DAN DOKTORATA

KNJIGA SAŽETAKA

PhD DAY 2018.

ABSTRACT BOOK

Sveučilište u Zagrebu, Medicinski fakultet / University of Zagreb, School of Medicine

Dan doktorata 2018 / PhD Day 2018

Knjiga sažetaka / Abstract Book

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ISBN: 978-953-176-848-1

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Sveučilište u Zagrebu – Medicinski fakultet
Zagreb, 2018.

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Preface

PhD Day (Dan doktorata) started in 2012 and quickly became an annual event of PhD students of both PhD programs (I – Biomedicine and Health Science, and II – Neuroscience) at the University of Zagreb School of Medicine. We are glad to see that this year 143 of our PhD students (128 from Biomedicine and Health Science, and 15 from Neuroscience) will present their ongoing research at the PhD Day in a form of published abstracts, posters and selected oral presentations. PhD Day, in the form of a one day symposium, is mandatory for all the 2nd-year and 3rd-year students. This symposium is a welcome opportunity for our PhD students (for some students this is their first chance to give an oral presentation) and their mentors to exchange ideas among themselves, to present their data to their teachers and experts and get either positive or negative feedback about their scientific work. Thus, this kind of gathering of PhD students, mentors and distinguished internationally recognized scientists provides a great occasion to share experiences and ideas in specific research field as well as in science in general. We are honored to welcome two renowned scientists this year: Ms. Anna Hoerder Suabedissen, a postdoctoral research scientist at the Department of Physiology, Anatomy and Genetics, University of Oxford, UK and Ms. Marija Balic, associate professor at the Department of Internal Medicine, Medical University Graz, Austria who will be giving lectures from their respective reserch fields.

We wish that this PhD Day will be fruitful and useful for all participants, and we hope that it will continue to be a traditional day at our institution which celebrates science, research, co-operation and scientific friendship.

Marijan Klarica

Marijan Klarica

PhD Day 2018 PROGRAMME – 25 May 2018

"Biološka" Hall, School of Medicine, Šalata 3, Zagreb

10.00 – 10.30	Opening / Greetings (dean, Prof. Batinić, Prof. Borovečki, Prof. Vukšić)
10.30 – 11.15	Anna Hoerder Suabedissen: Subplate through the ages: from development to adult connectivity
11.15 – 12.00	Marija Balić: Liquid biopsy in cancer patients, a tool for diagnosis, prognosis or treatment decision
12.00 – 13.15	Selected students' presentations:
	Ana Babić - Cognitive and metabolic effects of oral galactose in streptozotocin-induced rat model of sporadic Alzheimer's disease
	Darko Orešković - Expression of perineuronal nets in the human insula during fetal and perinatal development
	Irena Makovac - Presence of BRAF V600E mutation and lack of CPSF2 expression as prognostic markers for papillary thyroid cancer
	Magdalena Karadža - Molecular analysis of human papilloma viruses in females with high-grade cervical intraepithelial neoplasia and cervical carcinoma
	Iva Topalušić - Allergic diseases in childhood - developmental origin of health and disease hypothesis
13.30 – 14.30	Lunch break
14.30 – 16.00	Poster Session and Evaluation

RESEARCH EXCELLENCE LECTURES

SUBPLATE THROUGH THE AGES: FROM DEVELOPMENT TO ADULT CONNECTIVITY

Anna Hoerder-Suabedissen

Department of Physiology, Anatomy & Genetics,
University of Oxford, UK

Subplate neurons have an essential role in cortical circuit formation. They are among the earliest formed neurons of the cerebral cortex, are located at the junction of white and grey matter . They are necessary for correct thalamocortical axon growth towards the cortex, including crossing the pallial-subpallial boundary, correct areal targeting and formation of periphery related innervation patterns such as ocular dominance columns and formation of barrels in rodent somatosensory cortex . Recent transcriptomic studies have provided opportunities for monitoring and modulating selected subpopulations of these cells. Several markers including connective tissue growth factor and complexin 3 are suitable for identifying subplate neurons across a wide range of species during later developmental stages. Analyses of mouse lines expressing reporter genes have demonstrated a novel, extra-cortical site of subplate neurogenesis , and highlighted that the early born cells of the subplate compartment are continuous with the cells residing in layer 6b, adjacent to the white matter, in adult rodent brains. Moreover, layer 6b cells integrate into the mature cortico-thalamic circuitry in unique patterns that are distinct from those of the other cortico-thalamic projection neuron groups.

Hoerder-Suabedissen A, Hayashi S, Upton L, Zachary N, Casas-Torremocha D, Eleanor G, Sarada V, Kanold PO, Fracisco C, Kim Y, Molnár Z. 2018. Subset of Cortical Layer 6b Neurons Selectively Innervates Higher Order Thalamic Nuclei in Mice. Cereb Cortex.

Hoerder-Suabedissen A, Molnár Z. 2015. Development, evolution and pathology of neocortical subplate neurons. Nat Rev Neurosci. 16:133–146.

Kostovic I, Rakic P. 1980. Cytology and time of origin of interstitial neurons in the white matter in infant and adult human and monkey telencephalon. J Neurocytol. 9:219–242.

Pedraza M, Hoerder-Suabedissen A, Albert-Maestro MA, Molnár Z, De Carlos JA. 2014. Extracortical origin of some murine subplate cell populations. Proc Natl Acad Sci U S A. 111:8613–8618

LIQUID BIOPSY IN CANCER PATIENTS, A TOOL FOR DIAGNOSIS, PROGNOSIS OR TREATMENT DECISION

Marija Balić

Department of Internal Medicine,
Medical University Graz, Austria

There is a growing interest in analysis of biomarkers circulating in peripheral blood of cancer patients, and the term liquid biopsy has been used as a synonym for diverse circulating markers including circulating tumor cells (CTCs), cell free DNA and particularly circulating tumor DNA (ctDNA), but also traditional tumor markers or others. CTCs have been shown to play a central role in tumor dissemination and metastases, which are ultimately responsible for most cancer deaths. Technologies that allow for identification and enumeration of rare CTC from cancer patients' blood have already established CTC as an important clinical biomarker for cancer diagnosis and prognosis. Whereas it can be assumed that the clinical utility of CTC will be fully realized once CTC can be reliably cultured to and proliferated as a biospecimen for precision management of cancer patients and for discovery of novel therapeutics, ctDNA holds a greater promise for clinical diagnostics, as has been shown recently in EGFR mutated lung cancer or PIK3CA mutated breast cancer. Moreover, CTC and ctDNA allow for better understanding of molecular processes involved in the progress of disease, including epithelial to mesenchymal transition, cancer stem cells, and metastasis.

PRELIMINARY RESEARCH RESULTS

Basic Medical Sciences

EFFECT OF PENTADECAPEPTIDE BPC 157 ON VESICOVAGINAL FISULA HEALING IN RATS

PhD candidate: Domagoj Rašić

Part of the thesis: Effect of pentadecapeptide BPC 157 on vesicovaginal fisula healing in rats

Mentor(s): Assoc. Prof. Alenka Boban Blagaić, MD PhD, Marko Sever, PhD, research associate

Affiliation: University of Zagreb School of Medicine, Department of Pharmacology; University of Zagreb School of Medicine, Department of Pathology; University of Zagreb School of Medicine, Department of Surgery

Introduction: Vesicovaginal fistula is anomalous connection between the bladder and the vagina, which allows direct contact between these two separate organs, and represents a great difficulty in healing. It occurs as a complication of surgical procedures on urogenital system, as a complication of childbirth and radiation of tumors of the genitourinary tract. As such it represents medical, social and economical problem that undermines the quality of life of women. Formed vesicovaginal fistula requires long-term treatment often with a number of surgical procedures in the presence of incontinence and frequent recurrence.

Materials and methods: For the model of vesicovaginal fistula, female Wistar rats that weight 200 g were used. After lower median laparotomy in deep anesthesia, an incision was made on anterior wall of vagina and rear wall of urinary blader in the length of 4 mm and vesicovaginal fistula was formed. Pentadecapaptide BPC 157 dissolved in saline solution was applied in daily doses of 1 µg/kg, 10 ìg/kg, 100 ng/kg, 10 ng/kg intraperitoneally during the entire duration of the experiment starting 14 days afer surgery (7, 14, 21, 28, 42 days) and the control group of animals was given daily equivalent amount of 0.9% NaCl ip. The animals were in deep anesthesia during the sacrifice to measure the pressure required for leakage of fluid through fistula. After the sacrifice the diameter of the fistula, macroscopic changes and differences in fistula healing between groups was observed and compared. Tissue was taken for microscopic analysis.

Results: In general, unlike poor healing in controls, BPC 157 reduced fistulas diameters (significantly since 2 week period) leading to the complete closure. Finally, the fistula was successfully healed in all rats that underwent BPC 157 therapy (i.e., 6 week, 0+/0 mm diameter bladder/vagina), considerable volume needed to leakage of fluid through fistula (V: 5.3+/-0.3 mL), in rats treated intraperitoneally, unlike open defects in controls: 3.1+/-0.6 mm (bladder), 3.3+/-0.4 mm (vagina) diameter and small volume sustained before leakage (V: 1.2+/-0.4 mL). In the control group of animals urinary stones were found, while in the group treated with pentadecapeptide BPC 157 where was the closure of the vesicovaginal fistula and there was absence of urinary stones.

Discussion: For now, there is no adequate pharmacological therapy of vesicovaginal fistula and therefore we believe that the results of this study could lead to new therapies and thus contribute to the success of the treatment and improvement of the quality of life of the patients with vesicovaginal fistula. Also, the pilot study already carried out is a scientific contribution because it defines the model of experimental vesicovaginal fistula in the rat, which can also serve as an experimental model of bladder stones.

Acknowledgments: I would like to thank my mentors associate professor Alenka Boban-Blagaić, MD, PhD and research associate Marko Sever, MD, PhD as well as full professor Predrag Sikirić, MD, PhD for their great support and assistance

MeSH/Keywords: BPC 157, vesicovaginal fistula, rats

Poster code: R-01-03-003

COGNITIVE AND METABOLIC EFFECTS OF ORAL GALACTOSE IN STREPTOZOTOCIN-INDUCED RAT MODEL OF SPORADIC ALZHEIMER'S DISEASE

PhD candidate: Ana Babić

Part of the thesis: Therapeutic potential of oral galactose on cognitive and metabolic changes in 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: Sporadic Alzheimer's disease (sAD) is associated with dysfunction of the brain insulin receptor signaling and decreased glucose metabolism and energy in the brain which have become new targets in sAD therapeutic strategy. D-galactose, a C-4-epimer of D-glucose, could be considered as an alternative source of energy but can also stimulate secretion of glucagon-like peptide 1 (GLP-1), an incretin which stimulates insulin release and has its own neuroprotective activity. Our preliminary research in a streptozotocin-induced (STZ-icv) rat model of sAD demonstrated that one month of oral galactose treatment initiated immediately after STZ-icv administration successfully prevented the development of STZ-icv induced cognitive deficits. With this research we aimed to explore if oral galactose treatment could improve already developed cognitive deficit in the STZ-icv rat model and which mechanisms might be involved.

Materials and methods: Two-month oral galactose treatment (200 mg/kg/day) was initiated 1 month (Exp 1/early sAD stage) and 4 months (Exp 2/medium sAD stage) after STZ/buffer-icv injection (3 mg/kg) in 3-months old male Wistar rats. Cognitive performance was tested by Morris Water Maze (MWM) and Passive Avoidance (PA) test. The level of GLP-1 (active/total), insulin, glucose and galactose was measured by ELISA immunoassay in plasma and cerebrospinal fluid (CSF). Brain glucose metabolism was measured in vivo by [18F]-fluorodeoxyglucose positron emission tomography (FDG-PET) scanning. Data was analyzed by Kruskal-Wallis, followed by Mann-Whitney U test and 2-way ANOVA for repeated measures ($p<0.05$).

Results: STZ-treated animals showed cognitive decline in both experiments compared to their respective control (-85% PA, +93% MWM/Exp1; -78% PA, +71% MWM/Exp 2; $p<0.05$), while galactose treatment successfully improved cognitive deficit only in Exp 1 (+349% PA, -36% MWM; $p<0.05$). FDG-PET scan indicated a time-dependent mild decrement in glucose metabolism in the brain of STZ-treated rats compared to the controls in both experiments (-3% Exp 1; -12% Exp 2) which was increased (+14% Exp 1; +38% Exp 2) by oral galactose treatment in STZ-icv rats. The level of active GLP-1 was found decreased in plasma of STZ-treated rats compared to the controls only in Exp 1 (-51%, $p<0.05$) which was normalized by galactose treatment (+70%, $p<0.05$). Compared to the untreated controls, galactose-treated control rats demonstrated decreased levels of active GLP-1 in plasma (-80%, $p<0.05$) and total GLP-1 in CSF (-31%, $p<0.05$) in Exp 1 only. Plasma and CSF insulin levels were remained unchanged regardless the treatment in both experiments.

Discussion: Our preliminary results indicate that 2-month oral galactose treatment has a potential to normalize previously developed cognitive deficits in a STZ-icv rat model in early but not in medium-stage of experimental sAD. The mechanism of this cognitive improvement in early sAD stage might be the stimulation of GLP-1-mediated effects, as shown by increments in total/active GLP-1 plasma levels found previously decreased only in early stage of sAD in the STZ-icv rat model. Since oral galactose-induced improvement in brain glucose metabolism in the STZ-icv rat model was manifested both in early and medium stage of diseases, this mechanism does not seem to be the major contributor to the beneficial effects of oral galactose on cognitive impairment.

Acknowledgments: Supported by the Croatian Science Foundation; HRZZ-IP-09-2014-4639.

MeSH/Keywords: Alzheimer disease; streptozotocin; galactose; fluorodeoxyglucose F18; glucagon-like peptide 1; cognition; metabolism

Poster code: R-01-03-008

EFFECT OF PENTADECAPEPTIDE BPC 157 ON COLLATERAL CIRCULATION AFTER ACUTE OCCLUSION OF THE ABDOMINAL AORTA IN RATS

PhD candidate: Katarina Kašnik

Part of the thesis: Effect of pentadecapeptide BPC 157 on collateral circulation after acute occlusion of the abdominal aorta in rats

Mentor(s): Assoc. Prof. Alenka Boban Blagaić, MD PhD, Assoc. Prof. Zoran Brnić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: In this study, we investigated the effect of pentadecapeptide BPC 157 on rapid activation of collateral circulation after acute occlusion of abdominal aorta in rats.

Materials and methods: Female Wistar albino rats weighing 300 ± 20 g were used. Animals were randomized to control and pentadecapeptide BPC 157 treated group, and through time intervals of a) 1min b) 1h c) 6h and d) 24h. Each group had ten animals, and altogether 160 animals were used in the experiment. Deeply anaesthetised (diazepam 6 mg/kg i.p., thiopental 40 mg/kg i.p.) rats underwent abdominal aorta ligation with surgical suture at the level between the right iliolumbar artery and bifurcation of the aorta. The animals have etiher received an oral application of 10 μg/kg during pre-treatment assessment, or have received a dose of 10 μg/ kg of BPC 157 dissolved in saline as an intraperitoneal bath after surgery. The control groups received an equivalent volume of 0.9% NaCl. After induced occlusion at intervals of 1min, 1h, 6h and 24h, we analyzed the redistribution of collateral circulation using digital subtraction angiography (DSA). A fluoroscopy unit (Shimadzu type C-Vision Plus) and nonionic contrast medium (Iohexol; Omnipaque 350, GE Healthcare) were used to estimate collaterization after ligation. At intervals of 1min, 1h, 6h and 24h of ligation-time, angiograms were taken and analyzed using ISSA program, a PACS software (by VAMSTEC- Software Company, Zagreb). We used a VMS-004D USB Microscope (Veho) micro-camera to estimate branching of blood vessels in uterus and at distal part of descending colon.Walking test was performed in groups at 6h and 24h intervals. After sacrifice of probands, we have taken the samples of uterus, descending colon and leg muscles to pathohistologic analysis.

Results: Captured angiograms demonstrated that in the treated group there was much more collateral blood vessels. The main collaterals were anastomosis between left colic artery and middle colic artery, collaterals via ovarian artery and via lumbar arteries. Presentation of collateral blood vessels in the control group was significantly reduced and there was usually only one collateral blood vessel. Ilustratively, at 1 h interval, in rats underwent BPC 157 application a network of small interconnected blood vessels appears in colon and uterus reaching an increase ranging 30% of the initial values, while simultaneously controls exhibited a 30% decrease compared to the initial values.

Discussion: Pentadecapeptide BPC 157 counteracts the effects of acute occlusion of abdominal aorta at the bifurcation level. In control group, we observed much lower redistribution of collateral circulation at 1min, 1h, 6h, and 24h intervals using digital subtraction angiography (DSA), while in BPC 157 groups redistribution of collateral circulation (i.e. via anastomosis between left colic artery and middle colic artery, collaterals via ovarian artery and lumbar arteries) were observed.

MeSH/Keywords: ligation of abdominal aorta, pentadecapeptide BPC 157, collateral circulation, digital subtraction angiography

Poster code: R-01-03-037

THE EFFECT OF PENTADECAPEPTIDE BPC 157 ON CONSEQUENCES OF EPISCLERAL VEIN CAUTERISATION IN RATS

PhD candidate: Tamara Kralj

Part of the thesis: The effect of pentadecapeptide BPC 157 on consequences of episcleral vein cauterisation in rats

Mentor(s): Professor Predrag Sikirić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Glaucoma is an irreversible optic nerve neuropathy. This study aimed to evaluate the effect of BPC 157 on glaucoma-like features in the rat episcleral vein cauterization model. BPC 157, given locally or systemically, counteracts atropine mydriasis in rats.

Materials and methods: Female Albino Wistar rats, 220-250 g, randomly divided were used. After application of Ketamine-HCl 50–60 mg/kg + Xylazine-HCl 5–10 mg/kg, Tetrakain 0.5% drops, two dorsal episcleral veins and one temporal episcleral vein were isolated from the surrounding tissues. A cauterization was specifically applied to the selected vein. Rats recieved BPC 157 (Diagen doo, Ljubljana, Slovenia) dissolved in saline in dose of 10 ìg/kg and 10 ng/kg intraperitoneally or topically immediately before the surgery and then daily to the sacrification. Alternatively, rats received BPC 157 as posttreatmant 24 h after surgery at dose of 10 ìg/kg in same way of application as mentioned above. Controls received an equal volume of 0.9% NaCl. Intraocular pressure (IOP) (% of normal IOP), pupillar function were measured 24h, 4 and 6 weeks after procedure. Non-invasive IOP measurements were taken after local application of Tetrakain 0.5%, by aplanation tonometer Tonopen XL. Pupillar function was photographed by USB Veho Discovery VMS-004 Deluxe microcamera. Photographs were taken before and after application of BPC 157 or saline and analyzed with special software bought with camera for measurement of pupillar diameter (r=mm), range (C=mm) and surface (S=mm2). Camera was previously calibrated using graph paper. Vascularization of the eye fundus and presentation of papilla n. optici were analyzed with microcamera and Digital Widefield lupe 90D at 24 h, 4 weeks and 6 weeks after procedure. We focused our images on the vessels extending into and out of the optic disc. Retinal changes alterations in vessel caliber and tortuosity and optic disc pallor were observed and scored (Score 1–3). Microscopy analysis was performed after scarification performed at 24h, 4 and 6 weeks after surgery.

Results: 24 hours after surgery control rats exhibited the following values (r=1.3±0.1, C=7.3±0.4, S=6.0±0.2, IOP=125%). BPC 157 group values were: r=0.14±0.03, C=1±0.05, S=0.1±0.03, IOP=105%. At week 4 and 6 all BPC 157-rats exhibited only slight generalized vessel caliber irregularity (difference in diameter between arteries and veins) with a vaguely atrophic optic disc and an area not bigger than 1 optic disc diameter containing leaking of retinal vessels (Min/Med/Max 1/1/1). Controls exhibited increased disturbances: either moderate generalized vessel caliber irregularity (bigger difference in diameter between arteries and veins with moderate overfilling of veins) Min/Med/Max 2/2/3; with moderate atrophic optic discs Min/Med/Max 2/2/3; and an area not more than 3 optic disc diameters containing leaking of retinal vessels Min/Med/Max 2/2/3; or severe generalized vessel caliber irregularity (very big difference in diameter between arteries and veins with severe overfilling of veins) with severe atrophic optic disc and an area more than 3 optic disc diameters with leaking of retinal vessels (all Min/Med/Max 3/3/3). Microscopy analysis correlates well with ophthalmoscopy findings (i.e., BPC 157 groups diameter of the optic nerve remained close to normal unlike control's atrophy/thinning of the optic nerve).

Discussion: Preliminary results that we have obtained so far suggest that pentadecapeptide BPC 157 used intraperitoneally counteracts the observed effects of episcleral vein cauterization.

MeSH/Keywords: Glaucoma, episcleral vein cauterisation, pentadecapeptide BPC 157

Poster code: R-01-03-049

USE OF A STABLE GASTRIC PENTADECAPEPTIDE BPC 157 IN RATS WITH SUPERIOR MESENTERIC ARTERY LIGATION OR WITH SUPERIOR MESENTERIC VEIN LIGATION OR WITH SIMOULTANEUS SUPERIOR MESENTERIC ARTERY AND SUPERIOR MESENTERIC VEIN LIGATION

PhD candidate: Mario Knežević

Part of the thesis: Use of a stable gastric pentadecapeptide BPC 157 in rats with superior mesenteric artery ligation or with superior mesenteric vein ligation or with simoultaneus superior mesenteric artery and superior mesenteric vein ligation

Mentor(s): Assoc. Prof. Leonardo Patrlj, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: We focused on ligated superior mesenteric artery (SMA), superior mesenteric vein (SMV) and superior mezenteric artery and vein lesion in rats (SMA+SMV) and the therapy with the stable gastric pentadecapeptide BPC 157. Cytoprotective agents share an essential endothelium protection that would rapidly propagate to mucosal integrity in stomach cytoprotection studies. With the stable gastric pentadecapeptide BPC 157, as a cytoprotective prototype, we extended this beneficial effect on endothelium integrity.

Materials and methods: Rats underwent SMA, SMV or SMA and SMV- ligation were recorded throughout 30 min period (USB microscope camera): depending whether disappeared (empty) or accessible (filled) (scored 0-3), SMA, inferior pancreaticoduodenal artery (IPDA), arch of Riolan (AR) with gross lesion presentation, as well as depending on vein congestion presentation upon SMV ligation (scored 0-3), presentation of SMV cranial or caudal to ligation, inferior mesenteric vein (IMV) that bridges ligation site at the SMV with gross lesion presentation (duodenum, jejunum, caecum and ascending colon). Medication was bath (BPC 157 10 ug, 10 ng/kg/1ml bath/rat; equivolume saline (controls)) at 1 min ligation-time. Angiography (Shimatzu, DSA C Vision) was at 30 min ligation-time. After 30 minutes the animals were euthanized according to established standards. The specimen of the small intestine, cecum and ascending colon will be taken after the sacrifice for pathohistological analysis.

Results: Unlike progressive failure in control SMA-ligated rats (USB microscope camera, angiography recording), after BPC 157 application, we noted all vessels (IAPDA, AR) quickly propagating toward bypassing the hindering defect, and replete original SMA flow, the vasa recta vessels (VRV) reappear at the intestine surface. In angiography, SMA presentation was clearly shown. This goes to regain tissue integrity (intestinal preservation with only mild villous edema and no congestion or submucosal edema and no elevation of epithelium from lamina propria). Progressive failure appeared control SMV-ligated rats (SMV collapsed cranial to ligation; SMV congested caudal to ligation; IMV congested; the vasa recta vessels congested with hemorrhage). By contrast, after BPC 157 application, we noted SMV filled cranial to ligation and non-congested caudal to ligation, IMV non-congested and bridging ligation (obstruction) defect. Thereby, rescued original SMV flow, the vasa recta vessels reappear at the intestine surface without hemorrhage and prominent branching and ramification. Angiography showed SMV presentation. This goes to regain tissue integrity and duodenum, jejunum, caecum congestive hemorrhagic lesion largely attenuated. SMA+SMV-ligated rats have SMV collapsed cranial to ligation; SMV congested caudal to ligation; IMV congested; SMA collapsed, IPDA, AR poorly presented; the vasa recta vessels congested with hemorrhage. After BPC 157 application, we noted SMV filled cranial to ligation and non-congested caudal to ligation, IMV non-congested and bridging ligation defect. IPDA, AR quickly propagate toward bypassing the obstruction and rescue original SMA flow. Vasa recta vessels reappear at the intestine surface without hemorrhage and with prominent vessel branching and ramification. Angiography showed SMA and SMV presented. This goes to regain tissue integrity and duodenum, jejunum, caecum congestive hemorrhagic lesion mitigated.

Discussion: We demonstrated in rats that BPC 157 application during ischemia may be a particular therapy of SMA or SMV occlusion disturbances. After SMA/SMV/SMA+SMV ligation a consistent assessment reveals a rapid effect of BPC 157 administration that prevents and reverses the consequent lesions and reorganizes blood flow to activation of particular major collaterals (IPDA, AR, IMV). These findings are consistent with the previous BPC 157 beneficial effect on vessels "running" and lesions attenuation, noted in rats underwent perforated stomach lesion or ischemic colitis, ischemia and reperfusion.

MeSH/Keywords: Acute mesenteric ischemia, superior mesenteric artery and vein ligation, BPC 157

Poster code: R-01-03-057

CHRONIC DEEP VEIN THROMBOSIS: PENTADECAPEPTIDE BPC 157 EFFECT

PhD candidate: Marko Siroglavić

Part of the thesis: The effect of pentadecapeptide BPC 157 on chronic deep vein thrombosis in rats

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

Affiliation: University of Zagreb School of Medicine

Introduction: Deep vein thrombosis (DVT), with incidence of 1 on 1000 in adult population, presents a major health issue and large financial burden on health system. Previous research has shown the effect of pentadecapeptide BPC157 on acute DVT and on blood vessels, but it's effect on chronic DVT was not investigated.This research would determine the effect of BPC 157 on thrombi resolution in chronic DVT.

Materials and methods: This research would determine the effect of BPC 157 on Wistar rats (females, 200 g) following the ligation of inferior caval vein below the left renal vein, in time frame of 4 and 7 days. According to experimental protocol, treated animals will receive BPC 157 (1mcg/mL, 1ng/mL) following operation and on the fourth day as post-treatment therapy intraperitoneally, and control animals will receive 1mL of saline in aforementioned intervals.

Results: Pentadecapeptide BPC 157 was shown to reduce thrombi size after trombotic incident, protect the endothelium and increase the number of collateral vessels.

Discussion: This study has shown a significant beneficial effect of tested substance following a thrombotic incident.

