhanced expression of antiapoptotic proteins, enhanced tumour cell survival, and chemoresistence. 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.

Hypothesis: Genetic variability of the promoter region of the TLR7 gene is associated with affinity of development of non-small cell lung cancer, and the level of gene expression in tumor tissues, chemotherapy resistance, and clinical outcome of the disease.

Aims: 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.

Materials and methods: The collection of samples began in 2017 after obtaining the license of the Ethics Committee of UHC Zagreb. 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 lung cancer patients were collected at the UHC Zagreb, at the PDC Jordanovac, and healthy subjects at the Institute for Transfusion Medicine, Zagreb. Respondents involved in this research signed an informed consent.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. This part of the proposed research is a retrospective study. 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. Given that all respondents were collected in 2017, we expect that the survival monitoring period, for the purposes of this research, would be until the end of 2021, which is approximately 5 years for most respondents. For the purposes of analyzing the expression of the TLR7 and IFNγ gene expression, the tissues of the subjects with known genotype will be used. Tissue or histological preparations will be obtained from the excess tissue that is taken when taking a tissue sample for routine treatment of the patient.

Expected scientific contribution: 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 IFNγ, important regulators of inflammatory activation and immunological response modulators.

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

Poster code: T-02-09-138

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

PhD candidate: Cecilija Rotim

Part of the thesis: Assessment of adherence to a gluten-free diet in celiac disease patients 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 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 stucture. Today, celiac disease therapy is based on a strictly gluten-free diet and in most of the patients, leading to a satisfactory remission - a symptom, a serological and histological remission. The foundation of clinical success with these patients in their cooperation and follow the gluten-free diet.

Hypothesis: By introducing a new biomarker for the detection of gluten intake (GIP), it is possible to monitor more closely the adherence of a gluten-free diet to celiac patients in relation to current clinical practice, and to better assess the impact of the child's nutritional status on the clinical course and quality of patient's life.

Aims: The main aim is to determine the role of GIP in adhering to a gluten-free diet in adult patients with celiac disease, and to determine the relationship of compliance with parameters of nutritional status, disease activity and quality of life. Specific aims of this research are to determine the fecal concentrations of biomarkers GIP in patients with celiac and control group, to assess daily gluten intake by GIP and adhere to gluten-free diet by tissue transglutaminase and a structured questionnaire, to assess the activity of the disease, the quality of life and the nutritional status of patients with celiac disease and their connection with adherence to gluten-free diet, to analyze the association of GIP biomarker concentrations with tissue transglutaminase and serum biomarkers of inflammatory activity, to analyze the correlation of GIP biomarker concentrations with tissue transglutaminase and serum markers of inflammatory activity.

Materials and methods: This transient research will include patients in the Clinical Nutrition Clinic of the Clinical Hospital Center Zagreb. The study will include 80 adult patients with diagnosed celiac disease and 10 healthy volunteers as a control group. Excluding the criteria will be age <18 or> 65 years, systemic. All patients will sign an informed consent, and the research will be approved by appropriate ethical committees. Demographic and anthropometric data will be collected from the prepared questionnaire, available medical documentation and measurements. From the collected blood samples, a complete blood count will be determined, . The assessment of adherence to gluten-free diet and nutritional quality will be assessed by assessments by dietitians and validated diet diets and by measuring tissue transglutaminase. Nutritional status will be assessed using validated methods (NRS-2002, anthropometry) for evaluation of nutrition. The quality of life of patients will be evaluated with a validated questionnaire for cell patients - CDQ. GIP measurements in urine and stools will be determined by a commercial ELISA test (iVYLISA GIP, Biomedal SL).

Expected scientific contribution: This study for the first time introduces the measurement of the level of the new biomarker (gluten-based immunogenic peptide-GIP) into a regular clinical protocol for monitoring patients with celiac disease. Since this is a new and more reliable non-invasive method for assessing adherence to gluten free nutrition, better control of adherence to gluten-free diet, better control of disease activity and improved quality of life in patients with celiac disease can be achieved. Furthermore, applying this method could be stratified and adequately treated by those patients who adhere to gluten-free children but enter unwanted gluten from hidden sources.

Acknowledgments: I am greatfull to my mentor professor Krznarić for his unselfish support and guidance thorugh my research and I owe deep gratitude to the members of his team at Department of University Hospital Center of Zagreb

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

Poster code: T-02-09-142

Poster Title: Survival analysis of patient or graft after liver transplantation by machine learning methods

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

Affiliation: University of Zagreb School of Medicine

Introduction: Liver transplantation is a successful method of treatment of liver cirrhosis. 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. The process of matching recipients and donors relies on various scales and the experience of the transplantation team. A universal algorithm for predicting the outcomes of transplantation does not yet exist.

Hypothesis: It is possible to predict patient's survival after liver transplantation from a combination of recipient and donor characteristics by using statistical methods of survival analysis (Kaplan-Meier; Cox) and machine learning methods.

Aims: The aim of this research is to analyze the outcomes of liver transplantation using the methods of machine learning and statistic methods of survival analysis (Kaplan-Meier; Cox) in defined period of the study.

Materials and methods: The study will cover around 600 patients with liver transplants made in the period between April 2013 and end of December 2018 in Clinical Hospital Merkur, Zagreb, whose data will be analyzed up until the predefined end of the study, that is, until the end of 2019.

Expected scientific contribution: The obtained results may be used to improve the understanding of risk factors and may back decision-making during the planning of a liver transplantation.

MeSH/Keywords: liver transplantation, machine learning, survival analysis

Poster code: T-02-10-030

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

PhD candidate: Inga Đaković Bacalja

Part of the thesis: Prediction of methicillin-resistant staphylococcus aureus in infections after vascular surgical reconstructions

Mentor(s): Assist. Prof. Tomislav Meštrović, MD, Phd, Professor Zdenko Sonicki, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Infections after surgical revascularisation procedures increase postoperative mortality and morbidity significantly. Among these, methicillin-resistant Staphylococcus aureus infections are particularly frequent and severe, frequently accompanied by anastomotic disruptions, graft occlusions and bleeding. There were studies describing some risk factors for methicillin-resistant Staphylococcus aureus infections, but none of these, to our knowledge, created clinical prediction rules.

Hypothesis: It is possible to develop clinical prediction rules for MRSA infections compared to other pathogen infections in infections occurring after reconstructive vascular graft surgery.

Aims: The main goal of this study is to derive clinical prediction rules that would help clinicians as a prediction and decision-making tool during the treatment of patients with a high risk of methicillin-resistant Staphylococcus aureus infections after vascular reconstruction procedures.

Materials and methods: A retrospective analysis of risk factors for MRSA infections after vascular reconstructive surgery will be performed. The data will be collected retrospectively from medical histories and electronic database in University hospital centre Zagreb, Department of Surgery, Division for vascular surgery, in the period from 1996 to 2018. Preoperative, operative and postoperative variables will be included. A group of the variables of the observed patients will be explored by descriptive statistics, by data mining and by univariant statistical analysis. Central tendency measures will be used to describe distributions of numerical variables. Proportions of qualitative variables will be interpreted by contingency tables. To test numerical variables for normal distribution, a Shapiro-Wilk test will be used. The differences in proportions of the qualitative variables will be evaluated by chi-square test or Fisher's exact test while the differences in numerical variables will be evaluated by the Student t-test for normally distributed variables and by nonparametric Mann-Whitney U test for variables that are not normally distributed. Statistical significance of the results will be interpreted at the level 0.05. The data mining analysis will be performed: a) by using decision trees in Weka (J48 algorithm), and by using random forest in R ("Cforest" algorithm,). Discriminative power of the prediction models will be evaluated by ROC analysis. Variables chosen as relevant in data mining models with sensitivity equal or higher than 50%, specificity equal or higher than 70% and the area under the ROC curve equal or higher than 70% will be selected for further multivariate analysis, as well as the variables that were selected as significant in multivariate analysis. To create a multivariate prediction model for MRSA infection, a stepwise logistic regression will be used with the starting variables selected by univariate statistical methods and the data mining methods. Data analysis will be performed in „WEKA 3.8 “ data mining software (1) and „R 3.5.2“ statistical and data mining software (2). The discriminative ability of the selected variables will be evaluated and visualized in the „FreeViz“ module of the „Orange 3.20.1“ software (3).

Expected scientific contribution: To our knowledge, there has been no research creating prediction rules for MRSA infection after vascular reconstructive operations in comparison to other pathogens. To identify the specific risk factors for MRSA infection and the clinical prediction rules accordingly will enable better treatment approach by correction of the risk factors in preoperative period. These rules might encourage further investigations needed in this field.

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

Poster code: T-02-10-081

Poster Title: Association of tumor budding and tumor deposits and their relation to clinical-pathological parameters in patients with colorectal carcinoma

PhD candidate: Zdenko Bilić

Part of the thesis: Association of tumor budding and tumor deposits and their relation to clinical-pathological parameters in patients with colorectal carcinoma

Mentor(s): Assist. Prof. Mario Zovak, MD PhD, Alma Demirović, PhD, research associate

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

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

Hypothesis: Tumor deposits are more common in patients with colorectal cancer with a high-grade tumor budding.

Aims: General aim: Investigate tumor budding grade and its associations with the presence of tumor deposits in histopathological findings of tissue samples obtained from patients with colorectal cancer. Specific aims: 1.Determine presence and tumor budding grade on the tissue blocks stained with H&E and analyzed by PAN-CK staining. 2. Determine the presence and the size of tumor deposits on the tissue blocks stained with H&E. 3. Determine the association of tumor budding grade and the tumor deposits size. 4. Analyze the relation between tumor budding and tumor deposits to clinical-pathological parameters in patients with colorectal carcinoma.

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

Expected scientific contribution: The existence of a possible connection between tumor budding and tumor deposits may indicate the possibility that in the early stage of tumor progression, that connection is more likely to be due to direct spread of dedifferentiated tumor bud cells in the surrounding stroma and less likely to be due to lymphoid or perineural spread. Possible association of tumor budding and tumor deposits could further emphasize the importance of tumor budding as a

negative prognostic factor in patients with colorectal cancer. New knowledges could in the future affect the changes of therapeutic modalities in the treatment of patients with colorectal cancer.

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

Poster code: T-02-10-087

Poster Title: Impact of tumor characteristics and intraoperative factors on future liver remnant hypertrophy in ALPPS

PhD candidate: Ivan Romić

Part of the thesis: Impact of tumor characteristics and intraoperative factors on future liver remnant hypertrophy in ALPPS

Mentor(s): Assoc. Prof. Anko Antabak, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: ALPPS (Associated Liver Partition and Portal Vein Ligation for Staged Hepatectomy) is an innovative 2-stage surgical procedure for patients with advanced liver malignancies in whom inadequate future liver remnants after standard hepatectomy is expected. This is a physiologically demanding procedure which consists of portal vein ligation and liver parenchymal transection as the first stage resulting in adequate liver hypertrophy of the remaining tissue (7-14 days). The second stage is performed and includes the removal of the deportalized liver lobe. ALPPS research is still in the early phase of clinical-scientific evaluation, but results clearly show faster and more intensive hypertrophy of liver remnant in various studies. This has led to the gradual acceptance of ALPPS in hepatobiliary centers, particularly in Europe. Although, this procedure has narrow indications and some studies suggest a relatively high rate of postoperative complications, there are an increasing number of studies that show that technical advancement and better perioperative management improve ALPPS outcomes. The main goal of our retrospective multicentric study is the identification and analysis of disease characteristics and intraoperative factors (line of liver transection and type of synchronous intraabdominal resection) which affect the degree and intensity of remnant liver hypertrophy (measured by CT or MRI volumetry).

Hypothesis: Liver transection line in ALPPS has significant impact on intensity of future liver remnant hypertrophy. Additional intraabdominal resections during ALPPS have significant impact on expected future liver remnant hypertrophy and ALPPS outcome.

Aims: Main goal is to improve our understanding of liver hypertrophy after ALPPS, analyze outcomes of ALPPS and compare it with conventional methods of liver malignancy treatment.

Materials and methods: Our study includes patients from the University hospital centre Zagreb and from the ALPPS registry led by University hospital Zurich, Switzerland in whom ALPPS procedure was done for the treatment of liver malignancy. Only patients with an adequate follow-up period and liver volumetry results will be included.

Expected scientific contribution: The exact cause of intensive postoperative liver hypertrophy is still unknown, especially when compared to conventional methods of liver hypertrophy induction: ligation/embolization of portal vein branch. Results of our study would provide us scientific explanation of future liver volume hypertrophy induction and help us to define factors that positively or negatively affect liver hypertrophy between two stages of ALPPS. Some of these factors (transection line, additional resection, timing of additional metastasectomy) could be changed in order to improve outcomes. Beside intraoperative factors, we would study factors associated with liver condition, localization and size of the tumor and previous chemotherapy.

MeSH/Keywords: ALPPS; liver resection; hypertrophy; volumetry

Poster code: T-02-10-107

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 Universitiy Hospital Center; Ljudevit Jurak Department of Pathology and Citology, Sestre milosrdnice Universitiy 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 will be compared with TNM stage, age, sex, and survival.

Hypothesis: There is a greater immunohistochemical expression of MAGE-A10 and NY-ESO-1 in primary gastric cancer with metastasis to regional lymph nodes compared to nonmetastatic gastric cancer.

Aims: The study aimed to determine and compare the immunohistochemical expression of MAGE-A10 and NY-ESO-1 in primary gastric adenocarcinoma with and without metastasis in the lymph nodes, and to evaluate their association with the standard clinical-pathological features of surgically treated gastric cancer patients.