MeSH/Keywords: deep vein thrombosis, chronic, BPC157, inferior caval vein

Poster code: R-01-03-080

THERAPEUTIC EFFECTS OF PENTADECAPEPTIDE BPC 157 ON GENTAMICIN INDUCED TUBULAR NECROSIS IN RATS

PhD candidate: Ivan Vukoja

Part of the thesis: Effects of pentadecapeptide BPC 157 on gentamicin induced tubular necrosis in rats

Mentor(s): Professor Predrag Sikirić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Almost 10% of acute renal failure is caused by aminoglycosides. Because aminoglycosides are potent antibiotics which are used with lot of reserves it is desirable to research the effects of pentadecapeptide BPC 157 as nephroprotective agent. In this research we investigated the effect of pentadecapeptide BPC 157 as a therapy for gentamicin induced tubular necrosis in rats, caused by administration of high dose of gentamicin (100mg/kg b.w.). Precise effect of aminoglycosides in tubular epithelium is not known, but it is noted increased values of renal lipid peroxidase and reduced activity of superoxid dismutase and catalase as well as glutation peroxidase, glutation-S-transferase and values of glutation. There is also noticed increased activity of inducibile NO syntetase (iNOS) and core factor NF-êB. Commonly known dual role of NO-system should be relevant for crucially implicated homeostasis. NO-elicited events act as triggers by which mitochondria modulate signal transduction cascades involved in the induction of cellular defense mechanisms and adaptive responses, but the effects of NO on the electron transport chain might lead to mitochondrial dysfunction and pathology. We strongly believe that BPC as nephroprotective agent modulate hyperactivitiy of iNOS and NO as oxygen radical in tissues.

Materials and methods: Wistar albino male rats (150–200g b.w.), were used, randomly grouped as follows: I. control group, treated with gentamicin 100 mg/kg b.w. for 7 days, II. BPC 157 group with BPC 157 10ng/kg b.w. i.p. and gentamicin 100 mg/kg b.w. i.p. for 7 days, III. BPC 157 group with BPC 157 10ng/kg b.w. p.o. and gentamicin 100 mg/kg b.w. i.p. for 7 days, IV. BPC 157 group with BPC 157 10ìg/kg b.w. i.p. and gentamicin 100 mg/kg b.w. i.p. for 7 days, V. BPC 157 group with BPC 157 10ìg/kg b.w. p.o. and gentamicin 100 mg/kg b.w. i.p. for 7 days.

Results: Clinical status rate – two independent observers rated clinical status and overall condition and appearance of treated animals. BPC treated rats shown better score. Daily diuresis – BPC 157 treated rats had smaller daily diuresis in polyuric phase of aminoglycoside nephrotoxicity and anuric phase in BPC 157 treated rats wasn't noticed. Serum creatinine and electrolytes – values of serum creatinine were higher in control group comparing to BPC 157 treated rats. Pathomorphologic: right before immolation mass of the each animal was measured. After the immolation weight of both kidneys were taken, and average weight of kidney was divided by the mass of each animal. That ratio was lower in BPC 157 treated rats.

Discussion: We have noticed that pentadecapeptide BPC 157 has nephroprotective effect in aminoglycoside nephrotoxicity. Rats treated with pentadecapeptide BPC 157, without significant difference in applied dose (microgram or nanogram) and without significant difference in BPC 157 administration way (perorally or intraperitoneally), have better condition, clinical status and lower serum creatinine levels, while smaller daily diuresis during polyuric phase and anuric phase wasn't noted in BPC 157 treated rats. Control group has also higher kidney/body mass ratio. Therefore we conclude pentadecapeptide BPC 157 has the nephroprotective effect in aminoglycoside induced nephrotoxicity, what demands further research.

MeSH/Keywords: BPC 157, gentamicin, tubular necrosis, rat

Poster code: R-01-03-081

DNA PROMOTER METHYLATION STATUS AND PROTEIN EXPRESSION OF PTCH1, SHH AND IHH IN SEROUS OVARIAN CARCINOMAS

PhD candidate: Valentina Karin

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

Mentor(s): Assoc. Prof. Ljiljana Šerman, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: The Hedgehog (Hh) signaling pathway is an evolutionarily conserved pathway of signal transmission which plays a significant role in the normal embryonic development of invertebrates and vertebrates. In the adult organism, Hh signaling pathway is mostly inactive or poorly active whereas its hyperactivation is associated with carcinogenesis. Binding of the Hh ligands Sonic Hedgehog (SHh), Indian Hedgehog (IHh) and Desert Hedgehog (DHh) along with PTCH protein activates Hh signaling, resulting in increased activity of the GLI transcription factors that activate targeted genes. The status of Hh pathway components in serous ovarian carcinomas is poorly understood.

Materials and methods: Formalin-fixed paraffin-embedded samples of 11 low-grade serous ovarian carcinomas (LGSC), 40 high-grade serous ovarian carcinomas (HGSC) and 7 normal/benign ovarian tissues (controls) were used for this study. Expression of PTCH1, SHh and IHh proteins were examined using immunohistochemistry. DNA methylation pattern of PTCH1, SHh and IHh genes were analyzed by methylation-specific PCR (MSP).

Results: PTCH1 nuclear expression and expression of SHh and IHh were significantly higher in both LGSC ($p<0.001$, $p<0.001$ and $p=0.011$, respectively) and HGSC ($p<0.001$, $p<0.001$ and $p=0.003$, respectively) compared with normal/benign ovarian tissues. There were no statistically significant differences in expressions of SHh and IHh proteins and PTCH1 nuclear expression between LGSC and HGSC. PTCH1, SHh and IHh DNA methylation was observed exclusively in HGSC Š(6/40, 15%), (2/40, 5%) and (1/40, 2.5%), respectivelyĆ. No methylation was detected in any of the normal (control) ovarian tissue.

Discussion: A significant proportion of serous ovarian carcinomas exhibits increased expression of SHh and IHh proteins, which indicates that these Hedgehog signaling pathway components may be actively involved in pathogenesis of serous ovarian carcinomas. A significant proportion of serous ovarian carcinomas exhibits aberrant (nuclear) PTCH1 protein expression. Therefore, PTCH1 may play an active transcriptional role in LGSC and HGSC. A subset of HGSC may have a PTCH1 gene deregulation caused by its promoter methylation. Low methylation levels of SHh and IHh genes in HGSC and absence of methylation in LGSC and normal ovarian tissues indicate that there are alternative mechanisms of SHh and IHh activation. Further clinical studies should confirm the clinical and therapeutic relevance of the observed Hh 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, SHh, IHh

Poster code: R-01-05-005

MYELOID-SPECIFIC MOLECULAR MEDIATORS OF SUBCHONDRAL BONE DAMAGE IN ANTIGEN-INDUCED ARTHRITIS

PhD candidate: Nina Lukač

Part of the thesis: Call and molecular mediators of subchondral bone damage in antigen-induced arthritis

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

Affiliation: University of Zagreb School of Medicine

Introduction: Rheumatoid arthritis (RA) is a chronic autoimmune joint inflammation marked by cartilage and bone destruction, and subsequent permanent disability. Currently available therapeutics improved the prognosis, but still have limited effect on the attenuation and reversal of bone destruction. Using antigen-induced arthritis (AIA), animal model of RA, we found that mice deficient for Fas gene (Fas-/-) develop non-destructive arthritis, marked by lower frequency of myeloid (CD11b+Gr1+) cells in the synovial compartment. We aim to identify myeloid-specific molecular mediators driving bone-resorption in AIA, by analyzing differentially expressed genes in sorted myeloid population from wild-type (WT) and Fas -/- mice with AIA.

Materials and methods: AIA was induced by two subcutaneous doses of methylated bovine serum albumin (mBSA) in complete Freund's adjuvant, and intra-articular injection of mBSA. Bone resorption was assessed by μCT. Synovial cells were released by collagenase, labeled with anti-mouse CD45-FITC, CD11b-PE, Gr1-PECy7, B220/CD3/NK1.1/CD31/TER119-APC, and CD51-APCeF780, and CD11b+Gr-1+ population was sorted using BD FACSAria. RNA was extracted by Trizol, amplified by GeneChipTM WT Pico Kit, and hybridized to Affymetrix ST 2.0 arrays. Signal intensities were pre-processed, differential expression analysis was performed with Bioconductor project tools in R, while gene enrichment analysis was done using ToppGene. Differences in gene expression were confirmed by qRT-PCR in sorted populations and total joint tissue extracts.

Results: Synovial CD11b+Gr1+ population from Fas -/- mice with AIA is largely similar to this population in WT mice. Samples split into two hierarchical clusters dominantly represented predominantly by Fas -/- or WT samples. WT-dominant cluster revealed up-regulated genes related to cell cycle progression and mitotic activity, suggesting that majority of WT myeloid cells have higher proliferative activity. Mid1 and Erdr1 genes were down-regulated in joints from Fas -/- mice with AIA. According to PCR validation, Mid1 is clearly up-regulated in AIA in comparison to non-immunized mice.

Discussion: Inflammation in resorptive AIA is marked by higher myeloid proliferation potential. Mid1 gene is a potential novel mediator for targeting inflammation-mediated joint destruction in arthritis. Activation of this gene has already been reported for allergic airway inflammation, and dependent on death receptor TRAIL.

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

MeSH/Keywords: antigen-induced arthritis, bone destruction, Fas, myeloid cells, rheumatoid arthritis

Poster code: R-01-06-115

THE EFFECT OF PENTADECAPEPTIDE BPC 157 ON ISCHEMIC/REPERFUSION INJURIES IN RAT BRAIN

PhD candidate: Jakša Vukojević

Part of the thesis: The effect of pentadecapeptide BPC 157 on ischemic/reperfusion injuries in rat brain

Mentor(s): Assoc. Prof. Alenka Boban Blagaić, MD PhD, Assist. Prof. Danijela Kolenc, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Ischemic/reperfusion injuries are elementary pathophysiological findings in stroke, making it the third most common cause of death and the first cause of long-term disability. Pentadecapeptide BPC 157, has already been proven to have an effect on vessel integrity and protection, it interacts with the NO system and has healing properties in different tissues, all of which, make it a promising agent when it comes to cerebral ischemic/reperfusion injuries .

Materials and methods: In this experiment, ischemic/reperfusion injuries are induced using bilateral carotid artery occlusion (BCAO). The effect of BPC 157 on ischemic/reperfusion injuries was investigated in male Wistar rats. After an occlusion of 20 min, the rats were randomly divided into groups. The treated group received BPC 157 (10ìg/kg, I.P.) right after surgery, while the control group received saline (1ml, I.P.) immediately after surgery. To test the relation with the nitric oxide (NO) system, we created three new groups, to which we administered L-NAME (5 mg/kg, I.P.) alone, in combination with L-arginine (100mg/kg, I.P.) or BPC 157 respectively. After a reperfusion period of 24 hours, the neurological assessment was performed and samples were taken. Neurological assessment was conducted using the Morrison water maze test (MWMT) and beam walk test (BWT).

Results: In the MWMT the control animals had far greater memory loss and spatial orientation loss, while the BPC 157 treated group had almost no loss in the MWMT. In the beam walk test, we also observed substantial differences between the control and treated group, where the control group walked far worse than the BPC 157 treated group. The animals treated with L-NAME scored worst, of all groups, in the MWMT as well as in the BWT. When L-NAME was administrated along with L-arginine, it showed slight improvement, while the combination of L-NAME and BPC 157 abolished all the negative effects of L-NAME. The pathology findings concurred with the results obtained in the neurological assessment. Neuronal death was significantly lower in BPC 157 treated group after 24h as well as 72h. On top of this we conducted Real Time qPCR study where we evaluated the differences in RNA expression between the two groups, with multiple genes involved in various aspects of angiogenesis and injurie healing as well as NO sinthesys. All of the mentioned results where analyzed using parametric statistical methods ($p < 0,05$ was considered significant) and the sample size, 8 animals per group, was tested using power test analysis.

Discussion: Pentadecapeptide BPC 157 showed that it counteracts ischemic/reperfusion injuries, saving the rats from memory and orientation loss, as well as maintaining their motor capabilities. Along with that it can successfully counteract the negative effects of NO system inhibition and thereby confirming its close relation to the NO system. The results we present here are promising and prove that BPC 157 has potential as a neuroprotective agent in cerebral injuries, although further investigations should be conducted to confirm the full effects of BPC 157.

MeSH/Keywords: stroke; BCAO; pentadecapeptide BPC 157; nitric oxide; real time pcr

Poster code: R-01-08-047

PERINATAL AND EARLY POSTNATAL DEVELOPMENT OF BASAL TELENCEPHALON IN HUMAN

PhD candidate: Matea Baljkas Barković
Part of the thesis: Perinatal and early postnatal development of basal telencephalon in human
Mentor(s): Academician Ivica Kostović
Affiliation: University of Zagreb School of Medicine-Croatian Institute for Brain Research

Introduction: N. basalis is a part of a cholinergic neurons chain of mediobasal telencephalon, and in humans, it contains 70-90% of ChAT positive neurons. Complex of magnocellular nuclei of basal telencephalon plays the key role in cholinergic modulation of multiple areas of telencephalon, and especially cerebral cortex. Prenatal development of nucleus basalis has been thoroughly described in the early papers of I. Kostović and Zagreb research group. Perinatal development, basal telencephalon growth indicators, apart from the preliminary observation of this area by MR volumetry, as well as the possible perinatal and postnatal reorganization of cellular organization of nucleus basalis in human are not known. By using Magnetic Resonance Imaging and advanced computer programs for the morphometric analysis, surface and volume of different cerebral structures may be quantified.

Materials and methods: In this paper, we shall, for the first time, compare the development of basal telencephalon by histology analysis on the material of Zagreb Neuroembryological Collection and by in vivo and in vitro MR Imaging in the key moment of telencephalon development – late fetal, perinatal and postnatal period up to 1 year of age. The results of quantitative MRI analysis of the area of nucleus basalis and cortical areas, which indicate specific coordinated growth, shall be correlated to histology markers of microstructural reorganization of said areas.

Results: Based on the results of quantitative MRI analysis of the area of nucleus basalis and cortical areas, from the 20 in vivo and in vitro MRI samples processed so far, in the late fetal, perinatal and postnatal period (from 25 PCW up to 30 days of age), we presume that the changes of volume show developmental changes which are chronologically harmonized with the growth and perinatal reorganization of the cerebral cortex, especially of the frontal lobe in the same series. The study is stil in progress and the results are still not complete. Conclusions were made on a small sample. The study should be continued and data subjected to more detailed statistical analysis to have definitive conclusion. Regarding the changes of volume, cytoarchitectonic organization and histochemical properties of Nucleus basalis on the material of Zagreb Neuroembryological Collection, the data is still collected and there are not any preliminary results available.

Discussion: Nucleus Basalis is an important source of extrathalamic subcortical innervation of the cerebral cortex in brain of fetus and adult. The information on the normal development form a basic prerequisite for research of normal development, research of disrupted development after perinatal damage, especially hypoxic-ischemic episodes, and may be very helpful in researching disturbances of cerebral circuits innervation (Down syndrome, schizophrenia).

Acknowledgments: This work has been supported by CSF IK-4517

MeSH/Keywords: nucleus basalis, MRI, prematurus, cholinergic neurons, perinatal development

Poster code: R-01-08-071

CHANGES IN MICROSTRUCTURE OF WHITE MATTER SEGMENTS AS INDICATORS OF GROWTH AND DEVELOPMENT OF AXONAL PATHWAYS IN THE HUMAN PRETERM BRAIN

PhD candidate: Iris Žunić Išasegi

Part of the thesis: Analiza mikrostrukture prolaznih fetalnih zona i segmenata bijele tvari ljudskoga mozga histološkim metodama i magnetskom rezonancijom

Mentor(s): Academician Ivica Kostović

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

Introduction: Development of fiber pathways in the human brain is complex process in which transient fetal zones play important role in axonal growth, navigation, elongation and recognition of postsynaptic elements. Different classes of fiber pathways have distinct periods of intensive growth and development, more precisely defined within five segments of white matter by Von Monakow (I- corpus callosum with periventricular fibers; II- crossroads and sagittal strata; III- centrum semiovale; IV -gyral white matter; V- intracortical white matter), which leads to increase of vulnerability to noxious factors in sequential developmental periods. Well developed MRI techniques enabled us to visualize brain development in-vivo, and it became possible to diagnose various disorders of the fiber pathway growth in fetuses and prematurely born children. Fractional anisotropy (FA) and apparent diffusion coefficient (ADC) are used to represent microstructural changes in different pathologies of white matter, both in adult brain and developmental neurobiology, but never before in the context of a fine division of white matter by Von Monakow segments.

Materials and methods: MRI, using conventional and diffusion-weighted imaging (DWI), was performed in 34 preterm infants at term-equivalent age using a 3 Tesla MR scanner. The infants were divided into 3 groups on the basis of their age by birth expressed in postconceptional weeks (PCW): 1. extremely premature (22-28 PCW), 2. very premature (28-32 PCW) 3. control group (full-term birth). FA and ADC were measured radially along the cerebral wall, in I-IV segments in frontal and occipital lobe using Diffusion Toolkit and TrackVis programs. Segment V was not processed, due to the overlapping with cortex on MRI. Data were statistically processed in MATLAB® program, for comparison between two groups t-test was used, and significantly statistic results were considered if p?0.05.

Results: All segments differ mutually in FA and ADC values, except segment III and IV. There were statistically significant differences in FA values in frontal segments II, III, IV comparing control group and both premature groups; pronounced difference took place in segment II in occipital lobe. Considering ADC values, there were significant differences between full-term born children and both premature groups in segment III, both frontal and occipital, also with distinct difference in segment II in occipital lobe. Briefly, higher FA values and lower ADC values were found in control group, when comparing to groups of prematurely born infants.

Discussion: Maturational delay of white matter segments, expressed as differences in FA and ADC values, comparing to full-term-birth children, is present in group of extremely premature and very premature infants . Pronounced difference, manifested as lower FA and higher ADC values, in segment II indicates delay in the development and organization of microstructural elements in sagittal strata and periventricular crossroad, which contain complex vulnerabile cellular and fibrillar architecture, including optic radiation in occipital lobe. Further follow up in second year should discover if these changes disspear or become more subtle, implying that reorganization, as a consequence of brain plasticity, could play its role in postnatal brain development.

Acknowledgments: This work was supported by CSF-IP-09-2014-4517 and CSF-DOK-10-2015; co-financed by the European Union through the European Regional Development Fund, Operational Programme Cinanompetitiveness and Cohesion, grant agreement No.KK.01.1.1.01.0007, CoRE - Neur

MeSH/Keywords: white matter development, fractional anisotropy, apparent diffusion coefficient, human preterm brain

Poster code: R-01-08-113

EXPRESSION OF PERINEURONAL NETS IN THE HUMAN INSULA DURING FETAL AND PERINATAL DEVELOPMENT

PhD candidate: Darko Orešković
Part of the thesis: Izražaj perineuronskih mreža u fetalnom i perinatalnom mozgu čovjeka
Mentor(s): Professor Nataša Jovanov-Milošević, DVM PhD
Affiliation: University of Zagreb School of Medicine

Introduction: Perineuronal nets (PNNs) are lattice-like structures in the brain which are formed by the brain extracellular matrix condensing around certain neurons, as early as 12 PCW. Today it is well known that the perineuronal nets have a vital role in the fetal development and normal functioning of the brain. The insula is a region of the brain which, due to it's complexity, still eludes our full understanding. It is a structure which is not only involved with a wide variety of functions (both basic and higher) but also has a vast connectome connecting it to many different brain regions. The aim of this study was to determine the expression of perineuronal nets in the human fetal and perinatal insula using the Wisteria Floribunda Agglutinin (WFA), as a well-established method of visualizing them.

Materials and methods: The sample size was 30 fetal and 5 postnatal human brains aging from 12 PCW (when the insula still cannot be differentiated from the surrounding structures) up until 3 months postnatally.

Results: The results showed that the different laminar layers of the insula do in fact express the WFA reactivity differently and specifically. While there were no visible pericellular WFA staining in the Marginal Zone, the Cortical Plate showed a distinct pattern of WFA reactivity, where the PNNs were first visible around immature (probable) neurons at around PCW 28, with older specimens expressing them progressively stronger and more numerous, especially in the deeper layers. In the Pre-Subplate Zone, possible pericellular WFA reactivity can be seen in the deeper layers as early as 22 PCW. Convincing examples of pericellular WFA staining in the Subplate Zone can however be seen from 28 PCW onward, with the oldest specimens having intense diffuse WFA staining making the characteristic pericellular and perineuronal staining hard to observe. This layer is indeed one where the diffuse WFA staining can be seen most intensely throughout the observed periods. Lastly, in the Intermediate Zone, possible (albeit unconvincing) WFA pericellular reactivity can be seen even before 22 PCW. Both diffuse as well as pericellular (and perineuronal) WFA staining can be seen in this layer, especially in the upper layers, progressively more intensely throughout the fetal development, albeit more faintly compared to more superficial layers of the telencephalic wall.

Discussion: This investigation shows and describes the onset and the development of PNNs in the human insula, which, knowing the vast number of critical functions the PNNs perform in the normal as well as pathological brain, shows the direction where future investigative and therapeutical approaches could be performed.

MeSH/Keywords: Perineuronal nets, insula, fetal development

Poster code: R-01-08-129

THE PATHOHISTOLOGICAL PROFILE OF THE DRUG-RESISTANT MESIAL TEMPORAL LOBE EPILEPSY IN CROATIA

PhD candidate: Barbara Sitaš

Part of the thesis: Imunohistokemijska i histokemijska obilježja izvanstanične tvari hipokampusa osoba s farmakorezistentnom epilepsijom mezijalnoga temporalnoga režnja

Mentor(s): Professor Nataša Jovanov-Milošević, DVM PhD, Assist. Prof. Danijela Kolenc, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Epilepsy is one of the most common neurological diseases; 20 – 40% of patients have epileptic seizures that cannot be controlled with conventional antiepileptic therapy and thus present a special challenge in neurology and therefore, are usually candidates for neurosurgical treatment. The most common pathohistological diagnosis found in drug-resistant epilepsies are focal cortical dysplasia (FCD) and hippocampal sclerosis (HS).

Materials and methods: We retrospectively analysed patients' history from Referral Center for epilepsy, Department of Neurology; University Hospital Center Zagreb. Patients underwent surgical resection due to drug-resistant mesial temporal lobe epilepsy (MTLE) .

Results: In the period between 2011. – 2016. seventy-one (36 female and 35 male) patients were surgically treated. Pathohistologically 43 patients were confirmed as hippocampal sclerosis, HS type 1 predominantly. In samples of 25 patients FCD, coexisting HS and FCD or combination of HS, FCD with other malformations of neuronal migration or cortical development was found. Only five patients had FCD type 1 or 2. In 3 patients coexisting tumors (2 gangliogliomas and one dysembryoblastic neuroepithelial tumor) were present. The average age of partial seizures onset was 17,4 years. Medical history of febrile seizures (FS) in early childhood was positive only in a case of HS. During the treatment of disease, seizure control was not established with the combination of approximately seven antiepileptic drugs. Disease duration, until a neurosurgical treatment, was on average 21 years. After neurosurgical treatment, 42 patients were seizure free. The seizure-free rates were the highest in HS group while in HS 1 group only one patient was confirmed as not being seizure free.

Discussion: Almost all our MTLE patients had MRI HS, but pathohistologically in not that small group of patients coexistence of different types of FCD and HS was present, an association which according to literature is being increasingly recognized and thus implies a possible common pathogenesis. We should have taken into account possible, MRI extra-hippocampal abnormalities (ipsilateral thalamus and pallidum, widespread reductions in cortical thickness, especially motor cortex) since recent imaging studies of epilepsies show that these also have an impact on the seizure frequencies, epilepsy duration and postoperative outcome.

Acknowledgments: This work was supported by Referral Centre for Epilepsy, University Hospital Centre Zagreb,Department of Neurology. I would like to thank Assoc. Prof. Željka Petelin Gadže, M.D., PhD and other physicians of the Referral Centre for Epilepsy.

MeSH/Keywords: MTLE, hippocampal sclerosis, focal cortical dysplasia

Poster code: R-01-08-134

HYDROCEPHALUS IN PATIENTS WITH ANEURYSMAL SUBARACHNOID HEMORRHAGE TREATED WITH ENDOVACULAR COILING

PhD candidate: Ivan Jovanović

Part of the thesis: Predictors of hydrocephalus in patients with ruptured intracranial aneurysm treated with endovascular coiling

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

Affiliation: University of Zagreb School of Medicine

Introduction: Hydrocephalus after aneurysmal subarachnoid hemorrhage (SAH) is a well known complication, and has been reported to range from 6 to 67%. It is associated with more neurological impairment and mortality than subarachnoid hemorrhage without hydrocephalus. Our study is designed to identify factors predictive of hydrocephalus among patients with aneurysmal SAH who underwent endovascular treatment.

Materials and methods: Fifty-seven patients with aneurysmal SAH who were treated with endovascular coiling between 2013 and 2016 were retrospectively included in this pivotal study. Demographic data (age and sex) and neuro-imaging findings (CT and DSA images) were analyzed to determine the potential risk factors predictive of hydrocephalus. The initial and follow-up computed tomography (CT) images were reviewed, and the amount and distribution of blood and the occurrence of hydrocephalus were registered. Digital subtraction angiography (DSA) images were reviewed to determine morphological parameters (size and location) of ruptured intracranial aneurysms. Criteria indicating the occurrence of hydrocephalus were the presence of significantly enlarged temporal horns or ratio of frontal horn to maximal biparietal diameter more than 30% in computerized tomography.

Results: The female to male ratio was 2.4:1, the mean age of the patients was 56.2 years (range 31-85 years). Overall, 20 of the 57 patients (35%) included in this pivotal study developed hydrocephalus. Statistically significant associations among the following factors and hydrocephalus were observed: (1) intraventricular hemorrhage, (2) Fisher grade IV at admission and (3) radiological hydrocephalus at admission. The location and size of the ruptured aneurysms in posterior cerebral circulation, sex and age did not influence the development of hydrocephalus.

Discussion: Hydrocephalus after aneurysmal SAH seems to have a multifactorial etiology. Understanding predisposing factors related to hydrocephalus may lead to improved clinical outcomes in patients with aneurysmal SAH treated with endovascular coiling. Our preliminary data indicate that baseline CT findings can help identify patients with a high risk of developing hydrocephalus.

MeSH/Keywords: intracranial aneurysm, subarachnoid hemorrhage (SAH), endovascular treatment, hydrocephalus, neuro-imaging findings

Poster code: R-01-08-135

PERCEPTION AND REPRESENTATION OF TIME: DIFFERENCES IN DURATION DISCRIMINATION OF SHORT AUDIO STIMULI

PhD candidate: Ivan Šerbetar

Part of the thesis: Perception and representation of time: differences in reproduction, duration discrimination and production of short time periods

Mentor(s): Professor Zdravko Petanjek, MD PhD, Professor Predrag Zarevski, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Time estimation is crucially important for almost every human behavior. Brain uses knowledge of time's passage to anticipate sensory events and to arrange motor and cognitive actions accordingly. Over the decades, several brain regions, like striatum or cerebellum, were prospective candidates as the structures for the neural basis of time perception and representation. However, neither of structures nor processes have yet been discovered. The fundamental question in the field is how the nervous system encodes the events' duration in the milliseconds range, which is particularly relevant for instant perception and the actions produced and related to these events. In the broader context, the present doctoral research is aiming to establish relationships between several behavioral paradigms of perception and production of short time intervals in milliseconds and seconds range. Nonetheless, only the part of project concerning discrimination of intervals in a millisecond range will be presented. More specifically, the procedure for establishing the audio threshold will be tested.

Materials and methods: Ten subjects aged 20 – 25 yrs participated in the pilot experiment. Psychoacoustics - the MATLAB toolbox for auditory threshold estimation by Soranzo and Grassi (2014) was used to establish the discrimination threshold as the minimum detectable difference in duration between the two tones. Multiple Alternative Forced Choice task (nAFC, n – the number of alternatives) was employed in the Transformed up-down procedure. In this procedure the duration of stimuli was selected in real time during the experiment and each trial depended on the previous response. If the two subsequent responses were correct, the level of stimulus moved down toward the threshold, while after the first false response, the stimulus moved up. The standard interval was set to 200 msec and the initial comparison interval was 100 msec; step size or value of reduction/increment of the tone duration was fixed. Each subject performed 5 blocks, and each block contained 50 trials.

Results: In the present study, the adaptive procedure was used to determine the 75% difference threshold as an indicator of performance on time estimation. This threshold represents the mean difference in duration between the standard and the comparison interval required to produce 75% correct responses, with better performance being indicated by lower threshold values. Individual threshold values were computed on the basis of averaging arithmetically the various thresholds at the reversal points. The pilot data were significantly skewed and kurtotic for the first block of trials, but not for the others. The overall mean was M=39.79 without clear pattern of reduction or increment from the first to the last block. The SD was highest (20.35) in the first block with the lowering tendency toward the end of trials (10.92). Subjects' individual thresholds ranged from 23.51 to 56.03 but individual variability (SD) was lower than overall.

Discussion: The advantage of adaptive procedures is possibility to select the stimuli in real time while the experiment is running and the presented stimulus depends on the correctness of the previous stimulus. Adaptive procedures maximize the ratio between the stimuli near the thresholds and those far away; therefore, they are more efficient than non-adaptive procedures. Although very popular recently, 2-down 1-up procedure is not reliable according to some authors, especially when employed in a 2AFC task; however, the most robust alternative, the 3-down 1-up, tends to significantly lengthen the experiment. Despite technical flawlessness of the Psychoacoustics software, some issues like step size and number of steps should be considered to eventually lower the variability between blocks, mostly caused by attention and/or memory lapses.

MeSH/Keywords: time perception, transformed up-down procedure, audio threshold

Poster code: R-01-08-143

PRELIMINARY RESEARCH RESULTS

Clinical Medical Sciences

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): Assist. 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: There are no differences between the groups regarding age (P=0.4934, Independent samples t-test), BMI (P=0.1315, Mann Whitney test) and parity (P=0.231, Chi-square test). Area under the curve significantly increased over time in both groups (P<0,001, Wilcoxon test for paired samples) but there is no difference between the groups in the change in the mentioned parameter over time (P=0.859, Mann-Whitney test for independent samples).

Discussion: Area under the curve increased over time in both groups which is explained by pharmacological sympathectomy and subsequent arterioral vasodilation. However, there is no difference between the groups in the change of the mentioned parameter over time which leads to conclusion that there is no 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 (amplitude and pulse transit time) are needed for final conclusions.

MeSH/Keywords: photoplethysmography, sympathectomy, epidural analgesia

Poster code: R-02-01-021

IMPACT OF BONE MORPHOGENETIC PROTEIN 1-3 (BMP1-3) AND ITS ANTIBODY ON DERMAL SCARRING

PhD candidate: Anamaria Balić

Part of the thesis: Impact of bone morphogenetic protein 1-3 (BMP1-3) and its neutralizing antibody on dermal scarring in rats

Mentor(s): Assoc. Prof. Zrinka Bukvić Mokos, MD PhD, Assoc. Prof. Lovorka Grgurević, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Scarring and its accompanying esthetic, functional, and psychological sequelae still pose significant challenges in modern societies. We can expect around 30% of scars to undergo abnormal growth due to aberrations in physiologic healing resulting in hypertrophic scar or keloid formation, which are frequently accompanied by a number of disfiguring, devastating, functional, and social impairments that lead to a decreased quality of life. Currently, among commercially available products, there is no effective treatment option which can successfully overcome impaired skin healing. On the basis of prior findings, we hypothesize that bone morphogenetic protein 1-3 (BMP1-3), circulating Bmp1 gene metalloproteinase isoforms (mTld), represents a core pathway molecule essential in fibrosis and its targeting may be sufficient to limit fibrosis progression. As BMP 1-3 plays an important role in all stages of wound healing we utilized our understanding of BMP1-3 monoclonal antibody (moAb) mechanism of action in procollagen processing and tested it's potential against BMP1-3 to enhance skin scar appearance. Moreover, we investigated the effect of BMP1-3 inhibition in rat model of normal and rabbit ear model of hypertrophic scarring.

Materials and methods: We established a rat model of primary healing and treated it locally and systemically with different doses of BMP1-3 moAb. Two full-thickness, 6 cm linear skin incision were made on the dorsum of each rat at equal distances from the midline. The wounds were closed with 4-0 ethilon sutures. To prove the local efficacy of BMP1-3 antibody, rats were treated with subcutaneous injections. Wounds on the right side of each rat were treated with BMP1-3 antibody, while the left incision was injected with saline. Additionally, two groups were treated systemically, with saline and BMP1-3 moAb. Therapy was administered at days 1, 2, 5, 7 and 14. To prove the beneficial effect of BMP1-3 moAb on rabbit ear model of HS formation, we treated New Zealand albino rabbits with BMP1-3 moAb and BMP1 protein; precisely, wounds that were created on the ventral side of the ear down to the bare cartilage using 7 mm biopsy puncher. All wounds and scars were photographed postoperatively and on every second day of the experiment, to enable quantification utilizing the visual scale assessment. Histologic analysis was performed on day 21 and scar elevation index (SEI) was calculated.

Results: BMP1-3 moAb significantly accelerated wound healing, and improved scar appearance in contrast to control on day 21, but also in all animals at different time points prior to the day 21 when the animals were euthanized. BMP1-3 antibody significantly improved scar appearance comparing to control group in both locally and systemically treated rats. The average visual score was 1.5 on the control side and 2.2 on the side treated with BMP1-3 antibody administered locally, 2.4 systemically. SEI of the wound treated with saline was 1.57 while was significantly decreased (1.26 and 1.14) after both local and systemic application of BMP1-3 antibody. In rabbit ear model of HS BMP1-3 antibody significantly enhanced scar appearance. In contrast, BMP1 protein had an adverse impact on scar but according to grading system did not reach statistical significance Histological analysis, i.e. SEI proved BMP1-3 antibody capacity to reduce hypertrophy of the scar. Moreover, when BMP1 protein was injected, SEI was significantly increased

Discussion: In conclusion, we suggest that BMP1-3 represents the key profibrotic protein, playing an important role at all stages of wound healing and is needed to maintain the skin homeostasis. Targeting BMP1-3 protein as a fibrous mediator may provide a new therapeutic approach to the reduction or prevention of (hypertrophic) scarring with a functional and aesthetically acceptable outcome, without interrupting the wound healing process.

MeSH/Keywords: Scar, wound healing, collagen, BMP1-3, regeneration

Poster code: R-02-02-046

THE ASSOCIATION BETWEEN SEVERITY OF PSORIASIS AND OBESITY BASED ON THE ANALYSIS OF THE SERUM LEVELS OF TNF ALPHA, IL-6, RESISTIN, OMENTIN AND AMOUNT OF VISCERAL FAT

PhD candidate: Kristina Žužul

Part of the thesis: The association between severity of psoriasis and obesity based on the analysis of the serum levels of TNF alpha, IL-6, resistin, omentin and amount of visceral fat

Mentor(s): Assoc. Prof. Suzana Ljubojević Hadžavdić, MD PhD, Professor Drago Batinić, MD PhD

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

Introduction: Psoriasis is a chronic inflammatory skin disease associated with numerous comorbidities. Studies that have investigated the relationship of psoriasis to obesity proposed the association through a common pathophysiological mechanism of chronic low-grade inflammation. Adipose tissue, especially visceral fat, is an active endocrine organ that secretes adipokines involved in regulation of various metabolic processes. Until today, several adipokines have been identified that may contribute to the systemic inflammation present both in obesity and psoriasis, but their exact mechanism of action in both of these conditions is still not completely understood. This study will analyze the relationship of the serum levels of TNF alpha, IL-6, resistin, omentin and amount of visceral fat, with the severity of psoriasis.

Materials and methods: The study is being conducted at the University Hospital Centre Zagreb and University of Zagreb School of Medicine, and will include at least 50 patients with histologically verified plaque psoriasis and at least 25 age and sex adjusted healthy volunteers as controls. The patients are clinically examined, and severity of psoriasis is assessed according to PASI (Psoriasis Area and Severity Index). The following parameters are measured: height, weight, BMI, waist circumference, amount of visceral fat and serum levels of cytokines and adipokines. The amount of visceral fat is measured by bioelectrical impedance analysis (BIA) using Tanita BC-418 Segmental Body Composition Analyzer. Peripheral venous blood samples (5 mL) are collected and serum concentrations of TNF alpha, IL-6, resistin, and omentin determined with ELISA method, using commercial kits.

Results: Preliminary results on a sample of 24 patients and 12 age and sex-adjusted healthy volunteers show statistically significantly higher values of the amount of visceral fat in the patients compared to control group. There is also a correlation between PASI score to BMI, waist circumference and the amount of visceral fat. The serum samples for the analysis of concentrations of TNF alpha, IL-6, resistin, and omentin, which will be determined with ELISA method, are currently in the process of collection.

Discussion: These preliminary results are from a small number of patients and healthy volunteers. Therefore, in order to reach the final conclusion, it is necessary to wait for the final results of this study, which could help clarify the pathophysiology of psoriasis.

MeSH/Keywords: psoriasis, obesity, cytokines, TNF alpha, IL-6, resistin, omentin, visceral fat

Poster code: R-02-02-095

THE INFLUENCE OF EXERCISE ON REDUCTION OF SACROILIAC DISFUNCTION IN PREGNANCY

PhD candidate: Manuela Filipec
Part of the thesis: The influence of exercise on reduction of sacroiliac disfunction in pregnancy
Mentor(s): Assoc. Prof. Ratko Matijević, MD PhD
Affiliation: University of Zagreb School of Medicine

Introduction: Sacroiliac dysfunction (SID) is major pain syndrome in pregnancy. It is caused by specific structure and function of the sacroiliac joints and their adjustment during pregnancy. SID incidence is reported up to 76.6%. SID causes limitations in performing routine daily activities, social and sexual life reducing quality of life for pregnant women.

Materials and methods: Randomised controlled trial has been undertaken. Inclusion criteria were primiparous/multiparous, singleton/multiple pregnancy, gestational age between 10 and 34 weeks, age between 25 and 45 years. Exclusion criteria were surgical procedures in the area of the spine and pelvic, symptomatic appearance of sacroiliac dysfunction before pregnancy, ankylosing spondylitis and any other disease whose symptoms can cause symptoms of sacroiliac dysfunction. Study group has conducted four different exercises used in order to stabilize the pelvis performed twice weekly for 20 minutes under the supervision of a physiotherapist. The control group was complied with their normal lifestyle without specific education about exercises used in order to stabilize the sacroiliac joints. Visual Analog Scale (VAS) was used to assess the pain intensity while Quebec scale was used for assessment of the degree of disability in everyday activities.

Results: Analysis is based on 408 pregnant women included (207 in the study and 201 in control group). Statistical significance was found ($p<0.001$) in reduction of pain intensity with significantly reduced disability in study group ($p<0.001$). There was no statistical significance between both group regarding pain intensity and degree of disability before inclusion in the study ($p=0.06$; $p=0.79$). Results indicate a higher incidence of sacroiliacal dysfunction in primiparous women (84.70%) and in multiple pregnancies (86.53%). There was a statistically significant difference in the reduction of the pain intensity in primiparous compared with multiparous after six weeks of exercise ($p = 0.031$) while the degree of disability shows a statistically significant difference after three weeks of exercise at primiparous ($p = 0.005$), but this difference is lost after six weeks of exercise ($p = 0.383$). There was no difference in the reduction of the pain intensity as well as the degree of disability in singleton compared to multiple pregnancies. The results showed a strong positive correlation between the reduction in pain intensity and degree of disability after three ($r = 0.898$) and six ($r = 0.859$) weeks of exercise in the study group. Also, correlation analysis showed the correlation between increased pain intensity and degree of disability after three ($r = 0.840$) and six ($r = 0.938$) weeks of exercise in the control group.

Discussion: Our results supports effectiveness of exercise used in order to stabilize the sacroiliac joints during pregnancy, pointing importance of exercise in management of SID during pregnancy.

MeSH/Keywords: sacroiliac dysfunction, pregnancy, exercise

Poster code: R-02-03-006

EFFECTS OF LATERAL EPISIOTOMY ON THE PELVIC FLOOR AND SEXUAL FUNCTION DURING THE FIRST YEAR AFTER DELIVERY

PhD candidate: Krešimir Živković

Part of the thesis: Effects of lateral episiotomy on the function of pelvic floor and sexual function after vaginal delivery in primiparas

Mentor(s): Professor Slavko Orešković, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Episiotomy is an incision which extends the vaginal vestibule during fetal expulsion. Lateral episiotomy is widely used in obstetric practice, but is rarely mentioned in the literature and its effect on the pelvic floor and sexual function is unexplored. The available data largely support the theory of the negative or neutral effect of median and mediolateral episiotomy on the pelvic floor. The hypothesis of this study was that lateral episiotomy during delivery in primiparas has a protective effect on the emergence of urinary and anal incontinence, as well as sexual dysfunction during the first year postpartum. The aim of this study was to investigate the effect of lateral episiotomy on the pelvic floor in the primiparas as compared to the group of women without episiotomy but with minor perineal tears and intact perineum.

Materials and methods: Prospective study was conducted in University Hospital Sveti Duh and consisted of 2 groups of primiparas, with 100 examinees in each group. The first group consisted of women who had a delivery with lateral episiotomy (E) and another group of women who had a delivery with perineal tears of I. and II. degree and intact perineum (P). The study included primiparas with singleton pregnancies and spontaneous onset of labor with fetuses in cephalic presentation. An episiotomy was used in restrictive manner. Examinations of pelvic floor were performed at 5 and 8 months after delivery accompanied by specific questionnaires. International Consultation on Incontinence Questionnaire-Urinary Incontinence-Short Form was used to assess urinary incontinence, while Wexner Scale was used to assess fecal incontinence. Female Sexual Function Index was used to assess sexual function. The clinical examinations were consisted of: Pelvic Organ Prolapse Quantification system, cough stress test, transperineal sonography of anal sphincter and pelvic floor muscles and vaginal manometry. Non-parametric analyses were carried out. A p-value <0.05 was taken as statistically significant.

Results: Results showed that there were no significant differences ($p>0.05$) in emergence of stress incontinence by groups in two-time points (E: 16% and 13.5% vs. P: 20% and 14.9%). There were also no differences ($p>0.05$) in the occurrence of prolapse (E: 42% and 31.2% vs. P: 52% and 38.3%). The muscular strength of the pelvic floor was almost equal by groups as well as the occurrence of symptoms of anal incontinence and sexual dysfunction. There were certain relations between the ages of the examinees, use of oxytocin, weight of the newborn to poorer results of the questionnaires in episiotomy group. Obstetric and anthropometric variables were not associated with emergence of urinary and anal incontinence in episiotomy group. Significant correlations between questionnaires and clinical tests were mild and moderate.

Discussion: The effect of lateral episiotomy on the pelvic floor is comparable to delivery with perineal tears of I. and. II. degree and intact perineum based on the data of this research. Rates of stress urinary incontinence, anal incontinence as well as sexual dysfunction were similar in episiotomy and perineal tears group. No substantial associations between episiotomy and pelvic floor disorders have been found, which is in accordance with majority of researches on other types of episiotomy. Lateral episiotomy is a safe incision when it is indicated. Lateral episiotomy has a comparable effect on the pelvic floor and sexual function as well as other types of episiotomy. To conclude, lateral episiotomy has neither protective, nor negative effect on the onset of pelvic floor disorders and sexual dysfunction in the primiparas.

Acknowledgments: To my mentor Professor Slavko Orešković for guidance.

MeSH/Keywords: Episiotomy, Urinary incontinence, Anal incontinence, Sexual dysfunction, Primiparas, Perineum.

Poster code: R-02-05-001

MOLECULAR ANALYSIS OF HUMAN PAPILLOMA VIRUSES IN FEMALES WITH HIGH-GRADE CERVICAL INTRAEPITHELIAL NEOPLASIA AND CERVICAL CARCINOMA

PhD candidate: Magdalena Karadža

Part of the thesis: Molecular analysis of human papilloma viruses in females with high-grade cervical intraepithelial neoplasia and cervical carcinoma

Mentor(s): Professor Adriana Vince, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Cervical cancer is the second leading cause of death in women. It is associated with a permanent infection caused by oncogenic HPV types. All over the world dominant is HPV 16. There are multiple variants of the nucleotide sequence of HPV 16. Infection with certain variants more likely leeds to development of preinvasive and invasive lesions. HPV16 is the most common in women with high-grade cervical intraepithelial neoplasms and cervical cancer in Croatia. The objectives of the study were to analyze the distribution of high-risk HPV types in women with high-grade cervical intraepithelial neoplasms and cervical carcinoma, to analyze genomic variants and to determine the incidence of individual genomic variants of HPV 16 in existing premalignant and malignant changes.

Materials and methods: A total of 406 patients with cytological finding of CIN 2+ were treated in the Department of Obstetrics and Gynecology, University Hospital Center Zagreb, in the period from 01.12.2009. until 19.12.2013. After the obtained pathohistological findings, 33 subjects were excluded from the study due to inadequate samples.

Results: 373 samples were subjected to statistical processing. Distribution by pathohistological diagnosis was as follows: negative findings: 49 (13.1%), preinvasive changes totaled 219 (58.7%), CIN 2: 15 (4.0%), CIN 3: 204 (54.7%); invasive changes totaled 105 (28.2%), MIC: 24 (6.4%), carcinoma planocellulare: 45 (12.1%), AIS: 8 (2.1%), adenocarcinoma: 20 (5.4%), carcinoma adenosquamosum in situ: 1 (0.3%) and carcinoma adenosquamosum: 2 (0.5%). One sample was adenocarcinoma and MIC (0.3%), and 4 samples were CIN3 and AIS (1.1%). In preinvasive changes the most common type was HPV 16 which was isolated in 99 (45.2%) samples. If we add HPV 16 from multiple infections, then the number is higher - 127 (58%). Other most common types in preinvasive changes were HPV 31 in 21 (9.6%) samples, HPV 58 in 11 (5.0%) samples, HPV 33 in 9 (4.1%) samples and HPV 52 in 7 (3.2%) samples. Other types were represented in a smaller number. In 8 (3.7%) samples HPV was negative. Multiple infections in preinvasive changes were found in 43 (20.5%) samples. HPV 16 was also was dominant in samples with carcinoma planocellulare - 37 (53.6%), that is 46 (66.7%) if the HPV 16 from multiple infections were counted. Other types that were found are HPV 31 in 5 (7.2%), HPV 45 in 4 (5.8%) and HPV 18 in 3 (4.3%) samples. The rest were found in a smaller number. HPV was negative in 3 (4.3%) of the sample, and multiple infections were 14 (21.2%). In samples with adenocarcinoma, HPV 16 was isolated in 14 (38.9%), respecting 18 (50%) samples if HPV 16 from multiple infections were counted. HPV 18 was isolated in 10 (12.8%) samples. Multiple infections were in 7 (21.9%) of the samples, and 4 (11.1%) samples were HPV-negative. HPV16 LCR-E6-E7 variants were determined for 130 HPV16 positive samples. Overall, in 74 samples with CIN 3 and CIN 2, 41 genomic variants were found, 98.6% belonged to the European branch, while one (1.4%) variant belonged to the African branch. Observing carcinoma planocellulere and adenocarcinoma samples 95% isolates grouped in 41 genomic variants belong to the European branch, one isolate (2.5%) belongs to the North American branch (one carcinoma planocellulare sample), and one (2.5 %) belongs to the Asian-American branch (one adenocarcinoma sample).

Discussion: Cervical cancer is in the 10th place by incidence of cancer in women in Croatia. This represents rather serious issue, specially among fertile age women since in this group it is the third most common cancer. Therefore it is a significant public health problem that potentially negatively reflects in demographic statistics. In 2014, there were 307 newly discovered cases of cervical cancer, with highest rate in women aged 45-59 (86.1%). Incidence for ca in situ is the highest at age 30-34. According to current research, HPV 16 is dominant in all over the world. In our samples, HPV 16 was dominant too. The number of newly discovered patients since 2010 is relatively stationary, despite the introduction of a National Early Cervical Cancer Screening Program. Hopefully, the research results could contribute to changing the tactics of cancer prevention (vaccination) and to further reduce the incidence of cervical cancer.

MeSH/Keywords: human papilloma virus, high risk HPV types, genomic variants of HPV 16 , cervical cancer, cervical high-grade intraepithelial neoplasia, HPV vaccine

Poster code: R-02-05-045

PROGNOSTIC FACTORS IN ANTINEUTROPHYLLIC CYTOPLASMATIC ANTIBODIES ASSOCIATED VASCULITIS WITH KIDNEY INVOLVEMENT

PhD candidate: Matija Crnogorac

Part of the thesis: Prognostički čimbenici vaskulitisa povezanih s antineutrofilnim citoplazmatskim protutijelima koji zahvaćaju bubrege

Mentor(s): Professor Krešimir Galešić, MD PhD

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

Introduction: Anti-neutrophil cytoplasmatic antibodies (ANCA) – associated vasculitis (AAV) are small vessel vasculitides classified as microscopic polyangiitis (MPA), granulomatosis with polyangiitis (Wegener's) (GPA), eosinophilic granulomatosis with polyangiitis (Churg-Strauss) (EGPA) and renal-limited vasculitis (RLV). Renal involvement in AAV is characterized with pauci-immune crescentic glomerulonephritis on renal biopsy and mostly with rapidly progressive glomerulonephritis clinically. Several studies have looked at prognostic determinants of outcome in AAV, with different results. The aim of the presented study was to evaluate significance of clinical, serological and histopathological prognostic factors for renal and patient outcome in AAV patient cohort.

Materials and methods: Retrospective study included consecutive patients diagnosed with pauci-immune crescentic glomerulonephritis from January 2003. to December 2013. Clinical, laboratory and pathohistological data were analysed. Primary outcome was combined end-point patient death or progression to end-stage renal disease (ESRD). Secondary outcomes were patient survival and progression to ESRD (renal survival) singularly and disease relapse. Kaplan Meyer survival analysis and multivariate Cox proportional hazard regression analysis were used to explore difference between phenotypes and finding significant predictors regarding outcomes.

Results: Out of 81 patients, 40.7% patients reached primary end-point, 22.2% died, 29.6% reached ESRD and 16% relapsed during follow-up. Multivariate Cox proportional hazards regression adjusted analysis found higher BVAS (HR 1.08, 95% CI 1.01-1.17, p=0.042), higher baseline maximal serum creatinine (HR 1.02, 95% CI 1.01-1.03, p=0.04) and lower haemoglobin (HR 0.97, 95% CI 0.95-0.99, p=0.011) significantly associated with primary endpoint. Higher BVAS (HR 1.25, 95% CI 1.01-1.43, p=0.001) and lower haemoglobin (HR 0.95, 95% CI 0.91-0.99, p=0.008) were significantly associated with patient survival, while for renal survival, lower haemoglobin (HR 0.97, 95% CI 0.94-0.99, p=0.041) and the need for acute hemodialysis (HR 3.15, 95% CI 1.20-8.26, p=0.02) were significant predictors. On multivariate adjusted analysis, no significant predictors for disease relapse were found. Kaplan-Meier survival analysis found no difference between clinical, serological and pathohistological phenotypes for all of the endpoints. As for the results on serum C3 levels, low serum C3 levels were significantly associated with worse combined end-point patient and renal survival (HR 3.079; 95% CI 1.231-7.701; p=0.016), and on multivariate adjusted analysis association remained significant (HR 2.831; 95% CI 1.093-7.338; p=0.032). For both end-points individually low serum C3 levels were significantly associated with poorer patient survival (HR 6.378; 95% CI 2.252-18.065; $p < 0.001$; on multivariate adjusted analysis HR 4.315 95% CI 1.350-13.799; p=0.014) and renal survival (HR 3.207; 95% CI 1.040-9.830; p=0.043; on multivariate adjusted analysis HR 3.679; 95% CI 1.144-11.827; p=0.029). In our study there was no significant association between serological and patohistological phenotypes and serum C3 levels.

Discussion: Renal function at presentation, anaemia and BVAS should be included in prediction models for the outcomes for the AAV patients. Lower serum C3 levels at the diagnosis is associated with poorer patient and renal outcomes in AAV patients. Limitations of our study are in part due to its retrospective nature as well as the fact that comparison among the groups may have been underpowered to detect differences among some of the aforementioned groups due to the difference in number of patients and also smaller number of events. Though ANCA vasculitis are in nature systemic diseases (RLV excluded) we focused on those patients that presented with renal involvement. Strengths of our study are mainly the patient's long-term follow-up, use of hard clinical end points such as both patient and renal survival (ESRD), multivariate statistical analysis as well as good and detailed analysis of histological specimens including EM in all patients which strongly supports diagnosis of pauci immune GN. Our results contribute to better understanding of outcomes regarding clinical, serological and pathohistological presentations of AAVs and emphasize the need for further clinical, epidemiological and histological research.

Acknowledgments: To my supportive wife Maja

MeSH/Keywords: vasculitis, ANCA, complement, C3

Poster code: R-02-09-010

N-GLYCOSYLATION OF IMMUNOGLOBULIN G IN ADULT PATIENTS WITH IMMUNE THROMBOCYTOPENIA

PhD candidate: Ena Ranković

Part of the thesis: N- glycosylation of immunoglobulin G in adult patients with immune thrombocytopenia

Mentor(s): Assist. Prof. Dražen Pulanić, MD PhD, Professor Gordan Lauc, MD PhD

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

Introduction: Immune thrombocytopenia (ITP) is a disease in which thrombocytopenia occurs due to an acquired autoimmune disorder. Previous studies have shown that in various pathological conditions such as inflammatory and autoimmune diseases, changes in the composition of glycans occur, and that precisely these differences have a significant impact on the pathophysiology of various diseases.

Materials and methods: In this prospective study we have analyzed 20 adult patients with ITP and 8 subjects in the control group. Demographic data, former course of the disease, treatment lines, quality of life, intensity, localization, frequency of bleeding, and routine laboratory tests were evaluated. In order to analyze N-glycosylation of immunoglobulin G, blood samples were taken and stored and will be sent to the Genos laboratory for analysis.

Results: Of 20 patients with ITP, 50% were males. Median age was 40 years (range from 23-69). 70% of patients had primary ITP whereas in 30% patients secundary ITP was diagnosed. The median of previous therapy lines for ITP was 2 (range 0-7). Most patients were asymptomatic, while only 20% had milder signs of hemorrhagic diathesis (hematomas or petechia). The lowest platelet count was 1x109 /L, but no life-threatening bleeding was noted. Only 10% of patients had decreased levels of immunoglobulin G, all of whom had secondary ITP. Blood samples from 8 individuals from the control group were also collected. The median age in the control group was 41 years (range form 28-68). Of 8 individuals 62% were females. Chronic gastritis was noted in 25% of individuals, while malignant and autoimmune diseases were not observed. All of the subjects in the control group had normal platelet counts and no signs of hemorrhagic diathesis.

Discussion: So far we have been able to collect data of 20 patients with ITP who had various clinical manifestations, therapy responses and laboratory values. Analysis of IgG glycosylation in this population of patients in comparison with the control group could have significant implications for understanding the pathophysiology of the disease and could potentially become a useful biomarker for the diagnosis, treatment options and management of patients with ITP.