Materials and methods: A retrospective research will be conducted in which the archival materials of paraffin-embedded tissue blocks will be used together with all relevant patients clinical data. Tumor specimens will be obtained from patients operated on in the Department of Surgery, Sestre milosrdnice University Hospital Center, Zagreb, Croatia, from 2005 to 2014. It will include at least 104 samples randomized into two groups: 52 with lymph node metastasis and 52 without lymph node metastasis. Standard immunohistochemistry will be used to determine MAGE-A10 and NY-ESO-1 antigen expression in primary tumors. Descriptive and analytical statistical methods will be used in the statistical analysis of the results.

Expected scientific contribution: The results of the study could result in new diagnostic and prognostic markers or potential targets for immunotherapy and anti-tumor vaccination. The results might contribute to a better understanding of the role of MAGE-A10 and NY-ESO-1 in metastasis of gastric cancer, which can be an incentive to develop targeted therapies.

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

Poster code: T-02-10-120

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

PhD candidate: Alen Vrtarić

Part of the thesis: Relationship of vitamin D receptor polymorphisms and 25-hydroxy vitamin D concentrations with clinical trait and bone density in patients with psoriatic arthritis

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

Affiliation: University of Zagreb School of Medicine

Introduction: Vitamin D receptor (VDR) is important for biological response of vitamin D (1,25(OH)2D). Binding of vitamin D to VDR results in dimerization of the VDR receptor with retinoic acid receptor and activation of target genes dependent on vitamin D, resulting in activation or inactivation of transcription. Psychiatric Arthritis (PsA) is a chronic inflammatory rheumatic disease which belongs to a group of spondyloarthritis. It is closely related to skin disease, psoriasis. PsA is non-infectious arthritis typically affecting the peripheral joints, less frequent spine, while characteristic symptoms of locomotor system are dactylitis and entezitis. Among laboratory findings, the characteristic finding is seronegativity, i.e. the absence of rheumatoid factor in serum. The basic goals of treating PsA are to reduce the inflammation and thus the symptoms of arthritis (eg. pain, tenderness), maintain the joint structure and improve functional ability. Gene for VDR has 11 exons and contains four polymorphic domains. One of these polymorphic domain, rs731236 is located at the 3 'end of the gene and can be detected by restriction enzymes. The second polymorphic domain, rs2228570 is located in the initial codon of exon 2.

Hypothesis: Presence of polymorphic variants of polymorphisms rs2228570 and rs731236 and reduced concentration of vitamin 25(OH)D2 and 25(OH)D3 are associated with elevated activity of the disease and poorer functional abilities and reduced bone mineral density in patients with psoriatic arthritis.

Aims: General aim is to determine the association of VDR gene polymorphisms with vitamin 25(OH) D2 and 25(OH)D3 concentrations in patients with psychiatric arthritis.

Materials and methods: Patients diagnosed with psoriatic arthritis according to CASPAR (Classification of Psoriatic Arthritis) criteria that are treated at the Clinic for Rheumatology, Physical Medicine and Rehabilitation at Sestre milosrdnice University Hospital Center (ages 18-65) will be included. Blood samples will be taken during hospitalisation: one tube with an anticoagulant EDTA for DNA isolation and genotyping, while two tubes with clot activator will be collected for measuring concentrations of vitamin 25(OH)D2 and 25(OH)D3. In addition, the concentration of total and ionized calcium, CRP, protein, albumin, creatinine, PTH, phosphate, RF and anti-CCP will be determined from the serum sample. Samples will be stored up to -80 °C. Patients will be divided into two groups according to the DAPSA (Disease Activity Psoriatic Arthritis) index, which includes calculating the number of painful and swollen joints, the CRP concentration, and the patient's grade of disease activity and joint pain. The first group will be patients with remission and low activity of the disease (DAPSA index from 0 to 14) and in the second group of patients with moderate and high disease activity (DAPSA index> 14). Methods to be included in clinical diagnostics are: assessment of functional ability measured by HAQ-DI (Health Assessment Questionnaire Disability Index), TUG (Test Timed Up and Go test) muscle performance test, bone density measured by X-ray dual energy absorption (DXA) and an indirect measure of bone structure microarray, TBS (TrabecuLar bone score).

Expected scientific contribution: Determining the association between the VDR gene polymorphisms, concentrations of 25(OH)D2 and 25(OH)D3 with the degree of disease activity will allow the recognition of patients with increased appearance for a worse clinical picture of the PsA (reported by reduced functional capacity and mineral bone density). These results can be the basis of therapeutic interventions in patients with PsA.

MeSH/Keywords: vitamin D, psoriatic arthritis, VDR receptor, polymorphisms
Poster code: T-02-11-037

Poster Title: Oxidative stress evaluation in follicular fluid of women with unexplained infertility

PhD candidate: Ivana Zec

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

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

Affiliation: University of Zagreb School of Medicine

Introduction: Follicular fluid (FF) represents microenvironment surrounding the oocyte. It is composed of plasma constituents and molecules produced from the secretory activity of granulosa and theca cells. Assisted reproductive techniques include the use of exogenous gonadotropins for follicle growth and maturation. Cellular metabolism and exposure to exogenous prooxidants produce oxidants involved in intercellular signalling, steroidogenesis and follicle growth. Larger number of growing follicles can altered the balance between prooxidant and antioxidant due to its cellular metabolism. Oxidative imbalance causes macromolecule damage and thus can affect oocyte quality. Gonadotropin stimulation may have a direct impact on oxidation stress markers. However, there is a lack of studies comparing oxidative stress between natural modified and ovarian stimulation cycle. In this study, FF will be used for oxidative stress determination.

Hypothesis: The level of oxidative stress in follicular fluid in women with unexplained infertility is higher during ovarian stimulation compared to modified natural cycle in assisted reproductive technique and correlates with lower oocyte and embryo quality and lower pregnancy rate.

Aims: The main objective of this study is to determine if ovarian stimulation changes the oxidative stress parameters. Through the measurement of F2-isoprostanes, malondialdehyde, vitamin E and superoxide dismutase activity, the aims of this study are to determine: (i) whether the ovarian stimulation changes the parameters of oxidative stress, (ii) an association of oxidative status with the pregnancy outcome, the quality of an embryo and oocyte, (iii) the predictive value of F2-isoprostanes in assisted reproductive technology.

Materials and methods: This cross-sectional study will include women under the age of 42 with unexplained infertility. Study participants (N=60) will be divided into two groups, first with the modified natural cycle and the second with ovarian stimulation. The surplus specimen of FF will be collected during the oocyte retrieval from the women undergoing in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) treatment. Specimens with blood contamination or without isolated oocyte and women with acetylsalicylic acid therapy will be excluded. The concentrations of F2-isoprostane will be measured with ELISA, malondialdehyde and vitamin E with high-performance liquid chromatography. Superoxide dismutase activity will be determined spectrophotometrically. The study outcome will include the quality of oocyte and embryo and confirmed pregnancy at 12 weeks after IVF/ICSI procedure. The differences of quantitative variables between natural cycle and ovarian stimulation and differences between pregnant and non-pregnant group will be tested with Student t-test or Mann Whitney U test. Correlation of quantitative variables with embryo and oocyte quality and pregnancy outcome will be determined with Spearman correlation coefficient. Logistic regression analysis will be used to obtain the best predictive markers for IVF/ICSI outcome.

Expected scientific contribution: This study provides first results in F2-isoprostane concentrations in the follicular fluid under the influence of ovarian stimulation. The research will answer the question of whether hormonal ovarian stimulation significantly changes the oxidative status of follicular fluid, which can have a negative effect on the IVF/ICSI outcome.

Acknowledgments: This study was supported by Scientific Centre of Excellence for Reproductive and Regenerative Medicine, Croatia, and by the European Union through the European Regional Development Fund, KK.01.1.1.01.0008, Exploring New Platforms and Potentials

MeSH/Keywords: F2-Isoprostane, oxidative stress, in vitro fertilization, female infertility, follicular fluid

Poster code: T-02-11-070

Poster Title: Association of vitamins B1, B2, B6 and oligoelements Zn, Cu, Se, Mg concentrations with stage and course of illness in patients with chronic heart failure

PhD candidate: Marija Božović

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

Mentor(s): Assist. Prof. Matias Trbušić, MD PhD, Nora Nikolac Gabaj, PhD, research associate

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

Introduction: Heart failure (HF) is clinical syndrome caused by a structural and functional cardiac abnormality. There are several methods for illness monitoring in patients with chronic HF. Depending on measurement of the LVEF (left ventricular ejection fraction), patients can be classified into 3 groups. New York Heart Association (NYHA) classification is a functional classification and according to severity of symptoms and exercise intolerance patients are classified into 4 groups. Simple method for assessment of exercise capacity in chronic HF patients is six-minute walk test. It can be used for illness monitoring and prognosis in cardiovascular diseases. Diagnostic test often used for ruling-out HF and for monitoring illness is measurement of B-type natriuretic peptide (BNP) or N-terminal pro-BNP (NT-proBNP). In healthy heart main substrates for ATP production are fatty acids, but in patients with chronic HF there is switch in primary substrate utilization from fatty acids to glucose. In previous studies it has been observed that patients with chronic HF are deficient in micronutrients important for ATP production. Especially important micronutrients are vitamins B1, B2, B6 and oligoelements Zn, Cu, Se, Mg. Vitamin B1 deficiency ranges from 13% to 96% among chronic HF patients. For vitamins B2 and B6 just a few studies were preformed and established deficiency among population is 27% and 33%, respectively. Oligoelements also have an important role in energy producing reactions. Among chronic HF population, Zn, Se, Mg deficiency has been established and Cu level has been elevated.

Hypothesis: Lower vitamin B1, B2, B6 and oligoelements Zn, Mg, Se concentrations and elevated Cu concentration are linked with poorer stage and course of illness (poorer NYHA class, poorer LVEF, higher NT-proBNP concentration) in patients with chronic heart failure after one year follow up.

Aims: Main aim is to determine if there is association between stage and course of illness with vitamins B1, B2, B6 and oligoelements Zn, Cu, Se, Mg concentration in patients with chronic HF. Specific aims are to determine vitamins B1, B2, B6 and oligoelements Zn, Cu, Se, Mg status in patients with chronic HF and to determine if there is association between vitamins B1, B2, B6 and oligoelements Zn, Cu, Se, Mg concentration with six-minute walk test, and stage and course of illness expressed with NYHA classification, LVEF, and NT-proBNP.

Materials and methods: This study will be conducted in Sestre milosrdnice University Hospital Centre during two years period in two time points. Participants will be adults (18-80 years old) with the diagnosis of chronic heart failure. Exclusion criteria are conditions after stomach and bowels surgical procedure, inflammatory bowel diseases, pancreas and gut diseases, acute inflammation, kidney failure and systematic inflammatory diseases. In the first time point, by inclusion in the study, all patient data will be collected. For each participant six-minute walk test, echocardiography, measurement of LVEF, NYHA classification and blood sampling will be performed. 5 sample tubes will be collected for vitamins B1, B2, B6, oligoelements Zn, Cu, Se, Mg, total proteins, albumin, creatinine, C-reactive protein and NT- proBNP measurement. After one year six-minute walk test, echocardiography, measurement of LVEF, NYHA classification and blood sampling will be performed again. But only one blood sample tube will be collected for NT-proBNP measurement. Vitamins will be measured using high-performance liquid chromatography, and Zn, Cu, Se using atomic absorption spectroscopy. Course of illness will be monitored through changes in six-minute walk test, NYHA class, LVEF and NT-proBNP value between first and second time point.

Expected scientific contribution: This study will contribute in assessment of vitamins B1, B2, B6 and oligoelements Zn, Cu, Se, Mg status in patients with chronic heart failure and vitamins and oligoelements status association with stage and course of illness.

MeSH/Keywords: Chronic heart failure, vitamins deficiency, oligoelements, course of illness

Poster code: T-02-11-111

Poster Title: Characterization of biochemical parameters of intraosseous blood under ischemic and non-ischemic conditions in patients undergoing high tibial osteotomy

PhD candidate: Lucija Kučko

Part of the thesis: Characterization of biochemical parameters of intraosseous blood under ischemic and non-ischemic conditions in patients undergoing high tibial osteotomy

Mentor(s): Prof. Slobodan Vukičević, MD PhD, Univ. Prof. Dr. Catharina Chiari

Affiliation: University of Zagreb School of Medicine, Vienna General Hospital, University Hospital Centre Zagreb, Clinical Hospital Center "Sisters of Mercy"

Introduction: During preparation for the use of new bone regeneration drug OSTEOGROW in HTO patients it was aimed to use autologous blood coagulum (ABC) from the tibia and mix it outside the body with a bone morphogenetic protein 6 (rhBMP6) to form an implant and install it back into the tibia osteotomy wedge to accelerate bone healing. Surprisingly, the tibia ABC did not clot and then the patient's peripheral vein blood was used to form ABC. Since, in the HTO procedure the tourniquet of the upper leg is used to prevent the blood flow the tissues below the tourniquet are exposed to ischemic conditions that might affect the blood clotting after the release of tourniquet and the tissue reperfusion. In this study we will explore the potential underlying mechanism preventing the blood clotting in tibia of patients undergoing HTO procedure with and without tourniquet.

Hypothesis: Ischemia due to the use of tourniquet influences the coagulation of the IOB from patients undergoing HTO as compared to HTO patients prior to establish ischemic conditions.