MeSH/Keywords: immune thrombocytopenia, glycosylation, immunoglobulin G, biomarker, glycans

Poster code: R-02-09-012

ASSOCIATION BETWEEN CXCL9 AND CXCL10 GENE POLYMORPHISMS AND ACUTE GRAFT REJECTION AFTER LIVER TRANSPLANTATION

PhD candidate: Ana Ostojić

Part of the thesis: Association between CXCL9 and CXCL10 gene polymorphisms and acute graft rejection after liver transplantation

Mentor(s): Assist. Prof. Anna Mrzljak, MD PhD, Assist. Prof. Tomislav Kelava, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Despite the improvement and optimization of immunosuppressive protocols, acute cellular rejection (ACR) is still a frequent complication after liver transplantation. Recent studies showed that increased concentrations of chemokines CXCL9 and CXCL10 are associated with the ACR occurrence. The aim of this study was to examine the association of CXCL9 and CXCL10 single nucleotide polymorphisms with ACR after liver transplantation.

Materials and methods: DNA was isolated from the whole blood of 215 patients transplanted due to alcoholic liver disease from 1/2009 to 3/2017 in University Hospital "Merkur", Zagreb, Croatia. Polymorphisms of CXCL9 (rs10336) and CXCL10 (rs3921) were determined by polymerase chain reaction using commercially available TaqMan SNP assays. ACR was defined as biopsy proven (Banff score $\geq$3) within 6 months after liver transplantation. All patients received the same immunosuppressive protocol comprising of calcineurin inhibitors, steroids (discontinued after 3 months) and mycophenolate mofetil.

Results: 59 patients with ACR and 156 patients without ACR were included into the study. There were no statistically significant differences in age, sex or MELD score at the time of transplant between rejection and non-rejection groups. Genotypes were in Hardy-Weinberg equilibrium ($p>0.05$), with strong linkage disequilibrium ($D'=0.99$, r=0986) between CXCL9 and CXCL10. In the rejection group 22 (37.3%) patients had GG, 25 (42.4%) had AG and 12 (20.3%) had AA genotype of the CXCL9 polymorphism, with similar genotype distribution observed in the non-rejection group, whereas 54 (34.6%) patients had GG, 75 (48.1%) had AG and 27 (17.3%) had AA genotype. Lack of association between CXCL9 genotypes and incidence of ACR was found in codominant, dominant, recessive, overdominant or log-additive model ($p>0.05$). Similar results were obtained for CXCL10 genotypes.

Discussion: Single nucleotide polymorphisms of CXCL9 (rs10336) and CXCL10 (rs3921) are not associated with the incidence of acute cellular rejection after liver transplantation. Further studies should be performed in the future, in order to validate these results.

MeSH/Keywords: liver transplantation, acute cellular rejection, single nucleotide polymorphism

Poster code: R-02-09-017

CAROTID DISEASE IN PATIENTS WITH SYMPTOMATIC PERIPHERAL ARTERY DISEASE

PhD candidate: Ksenija Vučur

Part of the thesis: Prognostic significance of carotid disease in patients with symptomatic peripheral artery disease

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

Affiliation: University of Zagreb School of Medicine

Introduction: Peripheral artery disease (PAD) is a multifactorial syndrome and one of the most common manifestations of atherosclerosis. Polyvascular involvement in PAD patients (pts) is associated with an increased risk of all-cause mortality as well as cardiovascular mortality. Carotid disease and coronary artery disease (CAD) are common in PAD pts. The aim of the study was to investigate the prevalence of concomitant carotid disease in pts with symptomatic PAD and associated cardiovascular risk factors.

Materials and methods: In the retrospective research were included 319 consecutive pts with symptomatic PAD (Rutheford stages 2-6) who were hospitalized and treated in the Department of vascular disease, Clinic of cardiovascular disease in the University hospital Sister of Mercy between January 2010 and January 2016. Patients with malignancy and / or concomitant autoimmune disorders were excluded in the study. Demographic data and baseline clinical characteristics were recorded during the hospital stay and included general information, data about cardiovascular risk factors, biochemical and hematological laboratory data, and data on comorbidities and medications. The diagnosis of PAD was established by clinical examination, ankle brachial index measurement, duplex sonography and/or computed tomography or magnetic resonance, angiography, and confirmed with peripheral angiography using the criteria of the European Society of Cardiology and American College of Cardiology Foundation. The diagnosis of carotid disease was established by duplex sonography and pts with carotid stenosis of 50% or more or carotid occlusion were considered to have carotid disease.

Results: The mean age of the study population was 70±10 years, and 66.5% were men. The mean ankle brachial index was 0.58±0.14. Of 319 symptomatic PAD pts 277 (87%) had hypertension, 242 (76%) had dyslipidemia, 172 (54%) had diabetes mellitus, 138 (43%) had carotid disease, 134 (42%) had critical limb ischemia, 91 (28%) had CAD, 21% had anemia, and 173 (54%) were smokers. Median systolic blood pressure was 140 mmHg (interquartile range, IQR 130-155), and diastolic 80 mmHg (IQR 80-90). The mean eGFR was 63±18.3 mL/min/1.73m2. Compared to pts without carotid disease, pts with carotid disease were older (72±8 vs. 68±10, $p<0.01$). There was no difference in cardiovascular risk factors (hypertension, dyslipidemia, smoking, and diabetes mellitus) or CAD between pts with carotid disease and without carotid disease.

Discussion: Peripheral artery disease represents a major atherosclerotic burden. Pts with symptomatic PAD have a greater probability of having polyvascular disease, indicating an extensive and severe degree of systemic atherosclerosis. As PAD shares the same risk factors as other cardiovascular diseases, the coexistence of carotid disease and PAD is quite common.

MeSH/Keywords: Peripheral artery disease, Carotid disease, Major adverse cardiovascular outcome

Poster code: R-02-09-052

TRANSCATHETER AORTIC VALVE IMPLANTATION INFLUENCES PLATELET REACTIVITY

PhD candidate: Zvonimir Ostojić

Part of the thesis: The value of platelet aggregation tests in the evaluation of paravalvular regurgitation after transcatheter aortic valve implantation

Mentor(s): Assist. Prof. Joško Bulum, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Transcatheter aortic valve implantation (TAVI) is a globally approved method for treating severe aortic stenosis (AS) in selected patients. Paravalvular regurgitation (PVR) is the most common complication after TAVI with incidence as high as 70%. Although majority of PVR are usually mild, moderate or severe PVR is expected in up to 20% of cases. In those cases additional interventional procedures, such as balloon dilatation, can reduce PVR severity. Recent studies indicate that von Willebrand factor dependent platelet reactivity (PR) may help in PVR assessment beside fluoroscopy and echocardiography. Dual antiplatelet therapy is recommended after TAVI, therefore, we investigated correlation of PVR after TAVI and PR triggered by arachidonic acid, adenosine diphosphate (ADP) and thrombin using Multiplate® function analyzer ASPItest, ADPtest and TRAPtest, respectively. To the best of our knowledge, these tests have not been previously used in the assessment of PVR after TAVI.

Materials and methods: 26 consecutive patients with severe and symptomatic AS, undergoing TAVI procedure in our institution were enrolled in research. Patients' perioperative clinical characteristics were collected from medical records. All patients who did not have chronic therapy with aspirin and clopidogrel, received loading dose of aspirin (300 mg) and clopidogrel (300 mg) one day before the procedure, followed by their standard maintenance doses (aspirin 100 mg, clopidogrel 75 mg) for the next three months. Patients' PR was measured in five time points: just before induction of anesthesia (T1), after heparin administration (T2), 10 minutes after valve implantation (T3), 10 minutes after additional interventional procedures (if necessary) or 20 minutes after valve implantation (T4) and three days after the procedure (T5). PR was measured using impedance aggregometer (Multiplate® analyzer, Roche, Munich, Germany). PR was measured in response to three platelet aggregation agonists: arachidonic acid (ASPItest), ADP (ADPtest) and thrombin receptor activating peptide-6 (TRAPtest). Results are presented in Units (U), which is calculated from the height and slope of aggregation curve and represents best overall measure of PR. During TAVI procedure, all patients received unfractionated heparin at does 50-70 IU/kg with a target activated clotting time of 250-300 seconds.

Results: Mean patient age was 82.3 years with majority patients being male 57.5% (N=15). Mean valve area prior to procedure was 0.71±0.19 and mean transvalvular gradient 45.35±11.14 mmHg. All patients underwent successful TAVI procedure using either self-expandable (N=14, 53.8 %) or balloon-expandable valve (N=12, 46.2 %). Balloon dilatation following initial implantation was performed in 9 cases (34.6 %). Mean post-implantation gradient was 11.75±7.6 mmHg. Significant PVR, defined as more then mild, developed in 7 cases (26.9 %). Mean PR before TAVI (T1) was 24.3±22.6 for ASPItest, 47.2±26.7 for ADPtest and 93.1±31.4 for TRAPtest. There was no significant difference between basal measurements and after administering unfractionated heparin (T2). However, 10 minutes after valve implantation (T3), significant reduction in PR was observed in all three tests; ASPI 11.6±12.1 ($p=0.018$), ADP 27.7±16.6 ($p<0.001$) and TRAP 75.5±26.4 ($p=0.016$). Subsequent measurements (T4, T5), demonstrated further decline in PR, although non-significant compared to T3 (T5: ASPI 8.9±9.5, ADP 15.8±8.5 and TRAP 63.3±21.8). There was no significant difference in PR levels between patients with or without significant PVR.

Discussion: Our results show that successful TAVI procedure induces decrease in PR regardless of the platelet activation pathway. Decrease was observed 10 minutes after valve implantation and was sustained in the short postoperative period. We found no correlation with presence of significant PVR. Further research on a larger number of patients is needed to confirm these results.

MeSH/Keywords: Aortic stenosis, Transcatheter aortic valve implantation, Platelet reactivity

Poster code: R-02-09-072

ASSOCIATION OF PPAR GAMMA AND MTHFR GENE POLYMORPHISMS WITH CLINICAL MANIFESTATION OF METABOLIC SYNDROME IN PATIENTS WITH PRIMARY SJOEGREN'S SYNDROME

PhD candidate: Marija Miletić

Part of the thesis: Association of PPAR gamma and MTHFR gene polymorphisms with clinical manifestation of metabolic syndrome in patients with primary Sjoegren's syndrome

Mentor(s): Professor Jasenka Markeljević, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Sjoegren's syndrome (SS) is an interesting autoimmune disease (AID) in the light of the research of local and systemic immunity and early atherogenesis (AG). Patients with AID develop early and severe vascular atherosclerotic changes. Metabolic syndrome (MS) represents the connection between chronic inflammation and elevated CV risk in patients with AID. It has a significant genetic component, and there is data available that confirms relationship between AID, MS and certain gene polymorphisms. Polymorphism in the peroxisome proliferator-activated receptor gamma gene (PPAR-ã) is associated with various metabolic disorders, but also may play an active role in the pathophysiology of SS. Polymorphism in methylenetetrahydrofolate reductase gene (MTHFR) has been shown to result in hyperhomocysteinemia, who is considered to be an independent risk factor for several diseases such as atherosclerotic vascular disease and AID, including SS.

Materials and methods: The research will include a total of 90 patients suffering from primary SS (pSS). Patients are classified into two groups, based on the presence / absence of the MS. After anthropometric measurements (blood pressure, waist circumferences), peripheral venous blood samples are collected for the purpose of DNA isolation and biochemical analysis (fasting glucose in blood, lipid status). Genomic DNA is isolated from 3 ml peripheral blood, collected in EDTA vacutainers by standard procedure, and for the detection of polymorphisms we will use the polymerase chain reaction (PCR).

Results: At the moment we have 42 patients enrolled in the study. All of them are female with a mean age of 62,3 years. We excluded 32 patients (1 died, 9 rejected follow up, 22 developed another AID). We have analyzed the overall prevalence of MS, as well as the number of components of the MS present in the individuals in this study. In a total of 42 females enrolled, 30 (71,42%) were identified as having MS. The major components of the MS are abdominal obesity (83,33%) and hypertension (54,76%). Till now, 28 patients had already isolated DNA (archived during the previous study), and in 14 new patients we have isolate DNA from peripheral blood leukocytes. Genotyping will commence when half of the subjects will be enrolled.

Discussion: Presented results demonstrated higher prevalence of MS in patients with pSS. According to these results we recommend regular screening for MS in pSS patients. Many mechanisms had been suggested for the development of MS in pSS patients, but the association is not yet fully understood. We hope that the further analysis of the results will determine whether certain polymorphisms differ in their frequency among patients with pSS who develop MS, and patients with pSS who did not develop MS. It is important to define genetic predisposition in the development of MS in patients with pSS.

MeSH/Keywords: Sjoegren's syndrome, metabolic syndrome, atherogenesis.

Poster code: R-02-09-077

COMPARISON OF INTERATRIAL CONDUCTION TIME IN HEART FAILURE PATIENTS AND CONTROLS

PhD candidate: Dora Fabijanović

Part of the thesis: A mechanism-based approach to the evolution of heart failure with preserved ejection fraction

Mentor(s): Assist. Prof. Maja Èikeš, MD PhD, Professor Bart Bijnens, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Interatrial conduction time (IACT) is significantly prolonged in a subset of HFpEF patients (pts), as demonstrated in a small number of studies, while its distribution appears highly heterogenous in HFrEF. Normal values of IACT in non-HF pts are still largely unknown. Thus, we aimed to compare IACT between HF pts and normal controls and to assess abnormal IACT among patients with HF.

Materials and methods: A total of 71 HF pts (mean age 65±14 years, 45% male) diagnosed per the current guidelines were retrospectively assessed and compared to 27 age- and sex-matched controls. IACT was measured from echo Doppler traces as the difference between the time interval from the onset of the Q wave on the ECG to onset of A wave at mitral and tricuspid valve level.

Results: Mean IACT in the control group was 2.2±22.3 ms and 14.9±46.1 ms in HF pts (p=0.07). The 95th percentile (40ms) of IACT in the controls was used as the cut-off point to define abnormal IACT, i.e. interatrial dyssynchrony (IAD). IACT>40 ms was present in 22 (31%) of HF pts: 15 pts (48%) with HFpEF, 3 pts (30%) with HFmrEF and 4 pts (13%) with HFrEF, p <0.001. Along with a higher proportion of HFpEF pts in the IACT>40ms group, higher IAD was related to lower heart rate (p=0.03), female gender (p=0.02) as well as higher LVEF (p=0.003) and smaller LVIDd (p=0.01) (Table). There was no difference in age, SBP, BMI, incidence of diabetes, NYHA class, eGFR, E/e' or atrial volumes.

Discussion: This pilot study suggests a possible cutoff value of IACT measured by Doppler echocardiography, which might lead to better recognition of the subset of HF pts with significant IAD, however it requires confirmation on a larger cohort.

MeSH/Keywords: Interatrial conduction time, Heart failure, Interatrial dyssynchrony

Poster code: R-02-09-082

CORRELATION BETWEEN RESISTIN EXPRESSION, CEREBROVASCULAR SYMPTOMATOLOGY AND HISTOLOGICAL FEATURES OF ATHEROSCLEROTIC PLAQUE

PhD candidate: Ivana Jurin

Part of the thesis: Correlation between resistin expression and histological features of atherosclerotic plaque

Mentor(s): Marko Ajduk, PhD, research advisor

Affiliation: University of Zagreb School of Medicine

Introduction: Resistin is an adipocytokine, which was discovered during screening to identify potential targets of the insulin sensitizer in 3T3-L1 adipocytes. Peripheral blood mononuclear cells are the main source of resistin, while resistin secreted from macrophages infiltrating atheromas affects the endothelial function and can induce the migration and proliferation of vascular smooth muscle cells. Previous studies have suggested that these serum levels were elevated in obese people, patients with diabetes mellitus and in patients with atherosclerotic disease, especially coronary artery disease. A vulnerable plaque can be histologically characterized by having a large lipid-rich core, a thin fibrous cap with dense inflammatory cell infiltration, ulceration, thrombosis, and intraplaque hemorrhage. Plaque vulnerability identification could help to stratify subjects who have a greater risk for developing acute occlusive syndromes. There is a need for circulating biomarkers that can predict plaque vulnerability and risk of developing cerebrovascular symptomatology (stroke, transient ischaemic attack or amaurosis fugax). The primary objective of this study was to evaluate the correlation between serum resistin levels and histological features of atherosclerotic carotid plaques. The secondary objective was to evaluate the correlation between serum resistin levels and cerebrovascular symptomatology.

Materials and methods: This prospective cohort study included 78 consecutive neurologically asymptomatic and symptomatic patients scheduled for carotid endarterectomy. Resistin levels were determined from fasting blood samples prior to surgery. Pathohistological and immunohistochemical analysis of atherosclerotic plaques were then subsequently performed.

Results: Subjects who had cerebrovascular symptoms more frequently had a positive family history of cerebrovascular disease, plaques categorizes as AHA VI (32% vs. 17%) and a higher resistin serum levels compared to subjects with no cerebrovascular symptoms. Serum resistin levels were associated with AHA type VI plaques, with every 100 unit increase in resistin levels increasing the odds for this plaque type by 13%. Resistin was the only variable significantly associated with cerebrovascular symptoms; every 100 unit increase in resistin levels increased the odds for cerebrovascular symptoms by 2.3% (OR 1,023 [1.007, 1.039]). According to the AHA classification, there was a total of 38 plaques categorised as AHA VI, 7 plaques categorised as AHA VII and 28 plaques categorised as AHA V. The expression of resistin in carotid plaques was expressed as semi-quantitative: 0- without staining, 1- less, focal staining (0-25% of the cells positive), 2- stronger colouring (more than 25% positive cells). More than 25% of positive cells were noticed in the soft part of the plaque (extracellular lipids), foam cells and macrophages. No staining was noticed in the fibrose part of the plaque. According to our results, resistin was more expressed in plaques cathegorized as AHA VI. Plaques cathegorized as AHA VI were more frequently cathegorized as probably or definitely vulnerable. Serum resistin levels are significantly correlated with the expression of resistin in carotid plaque ($p<0.001$). The higher the resistin serum levels, the more likely it is that the expression of resistin in carotid plaque will be higher.

Discussion: Our study is, to the best of our knowledge, the first study to establish a positive association of serum resistin levels and plaque vulnerability determined by AHA classification. These results may have clinical implications in defining (screening) a group of patients with potentially vulnerable plaques in the carotid artery and a high risk of plaque rupture. However, this has to be confirmed in further large prospective studies and also in studies examining local resistin (and possibly other adipokine) concentrations in the plaque itself. Our results may be a precursor of better models containing several adipokines used to predict plaque vulnerability and possible ruptures.

MeSH/Keywords: resistin; atherosclerotic plaque; arterial disease, carotid

Poster code: R-02-09-087

IS MITRAL REGURGITATION A PREDICTOR OF LEFT ATRIAL THROMBOSIS IN PATIENTS WITH ATRIAL FIBRILLATION?

PhD candidate: Irena Ivanac Vranešić

Part of the thesis: Correlation of mitral regurgitation with presence of the left atrial thrombus in atrial fibrillation

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

Affiliation: University of Zagreb School of Medicine

Introduction: Atrial fibrillation is the most common sustained cardiac arrhythmia with significantly increased left atrial thrombus formation risk, hence thromboembolic risk. According to the risk severity, atrial fibrillation is divided into valvular (involving presence of mitral valve stenosis and mechanical valve prosthesis) and nonvalvular. Previous studies about mitral regurgitation impact on left atrial thrombus formation as well as on thromboembolism showed contradictory results. Our aim was to assess the relationship between the severity of the mitral regurgitation and the prevalence of left atrial thrombus in patients with atrial fibrillation.

Materials and methods: A retrospective observational study involving patients with atrial fibrillation, who underwent pre-cardioversion transesophageal echocardiography in our department from January 2011 until January 2015 was performed. The severity of mitral regurgitation was assessed by Doppler echocardiography and was classified as mild, moderate or severe. The presence of spontaneous echo contrast or left atrial thrombus was evaluated. We also analysed the anticoagulation status and thromboembolic risk according to the CHA2DS2-Vasc score system.

Results: We enrolled 450 patients (65% male), median age of 65 years (ICR 57-71). 176 patients had no mitral regurgitation, 182 had mild, 84 moderate and 8 severe mitral regurgitation. There was no statistically significant difference among the groups in their anticoagulation status, median INR was 1.79 (1-2.3) vs. 1.86 (1.27-2.38) vs. 1.9 (1.18-2.5) vs. 2 (1.2-2.2) respectively, p=0.22. More severe forms of mitral regurgitation were associated with higher CHA2DS2 -Vasc scores p=0.001. There was no statistically significant difference in spontaneous echo contrast 27.3% vs. 25.8% vs. 27.4% vs. 0%, p=0.39 or left atrial thrombus prevalence 10.2% vs. 14.3% vs. 16.7% vs. 12.5%, p=0.486. Multivariate analysis revealed that mitral regurgitation is not a significant predictor of left atrial thrombus, OR: 1.36, CI: 0.93-1.98.

Discussion: This study shows that mild and moderate mitral regurgitation are not independently associated with left atrial thrombosis in patients with atrial fibrillation. The study was underpowered for the analysis of the association between severe mitral regurgitation and left atrial thrombus formation, hence further research with larger cohort is needed to evaluate this association.

MeSH/Keywords: atrial fibrillation; mitral valve insufficiency; thrombosis; echocardiography, transesophageal

Poster code: R-02-09-102

THE PROGNOSTIC VALUE OF AGE IN PATIENTS WITH CONTINUOUS FLOW LEFT VENTRICULAR ASSIST DEVICES AND ASSOCIATION WITH ICD/CRT-D TREATMENT

PhD candidate: Nina Jakuš
Part of the thesis: Combined device therapy for advanced heart failure
Mentor(s): Assist. Prof. Maja Čikeš, MD PhD, Professor Frank Ruschitzka, MD PhD
Affiliation: University of Zagreb School of Medicine

Introduction: There is an increasing interest in exploring the association between implantable defibrillators (ICD and CRT-D) and outcomes in ventricular assist device (VAD) carriers. Through a VAD registry formed by 10 European heart failure centres, we aimed to investigate the baseline differences among VAD carriers subgrouped by age quartiles, the prognostic role of age and its association with ICD/CRT-D treatment.

Materials and methods: At current, the registry includes data on 246 patients with continuous flow LVADs (median age 56 (IQR 48-63), 83% male), 63% of which are also ICD or CRT-D carriers. For this subanalysis, patients were subgrouped in quartiles according to age. Median follow-up time was 1.3 years (IQR 0.4-2.0) from index date, defined as time of LVAD or ICD/CRT-D implant, whichever came later.

Results: The crude event rates for the primary outcome (all-cause mortality) increased with age (event rates per 100 person-years: Q1: 9.8 [5.1-18.8], Q2: 11.3 [6.3-20.5], Q3: 27.1 [17.5-42.0], Q4: 36.2 [24.8-52.8]). In unadjusted analysis, the hazard ratio for all-cause death was significantly higher in Q3 and Q4 compared to Q1 (HR [95% CI] for Q2, Q3 and Q4 was 1.15 [0.48-2.79], p=0.8, 2.48 [1.13-5.45], p=0.024 and 3.31 [1.55-7.06], p=0.002, respectively), remaining significant after adjusting for HF aetiology, INTERMACS class and prior cardiac surgery. ICD use was associated with reduced mortality, and age strata at LVAD implantation did not modify the association between ICD/CRT-D therapy and reduced mortality (interaction p=NS for all age quartiles).

Discussion: Although increasing age at LVAD implantation is associated with a higher risk of all-cause death, it does not appear to modify the treatment effect of ICD/CRT-D therapy in cf-LVAD carriers.

MeSH/Keywords: Heart assist device, implantable defibrillator, heart failure.

Poster code: R-02-09-105

LONGITUDINAL EVALUATION OF HEALTH-RELATED QUALITY OF LIFE (HRQOL) IN HODGKIN LYMPHOMA (HL) PATIENTS AND SURVIVORS

PhD candidate: Ida Hude

Part of the thesis: Global quality of life in Hodgkin lymphoma patients and survivors

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

Affiliation: University of Zagreb School of Medicine, Zagreb, Croatia; German Hodgkin Study Group, University Hospital Cologne, Cologne, Germany

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, global HRQoL (gHRQoL), 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 gHRQoL 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.

Materials and methods: 5,306 qualified patients of the HD13-HD15 trials (ISRCTN63474366, ISRCTN04761296, ISRCTN32443041) $\leq$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 gHRQoL from diagnosis to y5EOT with means and 95% confidence intervals. Treatment effects on gHRQoL in y2EOT and y5EOT were tested with multiple linear regression analyses adjusting for age, gender and baseline-gHRQoL. German reference data of Schwarz&Hinz (2001) are used for interpretation of results. GHRQoL is compared with QLQ-C30 item 30 (general quality of life).

Results: Analysis showed significantly worse baseline-gHRQoL 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, gHRQoL 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, gHRQoL 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 gHRQoL (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 gHRQoL. Age, physical and cognitive functioning at baseline were prognostic QLQ-C30-parameters for gHRQoL. 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 global HRQL analysis on a largest HL sample size published thus far; 2) the feasibility of gHRQL QLQ-C30 summary score in hematologic cancer is investigated for the first time. Results show that gHRQL is largely impaired among HL patients. Despite significant baseline differences, gHRQoL 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 gHRQL 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 gHRQL of survivors. The new gHRQL 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: This study was designed and initiated by the candidate during a 6 months collaboration visit to German Hodgkin Study Group in Cologne, Germany, owing to European Hematology Association Junior Short Term Collaboration Award. Special thanks to Mr. Horst M

MeSH/Keywords: Hodgkin Lymphoma, Quality of Life, QLQ-C30, summary score, survivorship

Poster code: R-02-09-110

HEART RATE IS ASSOCIATED WITH GLOMERULAR HYPERFILTRATION IN APPARENTLY HEALTHY SUBJECTS

PhD candidate: Ana Jelaković

Part of the thesis: Glomerular hyperfiltration as a predictor for renal impairment and increased cardiovascular risk in prehypertension

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

Affiliation: University of Zagreb School of Medicine

Introduction: Glomerular hyperfiltration (GHF) was associated with progression of kidney disease and hypertension (HT). It was reported that high metabolic risk is related to increase of GHF.Our aim was to analyze in apparently healthy subjects which factor(s) influence(s) GHF and determine(s) clinical course in long-term prospective study.

Materials and methods: Out of 954 subjects enrolled in ENAH follow-up study,371(137 m, 234w; mean age=46 years) were eligible for further analysis: 100 with optimal, 72 with normal BP,70 with PHT (high normal BP), and 129 with newly diagnosed untreated HT.Follow-up period was 77±12 months. Exclusion criteria were treatment with antihypertensive drugs,diabetes, pregnancy, eGFR<60 ml/min, CV or cerebrovascular incident, chronic terminal diseases,dementia, immobility and missing data.BP and heart rate were measured using Omron 6 device following the ESH guidelines.Uric acid, glucose, lipids, serum creatinine, hsCRP, leptin and adiponectin were determined; HOMA index was used to calculate insulin resistance and MDRD formula to estimate GFR. Albumin to creatinine ratio (ACR) was determined from the first morning spot urine. GHF was defined as eGFR above the cut off value of the 5th quintile of the whole group.