Aims: General aim is to define the coagulation differences of the IOB exposed to ischemia compared with the IOB not exposed to ischemia in patients undergoing HTO procedure. Specific aims of this research are 1) to determine differences of cellular components between the IOB and the PB from patients undergoing HTO surgery with and without lower leg ischemia, 2) to determine coagulation parameters between IOB and the PB from patients undergoing HTO surgery with and without lower leg ischemia and 3) to characterize the amount of ROS and myeloperoxidase (MPO) in the IOB and the PB from patients undergoing HTO surgery with and without lower leg ischemia.

Materials and methods: Patients older than 18 years of age, of both genders, undergoing HTO procedure in the Department of Orthopedics and Trauma Surgery at Vienna General Hospital and Clinic of traumatology, Clinical Hospital Center "Sisters of Mercy" will be included. The IOB from each patient will be collected during surgical procedures, prior the use of tourniquet and after using it to compare this two blood samples in different conditions: with and without ischemia. In all patients prior to surgery the PB sample will be taken for comparative analyses. Within the frame of the proposed study the following will be explored: complete blood count; coagulation parameters (PT, aPTT, concentration of fibrinogen); thromboelastography; determination of reactive oxygen species and myeloperoxidase.

Expected scientific contribution: We expect that proposed analyses between IOB and PB under described conditions will give us a clue on how to use the IOB from patients undergoing HTO procedure as a carrier for rhBMP6 which will surely influence the acceleration of bone healing within the first several months following surgery. Since this information has not been described in literature it will contribute to more general understanding on the use of IOB samples in patients undergoing different orthopaedic and trauma related procedures and eventually contribute to adding additional laboratory analyses of blood samples following patients treated under ischemic and non-ischemic conditions.

Acknowledgments: This work is supported by the project Reproductive and regenerative medicine - exploration of new platforms and potentials, GA KK01.1.1.01.0008 funded by the EU through the ERDF.

MeSH/Keywords: intraosseous blood, coagulation, reactive oxygen species, high tibial osteotomy

Poster code: T-02-11-139

Poster Title: Long-term effect of Bruton tyrosine-kinase inhibitor ibrutinib on left atrium function

PhD candidate: Matea Kolačević Zeljković

Part of the thesis: Long-term effect of Bruton tyrosine-kinase inhibitor ibrutinib on left atrium function

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

Affiliation: University of Zagreb School of Medicine

Introduction: Ibrutinib is an irreversible Bruton tyrosine-kinase inhibitor with established efficacy in the treatment of various B-cell malignancies in prospective studies. Different ibrutinib side effects have been found, but most of them were < 3rd degree of severity and mostly didn't require dose adjustment or therapy discontinuation. Also, there was an increase in the incidence of atrial fibrillation (6-16%) without clear pathogenesis, however there are indications that ibrutinib inhibits phosphoinositide-3-kinase (PI3K)-Akt signal-pathway expressed in the cardiomyocytes. Regardless of the molecular pathogenesis, the clinical effect of ibrutinib on the myocardium, especially the left atrium, has not been studied.

Hypothesis: Long-term (at least 12 months) ibrutinib therapy causes a decrease in left atrial tissue strain, measured by a decrease in total atrial strain by more than 10%.

Aims: Aim of this study is to determine the ibrutinib effect on echocardiographic parameters of left atrial function.

Materials and methods: This study was designed as a clinical, prospective, observational cohort study with a structured follow-up period of 12 months. All consecutive patients > 18 years-old with hemato-oncologic diseases (including chronic lymphocytic leukemia, Mantle-cell lymphoma, Waldenstrom macroglobulinemia) prescribed with ibrutinib therapy, who are able to understand and sign informed consent, will be enrolled. Primary objective is change of the left atrial function measured by the decrease of the left atrial tissue strain deformation > 10%. Standardized statistical methods and tests will be done using SPSS Version 22.0 or newer. Power test using Wilcoxon signed-rank test was made, with the assumptions: a) α (double-sided) = 0.05, b) β = 0.2, c) the proportion of patients with a decline in atrial function of atrial global strain > 10% is 30%, d) planned duration of follow-up = 12 months. Total number of patients required to be included in the study in order to perform statistically significant conclusions based on the results of the research is 27. Study protocol: all patients in whom the hematologist indicates ibrutinib therapy, an additional cardiology diagnostics will be carried out before the introduction of the therapy. Diagnostic assessment will include: medical history, clinical examination, 12-lead-ECG, transthoracic echocardiography, 24h-Holter-ECG and the standard laboratory findings (total blood count; creatinine; AST, ALT, LDH; Na, K, Ca, Mg; CK, hs-troponin I; NT-proBNP; PV, INR, APTV, fibrinogen). The same diagnostic assessment will be repeated at 3, 6 and 12 month follow-up visits after the introduction of ibrutinib therapy. Primary endpoint is a decrease in left atrial function measured by a decrease in total atrial tissue strain by more than 10% assessed by transthoracic echocardiography. Secondary objectives: systolic and diastolic left ventricular function; left atrium function as a reservoir (2D and 3D left atrium volume, dimensions in 2 echocardiography projections: PLAX and 4C); left atrium conduction function (P wave duration and PQ interval change in the 12-lead ECG); atrium function as a pump (ejection fraction of left atrium); incidence of atrial fibrillation.

Expected scientific contribution: The most important scientific contribution of this study is assessing the impact of long-term ibrutinib therapy on the left atrium function assessed with transthoracic echocardiography by determining possible significant reduction in global atrial strain, potentially revealing pathogenesis of already established increased incidence of paroxysmal atrial fibrillation among patients with ibrutinib therapy. An additional benefit is that during clinical monitoring, appropriate treatment and prevention of complications, preferably atrial fibrillation, can be performed on time.

MeSH/Keywords: ibrutinib; echocardiography; left atrium; function; long-term
Poster code: T-02-13-051

Poster Title: The association of interleukin 6 and disease activity with chronic fatigue, depression and quality of life in patients with primary Sjogren'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 Sjogren'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. In addition to the symptoms associated with glands function, SS patients 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 cytokine 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, with its insufficiently investigated pathophysiological mechanism. Chronic fatigue is the most common symptom in patients with primary Sjögren's syndrome. It affects many aspects of life and has a negative impact on the quality of life of patients. Often due to chronic fatigue, patients have musculoskeletal pain and tendency for depression, which further disturbs the daily functioning and quality of life.

Hypothesis: Our hypothesis is that interleukin 6 levels and disease activity in pSS are associated with chronic fatigue, depression and worsening the quality of life in patients with primary Sjögren's syndrome.

Aims: The main aim of this study is to determine the correlation between interleukin 6 levels and disease activity with chronic fatigue, depression and quality of life in pSS patients. Our specific aim is to determine the correlation between inflammation and interleukin 6 with presence of chronic fatigue, depression and to determine the prevalence of chronic fatigue and depression in patients with pSS.

Materials and methods: The study will include around 70 patients with primary Sjögren's syndrome who meet the revised internationally recognized classification criteria for diagnosis of Sjögren's syndrome (American-European Consensus Classification Criteria-AECCC). Patients will be female ages 18-65 and their identity will be protected by the code. Exclusion criteria will include other autoimmune diseases, acute and chronic inflammatory diseases and patients with psychiatric diagnosis. 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). For the assessment of disease activity, we will use two disease activity indexes; ESSPRI and ESSDA index that will be filled out with the assistance of clinician. After completing the questionnaires, we will take blood samples (8 ml) for biochemical analysis (CRP, ESR, fibrinogen, haptoglobin, protein electrophoresis, CBC, ANF, ENA) and for interleukin 6. In control groups we will have around 30 healthy individuals that are identical to age and sex without the presence of fatigue, sicca symptoms and other autoimmune diseases.

Expected scientific contribution: The scientific contribution is based on the assumption of the immunopathogenetic association of proinflammatory cytokine IL 6 with chronic fatigue, depression, anxiety, pain and lower quality of life in patients with pSS. Since in the majority of cases it is about middle-aged patients, the introduction of clinical and laboratory parameters in everyday clinical practice, assessment of chronic fatigue, depression, anxiety 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, depression, quality of life

Poster code: T-02-14-019

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

PhD candidate: Nada Tomić Sremec

Part of the thesis: The frequency, characteristics and clinical significance of unusual indirect immunofluorescence autoantibodies 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 visualise 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 immunofluorescesnce patterns have not been sufficiently investigated, and their significance in diagnostics of autoimmune diseases is unknown.

Hypothesis: Certain rare and unusual indirect immunofluorescence patterns have distinguishing characteristics and are linked to certain autoimmune clinical entities as well as to specific autoantibodies associated with those entities.

Aims: 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: In this study we will enroll at least 5000 serum samples of patients referred for routine ANA assessment. Sera will be handled according to routine procedures, and subsequently assessed for presence of ANA using indirect immunofluorescence on HEp-2 cells. All positive samples will afterwards be classified according to current international guidelines, which distinguish between 29 separate ANA patterns. Furthermore, all positive samples will undergo assessment for the presence of specific autoantibodies, which is routinely carried out using a microbead based multiplex system (ZEUS AtheNA Multi-Lyte® ANA-II Plus). Rare or unusual ANA patterns will for this purpose be classified as those appearing in less than 3% of all ANA positive samples. Patients with particular unusual ANA patterns will be compared to a control group comprised of other ANA positive patients, and possible differences in gender or age distribution, associated specific autoantibodies and clinical entities will be determined.

Expected scientific contribution: This study is expected to advance our understanding of antinuclear antibodies, especially of many rare indirect immunofluorescence patterns often seen in routine ANA testing. It has the potential to improve the methods of diagnostic evaluation of autoimmune diseases.

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

Poster code: T-02-14-027

Poster Title: Correlation of risk reduction procedures and surgical site infections

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 are important health care-associated infections (HCAI). Pre-, intra- and postoperative risks for acquiring surgical site infection are numerous and are connected with patients and healthcare procedures. They are preventable, therefor risk reduction can decrease additional morbidity, mortality and treatment costs. There are no unique prevention recommendations affecting all risks during surgical treatment.

Hypothesis: Lower surgical site infections' prevalence and frequency among overall HCAIs are associated with hospital structure and process indicators, risk reduction procedures, and are different for particular hospital and surgical speciality. Prevalence and frequency of surgical site infections among overall HCAIs in Croatian hospitals correspond to those in countries in the same World Health Organization category.

Aims: Aims of this study are to establish the burden of surgical site infections in Croatia, to establish correlation between hospitals' structure and process indicators, risk reduction procedures and surgical site infections, to determine if implemented risk reduction procedures have same effect on hospital and specific surgical speciality level. Additional aims of the study are to determine bacterial causative agents, antimicrobial susceptibility, and correlation between misuse of surgical antibiotic prophylaxis and bacterial resistance.

Materials and methods: Study design: multicentre cross-sectional point prevalence study of HCAIs and antimicrobial use. Study is a part of European Centre for Disease Control's (ECDC) study. National coordination will be conducted from Ministry of Health's Referral Centre for Nosocomial Infections in University Hospital Centre Zagreb. Web-based closed questionnaire will be forwarded to Nosocomial Infection Control Committees asking to identify applied risk reduction procedures. Study participants: acute hospitals in Croatia, acute wards and patients with HCAIs in participating hospitals. Exclusion criteria are acute psychiatric and emergency departments unless patients are monitored longer than 24 hours. Material and methods: Training material will be enclosed in participation invitation. Data will be collected in a single day for each ward by hospital infection control personnel from medical records without contact with patients and/or healthcare workers, and will be recorded in standardized hospital, ward and patient ECDC forms. HELICS/IPSE infections' definition will be used. Hospital data include institution's structure and process indicators. Ward data include ward speciality, ward's structure and process indicators and number of patients with HCAIs. Patient data include date of the admission, age, gender, type and duration of HCAI, association with the current ward, bacterial causative agent and antimicrobial susceptibility of the isolate. Antibiotic use data include generic name of the antibiotic, way of application, and indication. Measured outcomes will be prevalence and frequency of surgical site infections among HCAIs. Correlation of hospitals' structure, process indicators and applied risk reduction procedures in specific surgical speciality on outcomes will be investigated. Data will be recorded in closed web-based application using hospital codes and will not be identified to researcher. Structure data will be presented in ratios and will not be connectable to specific institution. Statistical analyses will be conducted in MedCalc (v11.4.2).

Expected scientific contribution: This study is first study on surgical site infection prevalence and prevention in Croatia. It is expected to give the answers to correlation of surgical site infections prevalence, frequency among overall HCAIs and risk reduction procedures, analogy of risks in each hospital and surgical speciality, and difference between risk reduction procedures on surgical site infection burden.