Results: Subjects with GHF were younger (38.1vs.48 13), had smaller waist circumfernc (87±17vs.92±16), lower BP (121/76 vs.131/81), total cholesterol (5.3±1.1vs.5.8±1.1) LDL-cholesterol (3.1±0.9vs.3.5±1.0)and leptin (C 5,1 (IQ2.8-10.7)vs.10.4(5.4-16.6) compared to others;all p<0.05. Neither metabolic factors nor BP values were associated with GHF.However, GHF was positively associated with HR in a way that every 1 beat/min increases odds for hyperfiltration for 7% (1.07 [1.02, 1.13]) at baseline for 6% at the end of follow up (1.06 [1.01, 1.10])

Discussion: Contrary to some reports from literature, our group of apparently healthy subjects with GHF did not have increased metabolic risk. Interestingly, according to our results heart rate is positively associated with GFH indicating that increased sympathetic activity might have important role.

MeSH/Keywords: glomerular hyperfiltration, prehypertension, heart rate, cardiovascular risk

Poster code: R-02-09-147

DETERMINING ANATOMICAL RELATIONS OF POSTERIOR FOSSA AND CRANIOCERVICAL JUNCTION IN PATIENTS WITH CHIARI MALFORMATION TYPE ONE

PhD candidate: Petra Barl

Part of the thesis: Determining anatomical relations of posterior fossa and craniocervical junction in patients with Chiari malformation type one

Mentor(s): Professor Josip Paladino, MD PhD, Assist. Prof. David Ozretić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Type I Chiari malformation is defined as prolapse of cerebellar tonsils exceeding 5 milimeters below the level of foramen magnum. Current investigations and antromorphic measurements indicate that, while the clival and supraoccipital lengths are shorter in patients harboring Chiari I malformation, the McRae's line tends to be longer. There are several surgical options for treatment of Chiari malformation, such as suboccipital craniectomy with or without CI laminectomy as well as performing the expansive duroplasty with cerebellar tonsil resection or shrinkage including removing arachnoidal adhesions. Various authors have demonstrated developmental changes of the viscerocranium regarding the skull base angulation and relations of angles and lengths of posterior fossa structures in regards to the development of the skull base structures. The aim of this study is to research the importance of sphenoclival angle concerning the development of Chiari I malformation and to further elucidate the best choice of surgical treatment by measuring the lengths and angles of anatomical relations in posterior fossa and craniocervical junction.

Materials and methods: This retrospective study aims to include MRI verified Chiari I patients (N:30) and the control group of patients with normal neuroradiological endocranial and craniocervical MRI (N:200). The observed parameters in mediosagittal, coronal and axial images are the sphenoidal angle, clival length, tentorial length, the distance between the tentorial incisura and basion, the level of tonsillar descent below the level of foramen magnum, the basion-opisthion distance, the distance between the tentorial edge and the anterior edge of the foramen magnum, the distance between the highest point of the dens axis in relation to the Chamberlain line and the AP foramen magnum diameter line, relation between the lowest point of cerebellar tonsils and the level of the foramen magnum, codependency of sphenoclival angle and descended cerebellar tonsils, interrelation of the base angulation angle and the dens axis position and the relation between the lowest cerebellar tosil point and the most cranial point of the atlas arch. Furthermore, the transverse diameter of the posterior fossa will be measured at the area of the posterior edge of the internal acoustic meatus, the pyramid-clival junction as well as the transverse diameter of the posterior fossa in the level of the transverso-sygmoid junction. Finally, the transverse diameter of the foramen magnum and the distance between lower potine border and basion will be measured. Descriptive statistical analysis will be performed for all of the measured variables.

Results: Preliminary results demonstrated that the clival length in Chiari I patients seems to be 30,28 mm in average which is shorter than the same measure in control group. The tentorial length in Chiari I patients measures 49,33 mm in average opposed to the 52,28 mm tentorial length in control group. Basion-opisthion measure in Chiari I patients was also shown to be longer than in the control group. Finally, the transverse diameter of the foramen magnum measures 21,22 mm in Chiari I patients while the same measure in control group adds to 23,82 mm.

Discussion: Comparing the results of this study to previously published articles, we have found that there are similar data in relations measurements of clival length, tentorial length, McRae's line length and the transverse diameter of foramen magnum in patients with Chiari I malformations as opposed to the control group of patients.

MeSH/Keywords: Chiari I malformation, magnetic resonance imaging, posterior fossa, craniocervical junction, foramen magnum, clivus

Poster code: R-02-10-023

THE INFLUENCE OF SIZE, TUMOUR LOCATION AND HISTOLOGIC CHARACTERISTICS OF INVASIVE DUCTAL CARCINOMA ON THERMOGRAPHIC CHARACTERISTICS OF THE BREAST

PhD candidate: Marko Mance

Part of the thesis: The influence of size, tumour location and histologic characteristics of invasive ductal carcinoma on thermographic characteristics of the breast

Mentor(s): Assist. Prof. Krešimir Bulić, MD PhD, Assoc. Prof. Anko Antabak, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Breast cancer is one of the leading health problems in developed countries with an increase in incidence of more than 70% in the last forty years. The effectiveness of treatment of breast carcinoma is inversely proportional to the size and spread of cancer at the time of diagnosis. Therefore, it is of vital importance to perform and improve the methods of early detection. There are many imaging techniques used to screen breast carcinoma including mammography, ultrasound, computed tomography, magnetic resonance imaging. Thermography is in the early stages of clinical use in early tumor detection and is currently not included as a screening method because the technology for early detection had not been precise enough in the past. Fortunately, new generation thermo-cameras offer advanced detection and are more precise than their predecessors. There have been varying results and claims regarding the use of infrared imaging in breast carcinoma patients and it is important to research the effect that size and distance from the skin has on the thermal properties of the skin; which has not been conducted thus far.

Materials and methods: This research, conducted at the plastic surgery ward, University hospital center Zagreb includes all female patients who require a breast core biopsy based on prior diagnostic procedures. The distance of the tumor from the skin will be determined by ultrasound examination, while the size and histologic characteristics by pathohistologic analysis. Thermography will be conducted before the core biopsy using the TrueIR Thermal Imager, Model U5855A, by Keysight Technologies,11900 Penang Malaysis and be performed according to the standardized protocols and analyzed by software for thermal imaging.

Results: The pilot study which included 27 patients or 25% of the total required patient group showed that the size of the carcinoma positively correlated with the change in Tmax (rho=0,424, P=0,027). It was also found that the distance of the carcinoma from the skin negatively correlated with the difference of the average temperature between breasts. These findings suggest that breasts with larger carcinomas have a higher skin temperature difference when compared to the healthy breast and that that the distance of the carcinoma from the skin affects the skins thermal characteristics.

Discussion: According to the latest epidemiological data, breast carcinoma is the third highest cause of death in the female population. In Croatia, there are between 2200 and 2600 newly diagnosed patients and an average of 1000 deaths per year. Standardized diagnostic procedures recognize only changes in breast morphology and only detect suspicious lesions when they reach a certain size. Thermography captures the real-time infrared radiation or thermal characteristics of a surface and the camera software converts this non visible light spectrum into a coloured visible picture. There have been many studies thus far on the use of thermography for detecting pathologic conditions in patients, and many have researched the use of this technology for breast cancer. These studies thus far were conducted on a small sample size , without strict exclusion criteria, and their results greatly differ. Kontos M et.at. who observed 63 patients concluded that thermography has a 25% sensitivity and a 85% specificity in the early detection of breast carcinoma and that this is not a good detection method. In a paper by Kolarić et.al. who observed 23 patients, they concluded that mammography has a 85% sensitivity and 84% specificity while thermography had a 100% sensitivity and a 79% specificity which suggests that thermography may have a useful place in the early detection of breast carcinoma. Due to the wide range of results and clams by various authors, it is important to research the effect that tumor size and distance from the skin has on thermographic characteristics of the breast; something that has not been done thus far to the best of our knowledge.

MeSH/Keywords: Thermography, Ductal Breast Carcinoma, Histologic Grade, Screening

Poster code: R-02-10-031

INFLUENCE OF IMPLEMENTING THE ARTIFICIAL CERVICAL INTERVERTEBRAL DISC ON THE BIOMECHANICS OF THE CERVICAL SPINE

PhD candidate: Ivan Domazet

Part of the thesis: Influence of implementing the artificial cervical intervertebral disc on the biomechanics of the cervical spine

Mentor(s): Assist. Prof. Miroslav Vukić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Implantations of the artificial cervical intervertebral disc (arthroplasty) came into use as a modern method of treatment of cervical spine diseases at the beginning of this century. When performing cervical arthroplasty the artificial cervical disc is implanted in the intervertebral space after discectomy. The advantage of arthroplasty is in sustaining the mobility of the functional spinal unit.

Materials and methods: This study is currently being performed at the Department of Neurosurgery, Medical School University of Zagreb. Static and dynamic cervical radiographs of patients who had cervical arthroplasty because of one-segmental cervical disc herniation are compared with the cervical radiographs of the same patients before the surgery, and with the radiographs of the control group. The group of patients who underwent cervical arthroplasty consists of the 25 patients. The control group consists of healthy volunteers. The range of motion of the cervical spine will be calculated after the computer analysis of the static and dynamic cervical radiographs by calculating Cobb angle of the whole cervical spine and of the functional spinal unit in flexion and extension and by summing up the results.

Results: After the static and dynamic radiographs have been analysed, preliminary results showed that the postoperative range of motion of the cervical spine after cervical arthroplasty is increased at operated functional spinal unit but also overall.

Discussion: Preliminary results of this study indicate that the range of motion of both the whole cervical spine and the operated functional spinal unit are increased after cervical arthroplasty.

MeSH/Keywords: Cervical spine, Cervical arthroplasty, Range of motion

Poster code: R-02-10-032

THE CORRELATION BETWEEN COLORECTAL ANASTOMOSIS LEVEL AND ANORECTAL FUNCTION

PhD candidate: Branko Bakula
Part of the thesis: The correlation between colorectal anastomosis level and anorectal function
Mentor(s): Assist. Prof. Žarko Rašić, MD PhD, Professor Dragan Jurčić, MD PhD
Affiliation: University of Zagreb School of Medicine; Clinical Hospital Sveti Duh

Introduction: Anterior resection of rectum is a standard surgical procedure for treating malignant tumors of rectum and rectosigmoid junction. Some degree of postoperative fecal incontinence is frequently seen. Clear pathophysiological mechanism is still not completely clear.

Materials and methods: In our study, patients diagnosed with operable carcinoma of rectum or rectosigmoid junction will undergo anterior rectal resection. Six months after surgery we will measure anal manometry, Jorge-Wexner´s incontinence score and obtain the questionnaires about incontinence (urgency, discrimination, number of stools). After all data were collected using adequate statistic methods we will study the impact of anastomosis height on level of anorectal disfunction.

Results: Eighteen patients so far have been evaluated in our study. Six of them had anastomosis in distal third of rectum, five of them had anastomosis in middle rectum and in seven patients the anastomosis was located in proximal third of the rectum. The mean number of daily stools were 3.5, 2.6 and 1.07 in groups with distal, middle and proximal rectal anastomosis respectively. Percentage of patients with urgency and discrimination disorder were 66%, 40%, 0% and 83%, 40%, 0% respectively. The mean value of Wexners incontinence score was 7, 3.8 and 0.28 respectively in above mentioned groups.

Discussion: So far the results of incontinence questionnaires met our expectations where we found that patients with lowest coloretal anastmoses had the worst anorectal function with highest Wexners score. After receiving the results of anorectal manometry we will be able to evaluate value of anorectal manometry in predicting anorectal dysfunction after anterior rectal resection.

MeSH/Keywords: rectal resection, incontinence, anal manometry

Poster code: R-02-10-033

SEROTYPE DISTRIBUTION AND ANTIMICROBIAL RESISTANCE OF INVASIVE PNEUMOCOCCAL ISOLATES IN CHILDREN < 5 YEARS IN CROATIA (2005 TO 2014)

PhD candidate: Iva Butić

Part of the thesis: Serotype distribution and antimicrobial resistance in invasive Streptococcus pneumoniae isolates in Croatia

Mentor(s): Professor Arjana Tambić-Andrašević, MD PhD, Professor Waleria Hryniewicz, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Pneumococcal infections in children remain a major medical problem associated with high morbidity and mortality. The aim of this study was to evaluate serotypes and antibiotic resistance in invasive pneumococcal strains in children <5 years in Croatia from 2005 to 2014.

Materials and methods: Invasive pneumococcal strains were collected through the Croatian microbiological laboratory network with country coverage >95%. Serotyping was performed by the Quellung reaction (Statens Serum Institute, Copenhagen). The antimicrobial susceptibility testing was performed by disc diffusion method according to EUCAST guidelines. MICs for penicillin and erythromycin were determined (E-test, Biomerieux, France). Macrolide resistant isolates were tested for mefA and ermB genes by PCR.

Results: Three hundred and five invasive pneumococcal isolates were isolated in a 10-year period. The most prevalent serotypes were 14 (84 isolates), 19A (44 isolates), 6B (40 isolates) and 23F (30 isolates), comprising 65% of all invasive pneumococcal isolates. Non-susceptibility to penicillin was 30,5%, mostly detected in serotypes 14 and 19A (Fig.1.). Macrolide susceptibility was tested in 80% of isolates. Resistance was 48%, mostly due to serotypes 14, 19A and 6B (Fig 2.). In 84 out of 118 tested isolates, genes for macrolide resistance were detected: 58 isolates (69%) were ermB positive and 26 isolates (31%) were mefA positive.

Discussion: Non-susceptibility to penicillin and resistance to macrolides was mostly associated with serotypes 14 and 19A. Serotype 14 is covered by all available vaccines, whereas serotype 19A is covered by 13-valent vaccine only (Fig.3.). Resistance to macrolides was mostly mediated by ermB gene.

MeSH/Keywords: pneumococcal disease, serotype, antimicrobial resistance, macrolide, ermB, mefA

Poster code: R-02-16-067

THE IMPACT OF IODINE INTAKE ON FUNCTIONAL PARAMETERS OF THE THYROID IN PREGNANT WOMAN

PhD candidate: Vedrana Gladić Nenadić

Part of the thesis: The impact of iodine intake on functional parameters of the thyroid in pregnant woman

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

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

Introduction: According to the recommendations of the WHO/ICCIDD /UNICEF optimal daily intake of iodine in pregnant women is 250 μg, corresponding to the concentration of iodine in the urin (UIC) between 150 and 249 μg/L. Studies have shown that even in the countries with longtime programs of USI, there is always a proportion of pregnant women who have insufficient iodine intake. The American Thyroid Association (ATA) issued a recommendation that all women during pregnancy need to take supplements rich in iodine at a dose of 150 μg per day. However, these recommendations are not routinely accepted in practice. Epidemiological studies conducted 2009. in Croatia has shown that a significant number of pregnant women had a median UIC below the recommended level of 150μg/L, suggesting insufficient iodine intake. Therefore, further research are needed to assess whether there is a need for additional iodine intake in pregnant women.

Materials and methods: The research will include 2 groups healthy pregnant women in the 1,.2., and 3. trimester of pregnancy: 1. Pregnant woman who do not take supplements containing iodine and 2. Pregnant woman who take supplements containing iodine. The research includes: 1. Questionnaire with medical history information about previous pregnancies, complications of pregnancy, delivery, personal history of previous thyroid disorders and severe disease, family history of thyroid disease,drug therapy, dietary supplements with iodine and eating habits and iodized salt; 2. Ultrasound examination of the thyroid to determine thyroid volume; 3. Sampling urine to determine UIC modifying the method of Sandell-Koltoff; 4. Collection of blood samples for determination of TSH, FT4,Tg,TPOAt,TgAb. TSH and FT4 in serum will be determined using the chemoluminiscency, and Tg and TPOAb, TgAt elektrochkemoluminiscency method.

Results: The present study included a total of 95 subjects: 65 who did not take supplements containing iodine (1. group) and 30 that had supplements containing iodine (2. group). The following parameters were analyzed in both groups: TSH, fT4 and results were presented as AM ± SD, and Tg and TPO At and the results were presented as median and range (interquartile 25-27). In the first group, subjects who are not taking supplements containing iodine had higher serum fT4 value (12.1±1.9 pmol/L) of those that did not had iodine supplements (11.1± 1.3 pmol/L),wich is statistically significant (p=0.002). There was no statistically significant difference in other parameters: TSH value in first group was 1.9±1.3 mIJ/L and in second 1.9±0.9 mIJ/L, and Tg value in first group was13.4 ìg/L(7.6-20.9) and in second 15.0 ìg/L (6.2-20.9).

Discussion: Preliminary results at this moment are not in accordance with the hypothesis set that pregnant women not taking iodine supplements have lower values of thyroxine in serum but the study is ongoing and it is necessary to collect enough subjects in both groups (the prediction number in each group is 100) and to sample other parametars. We expect that the results of the research will be helpful in determining the importance of taking iodine supplements during pregnancy.

Acknowledgments: I thank my mentor Assist. Prof. Tomislav Jukic for their helpful advice and support.

MeSH/Keywords: iodine deficiency,pregnancy,thyroid hormones,iodination of salt

Poster code: R-02-17-042

LEVELS OF THYROID HORMONES IN DIFFERENT PROSTATE CANCER GRADE GROUPS

PhD candidate: Petra Petranović Ovčariček

Part of the thesis: Thyroid function in patients with newly diagnosed prostate cancer

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

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

Introduction: Prostate cancer is one of the most common malignancies in men. It's clinical behavior range from dormant to very aggressive. Many studies are focusing to determine a feasible biomarker that will help differentiate between these various forms. The prostate gland has receptors for thyroid hormones, i.e. thyroxin and triiodothyronine, which are associated with cell proliferation and carcinogenesis. The aim of this study was to investigate the association between thyroid hormone levels and different Grade Groups of prostate cancer to potentially identify eventual biomarkers for aggressive prostate cancer. Our hypothesis was that thyroid hormone levels are higher in patients with histologically aggressive prostate cancer.

Materials and methods: Upon approval by Institutional Ethics Board, a prospective study was initiated. Eligible participants were men aged between 60 and 70 who will undergo radical prostatectomy due to biopsy-confirmed prostate cancer, with no evident distant metastases. Exclusion criteria were history of thyroid disease, patients on medications known to affect thyroid function, serious comorbidity, and receipt of iodine contrast within one year. Study participants were divided into two exploratory research groups according to postoperative pathohistological findings. Subjects with Grade Group 1 and 2 of prostate cancer formed the first group, while subjects with Grade Group 3, 4 and 5 formed the second group. Target accrual for each group is 70 patients. Preoperatively, serum levels of T3 (total triiodothyronine hormone), fT4 (free fraction of thyroxine hormone) and TSH (thyroid-stimulating hormone) were measured using Immunoassay CLIA-Chemiluminescent at XPI Siemens Immulite 2000. Anti-TPO (antibodies to thyroid peroxidase) and Anti-Tg (antibodies to thyroglobulin) were measured with ECLIA - Electrochemiluminiscent Immunoassay at Roche COBAS e411.

Results: So far, we have completed data for 84 subjects. Four subjects were excluded as they failed to meet eligibility criteria – three patients had positive thyroid antibodies, and in one patient radical prostatectomy was abandoned. There are 46 subjects in the first group and 34 in the second group. Mean T3 level in the first group of patients is 1,36 nmol/L (range 0,9-1,9 nmol/L), while in the second group 1,59 nmol/L (range 1,1-3,1nmol/L). Mean fT4 level in the first group is 14,30 pmol/L (range 9,9-19,7 pmol/l), while in the second 14,65 pmol/L (range 11,0-19,9 pmol/L). Mean TSH levels are the same in the first group and in the second group – 1,48 mIU/L (range 0,561-3,67 mIU/L), 1,48 mIU/L (range 0,435-2,86 mIU/L), respectively. Participants in the second group (34 subjects), have higher levels of T3 and fT4 compared to the participants in the first group (46 subjects).

Discussion: Preliminary results of this study indicate that thyroid hormones, namely T3 and fT4, could potentially serve as biomarkers for aggressive prostate cancers. However, the main limitation of this study was the small sample size which precludes definitive conclusion on this matter. Yet, this study can provide benchmark for future research on eventual use of thyroid hormone levels to predict clinical behavior of prostate cancer, thereby potentially allowing treatment stratification.

Acknowledgments: I would like to thank my mentor Tomislav Jukić for providing me support and guidance in this research.

MeSH/Keywords: thyroid hormones, prostate cancer, Grade Groups

Poster code: R-02-19-011

PLASMA MICRORNA CAN SERVE AS A POTENTIAL DIAGNOSTIC BIOMARKER IN MYELODYSPLASTIC SYNDROME

PhD candidate: Inga Mandac Rogulj

Part of the thesis: Expression of circulating microRNA (miR-125a, miR-125b, miR-126, mir-99b, miR-let7a) in patients with myelodysplastic syndrome

Mentor(s): Assoc. Prof. Slobodanka Ostojić Kolonić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Myelodysplastic syndromes (MDS) are clonal haematopoietic stem cell disorders characterised by ineffective haematopoiesis leading to blood cytopaenias and by progression to acute myeloid leukaemia (AML) in one-third of cases. Current prognostic models in patients with MDS incorporate a number of factors that are related to the characteristics of the patient, the biology of the disease, transfusion burden, but not much regarding molecular genetic characteristics. Somatic mutations present in MDS can serve as an additional diagnostic or prognostic factors, but there are scarce data about the role of microRNA (miRNA) as posttranscription regulators of gene expression. MiRNAs genes are encoded in human genome mostly in the introns area of coding or non-coding transcripts, some of them in close proximity and are cotranscribed in clusters. They are small (~22-nucleotide) noncoding RNAs that fine-tune gene expression by base-pairing with target mRNAs, leading to messenger RNA (mRNA) destabilization or translational repression. The ability of these small molecules to interact with thousands of mRNAs in physiological and pathological states has led to identification of specific microRNAs linked to various diseases. Recently, many research articles have been published about their role in cancerogenesis in several areas: pathogenesis, drug resistance, biomarkers, and potential role in therapy. Due to molecular mechanisms investigated micro RNA (miRNA) profiling of specific circulating micro RNAs (miRNAs) could represent new noninvasive molecular target for detection and prognosis of MDS. Its clinical effectiveness is likely to be affected by various methodological issues and qPCR platform used. The aim was to investigate the plasma expression levels of four miRNA (hsa-miR-125a-5p, hsa-miR-99b-5p, hsa-miR-126-3p, hsa-miR-125b-5p) in MDS patients and healthy controls.

Materials and methods: We have analyzed plasma samples of 4 healthy controls and 18 untreated MDS patients (12 male, 6 females, median age 69 yrs). According to IPSS, 3 patients were MDS high risk (16,5%), 15 patients were low and INT-1 risk (83,3%). Whole blood was drawn into EDTA containing tubes and processed to plasma separation, miRNAs extraction (miRNeasy Serum7plasma Kit), reverse transcription (miScript II RT Kit) and qPCR measuring (miScript SYBR Green PCR Kit/Custom PCR Array). Data normalization (cell miR-39-3p) and data analysis (web- based software) were done according to the manufacturer instructions (Qiagen)

Results: We did not find any statistically significant difference in microRNA profile expression of healthy controls and MDS patients (hsa-miR-125a = 0,5184; hsa-miR-99b = 0,2626; hsa-miR-126 = 0,4024; hsa-miR-125b = 0,1227). All MDS patients had higher expression of 4 specific microRNA (hsa-miR-125a =1.72; hsa-miR-99b = 1.43; hsa-miR-126 = 1.40; hsa-miR-125b = 0,72). In the group of MDS patients, the expression of hsa-miR-125a was two times higher than expression of hsa-miR-125b (1.72 vs. 0.72).

Discussion: Higher expression of plasma hsa-miRNA-125a in MDS patients has been previously described. As we know, the hsa-miRNA-125a is involved in the process of myeloid leukemogenesis, and experimental studies have shown its impact on microRNA claster (miRNA-125b, 99b, let7a). The clinical potential of these 4 plasma miRNAs in claster as a biomarker in MDS should be validated in a prospective study that includes a large cohort of patients.

MeSH/Keywords: microRNA, myelodysplastic syndrome

Poster code: R-02-19-040

THE ROLE OF SERUM INTERLEUKIN-7 LEVELS AS BIOLOGICAL MARKER IN BREAST BANCER

PhD candidate: Faton Sermaxhaj
Part of the thesis: The role of serum Interleukin-7 level as biological marker in breast cancer
Mentor(s): Assist. Prof. Natalija Dedić Plavetić, MD PhD
Affiliation: University of Zagreb School of Medicine

Introduction: Breast cancer is a major public health issue worldwide, whose incidence is increasing dramatically. There are some indicators that may be used to redirect the prognosis of this disease as well as tend patients more effectively. Specifically, since Interleukin-7 induces the growth and proliferation of breast cancer cells, as well as lymphangiogenesis; the use of Interleukin-7 as a potential biological marker could prove useful.

Materials and methods: The research participants will be patients diagnosed with early invasive breast cancer. 260 participants from Croatia and Kosovo will be included in the study, 200 patients with early breast cancer (100 from Croatia and 100 from Kosovo) and 60 healthy participants as the control group (30 from Croatia and 30 from Kosovo). To evaluate the IL-7 serum level, blood samples will be taken from patients diagnosed with breast cancer prior to surgical intervention. In addition, after the surgical intervention, the histopathological specimen examination will be performed according to routine practice, to investigate tumor size, histopathological grade, hormone receptors' status, nodal status, Ki-67 status and lymphovascular and perineural invasion.

Results: The study is currently in the phase of data collection and preliminary results expected to be finalized in May 2018.

Discussion: Limited number of studies about IL-7 and BC have been conducted, taking into account the heterogenous populations. This study could help elucidate IL-7 serum levels' correlations with routine clinicopathological characteristics. Hence, results of this study will contribute in research medium on the potential use of IL-7 as a biological tumor marker and could further assist the research on the role of IL-7 in BC pathogenesis and progression. It could help towards the future development of novel diagnostic and therapeutic tools. The research will also contribute towards evaluation and comparison of clinicopathological characteristics of BC patients in Kosovo as compared to Croatian cohorts. The difference in IL-7 serum levels could be a reflection of genomic and epigenomic influences.

Acknowledgments: I would like to thank my mentor Assist. Prof. Natalija Dedić Plavetić for her help and support.

MeSH/Keywords: Interleukin-7, interleukins, breast cancer.

Poster code: R-02-19-078

THE EFFECT OF DIFFERENT ACIDITY SOLUTIONS AND THE NUMBER OF PITTING CORROSION CHANGES ON STAINLESS STEEL 316L

PhD candidate: May Labidi

Part of the thesis: The role of pitting corrosion on the integrity of osteosynthetic stainless steel 316L implants

Mentor(s): Assist. Prof. Ivan Dobrić, MD PhD, Professor Janoš Kodvanj, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Usage of metal implants in orthopaedic and trauma surgery has been practiced for many decades. 316L stainless steel is one of the most frequently used and until now there are many uncertainties to be resolved about implant breakage, one of them is metal corrosion and specifically the most common pitting corrosion. Biological media affects the integrity of osteosynthetic 316L implants due to pitting corrosion microstructural changes, by assessment of surgical stainless steel 316L we would like to confirm the effect of bilogical liqud media (SBF-Stimulated Body Fluid) the microscopic changes due to pitting corrosion.