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

Poster code: T-02-16-065

Poster Title: Salivatory melatonin pooling for measuring circadian rhythm response in oftalmological patients

PhD candidate: Leon Rabatić

Part of the thesis: Salivarno zbrajanje uzoraka melatonina u praćenju cirkadijalnih ritmova oftalmoloških bolesnika

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

Affiliation: University of Zagreb School of Medicine

Introduction: Intrinsic photosensitive retinal ganglion cells (IPRGCs) are subgroup of retinal ganglion cells, which are found in the ganglionic part of the retina. A large number of IPRGC / circadian rhythms studies focus on the measurement of melatonin oscillations within twenty-four hour curves for studying minor changes in the phase. The relative novelty of the ophthalmological studies of IPRGCs / circadian rhythms in their standardization attempts are copied by the methology of previous works in measuring the fine oscillations of twenty-four-hour rhythms, although they are usually oriented by the displacement of relative absolute changes in the level of melatonin over longer periods of twenty-four hour rhythms, individual papers measure the difference of several weeks to months from certain ophthalmic interventions. The previous methodology of ELISA and similar methods requires a large number of antibodies, loading of laboratories, employees of the same, costs of the procedure itself, and greater probability of systemic error in the handling and analysis of samples. If the results demonstrate the salivary addition of melatonin samples as an equivalent ELISA method to measure relatively absolute changes in plasma melatonin for the purpose of ophthalmological and similar investigations in periods of time beyond twenty four hour oscillations, this work would potentially provide a methodology with a smaller regeneration-more efficient, statistically safer / stronger, and more economical research in this area. The smaller amount of waste for the environment and greater availability of the method as a clinical test for human pathological conditions. By inspecting the database bible: Web of science, PubMed, Scopus, and Google Scholar. A number of papers that measure eye-related or similar melatonin-related interventions beyond twenty-four hour intervals are reported, but they use questionnaires (Pittsburgh Sleep Quality Index, and other surveys) without measuring systemic changes in melatonin. Potentially one of the reasons why surveys did not use the salivary melatonin test were the deficiencies of the serum salivary melatonin method, the recommended method for measuring oscillations within twenty-four hourly curves.

Hypothesis: Salivary pooling of melatonin samples is an equivalent method as serial taking salivary melatonin to test the absolute response of circadian rhythms in ophthalmic intervention.

Aims: To demonstrate through preoperative and postoperative measurement of the level of melatonin suppression in patients with cataract, in parallel with the visual acuity, the effect of the operative procedure on the function of the IPRGC with two different methods.

Materials and methods: The study will analyze the concentration of salivary melotonin in patients originating from various parts of the Republic of Croatia in which senile cataract is diagnosed, and are referred to the cataract surgery operation according to local guidelines. The inclusion criteria are the senile cataract of one eye. Only one (operated) eye is involved in the study to avoid a confusing factor that could affect the results. The exclusion criteria are ocular diseases with retinal consequences. Also excluded are patients with severe systemic diseases such as diabetes, cancer, and sleep disorders. In the control group, 24 hours prior to cataract surgery, every hour from 20:00 to 22:00 samples are collected and analyzed individually. Then, 3 weeks after surgery, samples are collected again every hour from 20:00 to 22:00, which are analyzed individually. In the test group the procedure is the same but the samples will be mixed.

Expected scientific contribution: To demonstrate through preoperative and postoperative measurement of the level of melatonin suppression in patients with cataract, in parallel with the

visual acuity, the effect of the operative procedure on the function of the IPRGC with two different methods.

MeSH/Keywords: cataract, melatonin, circadian photosynchronization, refractive error, sleep quality

Poster code: T-02-18-050

Poster Title: The effect of surgical treatment on retinal microcirculation and function

PhD candidate: Raševljka Tadić

Part of the thesis: The effect of surgical treatment on retinal microcirculation and function

Mentor(s): Assist. Prof. Igor Petriček, MD PhD, Professor Zoran Vatavuk, MD PhD

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

Introduction: The open-angle glaucoma is progressive neuropathy with characteristic changes at the optic nerve and retinal nerve fibre layer (RNFL), retinal ganglion cells death (RGC) and visual field loss. In majority, IOP levels that are not tolerated by the individual eye lead to axonal damage with apoptotic death of RGC. Studies implicate possible reversal of retinal ganglion cell dysfunction after treatment which can be measured by electrophysiology. Optical coherence tomography angiography is a new imaging technique showing a reduction of retinal vessel density in glaucoma. Limited number of publications have investigated reversal of retinal microvasculature after trabeculectomy using OCT angiography and their results are not consistent.

Hypothesis: Surgical reduction of intraocular pressure in glaucoma patients increases peripapillary and macular vessel density and leads to reversal of retinal ganglion cell dysfunction.

Aims: General objective: Examine the effect of glaucoma surgery on retinal structural, vascular and functional parameters. Specific objectives: 1. Investigate the peripapillary and macula microvascular changes postoperativly using OCT angiography; 2. Evaluate changes in retinal function after surgery using pattern electroretinography; 3. Determinate relationship between retinal structural, vascular and functional parameters postoperatively.

Materials and methods: 170 patients with primary open angle glaucoma who will be scheduled for surgery will be included. Surgery is indicated if glaucoma progression is detected despite medical therapy. Exclusion criteria are media opacity, previous intraocular surgery, retinal or neurologic disease. Trabeculectomy using mitomycin will be performed. 170 patients with open- angle glaucoma with stabile IOP on medical therapy with similar amount of baseline visual field damage but without indication for surgery will be included as control group. Best- corrected visual acuity, biomicroscopy, gonioscopy, Goldmann applanation tonometry, fundus examination, pahymetry , perimetry by Humphrey Field Analyzer 3, SITA 24-2 (Carl Zeiss Meditec), spectral- domain SD - OCT (RT XR Avanti; Optovue inc.), OCT angiography (AngioVue 2017; Optovue Inc) and pattern electroretinography (RetiMax Advance plus, CSP) will be performed. on the same preoperative day and 1 and 3 months postoperatively. Statistical analysis will be conducted using SPSS.

Expected scientific contribution: The proposed research will contribute to the knowledge of the effect of surgical reduction of intraocular pressure on the microvasculature changes in glaucoma patients. Significant scientific contribution will be evaluation of the possibility of reversal of retinal ganglion cell dysfunction and its association with possible reversal of retinal microcirculation. The results could serve for better understanding the pathophysiology of the disease.

MeSH/Keywords: Glaucoma, Optical coherence tomography angiography, Trabeculectomy, Electrophysiology

Poster code: T-02-18-092

Poster Title: Clinical significance of tumor hypoxia in patients with HER-2 positive breast cancer treated with neoadjuvant therapy

PhD candidate: Petra Vuković

Part of the thesis: Clinical significance of tumor hypoxia in patients with HER-2 positive breast cancer treated with neoadjuvant therapy

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

Affiliation: University of Zagreb School of Medicine

Introduction: Introduction: HER-2 positive breast cancer (BC) is an aggressive subtype which accounts for up to 20% of all BC. The neoadjuvant treatment approach is currently considered the preferred approach for the locally advanced HER-2 positive BC with the advantage of allowing the evaluation of tumor response in vivo. Neoadjuvant treatment outcome (pathological complete response (pCR) and residual disease) has been shown to be prognostic for the long-term outcome of BC patients. Tumor hypoxia is one of the main microenvironment factors in the process of carcinogenesis and Carbonic anhydrase IX (CA IX) is considered to be a marker of tumor hypoxia. Bcl-2 is a mitohondrial antiapoptotic protein involved in apoptosis and oncogenesis which can be also associated with tumor hypoxia. Currently, there is little clinical data on the role of hypoxia in HER-2 positive breast cancer (BC).

Hypothesis: Hypothesis: Increased expression of hypoxia marker carbonic anhydrase IX (CA IX) and antiapoptotic protein Bcl-2 is associated with poorer response to neoadjuvant therapy and a lower percentage of complete pathological response (pCR).

Aims: The aim of this study is to determine the predictive and prognostic role of carbonic anhydrase IX (CA-IX) and antiapoptotic protein Bcl-2 in the neoadjuvant treatment of HER-2 positive BC. Primary objective is to analyze the association of CA-IX and Bcl-2 expression and achievement of pCR. Secondary objectives are to analyze the association of CA-IX and Bcl-2 expression and residual disease, disease-free survival (DFS) and overall survival (OS).

Materials and methods: Materials and methods: The research will be conducted at University Hospital for Tumors in Zagreb and include at least 100 patients treated in a period from 2016-2019. We will determine the expression of CA IX and Bcl-2 immunohistochemically on the paraffin-embedded core biopsy specimens of HER-2 positive BC patients treated with anthracycline-taxane based neoadjuvant therapy with the addition of trastuzumab +/- pertuzumab at the University Hospital for Tumors, Zagreb. We will explore the association between the expression of CA IX and Bcl-2 and neoadjuvant treatment outcome determined after surgery (pCR/residual disease). pCR will be defined as the absence of residual invasive cancer on the hematoxylin-eosin evaluation of the complete resected breast specimen and all sampled regional lymph nodes (ypT0/is ypN0). Immunohistochemical positivity will be defined by immunoreactivity score, presented as the product of the staining intensity and the percentage of stained tumor cells. Medical history data will be obtained from hospital program wMIS. Patient follow-up is going to be at least 6 months after surgery.

Expected scientific contribution: Expected scientific contribution: This study would contribute to better understanding of the HER-2 positive BC biology, explanation of the potential mechanism of resistance to standard neoadjuvant therapy and allow individualization and optimization of the therapy according to tumor characteristics.

MeSH/Keywords: Keywords: Breast cancer, HER-2, neoadjuvant therapy, CA-IX, Bcl-2, pCR, RCB

Poster code: T-02-19-122

Poster Title: Analysis of osteogenic differentiation of muscle remnants in hamstring tendons by electrotransfection of BMP-2 to improve tendon graft integration in ACL reconstruction

PhD candidate: Ida Matić

Part of the thesis: Analysis of osteogenic differentiation of muscle remnants in hamstring tendons by electrotransfection of BMP-2 to improve tendon graft integration in ACL reconstruction

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

Affiliation: University of Zagreb School of Medicine

Introduction: Tear of the anterior cruciate ligament (ACL) is amongst the most common knee injuries. Although surgical treatment is not necessary in all patients, a number of patients present with chronic anterior instability of the knee that prevents them from their everyday activities. When this is the case, the ACL anatomical surgical reconstruction is performed, aiming to replace the damaged ligament with an autograft tissue. The most used autograft type is hamstring tendon grafts, which exhibit favourable mechanical properties and are easy and safe to harvest. Not many studies have dealt with the enhancement of the graft biological properties, which would improve critical points of the post-ACL reconstruction, i.e. graft remodelling, maturation and ligamentization, thus enhancing knee mechanical properties and patient's recovery. During standard operative technique, muscle remnants are divided routinely from the tendon graft and are considered as “biological waste”. An article by Ćuti et al. from 2017, in which muscle and tendon-derived adult stem cells obtained during the ACL reconstruction were proliferated and examined for capacity of osteodifferentiation, showed the muscle tissue tendency to ossification, which would improve tendon-to-bone healing. In addition to that, the group by Rod et al. recently used an adenoviral construct to transduce bone morphogenetic protein-2 (BMP-2) to muscle progenitors from the hamstrings tendons. The muscle tissue transduced with Ad.BMP-2 showed high BMP-2 release and consequently its differentiation towards osteogenic lineage. However, compared to gene delivery with viral vectors, non-viral approaches are preferred for clinical translation, as these vectors are cost-efficient, safe and do not trigger the host´s immune responses. Electroporation has been used as a generalised method of non-viral transfection into muscle tissue, with very good results.

Hypothesis: Transfection of the hamstring muscle - tendon graft by electroporation with introduction of growth factor BMP-2 could promote bone development at the integration site, ligamentization and accelerate tendon to bone healing in ACL reconstruction.

Aims: The objective of this experimental study is to apply the growth factor BMP-2 by electroporation method on muscle hamstring remnants, with the aim of directing muscle tissue towards osteogenic differentiation and thus improving the result of the ACL reconstruction.

Materials and methods: In this in vitro experiment, muscle tissue remnants from hamstring tendons from 10 patients undergoing ACL reconstruction surgery will be harvested, by standardized clinical and radiological inclusion criteria. Muscle tissue will be transported the same day to the laboratory, where electroporation procedures will be conducted, using an optimized transfection protocol. The freshly isolated muscle tissue will be electroporated with plasmids harbouring the sequences of bone morphogenetic protein-2 (BMP-2). After washing, the tissue will be incubated in culture and harvested over 2, 14 and 21 days. The harvested tissue culture will be examined for several molecular parameters. The expression of several genes involved in bone differentiation (for example, runt-related transcription factor 2 (RUNX2), dentin matrix protein 1 (DMP1), bone sialoprotein etc), as well as expression of BMP-2, will be measured. The total protein content will be extracted and the presence of bone protein markers examined by ELISA.

Expected scientific contribution: The proposed experiment covers fundamental pilot studies and aims to open perspective towards a new research topic, which has been thus far studied only superficially and as such represents an innovation in the field of tissue engineering. The proposed approach has a high potential for clinical translation in improving ACL reconstruction.

MeSH/Keywords: BMP2, reconstruction of ACL, ligamentization, tendon to bone integration, electroporation

Poster code: T-02-20-128

Poster Title: Prognostic significance of the proteome profile of well differentiated papillary thyroid carcinoma

PhD candidate: Filip Matovinović

Part of the thesis: Prognostička vrijednost proteomskog profila dobro diferenciranih papilarnih karcinoma štitnjače

Mentor(s): Professor Vladimir Bedeković, MD PhD, Assoc. Prof. Lovorka Grgurević, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Papillary thyroid carcinoma is the most common malignancy of the thyroid gland. Its annual incidence is 14 per 100 000 and its mortality is 0,5 per 100 000. It is reported that the incidence of papillary thyroid carcinoma has risen three times in the last 30 years. The preferred method of treatment is total thyroidectomy, the complete removal of the thyroid gland. Although papillary thyroid carcinoma has a good prognosis, some cancers are aggressive and give local and distant metastases, which alters the surgery and worsens the prognosis. It is estimated that approximately one third of papillary thyroid carcinomas gave regional metastases at the moment of diagnosis, while 7 percent of patients develop regional metastases after surgery and 5 percent develop distant metastases. Preoperative identification of these patients is an important clinical problem. Proteomics is a relatively new method of research which is based on identifying and analyzing the proteome profile, which is the complete protein composition, of the chosen specimen. By using proteomics and analyzing papillary carcinoma tissue, our aim is to identify a subset of papillary thyroid carcinomas that are more aggressive and require an altered clinical approach.

Hypothesis: Differences in the proteome profile of well differentiated papillary thyroid carcinomas with and without regional metastases are linked with regional metastatic disease.

Aims: Our main aim is to determine the proteome profile of papillary thyroid carcinomas with and without regional metastases at the time of surgery. Specific aims are to compare the differences between these two proteome profiles and to compare the differences in protein expression in analyzed carcinomas with clinical outcomes in a 5 year follow-up.

Materials and methods: In this research, 70 specimens of papillary thyroid carcinomas will be used, 35 which did not present with metastases at the time of surgery and 35 that gave away regional metastases. The specimens are fixed in formaldehyde and incorporated in paraffin. Proteomic analysis will be performed on the specimens and the results will be analyzed using recursive partitioning, a new method of data analysis based on computational learning. A follow-up of a minimum of 5 years and clinical outcomes, disease recurrence, death or disease-free-survival will be noted for all patients and used in the final analysis.

Expected scientific contribution: This research will try to determine the existence of new biomarkers of metastatic disease in papillary thyroid carcinomas and their role in disease prognosis, that is, to preoperatively identify biologically more aggressive carcinomas.

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

Poster code: T-02-21-031

Poster Title: Effect of topical anesthesia on phonation process 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): Assist. Prof. Mario Bilić, MD PhD

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

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. According to current literature, usage of topical anesthetic in oral cavity, oropharynx and laryngopharynx during examination is optional. 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. Evaluation of phonation process and vibratory characteristics of vocal cords is assessed with subjective rating of the following parameters: glottal closure, amplitude, mucosal wave, non-vibrating portion, supraglottic activity, periodicity, phase closure, phase symmetry, phase shift, etc. Additionally, software production of digital kymogram through juxtaposition of the laryngeal images within the frames allows quantitative analyses of vocal-fold vibratory functions. Objective parameters are: open, closed, opening and closing quotient, etc. Potential influence of topical anesthetic on phonation process has not yet been defined. Anatomical (body mass index, Friedman staging system, etc) or psychological predictors (anxiety) for unpleasant and painful examination as well as predictors of difficult visualization have not been established.

Hypothesis: Usage of topical anesthetic during high-speed digital imaging doesn't influence phonation process in healthy subjects and subjects suffering from laryngopharyngeal reflux.

Aims: Aim of this study is to investigate the influence of topical anesthesia of oral cavity, oropharynx and laryngopharynx during high-speed digital imaging on phonation process by analysis of vibratory characteristics of vocal cords and other laryngeal structures and analysis of digital kymogram in healthy subjects and subjects suffering from laryngopharyngeal reflux. Also, identification of discomfort, pain and difficult visualization predictors according to anatomical and psychological characteristics of subjects.

Materials and methods: This single-blinded randomized controlled study will include 100 subjects (50 healthy and 50 suffering from newly endoscopically and anamnestically diagnosed laryngopharyngeal reflux), aged 18 to 45. Complete medical history will be obtained, otorhinolaryngologic and fiberendoscopic examination will be performed and in case of meeting criteria, informed consent will be signed. Depending on results of examination subjects will be stratified in two groups (healthy and those suffering from laryngopharyngeal reflux). Anthropometric measures, score of fiberendoscopic examination, blood pressure levels before and after examination, maximum phonation time and GAP-7 questionnaire score of subjects will be taken. All subjects will be examined with high-speed digital imaging in 2 consecutive days, with and without anesthesia, by the same experienced examiner. Order of examination type will be predetermined with random number generator. After each examination subject will be asked to fill out visual-analog scale (VAS) 100mm regarding pain and unpleasantness of examination. Examiner will also fill out VAS regarding difficulty and quality of visualization and compliance of subject during examination. Analysis of high-speed digital imaging recording and digital kymogram will be performed by 2 experienced examiners and above mentioned parameters will be quantified.

Expected scientific contribution: Results of this study could have impact on defining and optimization of high-speed digital imaging examination and standardization of scientific studies in which high-speed digital imaging is used. Identification of discomfort, painful and difficult

examination predictors creates possibility of anticipation of subjects who could benefit from usage of topical anesthetic. Stated presents novelty break in usage of topical anesthetic.

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

Poster code: T-02-21-077

Poster Title: Različite postoperativne vrijednosti kalcija i fosfata u bolesnika nakon subtotalne i totalne paratireoidektomije

PhD candidate: Josip Markešić

Part of the thesis: Difference in postoperative values of calcium and phosphate in patients after subtotal and total parathyreoidectomy

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

Affiliation: University of Zagreb School of Medicine

Introduction: Parathyroidectomy due to secondary hyperparathyroidism in patients on hemodialysis is indicated in 15-38% of all patients and leads to better quality of life along with reduction of patient mortality. Standard surgical technique consists of subtotal or total parathyroidectomy, with or without autotransplantation of the remaining part of the gland either in the sternocleidomastoid muscle in the neck or in the subcutaneous tissue of the forearm. Blood levels of parathyroid hormone significantly decrease after surgery, but only in 20% of patients remain permanently low. In most cases they start increasing again in patients with subtotal or total parathyroidectomy with autotransplantation of the remaining gland. Tertiary hyperparathyroidism occurs in cases of long lasting secondary forms of illness when autonomous secretion of parathyroid hormone occurs, as seen in 30% of patients on hemodialysis. It remains in question how much quality postoperative control of parathyroid hormone and serum calcium and phosphate levels is gained by leaving residues of enlarged parathyroid glands after the surgical removal of parathyroid adenomas

Hypothesis: Since secondary hyperparathyroidism in the hemodialysed patients precedes the emergence of tertiary hyperparathyroidism, we have assumed that despite the surgical removal of one or two adenomas of the parathyroid gland, no long-term control of the value of the parathyroid hormone, serum calcium and phosphate occurs because all parathyroid glands were initially increased. We assume that subtotal or total parathyroidectomy would be preferred in this population, with consequent better control of serum calcium and phosphate levels.

Aims: In order to test this hypothesis, in patients with tertiary hyperparathyroidism, who had only one or two surgically removed adenomas with the remaining parathyroid glands left intact, we plan to analyze the values of intact parathyroid hormone and serum calcium and phosphate, monitor their clinical course, dose of substitution therapy to control calcium and phosphate and total and cardiovascular mortality in the control interval relative to patients with secondary hyperparathyroidism and total or subtotal parathyroidectomy.

Materials and methods: Patients admitted into the Clinic for Ear, Nose and Throat and Head and Neck Surgery of the Clinical Hospital Centre Zagreb will take part in the study. To test all the goals of this research, the plan is to follow the clinical course of patients during hospitalization and total survival over a period of two years.

Expected scientific contribution: The results of this study will show the percentage of patients with secondary and tertiary hyperparathyroidism who have enlarged parathyroid glands that are not characterised as nodular hyperplasia, as independent predictors of disease relapse and poor postoperative control of calcium and phosphate. Furthermore, it will show when subtotal or total parathyroidectomy is required in these patients in order to improve the postoperative control of calcium and phosphate and consequently reduce cardiovascular mortality.

MeSH/Keywords: parathyroid hormone, calcium, phosphate, adenoma, secondary hyperparathyroidism, tertiary hyperparathyroidism, cardiovascular mortality

Poster code: T-02-21-119

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, Clinical Hospital Center Sestre milosrdnice

Introduction: Type 1 diabetes (T1D) reports a significant increase in incidence in children over the past decades.Diabetic kidney disease (DKD) is the leading cause of the end-stage renal disease and albuminuria is considered to be an early indicator of kidney damage in T1D. Although it takes years for DKD to develop its advanced stage ,kidney biopsy done 1.5 to 2.5 years after the onset of the disease, and in children who were still normoalbuminic, 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 . Renal resistance index (RI), measured by the Doppler ultrasound method, is an indicator of increased vasculature intrarenal resistance, that occurs in the impaired renal function of patients suffering from T1D . Tubular damage is important in the developing of DKD and kidney injury molecule 1 (KIM 1) in urine a promising tubular marker.

Hypothesis: Using a combination of indicators of glomerular (serum cystatin C), vascular (Doppler RI) and tubular (KIM 1 in urine) damage, it is possible to develop a model for early detection of kidney damage in children with type 1 diabetes.

Aims: Primary goal is to analyze values of serum cystatin C, Doppler RI, KIM 1 value in urine in type 1 diabetic patients and healthy subjects. Secundary goals are to determine the multivariate association of serum cystatin C, Doppler RI, KIM 1 in urine with urine albumin count, glomerular filtration and creatinine in serum in patients with T1D.

Materials and methods: We will include 75 patients with T1D and 75 healthy controls in this cross sectional study.Inclusion criteria for patients with T1D are both sexes, age 10-18 years, degree of pubertal development ≥ II by Tanner , duration of T1D ≥3 years if diagnosed before puberty and ≥ 2 years if diagnosed in puberty. The exclusion criteria for both groups will be acute urinary tract infection, glucocorticoid therapy, other kidney disease, orthostatic proteinuria, thyroid disease, diabetic ketoacidosis, renal artery stenosis, malignant diseases. In study group a blood sample will be taken for HbA1C,creatinine,lipids, and three first-morning urine samples for albumin/creatinine ratio will be collected. In both study and control group urinary KIM 1,serum cystatin C and Doppler RI will be done. Cystatin C will be determined by immunoephelometric latex method against cystatin C antibodies on the Nehelometer Analyzer BN II (Siemens, USA) with the original reagent. Doppler RI will be determined on the ultrasonic device Philips Affiniti 50G with convex probe C6-2 .KIM 1 in the urine portion will be analyzed by an ELISA(enzyme-linked immunosorbent assay).The main hypothesis will be checked by a quantum-quantile regression analysis. The statistical analysis will be made in NCSS 12 Statistical Software (2018). NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/ncss.

Expected scientific contribution: The results of this study are expected to contribute to the understanding of the role of glomerular (serum cystatin C), vascular (Doppler RI) and tubular markers (KIM 1 in urine) in early detection of initial renal impairment in children with T1D. The development of such a multivariate model can have a practical meaning in identifying a group of children with T1D who have the risk of DKD development, and direct them to preventive and therapeutic measures to prevent progressive damage

MeSH/Keywords: Cystatin C, Doppler renal resistance index, KIM 1, Type 1 Diabetes Mellitus, children

Poster code: T-02-24-001

Poster Title: Assessment of arterial stiffness with oscillometria considering dynamic or static type of exercise in children athletes

PhD candidate: Maja Vugrinec Mamić

Part of the thesis: Assessment of arterial stiffness with oscillometria considering dynamic or static type of exercise in children athletes

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

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.

Hypothesis: In this study we hypothesize that the children athletes have reduced arterial stiffness in comparison to children of the same age who are not engaged in sports, and arterial stiffness is dependant on type of sport.

Aims: The aim of the study is to determine difference of arterial stiffness between children athletes, in dependance of static and dinamic type of exercise, and to compare them with children who are not practicing active sport. We will compare their differences according to age and sex, changes of arterial stiffness parametars in dependance of duration and type of exercise, and other common cardiovascular risk factors that affects arterial stiffness.

Materials and methods: One hundred children athletes age 13-18 years will be included in this study. We will devide them into two equal groups according on type of exersice (high level of dynamic and low level of static load, and vice versa). Measurments will be 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. In the control group will be included one hundred healthy children who do not practice active sport, and will be examined in cardiology department becouse of the innocent murmor in precordium. Parameters arterial stiffness will be measured with validated device called Arteriograph. Before the measurments children and their parents will fullfill questionnare, and physical examination and antropometric measurments will be performed.

Expected scientific contribution: This is the first study in which arterial stiffness is measured accoriding on type of sport in children athlete. We are looking for how type of exercise (dynamic or static) affects arterial stiffness as risk factor for cardiovascular diseases. Measurments of arteral stiffness in children athletes could have pratical values as conformation of positive affect of exercise on childrens health.

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

Poster code: T-02-24-012

Poster Title: Disorders of the autonomic nervous system in children with chronic inflammatory bowel disease and irritable bowel syndrome

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, 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 characterised 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.

Hypothesis: Children suffering from chronic IBD and children suffering from IBS have the same incidence of ANS dysfunction.

Aims: The primary objective of the study is to determine the incidence of ANS dysfunction in children with IBD and IBS comparing to healthy controls. The secondary objectives are to determine the impact of nutritional status and body composition on ANS dysfunction in all three groups and to determine connection between quality of life and autonomic nervous system dysfunction in children with chronic bowel diseases.

Materials and methods: We plan to include 60 patients aged from 12 to 18 years (20 patients suffering from chronic inflammatory bowel disease, 20 patients suffering from irritable bowel syndrome and 20 healthy controls). In all subjects, an assessment of nutritional status with body composition and ANS testing will be performed. ANS function will evaluate 3 major autonomic domains: cardiovagal, adrenergic and sudomotor. Tests will include Valsalva maneuver, deep breathing, head-up tilt, and quantitative sudomotor axon reflex test. The severity and distribution of dysautonomia will be quantified using Composite Autonomic Severity Scores (CASS). In patients with chronic IBD the effect of autonomic dysfunction on quality of life using IMPACT-III questionnaire will be determined.