Materials and methods: Using fourty eight (n=48) stainless steel 316L implants samples divided into four groups of a certain number of samples after randomization: the first group not immersed, second immersed in SBF(saline 0.9% NaCl), third group immersed in SBF with added lactic acid to obtain a pH value of 5.0, and fourth group immersed in SBF with added lactic acid to obtain a pH value of 6.0 . The morphology and dimentions of corrosion defects analyzed by SEM- Scanning Electron Microscope, biomechanical tests performed at the beginning and end of the study. Evaluation of corrosion changes by the SEM will be held in four different stages at a magnification of 2000 times: first at day ten, second at day 28 (four weeks), third at day 168 (six months), and the last at day 365 (one year later). Evaluation intervals are in accordance with bone healing dynamics and changes in acidity. A control evaluation will be performed at day zero.

Results: After eight months of starting the research, the first three evaluations were obtained as planned. Pits larger than 1.00μm were measured. In the first group at day zero 14 pits were found, in four plates none were found. Dimentions from 3.90 to 21.14 μm. In the second group at day zero 15 pits were found, the largest measured was 30.92 μm, in three plates none were found, at day ten 13 pits were found; the largest was 17.74μm, 11 had one and one had two pits, at four weeks 28 pits were found, the largest 8.86 μm and many other smaller than 1.00 μm were found additionally in 8 plates, at six months 26 pits were found, the largest was 32.57 μm and many other smaller than 1.00 μm were found additionally in 11 plates. In the third at day zero 14 pits were found; the largest was 45.36 μm, at day ten 13 pits were found; the largest was 17.42μm, 11 had one and one had two pits, at four weeks 41 pits were found; the largest was 32.02 μm and many other smaller than 1.00 μm were found additionally in all12 plates, at six months 37 pits were found; the largest was 27.13 μm and many other smaller than 1.00 μm were found additionally in all 12 plates. In the fourth group at day zero 14 pits were found; the largest was 37.68 μm, in three plates none were found, at day ten 17 pits were found; the largest was 15.80 μm, 2 had no pits, 6 had one and 4 had more than one pit, at four weeks 41 pits were found; the largest 12.96 μm and many other smaller than 1.00 μm were found additionally in all 12 plates, at six months 42 pits were found; the largest was 18.44 μm and many other smaller than 1.00 μm were found additionally in all 12 plates.

Discussion: Initial- zero day- changes seen by the SEM were to be expected, since production imperfections are notified in metallurgic industries. From the preliminary results it could be understood that the more acidic groups (3rd and 4th) at one month of immersion already had numerically much more (more than double) changes than before immersion and ten days later, and at six months the most acidic (4th) group had even more changes, whereas dimentions of pits were not getting larger as time passed and in different acidic solutions. Many new pits developed in all three groups after the first month of immersion.

Acknowledgments: I thank the biochemistry department at Zagreb Faculty of medicine for collaborating.

MeSH/Keywords: Osteosynthetic implants, 316L, corrosion surgical stainless steel

Poster code: R-02-20-044

SPINOPELVIC PARAMETERS PREDICT DEVELOPMENT OF PROXIMAL JUNCTIONAL KYPHOSIS IN EARLY ONSET SCOLIOSIS

PhD candidate: Ozren Kubat

Part of the thesis: The effect of spinopelvic parameters on the development of proximal junctional kyphosis in early onset scoliosis

Mentor(s): Professor Domagoj Delimar, MD PhD, Ron El-Hawary, PhD

Affiliation: University of Zagreb School of Medicine; IWK Health Centre, Dalhousie University

Introduction: Proximal Junctional Kyphosis (PJK) is diagnosed radiographically using the proximal junctional angle (PJA) and clinically with the requirement for proximal extension of the upper instrumented vertebrae (UIV) during revision surgery. Our hypothesis was that abnormal spinopelvic alignment will increase the risk of developing PJK in children with EOS.

Materials and methods: Children treated with distraction based implants from 2 EOS registries (min 2 yr f/u) were included. Sagittal radiographs were used to measure spinopelvic parameters and PJA (angle between caudal endplate of the UIV to the cephalad endplate 2 vertebrae above UIV). Risk ratios were calculated and analyzed using chi squared testing.

Results: 135 children with EOS meeting inclusion criteria were identified. Etiologies included 54 congenital, 10 neuromuscular, 37 syndromic, 32 idiopathic, and 2 unknown etiology patients. N=89 rib-based and n=46 spinebased implant surgeries were performed with mean age 5.2 yrs, scoliosis 71°, kyphosis (TK) 39°, lumbar lordosis (LL) 52°, pelvic incidence (PI) 49°, and pelvic tilt (PT) 11°. 24 children required revision with proximal extension of UIV. Final f/u: 56° scoliosis, TK 42° kyphosis, LL=55°, and PT=13°. Pre-op TK>50° had risk ratio of 1.67 (CI: 0.98-2.83)* for final PJA>10°. Increased pre-op LL, PI and PT did not increase the risk for final PJA>10°. None of these pre-op parameters were associated with increased risk of revision with proximal extension. Final PI-LL>20° and PT>30° were each associated with increased risk for revision with proximal extension (risk ratios of 2.1* and 2.5*). *denotes statistically significant.

Discussion: For patients undergoing growth-friendly surgery, pre-op hyperkyphosis increased the risk for developing post-op PJK. Final post-op PI-LL>20° and final post-op PT>30° were each associated with increased risk for revision with extension of UIV. Pre-op thoracic kyphosis and post-op spinopelvic parameters influence the development of PJK in children undergoing growth friendly surgery for EOS.

MeSH/Keywords: Early onset scoliosis; proximal junctional kyphosis; spinopelvic parameters; growing rods; VEPTR

Poster code: R-02-20-058

OXIDATIVE STRESS AND ANTIOXIDANT STATUS IN PATIENTS WITH CHRONIC RHINOSINUSITIS

PhD candidate: Aigerim Zhumabayeva

Part of the thesis: Association between reactive oxygen species and aryl hydrocarbon receptor in chronic rhinosinusitis

Mentor(s): Professor Livije Kalogjera, MD PhD, Professor Claus Bachert, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Constant exposure to environmental toxicants, such as cigarette smoke, organic air pollutants, exogenous and endogenous chemicals, allergens and other environmental factors, is known to cause chronic inflammatory response in the upper airways' epithelium. ROS are highly protective molecules generated during normal cellular metabolism or from external sources . When the level of ROS production exceeds the capacity of cellular antioxidants, an oxidative stress occurs. Aryl hydrocarbon receptor is a ligand-activated transcription factor that represents an important link between environmental stimulators and immune-mediated inflammatory disorder seen in chronic rhinosinusitis. As it acts as a sensor that detects the presence of xenobiotics and endogenous substances, We hypothesize that the aryl hydrocarbon receptor signalling pathways might be able to regulate oxidative stress and to subsequently modulate an inflammatory response seen in chronic rhinosinusitis.

Materials and methods: The study will include 57 patients who will undergo endoscopic sinus surgery procedure. The sinus mucosal samples and serum of 36 patients consist of 12 patients who are suffering from CRSwNPs, 12 operated due to chronic rhinosinusitis without nasal polyps (CRSsNP) and 12 control patients. This questionnaire focuses. on allergy and smoking history, the frequency of sinus surgery, the sensitivity to non steroid antiinflammatory drugs and co-morbidities. Total oxidative stress and antioxidative capacity will be measured in serum of each study groups patients Data will be confirmed by 4 hydroxynonenal-ELISA. Oxidative stress-induce lipid peroxidation marker 4 hydroxynonenal and acrolein will be measured using monoclonal antibody by immunohistochemistry. AhR and AhRNT protein expression will be measured in tissue homoginates using Western blotting test. mRNA NADPH oxidase isotypes measured by qRL-PCR assay. AhR expression in tissue will be measured using human monoclonal antibody by immunohistochemistry. The research shall be designed as a case-control study. To compare all 3 study groups we propose to use Friedman test and to compare experimental data we could use Wilcoxon and Sign test.

Results: Total oxidative capacity level is higher in both phenotypes of chronic rhinosinusitis compared with healthy controls ($p=0.01$, $p=0.011$, $p<0.001$, respectively).Total antioxidative capacity mostly are negative (80%) in serum samples. In CRS patients with lower TAC level (20%) , TOC level were higher compared to healthy controls. In all tissue samples of study groups 4 hydroxynonenal was negative, acrolein was identified in stroma and epithelium line of the tissue in patients with CRS comparing to healthy controls .The more inflammaton status in tissue than higher intensity of acrolein were identified. AhR and AhRNT protein expression and mRNA NADPH oxidase isotypes expression results are coming soon.

Discussion: Identification of lipid peroxidation markers in epithelium line CRS tissue can confirm that oxidative stress occurs in inflammated tissue. TAC/TOC imbalance could demonstrate oxidative stress level that cause inflammation and correlation between allergy status, smoking and chemicals exposure and TOC value in patients with CRSwNPs and CRSsNPs could indicate exposure of xenobiotics to chronic inflammation in CRS. Measuring ARH and AhR nuclear translocator protein expression could indicate AHR transcription factor activity in CRS tissue that could show role of xenobiotics in pathophysiology of chronic inflammation. Expecting correlation between AhR expression and activity and NADPH oxidase could demonstrated regulation of transcription factor to oxidative status in CRS patients

Acknowledgments: In performing original research we had to take the help and guideline of some respected persons, who deserve our greatest gratitude. Performing of this investigation gives us much Pleasure. We are grateful to our clinical staff of Otorhinolaryngology an

MeSH/Keywords: chronic rhinosinusitis, aryl hydrocarbon receptor, oxidative stress, antioxidative status

Poster code: R-02-21-038

PRESENCE OF BRAF V600E MUTATION AND LACK OF CPSF2 EXPRESSION AS PROGNOSTIC MARKERS FOR PAPILLARY THYROID CANCER

PhD candidate: Irena Makovac

Part of the thesis: Presence of BRAF V600E mutation and lack of CPSF2 expression as prognostic markers for papillary thyroid cancer

Mentor(s): Professor Drago Prgomet, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Papillary thyroid carcinoma (PTC) is the most common form of thyroid gland malignancy with substantial increase in incidence last two decades. BRAF mutation is important in cell and tumor progression and is the most common genetic event in thyroid cancer. Many studies find correlation between the presence of BRAF V600E mutation and aggressive behavior of PTC. According to the studies, prevalence varies between 30% and 80% depending on geographic region and iodine consumption. Genome-wide expression analysis in PTC samples identified five genes (CPSF2, LARS, AURKC, TRNT1 and BCL11A) that were differentially expressed in patients with PTC-associated mortality, giving that CPSF2 expression has the highest predictive value. Low protein expression of Cleavage and Polyadenilation Specificity Factor Subunit 2 (CPSF2) is associated with increased cellular invasion, increased markers of thyroid cancer stem cells (CD44 and CD133 expression) and predicts a poorer clinical outcome.

Materials and methods: During period from 2006 to 2014 we collected PTC samples of 90 patients with metastasis to regional lymph nodes of the neck and 40 patients withouth metastasis, operated at the Clinical Department of ENT, Head & Neck Surgery, University Hospital Centre Zagreb. Sample size was calculated based on Altman's nomogram. The expression of CPSF2 protein will be evaluated by immunohistochemistry (dilution 1:100-200, Abcam) (Figure 1). The presence of the BRAFV600E mutation will be evaluated by Cobas® 4800 BRAF V600 Mutation Test, both retrospectively in formalin-fixed, paraffin-embedded (FFPET) human PTC tissue samples . Patients epidemiological and clinical data and histopathological data (histological type, tumor size, extrathyroid invasion, lymphovascular invasion, status of the cervical lymph nodes metastases and distant metastases) will be collected from archived histopathological findings and medical history. During statistical data analysis we will use descriptive and analytical methods, intended for biomedical statistics. The level of significance will be set at $p<0,05$.

Results: 130 patients with PTC were included, and 62 (48 %) were BRAFV600E positive. Tumour size was similar for BRAFV600E-positive and -negative tumours (21.3 vs. 23.2 mm, $p = 0.23$). BRAFV600E-positive patients were significantly older at first operation (mean age 45 versus 49 years, $p = 0.003$). BRAFV600E-positive PTCs had a higher rate of lymph node metastasis (44 vs. 29.4 %, $p = 0.004$) and extra-thyroidal extension (44 vs. 22 %, p 0.001). There was no difference between groups for vascular invasion or multifocality. Negative protein expression of CPSF2 was observed in 28(21.5%) of the 130 PTCs. In multivariate analysis, negative CPSF2 expression was significantly associated with cervical lymph node metastasis (OR 2.56, $p=0.28$), and distant metastasis (OR 3.48, $p=0.02$).

Discussion: BRAF V600E mutation and low protein expression of CPSF2 in PTC is associated with the development of metastasis and aggressive form of the disease. Negative protein expression of CPSF2 is independently associated with a poor clinical outcome in PTC. CPSF2 could be a useful prognostic marker for PTC in regions with a high prevalence of the BRAFV600E mutation.

MeSH/Keywords: Papillary Thyroid Cancer, BRAF, CPSF2

Poster code: R-02-21-060

MITOCHONDRIAL QUALITY CONTROL DISORDER IN PATIENTS WITH ISHAEMIC HEART FAILURE

PhD candidate: Tomo Svaguša
Part of the thesis: Mitochondrial quality control disorder in patients with ishaemic heart failure
Mentor(s): Assist. Prof. Filip Sedlić, MD PhD, Academician Davor Miličić
Affiliation: University of Zagreb School of Medicine

Introduction: Heart failure is one of the most common causes of comorbidity in Western countries. Its pathogenesis is not yet entirely known. The most common cause of heart failure is ischemic heart disease. Newer studies indicate that mitochondria could play a key role in the onset of this disease. Mitochondria generate energy in cells but also in pathological conditions can produce toxic compounds such as free radicals (ROS). ROS can further damage the cell and ultimately lead to heart failure.

Materials and methods: In our preliminary experiments, we used 4 samples of healthy myocardium that we purchased from the United States. The other 8 samples we used were human myocardial tissue obtained heart explantation of the diseased heart. All 8 samples were from cardiac samples of ischemic cardiomyopathy. Samples were obtained from patients who were treated with heart transplantation at UHC Zagreb. All participants have voluntarily signed informed consent.

Results: Our preliminary results did not show any statistical significance. Four genes responsible for normal mitochondrial functioning (Yme1L, TOM70, PINK1 and OPA1) showed a tendency to be statistically significant ($P = 0.13$). For most of the other genes we analyzed, there was a difference in their expression but the results of the statistical analysis were not at the level of the above-mentioned genes.

Discussion: This research is in correlation with previous assumptions. Mitochondria really have an impact on the development of cardiac disorders. Certain mitochondrial genes are differently expressed in ischemic cardiomyopathy than in healthy myocardium. Further research is needed to confirm this claim.

MeSH/Keywords: mitochondria, gene expression, heart failure

Poster code: R-02-22-131

EARLY DETECTION OF DIABETIC NEPHROPATHY IN CHILDREN

PhD candidate: Bernardica Valent Morić

Part of the thesis: Ambulatory blood pressure monitoring and biomarkers of renal damage in early detection of diabetic nephropathy in children

Mentor(s): Professor Bojan Jelaković, MD PhD, Assist. Prof. Gordana Stipančić, MD PhD

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

Introduction: The morbidity associated with type 1 diabetes (T1D) in mainly related to development of long-term vascular complications the most common of which is diabetic nephropathy (DN). Microalbuminuria as a marker of glomerular damage is the most widely used early indicator of DN. However, recent research has shown that it's predictive value is limited because many T1D patients become normoalbuminuric at follow-up. The current data also suggest that subtle but early elevations of blood pressure (BP) antedate the development of microalbuminuria thus playing a key role in DN development. Recently, it has been shown that tubular damage is an important factor in progression of DN with neutrophil gelatinase-associated lipocalin (NGAL) being the most promising tubular marker.

Materials and methods: In this cross-sectional study we have included 191 children and adolescents of both genders, aged 5-20 years with T1D of at least 1 year duration. A control group of 93 children was formed in order to establish a normative values of NGAL in urine (in progress). The exclusion criteria for both groups were: hypertensive patients, receiving medication that affects BP, pre-existent kidney disease, acute or chronic inflamatory disease and abnormal urinary sediment. In study group three first-morning urin samples for albumin/creatinine ratio (ACR) were collected along with additional sample for NGAL and urinary sediment analysis. A child was classified as microalbuminuric if 2 out of 3 samples were positive for albuminuria. Subjects with T1D were further classified in 3 categories: 1. low normal albuminuria (n=159), 2. high normal albuminuria (n=29) and 3. microalbuminuria (n=9) with cut-off values for ACR (mg/mmol) set for each category as <0,83, ≥0,83<2,5 and >2,5 for males and <1,1, ≥1,1<3,5 and >3,5 for females, respectively. Ambulatory blood pressure monitoring (ABPM) was performed using oscillometric device Mobilgraf MO1100120, I.E.M.GmbH. The monitor was programed to measure BP every 15 min during the day and every 30 min during the night. We analysed 24 hour systolic and diastolic BP.

Results: A total of 191 children with T1D were included in the study along with 93 non diabetic controls. There were no differences in antropometric parameters between two groups. Of 191 children with T1D in 143 (74,8%) ABPM was normal, in 4 (2,1%) showed white coat hypertension, in 12 (6,2%) masked hypertension, in 24 (12,6%) prehypertension, in 6 (3,1%) ambulatory hypertension and in 2 (1,0%) severe hypertension. Mean systolic BP in albuminuria group 1,2 and 3 was 115,0±8,06, 111,6±7,63 and 118,4±3,61 mmHg, respectively and for diastolic BP 68,0±6,46, 66,3±6,6 and 73,8±3,72 mmHg, respectively. We found significant difference for 24 hour systolic and diastolic BP between three albuminuria groups (p=0,014 and p=0,0035, respectively). There was a significant difference between albuminuria group 1 and 2 for systolic (p=0,0367), but not for diastolic BP (p=0,1458) while between albuminuria groups 2 and 3 both systolic and diastolic BP showed significant differences (p=0,0134 and 0,0015, respectively).Albuminuria group 1 and 3 differ only for diastolic BP (p<0,05).

Discussion: Among T1D children we found lower prevalence of white coat hypertension compared to general pediatric population, possibly because this children have regular hospital visits. The prevalence of other hypertension categories was in accordance with results of others. Our results also showed that T1D children with microalbuminuria compared to those with high normal albuminuria group had higher both systolic and diastolic BP values. This results are in concordance with finding of others reporting that rises in ACR parallel similar progressive incresses in ABP. Suprisingly, children with low normal albuminuria had significantly higher systolic BP compared to high normal albuminuria group which was not confirmed in previous studies. Currently we have no explanation for this inverse phenomenon, but this preliminary results are only a minor section of overall analyses planned and we have to wait for all results to provide final conclusions. An increse in BP detected by ABPM may precede microalbuminuria and have a key role in the development of DN. Early documentation of elevated BP values in ABPM might warrant the use of renoprotective agents even before development of microalbuminuria.

MeSH/Keywords: ambulatory blood pressure monitoring, albuminuria, type 1 diabetes, children

Poster code: R-02-24-014

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

Introduction: Retinopathy of prematurity (ROP) is one of many chronic complications affecting very premature infants. ROP can be mild or progress to blindness. Normal retinal vascularization is delayed by premature birth. Vascularization continues after a period of time in an exaggerated manner as ROP phase 2. Ophthalmic examinations are performed to detect severe cases requiring treatment. Stress and pain of such mandatory diagnostic procedures have immediate effects and long-term consequences mostly on neurological development. Efforts to reduce stress and pain are obligatory part of contemporary neonatal intensive care. In very premature infants weight loss after birth is unavoidable, 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 of the study 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 24 infants. A week within weight gain acceleration immature avascularized retina is detected in all infants. ROP phase 2 emerges two weeks after weight gain acceleration in most infants and by the time of three weeks after weight gain acceleration it is detected in all cases. Severe ROP requiring treatment is not detected during observed period. There is no association of oxygen therapy cessation 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. Such association contributes to knowledge on pathogenesis of ROP with applicability in clinical practice. Weight gain acceleration might serve as an indicator to monitor the disease, with possible reduction of required ophthalmic examinations and exposure of newborn to stress, with timely diagnosis.

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-02-24-070

FREQUENCY AND TYPE OF RENAL DAMAGE IN CHILDREN AND YOUNG ADULTS WITH HAEMOPHILIA A AND B

PhD candidate: Zrinko Šalek

Part of the thesis: Frequency and type of renal damage in children and young adults with haemophilia A and B

Mentor(s): Assoc. Prof. Ernest Bilić, MD PhD, Professor Danko Milošević, MD PhD

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

Introduction: Haemophilia is rare, recessive X-linked inherited bleeding disorder with the incidence of 2-4 new new-born boys per year. There are many mutations of factor VIII and IX genes that cause haemophilia. Deficiency of FVIII activity is called haemophilia A, while deficiency of FIX is haemophilia B. They are classified according to the level of FVIII/FIX activity into three groups, severe, moderate and mild. Haemophilia A/B are treated today with plasma-derived and recombinant factor VIII/IX concentrates. Today we have better health care for the patients with haemophilia, and new uncertainties arise such as kidney pathology. The literature about haemophilia and kidney diseases is very rare, especially in children and young adults with haemophilia.

Materials and methods: After receiving informed consent, we have enrolled 29 (n = 29) paediatric patients in our prospective study so far. Expectedly, the majority of the patients have decreased FVIII activity – haemophilia A (n = 18) versus 11 patients (n = 11) with haemophilia B. According to the severity of haemophilia the patients were divided into two groups. The first group included the patients with moderate haemophilia (n = 4) and another group of severe haemophilia (n = 25).

Results: For everyone except one patient (n = 28) we tested serum creatinine concentration. The mean value of the serum creatinine is 46 μmol/L. The maximal value is 78 μmol/L while the minimum was 26 μmol/L. All the patients had FVIII and FIX activity tested. For the patients with haemophilia A the mean FVIII activity value is 0.049 kIU/L. The minimum FVIII activity value was 0.02 kIU/L, while the maximum FVIII activity value was 0.11 kIU/L. For the patients with haemophilia B the mean FVIX activity value is 0.046 kIU/L. The minimum FVIX activity value was <0.2 kIU/L, while the maximum FVIX activity value was 0.09 kIU/L.All the patients were tested for FVIII/FIX antibodies and they were all negative.

Discussion: Once we collect all the patient's data (including young adults) we can discuss the final results. We expect the results of our study to show if early kidney damage exist in children and young adults with haemophilia A and B and which the most important risk factors for the development of kidney damage are.

Acknowledgments: I would like to thank my mentor Associate Professor Ernest Bilić, MD, PhD and commenter Professor Danko Milošević for their support and scientific guidance.

MeSH/Keywords: haemophilia, bleeding, kidney damage, children

Poster code: R-02-24-100

NATIONAL REGISTRY FOR CHILDREN WITH INFLAMMATORY BOWEL DISEASE

PhD candidate: Lana Ivković

Part of the thesis: The role of national registry for children with inflammatory bowel disease in determining incidence and pediatric disease phenotype in Croatia

Mentor(s): Professor Sanja Kolaček, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Inflammatory bowel diseases have relapsing character and primarily affect gastrointestinal system, but may also occur in other organs and organ systems such as skin, joints, liver and gall bladder, eyes, pancreas, and rarely in lungs and kidneys. They are characterized by genotypic and phenotypic diversity. Disease in children is more aggressive and extensive than in adults and changes phenotype over time from mild to severe form. Due to many disease specifics regarding pediatric population, the only way for long-term systematic monitoring of children is through the patients registry. According to previous studies, national registries were established to determine national incidence and prevalence of disease as well as disease phenotype, and their most important value is possibility of long-term patient follow-up and tracking outcome of disease – development of malignancy and mortality level.

Materials and methods: In cooperation with pediatric gastroenterologists, we established national registry for children with inflammatory bowel disesase. It is formed as a web database. All-newly diagnosed children according to revised Porto criteria under the age of 18 were enrolled during one year period in Croatia. Database consists of all required details (age, sex, anthropometric measures, symptoms, extraintestinal manifestations, disease phenotype, results of laboratory, radiology, endoscopic and histological assessment, and disease activity index).

Results: Trough period of one year we enrolled 53 patients in national registry. There were 28 patients from Children's Hospital Zagreb, 6 patients from University Hospital Centre Zagreb, 6 patients from University Hospital Center Osijek, 5 patients from Clinical Hospital Center "Sisters of mercy", and 4 patients from Clinical Hospital Center Rijeka and Clinical Hospital Center Split. 58% of them were male, and 42% female. Most commonly diagnosed form of inflammatory bowel disease was ulcerative colitis (28 patients, 10 females, 18 males). Crohn's disease was diagnosed in 20 patients, and inflammatory bowel disease unclassified in 5 patients. Median of age at time of diagnosis was 14,75 years.

Discussion: Incidence and prevalence of inflammatory bowel disease is globally rising. 25% of inflammatory bowel disesase is diagnosed in children and adolescents. Most common age at diagnosis is varying in different studies, ranging from 9 to 14 years. In our case, the median age at diagnosis was 14,75 years. Unlike some studies showing that increase in incidence of inflammatory bowel disease is caused primarily by the increase in the number of Crohn's disease patients, data from our registry showed that ulcerative colitis is more often diagnosed than Crohn's disease. Also, 64% newly diagnosed patients with ulcerative colitis were male. Inflammatory bowel disease – unclassified was found in 9% of patients which is consistent with data from the EUROKIDS registry. Further evaluation of data is needed to determine the incidence of inflammatory bowel disease in Croatia as well as the existence of the north-south gradient.

MeSH/Keywords: inflammatory bowel disease, registries, child, Croatia

Poster code: R-02-24-121

TRANSITION FROM PEDIATRIC TO ADULT CARE IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE

PhD candidate: Tena Trbojević

Part of the thesis: Effect of transitional care on quality of life and disease activity in adolescents with inflammatory bowel disease

Mentor(s): Professor Sanja Kolaček, MD PhD, Iva Hojsak, PhD, research associate

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

Introduction: In 25-30% of all patients the diagnosis of inflammatory bowel disease (IBD) (Crohn's disease (CD), ulcerative colitis (UC) and IBD unclassified (IBD-U)) is established in childhood and in adolescence. Pediatric patients, at some point, are transferred to the adult health care where autonomy and responsibility are required. However, adolescents with IBD are mostly insufficiently competent in respect to disease management skills therefore leaving pediatric facility could lead to loss to follow-up and poor disease outcomes. To enable adolescent patients bridge the gap between pediatric and adult services, organized transition is required, whereby for a defined period of time health care is provided by both, pediatricians and adult specialists. Transition is defined as purposeful, planned movement of adolescents and young adults with chronic physical and medical conditions from child-centered to adult-oriented healthcare systems.

Materials and methods: IBD patients with pediatric-onset of disease who are ending pediatric care are dived in two groups. Group 1 includes patients who are transferred to adult health care system without organized transition whereas group 2 includes patients who are transferred to adult care through organized transition. Quality of life, activity of disease by disease-specific indexes, number of relapses and number of hospitalizations are evaluated in both groups. Organized transition is provided by both, pediatrician and adult specialists.

Results: Organized transition, provided by pediatric gastroenterologist and adult gastroenterologist, was implemented. In first year of implementation of this kind of care, 26 patients (age 18-21 years, male:female=9:17, CD:UC=20:6) transferred from three pediatric medical centers in Zagreb to adult care centers through organized transition. At the moment preliminary results are being analysed. We conducted as well survey of 27 adult IBD patients with pediatric-onset of disease who were transferred from our pediatric center into the adult care without organized transition, with the aim to determine their long-term prognosis (> 5 years) after transfer and to characterize their transfer experience. We expect that our results would expand knowledge about the role of transition care in IBD patients.