Expected scientific contribution: This study will determine incidence, significance and characteristics of ANS dysfunction in children with IBD compared to children with IBS and controls. The results will have scientific and clinical significance; scientific because it will help to understand the extent to which the symptoms are the consequence of autonomic dysfunction, and clinically because it will open the possibility of therapeutic intervention.

MeSH/Keywords: inflammatory bowel disease, irritable bowel syndrome, brain-gut axis, autonomic nervous system

Poster code: T-02-24-016

Poster Title: Calcium, oxalate, citrate and glycosaminoglycans excretion and their ratios in healthy children and children with idiopathic urolithiasis: the influence of age

PhD candidate: Daniel Turudić

Part of the thesis: Calcium, oxalate, citrate and glycosaminoglycans excretion and their ratios in healthy children and children with idiopathic urolithiasis: the influence of age

Mentor(s): Assoc. Prof. Ernest Bilić, MD PhD, Assist. Prof. Mila Lovrić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: The interaction of calcium (Ca), oxalate (Ox), citrate (Cit) and glycosaminoglycans (GAGs) ions in the urine can lead to the formation of calcium oxalate (CaOx) stones. The risk of urolithiasis changes with age according to urine excretion of stone participants.

Hypothesis: The hypothesis of the study is that with the occurrence of puberty the relationship of urolithiasis promoters / inhibitors (Ca, Ox, Cit, GAG) could be changed to monitor endocrine changes.

Aims: GENERAL OBJECTIVE: To obtain new insights and a better understanding of the Ca, Ox, Cit, GAG and Ca / Cit, Ox / Cit, Ox / GAG, Ox / (Cit × GAG), Cit / GAGs of children with urolithiasis and of healthy children before and after puberty. SPECIFIC OBJECTIVES: For variables whose values for healthy and children with urolithiasis are statistically significant, ROC analysis and Weka classifiers determine the values that best separate healthy children from urolithiasis.

Materials and methods: Only children with proven idiopathic CaOx urolithiasis will be included in the study (61) excluding all diseases that can change urinary promoter / inhibitor values. Children are additionally genetically tested to exclude 60 known pediatric congenital / metabolic mutations. The study will analyze age and gender data, urinary excretion of urolithiasis promoters / inhibitors (Ca, Ox, Cit, GAGs). The control group consists of 25 healthy children matched by age and gender. The data obtained by counting will be shown as numbers and percentages, and analyzed using the $\chi 2$ test. Data obtained by measurement are presented as arithmetic mean, standard deviation (SD), median and interquartile range (IQR), and analyzed using parametric or nonparametric tests, depending on the data distribution. All applied tests are bi-directional and p values ≤ 0.05 are considered statistically significant. The practical significance of the selected variables and the optimal criteria for distinguishing healthy children and urolithiasis children are further analyzed using ROC analysis and JRIP and J48 algorithms (Artificial intelligence, Weka Data Mining Open Source Software).

Expected scientific contribution: The basic scientific contribution would be to determine whether there is an age difference in the secretion of urolithiasis promoters / inhibitors related to puberty. Preliminary work results indicate that only a complex interaction between multiple promoters / inhibitors can result in a common value that reflects the influence of all variables in the ratio.

Acknowledgments: Accordingly with the mentor (Prof. dr. sc. Bilic), the doctoral thesis is changing.

MeSH/Keywords: calcium, oxalate, urolithiasis, children

Poster code: T-02-24-043

Poster Title: Diagnostic value of digital breast tomosynthesis in patients with nipple discharge

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. 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.

Hypothesis: DBT could improve characterization of suspicious lesions, increase cancer detection rate and could be useful in the nipple discharge evaluation.

Aims: Primary objectives are to determine the sensitivity, specificity and predictive values of DBT for the evaluation of patients with nipple discharge. Secondary objectives are to compare the diagnostic value of DBT and other diagnostic imaging methods (mammography, sonography, and breast magnetic resonance imaging) used for the evaluation of patients with nipple discharge, to determine the correlation between morphologic characteristics of lesions detected by DBT and the final histopathologic results and to determine the incidence of malignancy associated with nipple discharge.

Materials and methods: The study is prospective and approved by the Ethical Committee of the University Hospital Centre Zagreb. 200 patients with nipple discharge will undergo DBT, breast sonography and discharge cytology. Suspicious lesions will undergo core needle biopsy, stereotactic vacuum-assisted biopsy or fine needle aspiration. Patient with pathologic nipple discharge will undergo breast MRI.

Expected scientific contribution: Currently, there is no published literature that discusses the diagnostic accuracy of DBT compared with digital mammography in the specific setting of nipple discharge, and there is no literature on the role of DBT for the evaluation of 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: T-02-25-102

Poster Title: Correlation between cholin peak and apparent diffusion coefficient at multiparametric magnetic resonance and calcium sensing receptor expression in breast cancer

PhD candidate: Iva Bušić Pavlek

Part of the thesis: Correlation between cholin 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

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

Hypothesis: Elevated choline levels and the reduced apparent diffusion coefficient in breast cancer obtained by multiparametric Magnetic resonance imaging , positively correlate with the expression of Calcium-sensing receptor.

Aims: The purpose of the study is to analyze the association of choline and apparent diffusion coefficient of invasive breast cancer obtained by multiparametric MR with CaSR on carcinoma breast tissue.

Materials and methods: Preoperative examinations with contrast magnetic resonance imaging and breast cancer tissue specimens stained for immunohystochemical analyses of CaSR expression will be used.

Expected scientific contribution: If there is a positive correlation between the data obtained by multiparametric MR analysis and the CaSR appearance, it would be possible to have noninvasive estimates of the tumor's immunohistochemical characteristics.

Acknowledgments: Thank you to my mentors Associated professor Maja Prutki,MD, PhD and Zlatko Marusic,MD, PhD, Pofessor Ivica Sjekavica, MD, PhD, Head of the department of Radiology and my colleagues at the Department for Breast Imaging for their help and support.

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

Poster code: T-02-25-105

Poster Title: Influence of the morphology of the vastus medialis obliquus muscle on the value of the lateral patellar tilt in patellofemoral instability

PhD candidate: Marko Šimunović

Part of the thesis: Influence of the morphology of the vastus medialis obliquus (VMO) muscle on the value of the lateral patellar tilt in the patellofemoral syndrome

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. It is known that the VMO plays an important role in the stability of the patello femoral joint. VMO along with vastus medialis longus (VML) is an integral part of vastus medialis (VM), which makes up one of four muscles of the quadriceps. The idea of the VM as two distinct muscles was first described by Lieb and Perry in 1968. VMO originates from the distal third of the adductor magnus tendon and inserts at the distal quadriceps tendon and medial part of patella. 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, but their clinical significance and value of pathological value are not know, and they are not suitable for comparative research. In current practice most often use the modified measurement procedure by Fulkerson, and measurement by Sasaki-Yagi, however, they are both dependant on femorotibial rotation, which in particular jeopardizes the credibility of measurements. According to the mentioned conservative therapy is based on strengthening the VMO. 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 imiging (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, which is also much more sensitive to show bone abnormality of knee. As far as we know, no one has examined the influence of VMO on PF instability measuring CSA on CT scans.

Hypothesis: Morphology of the vastus medialis obliquus muscle affects the value of the lateral patellar tilt in patellofemoral instability.

Aims: To examine the influence of the morphology of the oblique vastus medialis muscle on the patellar tillt angle using a new, more objective measuring protocol.

Materials and methods: Retrospective cross-sectional research on axial CT scans of the knee taken at KBC-Zagreb. Following the application of exclusion criteria, parametric and nonparametric tests will be used to assess the correlation (with a statistical significance of 5%, statistical strength of 80%) of the impact of morphology (cross-sectional area) of VMO on the value of patellar tillt obtained by standard measurements according to Fulkerson and Sasaki-Yagi as well as the new method which looks at the connection of measurement methods between same patient follow up measurements, age, gender, foreign body, as well as angles of rotation of the knee.

Expected scientific contribution: To examine the influence of VMO morphology of patellar tillt in patellofemoral instability, and consequently the foundation of the conservative therapy in question with the proposal of a new, more objective method of tilt measurement.

MeSH/Keywords: knee; CT-imaging; patellar tilt angle; patellofemoral instability, VMO

Poster code: T-02-25-116

Poster Title: The role of changes in composition of perfusion fluid in graft function and survival

PhD candidate: Zoran Zimak

Part of the thesis: The role of changes in composition of perfusion fluid in graft function and survival

Mentor(s): Professor Željko Kaštelan, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Kidney transplantation is the best treatment method for the final stage of chronic kidney diseases. Perfusion solutions are special fluids used for organ perfusion, preservation and transport.

Hypothesis: Changes in the composition of perfusion fluid affect graft function and graft survival.

Aims: The purpose of this study is to determine whether or not a change in composition of perfusion fluid used during the organ retrieval has an effect on graft function and overall graft survival.

Materials and methods: This study will be performed on minimally 100 samples of perfusion fluid. Data on volume and kind of perfusion fluid will be obtained from Eurotransplant Kidney report. This study will analyze two types of perfusion fluids: UW (University of Wisconsin) and HTK (Histidine-tryptophan-ketoglutarate) fluid.

Expected scientific contribution: In this study we will investigate changes in composition of the same perfusion fluid. With this we will obtain important data on kidney metabolism during cold ischemia period, which can have a significant effect on its function and overall survival after kidney transplantation. This kind of study hasn't been performed yet, and it represents an original and important contribution to transplantation medicine.

MeSH/Keywords: Perfusion fluid, UW, HTK

Poster code: T-02-28-018

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 researsches supporting theories that (micro)environmental and (EPI)genetic factors lead to its development. Potential biological markers in ejaculate can be identified for pure seminoma group od 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 biological molecular markers of testicular seminoma.

Hypothesis: Seminoma tumors show hypomethylation in the methylation pattern of the promoter regions of the OCT3/4 and NANOG genes in the cell free DNA fraction (cfDNA) isolated from the semen of the patient.

Aims: The main aim is to determine the methylation patterns of cfDNA promoter regions of OCT3/4 and NANOG in the semen of patients with seminoma. The specific aims are to analyze the degree and pattern of methylation cfDNA of OCT3/4 and NANOG genes in preoperative and postoperative semen samples of the patients with seminoma, immunohistochemical analysis of OCT3/4 and NANOG protein expression in tumor tissue and surrounding non-tumor tissue, comparison of obtained DNA methylation data and gene expression data at protein level and comparison of DNA methylation data obtained with patient age, tumor size and TNM.

Materials and methods: A prospective research will be carried out for a period of 3 years. The power test was performed according to the epidemiological study rules and for a strength of 0.90, 14 samples per group will be enough, ie 14 patients and 28 samples. A potential candidate will be notified about the project and after signing the written informed consent, a sample of ejaculate will be collected. The patient population will be recruited from a male population with suspicion of a testicular tumor from University hospital "Sestre milosrdnice", Urology Clinic. Excluding criteria will be any histopathological diagnosis other than pure seminoma. One sample of semen from the patient will be taken preoperatively and one semen sample will be postoperatively. cfDNA will be isolated at the Laboratory for Epigenetics and Molecular Medicine, Department of Medical Biology. DNA methylation analysis will be performed according to the usual methodology. The pathohistological diagnosis of seminoma and tumor tissue sampling will be done at Clinical Institute of Pathology and Cytology Ljudevit Jurak, University hospital "Sestre milosrdnice", immediately after orhidectomy. Morphometric analysis of the IHC signal will be performed on a light microscope according to standard protocols. Using a light microscope, the whole tumor will be examined and IHC reaction will be evaluated on one sectional tumor with highest reaction, "hot spot". 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 also use statistical programs such as Statistics 12.0 (www.statsoft.com) and StatsDirect version 3.0.171 (www.statsdirect.com).

Expected scientific contribution: This research is expected to provide a scientific contribution to the development of epigenetic biomarkers in liquid biopsies from semen of the patients with testicular cancer and to understand the biology of testicular tumors, asses the degree of risk for patients, and also to improve the outcomes of disease monitoring.

Acknowledgments: This research is carried out within the framework of the project "Epigenetic Biomarkers in Blood and Semen of the Patients with Seminoma", Episem Project, approved by the Croatian Science Foundation (HRZZ), HRZZ Research Projects IP-06-2016

MeSH/Keywords: seminoma, methylation, cfDNA, OCT 3/4, NANOG, genes

Poster code: T-02-28-088

Poster Title: Immunohistochemical expression of PTEN and ERG proteins in prostate cancer specimens obtained with MRI guided prostate biopsy

PhD candidate: Ivan Pezelj

Part of the thesis: Immunohistochemical expression of PTEN and ERG proteins in prostate cancer specimens obtained with MRI guided prostate biopsy

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

Affiliation: University of Zagreb School of Medicine

Introduction: Prostate cancer (PCa) is a major cause of disease and mortality among men with 307 000 deaths worldwide in 2015. making it the fifth most common death cause among malignancies. Nearly 1 100 000 newly diagnosed cases where reported with an incidence ranging from 111,6 per 100 000 in Australia and New Zealand to 4,5 per 100 000 in south Asia. Traditionally prostate cancer was diagnosed with prostate specific antigen (PSA) serum levels and rectal exam findings which lead to an ultrasound navigated systemic transrectal prostate biopsy. Recently, prostate MRI has been included in the diagnostic protocol not just for staging but as a tool to perform guided prostate biopsies. In determining the optimal treatment protocols patient age, comorbidities and cancer risk stratification are used. The European Association of Urology uses PSA levels, T stage and Gleason score as factors for cancer risk stratification. The Prostate Imaging Reporting and Data System (PI-RADS) determines the likelihood of clinically significant prostate. The score is given for each lesion, with 1 being most probably benign and 5 being highly suspicious of malignancy. The phosphatase and tensin homolog gene (PTEN) is a tumor suppressor gene located on chromosome 10q23.3. Immunohistochemical staining of PTEN loss at the time of the initial biopsy seems to predict time to metastasis development, prostate cancer-specific mortality and, for the first time, castration-resistant prostate cancer, and response to androgen deprivation therapy after radical prostatectomy. ERG (ETS Related Gene) is an oncogene located in 21q22.2 and is frequently involved as a fusion protein with transmembrane protease, serine 2 (TMPRSS2), a protein encoded by TMPRSS2 gene located in 21q22.3 [20]. Recurrent translocations resulting in TMPRSS2: ERG fusion is associated with a greater probability of lethal prostate cancer, poorly differentiated tumors and higher stage diseases with pelvic lymph node involvement.