Discussion: Impact evaluation of organized transition on disease outcomes of IBD patients with pediatric-onset should give us new information for improving quality of care and disease management in IBD patients.

Acknowledgments: I would like to thank my mentors and colleagues. This research is financially supported by five pharmaceutical companies: AbbVie, Dr. Falk Pharma/Würth, MSD, PharmaS and Alvogen/Hospira

MeSH/Keywords: inflammatory bowel disease, transition, adolescent

Poster code: R-02-24-137

EVALUATION OF SUBCENTIMETER NECK LYMPH NODES WITH DIFFUSION WEIGHTED MRI IN HEAD AND NECK SQUAMOUS CELL CARCINOMA (HNSCC)

PhD candidate: Andrijana Jović

Part of the thesis: Evaluation of subcentimeter neck lymph nodes with diffusion weighted MRI in head and neck squamous cell carcinoma

Mentor(s): Assist. Prof. David Ozretić, MD PhD, Assoc. Prof. Mirko Ivkić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Lymph node metastasis is an important prognostic factor in patients with head and neck squamous cell carcinoma (HNSCC). An accurate assessment of lymph node metastasis is an important prerequisite for staging and proper treatment planning. Computed tomography (CT) and/or magnetic resonance imaging (MRI) are frequently used to preoperatively assess lymph node status in patients with HNSCC using morphologic criteria. Aim of our study is to determine whether diffusion weighted MRI can distinguish metastatic from metastatic free lymph nodes smaller than 10 mm in patients with HNSCC and clinical N0 neck.

Materials and methods: From October 2016 until April 2018 35 patients with biopsy proven HNSCC and clinically negative for lymphadenopathy underwent MRI with DWI of the neck. The study protocol was approved by the local ethics committee; informed consent was obtained from all patients. All MRI examination were performed with 1.5 T scanner (Espree, Siemens, Germany) with head and neck coil. DWIs were performed with the following imaging parameters: TR=7600 ms, TE=90 ms, slice thickness 4 mm, FoV 380 mm, matrix size 128x128, voxel size 2x2x4 mm. ADC was calculated using 3b values; 0, 500 and 1000 s/mm². From DWI images we measured apparent diffusion coefficient (ADC) of the lymph node by manually placing ROI in lymph node. All measurement were done on Leonardo Siemens workstation. The patients had neck surgery including neck dissection. Lymph nodes from dissection specimens were marked according to the anatomical neck level and sent to the pathologist. Results of pathologist analysis were defined as positive or negative. We compared measurement results from the MRI images with the results of the pathological examination. Statistical analysis were performed using MedCalc statistical software. Primary statistical analysis was done by mean ± standard deviation between metastatic and benign nodes. To find the optimal ADC threshold value to differentiate metastatic from benign lymph node we used ROC Curve. We calculated sensitivity, specificy, NPV, PPV and the area under the curve with 95% confidence interval.

Results: We measured 104 lymph nodes, 56 benign and 48 malignant. The mean minimum axial diameter was greater in metastatic lymph nodes (mean ± SD, 8.4±1.3 mm; range, 5.2–10.7 mm) than in benign lymph nodes (mean ± SD, 5.5 ± 2.4 mm; range, 5.2–10.0 mm). For malignant lymph nodes mean ADC value was 0.870×10^{-3} mm²/sec (range: 0.764–0.992 mm²/sec). In benign lymph nodes, ADC maps showed an average value of 1.182×10^{-3} m²/sec (range: 0.973–1.375 $\times 10^{-3}$ mm²/sec). Area under the ROC curve , with standard error and 95% confidence interval was 0.958, 0.846 to 0.996 (Z=15.582, P=0.0001). The optimal cut off value was 0.947 for distinguishing metastatic from metastatic-free lymph nodes. For optimal ADC sensitivity, specificy, PPV, NPV and accuracy was 88.46%, 93.75%, 51.6%, 99.1%, 92%, respectively.

Discussion: Few studies have reported the ability of diffusion-weighted imaging (DWI) to distinguish metastatic free from metastatic lymph nodes in patients with HNSCC. The main advantage of DWI is its sensitivity to microscopic pathological alterations before they become visible on conventional MRI; thus, DWI could remedy the morphological criteria limitations. But those studied didn't noted if lymph nodes had central necrosis or not. Central necrosis strongly affect to results of measuring ADC value. ADC value is high in central necrotic part so all measurements should be done by placing region of interest (ROI) within solid part of lymph nodes. Also, other studies had patients with different stages of HNSCC, and examinations were done on different straight of MRI field 1.5T and 3T. We are doing our study on the same machine and with patient all clinically negative for lymphadenopathy. Few studies were done with small number of lymph nodes so results are limited. We are planning to have more than 100 lymph node to have better accuracy DWI and ADC are strongly sensitive to artefacts so technical procedure is highly depended on collaboration with patients. Taking everything mentioned above in account, we believe to get good and presentable results of our study.

MeSH/Keywords: Cancer of head and neck, Diffusion magnetic resonance imaging

Poster code: R-02-25-009

ASSOCIATION OF GALLSTONE DISEASE WITH PERSONALITY DIMENSIONS

PhD candidate: Tatjana Jukić
Part of the thesis: Association of gallstone disease with personality dimensions
Mentor(s): Branka Aukst Margetić, PhD, research associate
Affiliation: Neuropsychiatric Hospital Dr. I. Barbot, Popovača

Introduction: Studies have shown that persons with gallstones have a higher risk of developing depression and emotional instability. Also, most previous research indicates connectivity personality traits with disturbances in lipid and glucose metabolism, which is indirectly associated with the metabolic risk factor for development of gallstones. Until now personality in gallstone disease was not measured by the validity criteria and a consistent personality profile of gallstone patients has not yet be established. For the assessment of personality, the Temperament and Character Inventory (TCI) was chosen, being one of the most widely used instruments for the assessment of personality in current research. The aim of this study is to determine if there is difference in personality dimensions in group with gallstones and control group without gallstones.

Materials and methods: It is planned to examine 300 patients (150 patient with ultrasound verified gallstones and 150 control subjects without gallstones matched for age, gender, body mass index, waist-hip ratio, glucose and lipid status). Inclusion criteria are the presence of gallstones. According to these criteria participants are assigned in two groups: participants with gallstones and participants without gallstones (control group). Both groups are measured body mass index, waist-hip ratio, blood glucose and lipid status. Differences between groups are analyzed by the test set including: Dimensions of personality will be assessed with Temperament and Character questionnaire (TCI-120). As depression highly correlates with temperament dimension Harm Avoidance, Center for Epidemiologic studies Depression Scale (CES-D) is used to measure depression as the control variable. Sociodemographic data were assessed with questionnaire.

Results: The research is still in progress, and currently is in the phase of data collecting. Up to now we completed the data for 58 participant, 32 (55%) with gallstones and 26 (45%) without gallstones in control group. Data collected from study participants are still not sufficient for statistical analysis, but for now indicated that participants with gallstones have higher levels of personality dimensions Harm Avoidance or Novelty Seeking compared to the control group.

Discussion: This is the first study to examine the relationship of gallstone disease with personality traits. Preliminary results show that there might be association between gallstones and personality dimensions Harm Avoidance and Novelty Seeking. When data from more participants are collected and analyzed, more appropriated results might be given. We hope that results of this study will show whether persons with gallstones differ from those who do not have, and are they the metabolic risk factors for the development of cholesterol gallstones related to the dimension of personality. This research also emphasize the need for prevention in the field of psychological health because of long-term mental problems can lead to the formation of somatic disturbances.

MeSH/Keywords: gallstone disease, persnality dimensions

Poster code: R-02-25-133

EFFECTIVENESS OF A NEW BREAST SHIELDING DEVICE IN ABDOMINAL CT - PRELIMINARY RESULTS.

PhD candidate: Nikola Ivan Leder

Part of the thesis: Effectiveness of breast radiation protection by circumferential shielding of thorax in computerized tomography of the abdomen

Mentor(s): Assist. Prof. Jelena Popić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: During CT scanning of the abdomen, a significant radiation dose is absorbed by the radiosensitive breast tissue, even though it is outside the primary imaging plane. The absorbed dose is dependent on several factors, including various imaging parameters, and the patient's anthropomorphic attributes. To emphasize the importance of this exposure, literature data demonstrates that unshielded breast during regular abdominal CT examinations is exposed to a dose as high as 3 mGy, while epidemiological studies show that exposure of breast tissue by 0,01 Gy fractionally increases risk of breast cancer by 13,6%. To date, several studies have demonstrated that using breast radiation shielding devices significantly lowers the radiation dose absorbed by breast tissues, however all these studies used frontal shielding devices, akin to a lead bra. During CT scanning the patient is exposed to an X-ray beam which rotates 360 degrees around the body, so to achieve significant dose reduction in breast tissues, we have constructed a shielding device which encompasses the entire circumference of the patients' upper thorax.

Materials and methods: Our custom tailored shielding device covers the entire thoracic circumference, unlike the commercially available anterior breast protection devices. This kind of shielding device provides both radiation shielding equivalent to a lead apron and physically compresses breasts cranially thus providing additional protection according to the inverse square law. Since there are no comparable studies in available literature, we have performed a pilot study on 28 participants, randomly split into three groups: no protection, anterior protection and complete thoracic circumference protection. Study participants were patients ordered for regular abdominal CT examinations in our institution, with signed informed consent for study inclusion. Only patients with no anatomical anomalies were included in the study (prior surgery, trauma, implants). Radiation dose was measured using TL dosimeters placed on the surface and below the shielding device. Our shielding device is custom tailored from a standard frontal lead apron with protection equivalent of 0,5 mm of lead. It is tailored as a short shirt with frontal touch fastener to accommodate various body sizes.

Results: Our pilot study demonstrated expected results according to the literature data, in some patients showing even significantly higher doses than expected measured on unshielded breasts. WIth application of our shielding devices, we demonstrated an average reduction in radiation dose measured on the surface of the breast with anterior shielding of 42% and in complete shielding with our custom device of 53% as opposed to doses measured on unshielded breasts.

Discussion: Data gathered from our pilot study confirms literature data that breasts are exposed to a significant amount of ionizing radiation during abdominal CT examinations, even though they are outside the primary imaging plane. We have also confirmed that applying a breast shielding device significantly reduces the radiation dose to the breast and that using our custom shielding device which envelops the entire upper thorax provides additional protection value. During our planned study, to achieve statistical significance we will use the same grouping for our participants with 30 participants in each group. In vitro studies on an anthropomorphic phantom will also be performed to enable dosimetry within the body of the phantom to attempt and calculate the amount of radiation spread to the breasts from outside and inside the patients body to further confirm the feasibility of using breast shielding devices.

MeSH/Keywords: Tomography, X-Ray Computed; Breast; Radiation Protection; Radiation Dosage

Poster code: R-02-25-141

TOXICITY OF INFRADIAPHRAGMAL RADIOTHERAPY IN PATIENTS WITH LYMPHOMA

PhD candidate: Lea Galunić Bilić

Part of the thesis: Efficacy and side effects of infradiaphragmal radiotherapy in patients with lymphoma

Mentor(s): Assoc. Prof. Fedor Šantek, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Lymphomas are very radiosensitive and radiotherapy (RT) was the first treatment modality that enabled cure. It is the most effective single modality for local control of lymphomas and is an important part of the treatment for many patients. However, as a local form of the treatment, the curative intention is possible only if all lymphoma tissue (macroscopic disease evident at the time of simulation and potential sites of microscopic disease) can be incorporated into the volume to be irradiated with the prescribed total irradiation dose. Radiotherapy can be used as an effective single treatment modality in the management of lymphoma. It can be used as a consolidation therapy or as salvage after the failure of chemotherapy. In the past two decades irradiation techniques have been improved in order to spare the critical tissues and to reduce toxicity. Study data are nowadays still mostly derived from the patients who received supradiaphragmal radiotherapy, therefore there is no agreement about the best management approach for the patients with infradiaphragmal lymphoma.

Materials and methods: Between January 2006 and December 2014 patients with proven Non-Hodgkin's lymphoma were treated with external beam radiotherapy to the infradiaphragmal region: as adjuvant treatment to chemotherapy (22 patients), as curative treatment for residual mass (26 patients) or as salvage treatment for refractory disease (13 patients). The dose administered was between 4 Gy to 47.5 Gy at the rate of 1.8-2 Gy per daily fraction. Irradiation was performed on mega-voltage equipment. Patients were irradiated with 2D technique or 3D conformal radiotherapy (3DCRT). A follow-up could be obtain on all patients.

Results: We studied 28 females and 33 male patient. Median follow-up was 54 months. The in-filed and out-of-field recurrence rates were 2 and 22 % respectively for the entire group and 8 and 31% respectively for the palliative group. Acute toxicity experienced 24 (39%) patients. It was nausea, fatigue and abdominal cramps. In three patients (5%) treatment was inerrupted because of fatigue in one patient, nausea in one patient and leukopenia in one patient. In four patients (7%) treatment was suspended: in two patients because of nausea and vomiting, in one patient because of nausea and diarrhea, and in one patient because of leukopenia. In 4 (7%) patients we don't know how they endured the treatment. Long term side-effects were noticed in 6 (10%) patients. One patient have renal disfunction, one gastritis, one diabetes mellitus and one secundary leukemia. Those patients required treatment because od toxicity. One patient (2%) died due to secondary cancer in the field of irradiation.

Discussion: Lymphoma treatment in the abdominal area is the issue of existing controversy. Although effective, RT is a neglected modality of the treatment due to the appearance of new drugs and because of the fear of side effects after irradiation. Radiation has been shown to be effective in the treatment of all stages and forms of lymphoma, but there is no consensus on the field size or the administered dose in the abdominal area. All current knowledge, radiation guidelines, and radiation side effects are for supradiaphragmal fields. This analysis demonstrates that radiotherapy can be effective in achieving local control of the lymphoma patients, and that the toxicity was very low in abdominal region.

MeSH/Keywords: Non-Hodgkin's disease, Radiation toxicity, Radiotherapy

Poster code: R-02-26-111

HYPERTROPHY AND HYPERPLASIA OF LEYDIG CELLS IN MEN WITH NON-OBSTRUCTIVE AZOOSPERMIA

PhD candidate: Dinko Hauptman

Part of the thesis: Hypertrophy and hyperplasia of Leydig cells in men with non-obstructive azoospermia

Mentor(s): Professor Željko Kaštelan, MD PhD, Professor Davor Ježek, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Non-obstructive azoospermia (NOA) is a type of male infertility caused by failure of spermatogenesis at the testicular level. Biopsies of men with non-obstructive azoospermia often reveal histological changes described as enlarged islets of Leydig cells. Combination of cell growth (hypertrophy) and cell proliferation (hyperplasia) of Leydig cells could be caused by a disruption of the hypothalamo-hypophyseal-gonadal axis's negative feedback mechanism, causing "bombardment" of Leydig cells with gonadotropins. This condition is characterized histologically by an increased total volume of Leydig cells and presence of atypical nuclei with more numerous nucleoli. Generally, hyperplastic Leydig cells tend to more closely resemble their derivative mesenchymal cells than mature Leydig cells. Interestingly, one study found that the changes previously described as Leydig cell hyperplasia may in fact be hypertrophic in nature, contrasting previous literature and raising questions about the true nature of the interstitial histological anomalies in azoospermic men.

Materials and methods: We plan to perform stereological analysis from samples of testicular biopsis in men with non-obstructive azoospermia. As control group we shall include patients with obstructive azoospermia (OA) who have normal spermatogenesis. A total od 72 patients will be included in this study. Control group will consist of 24 patients. Samples shall be fixed and stained using hemotoxylin and eosin staining and shall be graded on an infertility scale based on spermatogenic success. One-hundred to two-hundred microscopic fields shall be analysed for each testis. A microscope with a 42-point grid shall be used to determine the volumes of seminiferous tubules, interstitial space, and Leydig cells. Semithin sections shall be obtained using a microtome and shall be stained using toluidine blue.

Results: So far we examined 30 patients with non-obstructive azoospermia and 10 patients with obstructive azoospermia as control group. Volume and number of Leydig cells in men with NOA are profoundly heterogeneous. Patients with NOA have increased Leydig cell volume and number, though these results were not statistically significant. We expect more details after analysing all patients included in this study

Discussion: Histological analysis of testicular parenchyma in men with non-obstructive azoospermia revealed remarkable heterogenity amongst samples. Upon qualitative analysis, alterations in testicular parenchyma are apparent. In patients with NOA, seminiferous tubules are atrophied and largely devoid of mature spermatids. Leydig cell total volume is noticeably expanded in some NOA patients. Stereological analysis indicates that men with NOA seem to have increased volume and number of Leydig cells when compared to control, though further research must be done to determine significance.

Acknowledgments: I would like to thank Professor Željko Kaštelan and Professor Davor Ježek for guidance with this project.

MeSH/Keywords: hypertrophy, hyperplasia, Leydig cell, azoospermia

Poster code: R-02-28-002

UROTHELIAL CARCINOMA OF UPPER URINARY TRACT, ENDEMIC NEPHROPATHY AND HLA-DRB1 GENES IN THE CROATIAN POPULATION

PhD candidate: Damir Dittrich

Part of the thesis: Urothelial carcinoma of upper urinary tract, endemic nephropathy and HLA-DRB1 genes in the Croatian population

Mentor(s): Professor Željko Kaštelan, MD PhD, Professor Zorana Grubić, MD PhD

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

Introduction: Endemic nephropathy (EN) is a chronic tubuloinsterstitial renal disease. EN is associated with the development of cancer of the upper urynari tract (UUC). The purpose of the present study is to investigate the differences between EN patients with and without UUC given the prior history (age, age at onset of the disease, gender), and the diversity of HLA genes between these two subgroups.

Materials and methods: The study include 83 patients aged 58-88 years treated at the General Hospital „Dr. Josip Benčević" Slavonski Brod, divided into 2 subgroups (patients with/without UUC) and 150 healthy matched controls. Diagnosis of EN was established on the basis of medical history, clinical and laboratory parameters. Diagnosis of UUC was also established on the basis of red blood cell and/or malignant cells in urine, intravenous urography or CT, and MR urography. Five milliliters of peripheral blood with EDTA of all patients treated at the Urology Department and Dialysis Department in the period from 2005 to 2018, in the hospital, „Dr. Josip Benčević" in Slavonski Brod, was collected for HLA typing. Five mL of peripheral blood with EDTA was also obtained from control subcjects. All individuals were tested for HLA-DRB1 genes using Polymerase Chain Reaction – Sequence Specific Oligos probe (Luminex technology). Gene frequencies were calculated by statistical program (Genera Program; http://geneva.unige.ch/ahpd/). The frequencies were compared by X2 test. P value less then 0.05 was considered as significant.

Results: Thirteen different HLA-DRB1 genes were observed among EN patients as well as among controls. The most frequent HLA-DRB1 gene in patient's group was HLA-DRB1*11 (20.7%), while among controls two HLA-DRB1* genes (DRB1*11 and DRB1*16 were observed with the same frequency (15.9%, each). Comparison between tested groups revealed signisicant P value for DRB1*16 which was significantly more present among patients in comparison to controls (15.9% vs. 8.3%; P=0.0144).

Discussion: It is well established association between HLA genes with different autoimmune disease as well as with some carcinoma. Our very preliminary data demostrated existance of positive association between DRB1*16 gene and EN in our population. The HLA-DRB1*16 gene is one of the most frequent HLA-DRB1 genes in the Croatian population and its frequencies increased from the North to the South of Europe. The obtained data needs to be prooved and it is also necessary to analyze the distribution of HLA-DRB1 genes among patients with and without UUC.

MeSH/Keywords: endemic nephropathy, HLA-DRB1 genes, upper urinary tract carcinoma

Poster code: R-02-28-085

THE ASSOCIATION OF PROSTATIC INTRAEPITHELIAL NEOPLASIA AND INCIDENCE OF PROSTATE CANCER IN REPEATED PROSTATE BIOPSY

PhD candidate: Igor Grubišić

Part of the thesis: The association of prostatic intraepithelial neoplasia and incidence of prostate cancer in repeated prostate biopsy

Mentor(s): Assist. Prof. Borislav Spajić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Prostate cancer is the most common malignant tumor in Europe in men over the age of 70, especially in developed countries. According to the data of the Croatian Institute for Public Health in Croatia, prostate cancer is the second most frequent cancer of men in 15% of the population and the second cause of the death of male population suffering from malignant disease. Over time, more histologically changes in prostate tissue have been proclaimed by precancerous prostatic changes: Adenosis, Proliferative Inflammatory Atrophy (PIA) and Prostatic intraepithelial neoplasia (PIN). In the 1960s Mc Neal described prostate intraepithelial neoplasia for the first time, under the name intraductal dysplasia, and in 1986. Mc Neil and Bostwick described and categorized it more closely. The PIN is predominantly in the peripheral prostate area (75-80%), rarely in the transitional zone (10-15%), and very rarely in the central zone (5%) of the prostate, which coincides with the localization of prostate cancer. Other clinical parameters such as PSA, DRE finding, prostate volume, and patient age are still the basis for discussion, is the PIN an independent prostate cancer predictor.

Materials and methods: It is a retrospective study which uses the data from the archives of the findings of the Urology Clinic and the Clinical Institute of Pathology UHC Sestre milosrdnice. The identity of the patients involved in the research will be protected. The patient's personal data will be replaced by a clinical number and known only to the researchers. The findings are in the period from 01.01.2005. to 31.12.2014. Year in which there are about 5000 patients who have been subjected to clinical treatment and prostate biopsy due to suspected prostate cancer. The research group includes the following criteria: - in the first biopsy of prostate diagnosed PIN; - PSA less than 10 ng / ml; - without ASAP finding; - normal DRE; - in repeated biopsy, not more than three years after the first biopsy, diagnosed prostate cancer. The control group includes patients from the same archives that have the following characteristics: no PIN in the first biopsy, patients with PSA values <10 ng / ml, patients without ASAP findings, patients with a normal DRE, prostate cancer on repeated biopsy. All prostate biopsies were performed on Siemens SONOLINE Prima, with a 5-7.5 MHz rectal probe, and a biopsy „gun" of Magnum Bard and a biopsy needle „Bard magnum biopsy needles" at the Clinic for urology of the Clinical Hospital Center Sestre milosrdnice.

Results: We identified 95 patients in both groups. Control group have 9% of patients with prostate cancer on repeated biopsy, and research group have 32% of patients with prostate cancer on repeated biopsy. Prostatic intraepithelial neoplasia (PIN) on first prostate biopsy have much higher rate of prostate cancer on repeated biopsy. Our first results demonstrate that patients with bilateral PIN and/or localised PIN on the prostate apex on initial biopsy are also at greater risk for prostate cancer. All wanted results are not finished because we are going to compare age, PSA value and position of PIN in both groups.

Discussion: The role of PIN as independent risk factor for prostate cancer is still controversial. According to the EAU Guidelines, since 2010 the PIN finding is not an independent lesion that increases the risk of prostate cancer, and the repeated biopsy is indicated only in certain specific and clearly defined cases but its role as a risk factor remains uncertain. According to European guidelines for re-biopsy indications, there is an increase in PSA or PSA constant high values, suspicious digitorectal finding, ASAP, multiple PIN findings in extended prostate biopsy. Many studies reported on the positive predictive value of PIN, and reported detection rate was 2,3 -100%. Our first results demonstrate that position of PIN findings and bilateral PIN findings have much higher rate of prostate cancer on repeated biopsy than the benign prostate findings (32% vs 9%). A strength of this study is that all biopsis were performed in one center and pathological material was recorded, obtained and interpreted in also single institution. The presence of PIN in prostate apex and/or bilateral pin is independent risk factor for prostate cancer.

MeSH/Keywords: Prostate cancer, PIN, PSA, prostate biopsy, premalignant lesions

Poster code: R-02-28-112

DIAGNOSTIC VALUE OF SERUM CHEMOKINE CXCL13 CONCENTRATION IN PATIENTS WITH PROSTATE CANCER

PhD candidate: Marjan Marić

Part of the thesis: Diagnostic value of serum chemokine CXCL13 concentration in patients with prostate cancer

Mentor(s): Professor Željko Kaštelan, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Prostate cancer is second most common cancer in men. Usually is adenocarcinoma, and for patologicall scoring Gleason score (GS) is used and also International Society of Urological Pathology (ISUP) classification in 5 grades. Chemokines are low molecular weight basic proinflammatory proteins implicated in a variety of diseases. They have been associated with acute and chronic inflammation as well as with immunologically mediated diseases and various malignant diseases. Few recent studies showed biological and clinical significance of receptor CXCR5 and it's chemokine ligand CXCL13 in prostate cancer.

Materials and methods: Study is designed as a case-control study which so far included 80 patients (40 with biopsy proven prostate adenocarcinoma and 40 with normal prostatic tissue on transrectal prostate biopsy specimen (control group)). Chemokine concentration was measured according to standardized enzyme immunotests.

Results: A total of 80 patients was included in the study, with median age of 64 years (IQR 52-83). Half of them (40 patients, 50%) have adenocarcinoma on prostatic biopsy, with median Gleason score of 6.5 (IQR 6-7). Median chemokine CXCL13 concentration in patients with prostate cancer was 72.1 (IQR 61-90-2), while in control group was 70.8 (IQR 63.1-84.15). Median PSA value in adenocarcinoma group of patients was 9.415 (IQR 6.11-13.4), and in control group was 8.45 (IQR 5.16-16.17). Groups didnžt differ statistically regarding CXCL13 or PSA concentration ($p>0.05$).

Discussion: More complex statistic tests and higher sample number are neccessary to evaluate if there is diagnostic value of CXCL13 in prostatic adencarcinoma. Also, further analysis will determine if serum concentration of CXCL13 and/or multimarker approach (CXCL13 and PSA) is better predictor of more aggresive form of prostate cancer than prostate specific antigen (PSA) alone.

MeSH/Keywords: chemokine CXCL13 prostate cancer

Poster code: R-02-28-116

SHAME AND NARCISSISM

PhD candidate: Marija Eterović

Part of the thesis: The association between shame and grandiose and vulnerable narcissistic personality traits

Mentor(s): Professor Vesna Medved, MD PhD, Vedran Bilić, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: Theorists have long suggested that different dimensions of narcissism can be roughly categorized into narcissistic grandiosity and narcissistic vulnerability. Although this heterogeneity has been noted by many clinicians, empirical research had emphasized the grandiosity component while largely ignoring or excluding vulnerability. Despite the growing interest in narcissism, there are many unanswered questions in the literature, including interactions between narcissism and shame, which has been acknowledged as a central emotion in narcissism across different psychotherapy approaches. Additionally, shame is typically assessed using the total shame score on self-report measures of shame and guilt. In that way, only the conscious shame is measured. Implicit (unconscious) shame, which is typical for grandiose narcissists, remains overlooked.

Materials and methods: A minimum number of 300 students will be enrolled into the study. 1. Translation into Croatian language using forward- and back-translation processes, adaptation, and preliminary validation of the self-report measures of shame- and guilt-proneness (Test of Self-Conscious Affect, TOSCA), grandiose narcissistic personality traits (Narcissistic Personality Inventory, NPI), and vulnerable narcissistic personality traits (Hypersensitive Narcissism Scale, HNS). 2. Adding the situations of the increased intensity of shame-inducing scenarios to the original situations of the measure of shame- and guilt- proneness (TOSCA), and applying them, together with other self-report measures and demographic questionnaire to all subjects. 3. Examination of the associations of indicators of grandiosity, as well as vulnerability, with indicators of shame-proneness, with variability of the responses that indicate shame-proneness, and with sensitivity to the increased intensity of shame-inducing situations.