Hypothesis: Immunohistochemical expression of PTEN loss and TMPRSS2: ERG fusion in prostate cancer specimens obtained with MRI guided prostate biopsy correlates positively with risk groups as defined by the EAU and PI-RADS score.

Aims: To determine immunohistochemical expression of PTEN and ERG proteins in prostate biopsy samples, depending on tumor localization in the prostate, PIRADS score and risk stratification according to the EAU guidelines.

Materials and methods: Prostate biopsy samples 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.

Expected scientific contribution: Previous research has shown PTEN and ERG expression are associated with poor prognosis in patients with prostate cancer. Determining the link between the genes in prostate biopsy samples with risk groups as defined by the EAU and PIRADS score could help predict treatment outcome.

MeSH/Keywords: prostate cancer, PTEN deletion, TMPRSS2-ERG fusion, PI-RADS score

Poster code: T-02-28-104

Poster Title: Antinuclear antibodies and markers of apoptosis in multiple sclerosis

PhD candidate: Josip Sremec

Part of the thesis: Antinukelarna antitijela i biljezi apoptoze u multiploj sklerozi

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 a disease of a proposed, yet insufficiently elucidated autoimmune origin. As such, it bears some resemblances to systemic autoimmune diseases. One of those is an increased positivity for antinuclear antibodies (ANA), which are commonly associated with those diseases. Research of systemic autoimmune rheumatic diseases shows that those antibodies are often not in the pathogenic sequence of those diseases, but are rether a consequence of faulty or enhanced apoptotic processes in the body, and subsequent exposure of cell antigens to the immune system. Studies of MS have shown altered apoptosis not only in the CNS, but on the peripheral level as well (where it pertains to removal of harmful lymphocyte clones). It is possible that occurrence of ANA is a result of altered or enhanced apoptosis.

Hypothesis: The production of ANA in MS is a consequence of enhanced or altered apoptosis either in the CNS or on the peripheral level. It correlates with clinical features of the disease and with levels of soluble apoptosis factors sFas and sFas ligand.

Aims: Aim of this study is to determine the potential correlation of ANA and soluble apoptosis markers sFas and sFas ligand, and to assess correlation of ANA and aforementioned markers with disease characteristics.

Materials and methods: In this study, we will include 52 patients with CIS or RRMS (relapsing-remitting MS). The patients will be recruited during their hospital stay in „Sveti Duh“ university hospital. Patients with confirmed diagnosis of SARD or a clinical suspicion regarding one of those diseases, as well as patients currently on DMT therapy will be excluded. Data regarding disease duration, initial and current simptomatology, number of relapses in the first two years after diagnosis and total number of relapses (where applicable) will be obtained from patients' medical histories. Data on intrathecal immunoglobulin production will be obtained where available. Time elapsed from the previous relapse will be taken into account. All patients will have undergone magnetic resonance imaging, focusing on brain and cervical medulla, including the assessment after gadolinium administration. Lesion distribution will be determined, as well as the existence of gadolinium-enhancing lesions. A serum sample will be obtained from venous blood using standard procedures. In patients undergoing diagnostic lumbar puncture, a small additional sample (0.5 mL) will be collected. The presence of ANA will be assessed using indirect immunofluorescence assay on HEp-2 cells, and immunofluorescence patterns will be classified according to current recommendations. ANA+ samples will additionally be analyzed for the presence of specific autoantibodies (dsDNA, SSA, SSB, Sm, RNP, Scl-70, Jo-1, centromere B and histones) using the ZEUS AtheNA Multi-Lyte® ANA-II Plus microbead based system. Levels of circulating soluble apoptotic molecules sFas and sFasL in sera and CSF of patients will be determined using the BioLegend LEGENDplex™ microbead based system, in which quantification is done using flow citometry. Statistical analyses will be carried out using Statistica software.

Expected scientific contribution: This study is expected to elucidate the possible origin of ANA positivity in MS, as well as to improve our understanding of apoptotic processes in that disease. Possible association of apoptosis levels and ANA positivity in the context of a disease in which there is no known possible role of ANA antibodies in the pathogenic sequence could improve the knowledge about those antibodies in other autoimmune diseases.

MeSH/Keywords: multiple sclerosis, antinuclear antibodies, autoimmunity, apoptosis

Poster code: T-02-30-006

Poster Title: Influence of genetic polymorphisms of UGT enzymes and drug transporters on lamotrigine concentrations

PhD candidate: Ivana Šušak Sporiš

Part of the thesis: Utjecaj genskog polimorfizma enzima UGT1A4 i UGT2B7 te transportnih proteina ABCB1 i ABCG2 na serumsku koncentraciju lamotrigina

Mentor(s): Assoc. Prof. Nada Božina, MD PhD

Affiliation: Department of Neurology, University Hospital Dubrava; Department of Laboratory Diagnostics, University Hospital Center Zagreb, University of Zagreb School of Medicine

Introduction: Lamotrigin is a second generation anti-epileptic drug that has been widely used for the treatment of patients with epilepsy. Lamotrigine is metabolized in the liver by UGT-mediated glucuronidation, primarily by UGT1A4 and UGT2B7 and is excreted renally as glucuronide conjugate. Pharmacokinetics of lamotrigine 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, weight, smoking, pregnancy and concomitant medications. The coadministration of inducing drugs increases the systemic clearance of lamotrigine, whereas administration of an inhibitor such as valproic acid significantly reduces the elimination of lamotrigine. Therefore, therapeutic drug monitoring is often necessary to prevent the occurrence of unwanted reactions or toxicity as well as changes in drug efficacy. Along with clinical and environmental factors, genetic predisposition has been recognized to be a relevant factor for interindividual variability in drug response. Genetic polymorphism in genes encoding drug-metabolizing enzymes UGT1A4 and UGT2B7 as well as transport proteins ABCB1 and ABCG2 may be an important source in interindividual variabilitiy in lamotrigin metabolism, but available data are insufficient and contradictory.

Hypothesis: Genetic polymorphisms of metabolic enzymes UGT1A4 and UGT2B7, in addition to genetic polymorphisms of transporters ABCB1 and ABCG2 can significantly affect the bioavailability of lamotrigine in patients with epilepsy undergoing lamotrigine monotherapy or lamotrigine polytherapy with valproate.

Aims: The aim of this study is to investigate the role of genetic polymorphisms of metabolic enzymes UGT1A4 and UGT2B7, in addition to transporters ABCB1 and ABCG2 in interindividual variability of lamotrigin bioavailability among patients with epilepsy.

Materials and methods: The study will include at least 150 patients 18-70 years old with diagnosis of epilepsy undergoing lamotrigine monotherapy or lamotrigine polytherapy with valproic acid. Patients will be divided into two groups. In one group will be patients on lamotrigine monotherapy, while in the second group will be patient on lamotrigine and valproic acid polytherapy. Concentrations of lamotrigine in mono- and polytherapy will be analyzed 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. The therapeutic drug monitoring will be 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.

Expected scientific contribution: Due to significant interindividual variability in lamotrigine concentrations, as well as the range variability of interaction between lamotrigine and valproate, 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, pharmacogenetics, UGT1A4, UGT2B7, ABCB1, ABCG2, therapeutic drug monitoring

Poster code: T-02-30-069

Poster Title: Transcranial sonography in the evaluation of patients with Parkinson's disease

PhD candidate: Mislav Budišić

Part of the thesis: The role of transcranial sonography of brain parenchyma in the evaluation of patients with Parkinson's disease

Mentor(s): Professor Arijana Lovrenčić-Huzjan, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Parkinson's disease is the second most common neurodegenerative disease. The diagnosis is mostly made by clinical examination with average clinical accuracy of around 75% in the early stages of disease. 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. 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 major depression disorder and suicidal ideations.

Hypothesis: Findings of brain parenchyma sonography correlates with clinical aspects of the Parkinson's disease.

Aims: Aim of the study is to differentiate patients with Parkinson's disease from healthy subjects and to find correlation of sonography findings and clinical aspects of the disease (duration, severity, lateralization of affection, presence of depression and/or suicidal ideation). Goal of the study is also to determine intraobserver reliability for this method.

Materials and methods: We will include 40 patients with diagnosis of Parkinson's disease (MDS diagnostic criteria) and 40 healthy controls. Informed consent will be obtained before entering the study. Two independent physicians blinded on the results of each other will perform BPS and complete neurological examination. Severity of the disease and presence of depression will be measured with UPDRS III part scale and Hamilton depression scale. BPS will be applied transtemporaly by standardized protocol; SN displayed, manually encircled and measured. The echogenicity of the RN will be rated semi quantitatively on a three-point (not visible, slightly echogenic/interrupted RN, normal RN echogenicity). Appropriate statistical analysis will be used.

Expected scientific contribution: Routine usage of BPS in evaluation of Parkinson's disease patients might add to clinical accuracy in establishing diagnosis. 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: T-02-30-084

Poster Title: Association of chemokines CCL20, IL-8 i CXCL10 with Epstein Barr virus, and their role as prognostic and diagnostic markers in multiple sclerosis

PhD candidate: Jelena Košćak Lukač

Part of the thesis: Association of chemokines CCL20, IL-8 i 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). Different chemokines are considered to take part in pathogenesis of MS including CCL20, IL-8 and CXCL10. Studies have shown that these chemokines 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. Epstein Barr virus (EBV) is considered to be an important etiologic factor in MS. Studies have shown that there is correlation between activity of various diseases, especially nasopharingeal carcinoma, and levels of EBV antibodies and CCL20, IL-8 and CXCL10.

Hypothesis: Increased serum levels of chemokines CCL20, IL-8 and CXCL10 are characteristic for MS and are associated with clinical characteristics of the disease, levels of EBV antibodies and viral activity.

Aims: Aim of this study is to investigate correlation between EBV and chemokines CCL20, IL-8 and CXCL10 in patients with MS, their association with clinical characteristics of MS and their role as prognostic and diagnostic markers in MS.

Materials and methods: 35 patients with relapsing-remitting MS (RRMS) and 35 controls will be enrolled in the study, following ethical approval and written informed consent. Serum samples of patients and controls will be collected and stored at -20°C. Measurement of levels of chemokines CCL20, IL-8 and CXCL10 in serum will be performed using bead-based immunoassay with flow cytometric anaylte quantification. Serum levels of anti-EBNA IgG, anti-VCA IgG, anti-VCA IgM and anti-EA(D) IgG will be measured using CLIA (chemiluminescence immunoassay) method. Furthermore, EBV DNA load in biological samples will be assesed using real-time polymerase chain reaction. In group of selected patients during relapse and afterwards during remission, gene expression of CCL20, IL-8 and CXCL10 will be analysed. Serum levels of chemokines, anti-EBV antibody titres and viremia will be compared between patients and controls. Distribution of the variables will be analysed considering clinical characteristics of the disease, age and sex of the patients. Selection of the statistical tests that will be used will depend on distribution of the results. Differences will be considered significant if $p<0.05$.

Expected scientific contribution: Studies that have been performed so far show that chemokines CCL20, IL-8 and CXCL10 have important role in pathogenesis of MS and that EBV infection takes part in regulation of expression of these chemokines, but this connection has not been investigated in MS yet. Aim of this study is to investigate correlation between EBV and chemokines CCL20, IL-8 and CXCL10 in patients with MS in order to contribute to better understanding of pathogenesis of MS. Furthermore, diagnostic and prognostic value of the chemokines in MS will be investigated.

MeSH/Keywords: multiple sclerosis, chemokines, EBV

Poster code: T-02-30-086

Public health and health care - PhD thesis proposals

Poster Title: Epidemiological characteristic of influenza associated severe acute respiratory infection during influenza season

PhD candidate: Zvjezdana Lovrić Makarić

Part of the thesis: Epidemiological characteristic of influenza associated severe acute respiratory infection during influenza season

Mentor(s): Bernard Kaić, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Epidemiological surveillance for influenza in majority of the countries is based primarily on the data of influenza-like illnesses from the primary care physicians, presenting only mild disease due to influenza. Data about severe disease associated with influenza from inpatient hospital settings, presenting as severe acute respiratory infection (SARI), is lacking.

Hypothesis: There are no differences between influenza positive severe acute respiratory infection (SARI) cases and non-influenza SARI cases.

Aims: We aim to compare demographic and clinical characteristics as well as outcomes of influenza positive SARI cases to those of influenza negative SARI cases and to identify predictors associated with intensive care unit admission among SARI cases. Additionally, our aim is to determine the proportion of influenza that causes severe acute respiratory infection during season and its seasonal variation, in SARI patients.

Materials and methods: Study will be conducted using the data from the Croatian Institute of Public Health. Epidemiological surveillance of severe acute respiratory infections was established in Croatia during the 2018/2019 influenza season in selected sentinel hospitals. SARI case is defined according to the standardised definition of European Centre for Disease Prevention and Control. Case is a person presenting with an acute respiratory illness with onset during the previous 7 days requiring overnight hospitalization that includes: history of fever or measured fever of ≥ 38°C, and cough, and shortness of breath or difficulty breathing. SARI cases will be identified among patients hospitalised for at least 24 hours in one of the sentinel hospitals. Influenza laboratory confirmation will be done using RT-PCR. All positive samples will be typed for influenza A/B.

Expected scientific contribution: This research will contribute to determine the attribution of influenza virus in patients with severe acute respiratory infections and contribute to the improvement of the existing influenza surveillance in Croatia. Furthermore, comparing the characteristics of influenza positive SARI cases and influenza negative cases and identifying predictors of admission to the intensive care unit, it will provide a more accurate risk assessment in patients hospitalized under severe acute respiratory infection.

MeSH/Keywords: influenza; sentinel surveillance; intensive care units; severe acute respiratory infections

Poster code: T-03-01-117

Poster Title: Association of symptoms of anxiety and depression with work ability in women with breast cancer

PhD candidate: Ivana Prga

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

Mentor(s): Assoc. Prof. Darko Marčinko, MD PhD, Bojana Knežević

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

Introduction: Cancer diagnosed in the working-age population is increasing. Nearly 50% of women with breast cancer have anxiety, depression, or both in the year after diagnosis. The prevalence of long-term symptoms of anxiety varies from 17.9% to 33.3% and of depression from 9.4% to 66.1%, which is higher than in the general female population and among men cancer survivors. Overall 63.5% of cancer survivors return to work with the mean duration of absence from work of 151 days and with a reduction in physical or mental work ability up to 26%. Breast cancer survivors reported higher levels of work limitations compared to the non-cancer group of employed workers. Being able to work is in the interest of both society at large and the individual. For cancer survivors, return to work is a part of social recovery and a step towards improving the quality of life. Quality of life is higher among women who are working at least some hours per week compared to women who are not working. As most of the research on the work ability of cancer patients were conducted in developed countries, there is a need for conducting research in low- and middle-income countries where factors affecting work ability may be diverse. A better understanding of psychological problems in women with breast cancer as a factor that has an impact on their work ability is a step forward to supportive interventions that can improve the physical and psychological health of women, their quality of life and potentially prevent long-term sickness and inability to work.

Hypothesis: Symptoms of anxiety and depression are negatively associated with work ability in women with breast cancer.

Aims: To investigate the association of symptoms of anxiety and depression with work ability in women with breast cancer and to examine socio-demographic, psychological and clinical factors in women with breast cancer and their association with work ability and quality of life.

Materials and methods: This prospective study will be performed at the Department of Oncology, University Hospital Center Zagreb. The study group will include 77 women newly diagnosed with breast cancer (stage I, II or III), aged 20 to 60 years, employed at least six months at the time of diagnosis and with no personal history of psychotic disorders. Study group will be asked to complete the Work Ability Index questionnaire, State-Trait Anxiety Inventory, Beck Depression Inventory-II, European Organization for Research and Treatment of Cancer (EORTC) QLQ-C30 core questionnaire (version 3) with the breast module (EORTC QLQ-BR23) at two assessment points: 1-3 months after breast cancer diagnosis and 12-15 months after. Sociodemographic data and clinical data will be collected. Appropriate descriptive analyzes and inferential tests will be performed. The reliability of the instruments will be calculated using the coefficient of internal consistency (Cronbach alpha coefficient). The normality of distribution of the results obtained will be verified using the Kolmogorov-Smirnov test. The association of work ability and quality of life with sociodemographic, psychological and clinical factors will be tested by calculating bivariate correlation coefficients and hierarchical multiple regression analysis.

Expected scientific contribution: This study provides data on the association of symptoms of anxiety and depression with work ability in women with breast cancer. The results of this study can be used for development of the return to work early risk assessment model for women with breast cancer. There is also a possibility for designing return to work planning system with the support of occupational health specialist and active psychosocial support with an influence on work ability of women with breast cancer and certainly on their quality of life.

MeSH/Keywords: breast neoplasms, anxiety, depression, work ability
Poster code: T-03-02-026

Poster Title: Working Alliance Inventory-Short Revised (WAI-SR): Psychometric properties in Croatian primary care multimorbid patients

PhD candidate: Vanja Lazić

Part of the thesis: Working Alliance Inventory-Short Revised (WAI-SR): Psychometric properties in Croatian primary care multimorbid patients

Mentor(s): Assist. Prof. Stanislava Stojanović-Špehar, MD PhD, Professor Jean Yves Le Reste, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Therapeutic alliance consists of three distinct aspects: 1. therapeutic goal - the agreement of the therapist and patient on the goals of therapy,2. therapeutic task- the agreement of patient that tasks of the therapy will address his/her problem, 3. therapeutic bond - the quality of the interpersonal bond between doctor and patient. The Working Alliance Inventory- Short Revised (WAI-SR) has been developed as a tool to measure therapeutic alliance in mental health setting. This tool has been selected using a systematic review of literature and RAND UCLA appropriateness method as the most promising tool to measure therapeutic alliance in family medicine as well as in multimorbid patients by the European general practitioner research network (EGPRN).

Hypothesis: WAI-SR tool has the same psychometric characteristics in family medicine setting and in multimorbid patients as in the original population. There is a difference in the quality of aspects of therapeutic alliance between standard and multimorbid patient groups in family medicine.

Aims: The aims of this research are: 1) to assist the creation of strong tools for evaluation of core competencies family doctors in Europe, 2) to create a necessary tools and consensus for international collaborative study of multimorbidity and its components according to the EGPRN definition, 3) to test the reliability of the WIA-SR for patients in family medicine setting and in multimorbid patients group, and 4) to explore the relationship of the quality different aspects of the therapeutic alliance and multimorbidity in family medicine.

Materials and methods: A group of 3-6 family doctors will be asked to participate in the study and an informed consent will be obtained from them. Following a patient visit to one of the participant doctor's practice, selected according to a randomization scheme, a patient will be asked to participate in the study. in this event, a tablet containing the electronic questionnaire will be handed by the researcher to the patient and to the doctor, after the patient leaves the doctor's office. The questionnaire will contain informed consent form for participants and the Croatian translation of the WAI-SR. The WAI-SR is a 12 item questionnaire measuring the Goal-Task-Bond components of the therapeutic alliance (four items each) on a 5-point Likert scale (1="never", 5="always"). The WAI-SR was translated to Croatian in a forward-backward process with Delphi consensus procedure and cultural cross-validation. The questionnaire will also contain the therapeutic alliance item: "Do you have a good therapeutic alliance with your doctor/patient? " and the item on multimorbidity based on the EGPRN definition of multimorbidity. Patients will be assigned to two groups, according to the presence of multimorbidity, as determined by the multimorbidity item answered by their family doctor. Recruitment will proceed according to the scheme until the recruited number of patients exceeds 400 and at least 200 patients are included in the multimorbidity group. The collected data will be examined to determine the characteristics of the WAI-SR in the new population of family medicine and multimorbid patients. The quality of the aspects of multimorbidity will be compared within and between the two patient groups.

Expected scientific contribution: Validation of the WAI-SR tool in family medicine setting and especially in multimorbid patients is an important step towards creating a knowledge and tool base for the research of doctor-patient relationship in family medicine, comparability of international research on this topic, and in expanding the understanding of the complex interrelation of the identified factors that modify the outcomes of multimorbid patient care. Better understanding of the

different aspects of therapeutic alliance in family medicine and multimorbid patients will allow for the creation of informed intervention strategies aimed at improving the outcomes of care.

MeSH/Keywords: WAI-SR, working alliance, therapeutic alliance, family medicine, multimorbidity

Poster code: T-03-04-140

Poster Title: Factors of migration of bisphenol A and phthalates into waters from plastic bottles

PhD candidate: Rea Janda

Part of the thesis: Factors of migration of bisphenol A and phthalates into waters from plastic bottles

Mentor(s): Professor Ksenija Vitale, MD PhD, Assoc. Prof. Šime Ukić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Bisphenol A is used as an intermediate in the manufacture of polycarbonate plastics and epoxy resins, while phthalates are added to plastics for the purpose of softening. Phthalates can also occur as impurities in the raw material, which can be transferred to the final plastic material. The major problem of bisphenol A and phthalates is their ability to act as endocrine disruptors. In addition, they also affect lungs, liver, kidneys and reproductive system. Literature data show that bisphenol A and phthalates can migrate from plastic packaging to water.

Hypothesis: Various environmental storing conditions of bottled waters cause migration of bisphenol A and phthalates from plastic packaging to water.

Aims: The aim of this study is to qualify and to quantify bisphenol A and phthalates in commercially available water in the bottle on the Croatian market and determine whether the bisphenol A and phthalate concentrations comply with Croatian, European and world rulebooks. The purpose of this study is threefold. The first is to examine the existence of migration of bisphenol A and phthalates in selected water samples and plastic packaging at room temperature. The second purpose is to determine the migration kinetics of bisphenol A and phthalates at different temperatures from -20 to +75 °C. The last purpose is to examine the influence of carbon dioxide concentration, total hardness and pH on bisphenol A and phthalate migration kinetics.

Materials and methods: In this study commercially available bottled waters on the Croatian market will be used. Specifically, natural mineral, natural spring and table waters in 0.5 l plastic packaging and water from the public water supply of the City of Zagreb. Waters will be divided into groups according to the mass of empty bottle, water type and carbon dioxide content. To ensure the same water quality of a specific manufacturer, water packages with the same LOT number will be purchased. Waters in bottles will be exposed to varying temperatures, from -20 to +70 ° C for six months. Every 15 days, concentrations of bisphenol A and phthalates will be determined in test waters using GC/MS methods. Also, the initial and final concentration of phthalate, bisphenol A and carbon dioxide, as well as pH and total hardness will be determined. The obtained results will be processed using descriptive statistical methods and regression analysis. Additionally, the obtained results will be compared with Croatian, European and world regulations.

Expected scientific contribution: This study will associate harmful chemical factors, bisphenol A and phthalates, and their ability to migrate into water from plastic packaging, thereby contributing to the public health approach to the health of potentially exposed population associated with the harmful effects of bottled waters.

MeSH/Keywords: bisphenol A, phthalates, water, plastic bottles, Croatia

Poster code: T-03-06-141

Poster Title: Balneological potential of Croatian thermomineral waters

PhD candidate: Damir Andabaka

Part of the thesis: Balneological potential of Croatian thermomineral waters

Mentor(s): Prof. Jagoda Doko Jelinić, MD, PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Natural remedies have beneficial impact on human health, life quality, and healing and rehabilitation form different diseases. Croatia is rich in thermomineral waters which are one of the natural remedies. Balneology is the study of therapeutic use of thermomineral waters. In many countries is an independent specialty (Turkey, Spain, and Italy) or competencies (Germany, Austria, Serbia, Hungary, Portugal, and France). In the recent years some systematic reviews and clinical studies show positive effect on various chronical diseases. Different European countries have made balneological analysis of their thermomineral waters (Poland, Slovenia, Russia, Hungary, Serbia e.g.) and many of these waters are used as health resorts or as bottled water. For example Germany has about 100 health and spa resorts and Russia around 300. Germany has curative mineral water classification since 1911. According to this qualification a mineral water is designated as curative when it contains at least 1.0 g/l of dissolved minerals. Research of thermomineral water in Croatia is related mainly with bottled waters also with presence of radon and radionuclide in some thermal waters. Balneological classification of some springs has been made about 50 years ago.

Hypothesis: Thermomineral waters in the Republic of Croatia have a great balneological potential for use in balneotherapy and spa therapy according to their chemical composition.

Aims: Aim of this paper is to conduct balneological analysis of all springs of thermomineral waters in Croatia in order to make classification according to chemical composition and to give opinion for use in balneo and spa therapy.

Materials and methods: With this research all accessible springs of thermomineral waters in Croatia will be analysed. According to archive there is about 120 springs of thermomineral waters, some are natural and some drilled during period of oil investigation. Results of balneological analysis for some springs from archive of Laboratory for water analysis and balneoclimatology Department of Environmental and Occupational Health and Sports will be used to estimate if there is a change in chemical composition. Physical and chemical analysis will be carried out in our laboratory on these parameters: anions, cations, gases, temperature, pH, electrical conductivity, dry residue, sulfuric control, $KMnO_4$, silicon acid according to methods used in laboratory. Our laboratory is accredited for analysis of surface, ground, thermomineral and wastewater and certified by Ministry of Environment and Energy.

Expected scientific contribution: Suggested research will provide balneological analysis of all springs of thermomineral water, their classification according to chemical composition and making register. Balneological analysis will provide opinion for possible use of springs in balneotherapy and spa therapy or rehabilitation after surgery. Optained results would significantly contribute to possible usage of thermomineral waters in balneotherapy, health tourism or as a source of geothermal energy, also as basis for legislation, because Croatia has no legislation for use of thermomineral waters in balneotherapy or spa.

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

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