Results: Narcissistic grandiosity is associated with rigid and totalistic denial of shame. On the contrary, narcissistic vulnerability is associated with flexible and non-totalistic affirmation of shame.

Discussion: Shame seems to be the underside of narcissism. However, only vulnerable narcissists acknowledge shame, while grandiose narcissists defend against that painful emotion – they deny it in a rigid and totalistic manner, which is reflected in their pattern of responses on the original and modified measures of shame and guilt. That pattern of responses may serve as an indicator of implicit shame, which is typical for narcissistic grandiosity. The proposed research represents the first attempt of objectivizing implicit shame by self-report measures of shame and guilt. The methodological novelty of our research is exploring the pattern of responses on the original measures (measuring variability of participant's responses, apart from their total scores), and their modification (addition of shame-inducing scenarios of increased intensity to the original measure). By measuring both grandiose and vulnerable component of narcissism in each respondent and examining them as dimensions, as opposed to categories (we do not group participants into grandiose or vulnerable category, but examine how grandiose and how vulnerable each participant is), we generate a more clinically relevant narcissism construct. We contribute to understanding the complex interplay between shame and narcissism by examining the differential association of grandiose and vulnerable component of narcissism with proneness to shame.

MeSH/Keywords: shame, narcissism, grandiosity, vulnerability

Poster code: R-02-29-018

CORELLATION BETWEEN PLATELET MAO-B ACTIVITY AND SEROTONIN CONCENTRATION WITH DEPRESSION RATING SCALE SCORES IN MEDICATION-NAIVE DEPRESSED PATIENTS

PhD candidate: Anja Maravić

Part of the thesis: Effects of vortioxetine and escitalopram on plasma BDNF concentration, platelet serotonin concentration and platelet MAO-B in medication-naive depressed patients

Mentor(s): Assist. Prof. Marina Šagud, MD PhD, Nela Pivac, PhD, research advisor

Affiliation: University of Zagreb School of Medicine, University Hospital Centre Zagreb, University Psychiatric Hospital Vrapče, Ruđer Bošković Institute, Zagreb

Introduction: Effects of two antidepressants, vortioxetine and escitalopram on plasma BDNF concentration, platelet serotonin concentration and platelet MAO-B (monoamine oxidase B) activity haven't been compared so far.

Materials and methods: We will include 120 medication-naive patients with first or recurrent depressive episode randomized to treatment with vortioxetine or escitalopram. Severity of depressive symptoms and cognitive functioning will be evaluated using psychiatric and psychological scales. BDNF will be measured with enzyme linked immunosorbent assay. Platelet serotonin concentration and MAO-B activity will be measured spectrofotofluorimetrically. Biochemical and clinical parameters will be determined at baseline, after 1 and after 4 weeks of treatment.

Results: Preliminary results of the baseline data are reported for 86 patients enrolled so far. In the entire sample there was no association between baseline platelet MAO-B activity and platelet serotonin concentration and HAMD (Hamilton Depression Scale) and MADRS (Montgomery-Asberg Depression Scale) total scores (when divided in mild, moderate and severe depression). The only difference was in MAO-B activity between male and female patients, where women had higher activity (p=0,011). In addition there were no significant differences detected in MAO-B activitiy and serotonin concentrations between smokers and non-smokers.

Discussion: This preliminary results of baseline data showed no corellation between platelet MAO-B activity and serotonin concentrations with depression rating scale scores. Further analysis will show the effects of two antidepressants, vortioxetine and escitalopram on these parameters which is the primary aim of this research

MeSH/Keywords: depression, depression rating scales, serotonin, MAO-B

Poster code: R-02-29-107

PSYCHOSOCIAL CHARACTERISTICS IN WOMEN ENGADED IN *IN VITRO* FERTILIZATION

PhD candidate: Dunja Jurić Vukelić
Part of the thesis: Psychosocial characteristics in women engaded in in vitro fertilization
Mentor(s): Assist. Prof. Zorana Kušević, MD PhD
Affiliation: University of Zagreb School of Medicine

Introduction: Psychological problems that sometimes occur with infertility often remain unrecognized. Studies have shown that some of the psychological disorders such as depression and anxiety can have a negative effect on the outcome of IVF and can also be the reason of drop out from further treatment of infertility. In recent literature there is little work linking psychosocial factors with infertility and assisted reproductive techniques, and the results are often contradictory. Because of the potential benefits of interdisciplinary treatment of infertility, this study aims to determine the prevalence of alexithymia, depression and anxiety and their impact on the outcome of IVF.

Materials and methods: Research was conducted individually, using: - Sociodemographic questionnaire, including questions about age, level of education, psychiatric and other chronic diseases in personal and family medical history, relationship with the partner, number of children, duration of infertility, number of IVF, question about attending psychotherapy and question was there any stressful event during IVF process, more stressful than procedure alone. - Revised NEO Personality Inventory (NEO PI-R), 60 items personality inventory which assesses the Big Five personality traits: Extraversion, Agreeableness, Conscientiousness, Neuroticism, and Openness to Experience. - Toronto Alexithymia Scale questionnaire–20 (TAS-20) is a 60 items inventory of deficiency in understanding, processing, or describing emotions. - Clinical Outcomes in Routine Evaluation – Outcome Measure (CORE-OM), a scale designed as a measure of general psychological distress. It has 34 items covering problems (depression, anxiety, physical problems and trauma), well-being, functioning (general and social) and risk behaviour. - Religiosity questionnaire consists of 24 items relating to three dimensions of religiosity: spirituality, ritual dimension and impact of religion on behavior dimension.

Results: The mean age of the participants was 28 years, with 2 years of duration of infertility and low comorbidity. In line with previous research, although undergoing an IVF-treatment is an emotional and physical burden, woman entering an IVF procedure are, in general, psychologically well adjusted, with the exception of score above the norm on measures of anxiety – excessive concern, tension and fear of uncertainty.

Discussion: Reported symptoms of anxiety probably reflect reaction to uncertainty about the treatment outcome. Consequently, it is important to assess respondents' psychological reactions during different phases of the IVF procedure in order to react promptly and in an appropriate manner.

Acknowledgments: prof. dr. sc. Dinka Pavičić Baldani, prof. dr. sc. Marina Šprem Goldštajn, prof. dr. sc. Hrvoje Vrčić, prof. dr. sc. Snježana Škrablin Kučić, doc. dr. sc. Marina Šagud, prof. dr. sc. Nataša Jokić Begić, prof. dr. sc. Rudolf Gregurek, doc. dr. sc. Marijana

MeSH/Keywords: in vitro fertilization, anxiety, depression, personality traits, religiosity

Poster code: R-02-29-139

HYPOVITAMINOSIS D AND NEUROPATHY IN PATIENTS WITH CHRONIC GRAFT-VERSUS HOST DISEASE (cGvHD) – PRELIMINARY RESULTS

PhD candidate: Branimir Ivan Šepec

Part of the thesis: Prevalence and characteristics of neuropathy in patients with chronic graft-versus host disease

Mentor(s): Assoc. Prof. Ervina Bilić, MD PhD, Assist. Prof. Dražen Pulanić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Chronic Graft-versus Host Disease (cGvHD) is a major late complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT), which affects from 30 to 70% of long term survivors. Most frequently involved organs are the skin, eyes, mouth, lungs, gastrointestinal tract, liver, but also joints/fascia and genital tract. Although neurologic manifestations in cGvHD patients may involve the central nervous system (vasculitis, demyelination, encephalitis), the peripheral nervous system (polyneuropathy), the neuromuscular junction (myasthenia gravis) or the muscle (myositis), there are no neurologic diagnostic criteria for cGvHD yet. Polyneuropathy in cGvHD is well recognized, but the underlying pathophysiologic mechanisms are still to be discovered. Our goal in this study is to detect factors that promote the development of polyneuropathy in patients with cGvHD hoping to improve their treatment or alleviate their difficulties.

Materials and methods: In the period between 11/2016 and 02/2018, 30 different patients (15 male and 15 female, median age 45.63 years, range 19 to 68 years) with cGvHD were neurologically examined. Every patient underwent electroneurographic (ENG) and quantitative sensory testing (QST). Also, the serum concentration of 25-hydroxi vitamin D was determined (ECLIA Roche method, concentrations <75nmol/L were considered as deficiency).

Results: ENG testing showed a lesion of large nerve fibers in 19 (63.3%) patients. Twelve (40%) of these patients had pure motor neuropathy, 7 (23.3%) patients sensorymotor neuropathy, whereas no patient had sensory neuropathy alone. All patients with large fiber neuropathy showed an affection of motor nerve fibers. QST showed an affection of small nerve fibers in 21 (70.0%) patients. Nine (30%) of these patients had isolated A delta fiber neuropathy, while two (6.7%) patients had isolated C fiber neuropathy. Combined A-delta and C fiber neuropathy was found in 10 (33.3%) patients. The majority of patients (63.3%) with small fiber neuropathy had a lesion of of A-delta fibers. Altogether, in 27 (90.0%) patients some form of polyneuropathy was detected. The median of 25-hydroxy vitamin D concentration was 43.5nmol/L (range 11-143nmol/L). In 25 (83.3%) patients vitamin D deficiency was proven. There was weak to moderately strong correlation found between vitamin D deficiency and damage of A-delta fibers as well as a weak to moderately strong correlation between vitamin D deficiency and damage of C fibers (τb=0.402, p=0.03 and τb=0.365, p=0.049 respectively).

Discussion: The preliminary results of this study indicate a connection between vitamin D deficiency and polyneuropathy. This connection is more prominent for vitamin D deficiency and a lesion of A delta fibers. These results should be confirmed by follow-up examinations of these patients after vitamin D supstitution and by analysis of the same parameters in a control group of haematologic patients without cGvHD.

Acknowledgments: Ervina Bilić, Dražen Pulanić, Lucija Kraljević, Snježana Švedi, Milica Jug, Valentina Delimar

MeSH/Keywords: Hypovitaminosis D, polyneuropathy, cGvHD. small fiber neuropathy

Poster code: R-02-30-138

PRELIMINARY RESEARCH RESULTS

Public Health and Health Care

ALLERGIC DISEASES IN CHILDHOOD – DEVELOPMENTAL ORIGIN OF HEALTH AND DISEASE HYPOTHESIS

PhD candidate: Iva Topalušić

Part of the thesis: Prevalence of allergic symptoms in primary school children in the city of Zagreb and Natural Park Lonjsko Polje

Mentor(s): Assoc. Prof. Asja Stipić Marković, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: According to the Developmental origin of health and disease hypothesis, environmental pressure at critical, or early period of development can evoce changes in the gene regulation affecting allergy development. The aim of this study was to explore the prevalence of allergic diseases among adolescents in the area of the Natural Park Lonjsko Polje, Croatia, a special ornithologic reserve and the largest swamp area in Europe.

Materials and methods: Original ISAAC (international study of allergy and asthma in childhood) questionnaires, consisting of questions on child´s demographic characteristics, core modules on wheezing, atopic dermatitis and allergic rhinitis and supplementary modules were completed by parents of 13-14 year-old (13 years 0 months- 13 years 11 months) children from five elementary schools of the surrounding area of The Natural Park Lonjsko Polje. A total number of 160 questionnaires were returned and analysed. After cmpleting the questionnaire, skin prick tests with common inhalant allergens (Dermatophagoides pteronyssimus-DP, Dermatophagoides farinae-DF, grasses, Ambrosia artemisiifolia-AA, early and late blooming trees and cat dander) were performed using standard procedure among 56 children, according to the guidelines of the European Academy of Allergology and Clinical Immunology and ISAAC Phase Two Mannual. The result was considerred positive if the diameter of the wheal was 3 mm or greater. A histamine solution10 mg/ml as positive control, as well as negative control were used. Antihistamines, history of severe, systemic allergic reactions and skin changes of the testing area were the excluding criteria.

Results: 75 children (47%) were boys and 85 (53%) were girls. 88% of children live or have lived earlier in their lives in small villages and suburbs with a lot of green areas. Prevalence of wheezing in a 12- months period was 0.6%, of itchy rash on predilectic areas for atopic dermatitis was 4.3%, while 12- months prevalence of simptoms of allergic rinitis was 9.4%. 42% of children had positive skin test on at least one inhalant allergen, while 32% of them were polisensitized. 30 % were sensitized to DP, 21% to DF, 16% to AA, 12% to grasses, 18% to early, 20% to late blooming trees and 9% to cat dander.

Discussion: Results on 13-14-year-age group show lower prevalence of allergic disease symptoms in comparison with 6-7- years- age group. Compared with results in the costal part of Croatia, in which in the seven years period there was observed a significant increase in the current prevalence of all symptoms related to asthma, AD and AR (in a school year 2008/2009 a 12- months prevalence of Wh was 14%, of AD 5.9% and of allergic rhinoconunctivitis 25%), our results show lower prevalence as well. These result might show protective role of the lifestyle in this area. Results of this study showed lower prevalence of the skin sensibilization to inhalant allergens compared with the urban region. Sensibilization rate in the city of Zagreb Croatia reached 50% in the year 2004, with the similar allergen profile as in NPLP. Our further research will involve comparison with the new data from the city of Zagreb and correlation with risk factors.

MeSH/Keywords: epidemiology, allergy, ISAAC, early development

Poster code: R-03-01-094

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
Affiliation: University of Zagreb School of Medicine

Introduction: Aim of this study is 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. While it is well known that regular physical activity improves general health and reduces the risk of premature mortality, effects of vigorous training are still unclear. Still, a large retrospective multinational study which included 15 174 Olympic medalists found that they have lower mortality than their general populations; however, that study did not examine causes of death of the athletes.

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 with presented medical documentation when available. For deceased Olympic medalists, medical documentation was requested. Croatian Bureau of Statistics (CBS) provided data about the overall and disease specific mortality of the Croatian male population standardized by age and time period. Overall and disease-specific standard mortality ratios (SMR) with 95% confidence intervals (CI) were calculated to compare the mortality rates of Croatian Olympic medalists and the general population.

Results: Among 233 Olympic medalists, 57 died before the study's endpoint. Cardiovascular diseases were the most common main causes of death (33.3%), followed by neoplasms (26.3%) and external causes of death (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) when compared with the general Croatian male population.

Discussion: Croatian male Olympic medalists benefit from lower overall and cardiovascular mortality rates in comparison to the general Croatian male population.

MeSH/Keywords: Mortality, Athletes, Causes of death

Poster code: R-03-01-130

OUT-OF-HOSPITAL CARDIAC ARREST OUTCOMES IN CROATIA

PhD candidate: Damir Važanić

Part of the thesis: Analysis of out-of-hospital emergency medical service system regarding the outcome of resuscitation procedure

Mentor(s): Assoc. Prof. Ingrid Prkačin, MD PhD, Assist. Prof. Višnja Nesek Adam, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Approximately 8.000 people suffer from an out-of-hospital cardiac arrest (OHCA) in the Republic of Croatia every year. OHCA survival rates generally remain low despite major advances in resuscitation. The reported survival rate after an OHCA varies, ranging from 1,8%- - 21,5%. There are many factors influencing the OHCA outcome. Incidence and survival rate is well known in many countries, but there are still no data about OHCA in Croatia. The quality of a community chain of survival may directly reflect out of hospital cardiac arrest. Detailed analysis should identify the gap in chain of survival and should provide tool for improving survival rate.

Materials and methods: The aim of this study is to determine survival rate of patients with out of hospital cardiac arrest who received cardiopulmonary resuscitation in the Republic of Croatia. An observational study performed between October 1, 2017 and December 31, 2017 includes all adult patients with OHCA in Croatia who are treated by Emergency medical services (EMS). OHCAs were collected from the Croatian Institute of Emergency Medicine database and Utstein cardiac arrest data collection form. Descriptive data presentation was used in the analyses. Data were presented in the form of absolute frequencies and percentages and the central tendency measures. Testing of correlations in the observed categories was performed by logical regression.

Results: During the observational period total of 1763 adult patients without signs of circulation were assessed by EMS in Croatia and 760 (43%) adult patients were resuscitated by EMS personnel. Outcome measured in return of spontaneous circulation (ROSC) until emergency department admission were reported in 126 (17%) cases. Shockable rhythm vs. non-shockable rhythm (OR: 5,832, 95% CI: 3,621 - 9,392, p<0,001) and bystander witnessed cardiac arrest (OR: 8,213, 95% CI: 2,554 - 26,411, p<0,001) are significantly associated with a higher probability of survival. There were no significant difference in correlation with day or night shift, etiology of cardiac arrest and bystander CPR variables.

Discussion: Out of hospital cardiac arrest survival rate in Croatia is 17% until emergency department admission. In this study we identified that shockable rhythm and bystander witnessed cardiac arrest has impact on better survival. Improving community bystander CPR rates is in important step towards improving OHCA outcome.

MeSH/Keywords: out-of-hospital cardiac arrest, outcome, emergency medical services, survival

Poster code: R-03-02-122

BURDEN IN FAMILY MEMBER CAREGIVERS OF PATIENTS WITH DEMENTIA

PhD candidate: Jelena Lucijanić

Part of the thesis: Quality of Life and Burden in Family Member Caregivers of Patients with Dementia

Mentor(s): Professor Vesna Jureša, MD PhD, Miroslav Hanževački, PhD, research associate

Affiliation: University of Zagreb School of Medicine

Introduction: With aging of population dementia is becoming growing global health problem affecting both patient with dementia and his/her family members. Patient's family should be included in the disease management process due to long duration of disease. Although health care is focused on a patient, additional care should be given to caregivers-family members. People that care about family members with dementia are exposed to additional emotional, physic, social and financial stressors. We aimed to estimate caregiver burden in a population of caregiver family members of patients with dementia and to estimate patient- and caregiver-associated factors affecting caregiver burden.

Materials and methods: Up to this time-point a total of 78 families that include patient with dementia and one member that is a dominant caregiver were enrolled. It is planned to include a total of 130 families for the final analysis. The study is conducted in cooperation with family doctors in Health Care Center Zagreb-West. Zarit caregiver burden scale was used to estimate caregiver burden. Mini Mental State Examination was used to estimate stage of dementia and Barthel Index was used to estimate functional status of patient with dementia. Caregiver also answered questions about demographic and socioeconomic characteristics and present morbidities.

Results: Preliminary results of 78 families enrolled so far showed that most caregivers are women 52/78 (66.7%). Median caregiver age was 59.5 years with 33/78 (42.3%) of caregivers being older than 65 years and 12/78 (15.4%) being older than 80 years. Median of caregiver burden was 26.5 IQR (17-34.5). Most of caregivers had mild to moderate burden [39/78 (50%)], followed by no to mild burden [25/78 (32.1%)], moderate to heavy burden [12/78 (15.4%)] and least number of caregivers had heavy burden [2/78 (2.6%)]. Higher caregiver burden was associated with loger duration of caregiving (Rho 0.27; P=0.015), longer duration of dementia (Rho 0.35; P=0.002), worse functional status of patient with dementia (Rho -0.26; P=0.022) and enrolled in daily hospital care program for dementia (33 vs 25; P=0.035).

Discussion: Our preliminary results suggest that most of caregivers estimate their burden as mild to moderate. Most of caregivers were women and our results are in line with the role of women in the family as a person caring for other members. As expected, longer duration of dementia, longer duration of caregiving and worse functional status of a patient were associated with higher burden. We did not expect to detect an association between need for daily hospital care enrollement and higher burden and we plan to focus on further explore this relationship when more recruitment of more families into our study.

MeSH/Keywords: Caregiver burden, dementia, Croatia, family medicine

Poster code: R-03-04-061

PHD THESIS PROPOSALS

Basic Medical Sciences

DNA DAMAGING AND DEMETHYLATION PATHWAYS IN DEVELOPMENT OF CALCIFIC AORTIC VALVE STENOSIS

PhD candidate: Dubravka Šušnjar

Part of the thesis: DNA damaging 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: Recent findings suggest an important role of DNA damaging and DNA demethylation pathway in cardiovascular diseases. Among others this is reflected by their active role in endothelial dysfunction and phenotype transdifferentiation of vascular smooth muscle cells in atherosclerotic lesions. Due to common pathogenic features of atherosclerosis and calcific aortic stenosis (CAVS) it is expected that DNA damaging, and DNA demethylation pathways are also actively involved in the development and progression of CAVS.

Hypothesis: We except higher expression of DNA glycosylases and DNA demethylation enzymes in peripheral blood and aortic valve tissue of CAVS patients versus their expression in peripheral blood of voluntarily healthy subjects and AVI patients as well as AVI and control aortic valve tissue samples.

Aims: The aims of the proposed study are: (a) determination of expressional pattern of DNA glycosylases (OGG1/2, NTH1, NEIL1/2/3, SMUG1, TDG, UNG2), Apurinic/Apyrimidinic Endonuclease 1/Redox Factor-1 (Ape1/Ref-1) and DNA demethylation enzymes (TET1/2/3) in stenotic CAVS and control aortic valve tissue; (b) determination of biomarker potential of Ape1/Ref-1 in peripheral blood of CAVS patients; (c) determination of global DNA methylation/hydroxymethylation (5-mc/5-hmC) level in CAVS and control aortic valve tissue samples, and (d) comparison of obtained gene/protein expression values with clinical and demographic data of CAVS patients.

Materials and methods: Study will encompass healthy voluntary subjects (n = 100), adult patients undergoing valve replacement for severe calcific aortic valve stenosis (n= 100) and aortic valve insufficiency (AVI, n = 50) as well as pathologically unaltered aortic valve tissue samples obtained from heart transplant recipients (n= 15-25) and autopsy procedure (n= 15 - 25). Part of the each aortic valve will be embedded in FFPE tissue blocks for immunohistochemistry while the other part will be used for protein, DNA and RNA isolation for western-blotting and qRT-PCR (OGG1/2, NTH1, NEIL1/2/3, SMUG1, TDG, UNG2, TET1/2/3 and Ape1/Ref-1 protein and mRNA expression) as well as global DNA methylation (5-mC) and hydroxymethylation (5-hmC) analysis with appropriate ELISA assay. ELISA analysis will also be used for protein (Ape1/Ref-1) analysis of plasma samples obtained from healthy voluntary subjects and preoperative and postoperative (3-6 months) peripheral blood samples of CAVS and AVI patients. Statistical analysis will be performed with GraphPad Prism statistical software. In brief: Normal variable distribution will be checked by Kolmogorov-Smirnov/Shapiro Wilks test. The association between demographic and clinical data with CAVS disease will be determined using the student T test or ÷2/Fisher exact analysis while the comparison between the groups will be performed using independent Student T test, one-way ANOVA or Mann-Whitney U and Kruskal-Wallis test, correspondingly. Association of gene/protein expression data with CAVS disease will be determined by the logistic regression analysis and the interrelationship between quantitative gene/protein expression data and individual clinical parameters of CAVS patients will be established by Pearson correlation coefficient and linear regression analysis. Significance will be set at P (two tailed) < 0.05. Bonferroni's correction for multiple comparison will also be applied where appropriate.

Expected scientific contribution: Determination of currently unknown biomarker potential and expressional pattern of DNA damaging (DNA glycosylase, APE1/Ref-1) and DNA demethylation pathway (TET 1/2/3) in peripheral blood and aortic valve tissue of CAVS patients compared to control and AVI tissue samples will further enhance our understanding of biological pathways responsible for etiology and clinical manifestation of CAVS

MeSH/Keywords: aortic valve, aortic valve stenosis, aortic valve insufficiency, Demethylation, DNA

Poster code: T-01-05-076

EPIGENETIC BIOMARKERS OF TESTICULAR TUMORS; CELL FREE DNA METHYLATION OF RASSF1A AND PRSS21 GENES IN BLOOD AND SEMEN OF PATIENTS WITH TESTICULAR NONSEMINOMA

PhD candidate: Jure Krasić
Part of the thesis: Epigenetic Biomarkers of Testicular Tumors; cell free DNA methylation of RASSF1A and PRSS21 Genes in Blood and Semen of Patients with Testicular Nonseminoma
Mentor(s): Assist. Prof. Nino Sinčić, MD PhD
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 (cfDNA) 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.

Hypothesis: Cell-free DNA methylation of RASSF1A and PRSS21 in patients blood and ejaculate can be used as a biomarker of testicular non-seminoma.

Aims: To determine the possibility of using the methylation status of cell-free DNA of the RASSF1A and PRSS21 gene promotional regions in the ejaculate and blood of patients as a biological molecular marker of testicular non-seminoma. This will be accomplished by: -Collecting and comparing the relevant anamnestic and other clinical data of healthy volunteers and patients and collect biological samples; -Isolating cell-free DNA (cfDNA) from samples of healthy volunteers and patients, and cellular DNA from tumor tissue of patients with the best established protocols; -Determining and comparing the pattern and degree of methylation of the DNA promoter regions of the RASSF1A and PRSS21 genes; -Quantifying and comparing the expression of the RASSF1A and PRSS21 gene at the protein level in the tumor tissue of the patient in comparison with the surrounding healthy tissue and correlate the expression with the DNA methylation status; -Making an assessment on the potential of cell-free DNA methylation in blood or ejaculate as biomarker in patients with testicular non-seminoma

Materials and methods: Blood and ejaculate samples will be collected from 35 healthy volunteers and used as the control group. Non-seminoma patients will have blood and ejaculate taken pre and post-operation. Ten healthy volunteers will be used as the optimization group to establish the protocols. Cell-free DNA will be isolated by kit with silica-based columns and by phenol-chloroform extraction, and the two will be compared for efficiency. Cell-free DNA concentration will be measured using the ddPCR. Genomic tumor DNA will be isolated by house protocol from paraffin blocks. Analysis of DNA promotor methylation of RASSF1A and PRSS21 from cell-free DNA and genomic tumor DNA will be analysed with the PyroMark Q24 Advanced system. Protein localization and expression will be analysed by immunohistochemistry on non-seminoma tissue slides and will be analysed semi-quantitatively by morphometric analysis. The data obtained will be analysed with the appropriate statistical tests in the program Statistica.

Expected scientific contribution: Scientific contribution is expected in the development of epigenetic biomarkers on cfDNA in liquid biopsies of surrogate tissues of patients with TGCT and oncologic patients in general.

Acknowledgments: This publication was supported by the European Union through the European Regional Development Fund, Operational Programme Competitiveness and Cohesion, under grant agreement No. KK.01.1.1.01.0008

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

Poster code: T-01-05-123

DUAL FUNCTION OF SUFU IN THE INTEGRATION OF WNT AND HEDGEHOG SIGNALING PATHWAYS IN THE INTRAUTERINE GROWTH RESTRICTION PLACENTAS

PhD candidate: Ida Marija Šola

Part of the thesis: Dual function of Sufu in the integration of Wnt and Hedgehog signaling pathways in the intrauterine growth restriction placentas

Mentor(s): Assoc. Prof. Ljiljana Šerman, MD PhD, Assoc. Prof. Krunoslav Kuna, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Wingless (Wnt) and Hedgehog (Hh) are evolutionary conserved signaling pathways that play substantial roles in cell differentiation, proliferation, stem cell maintenance and embryonic development. Wnt signaling pathway is essential for decidualisation, implantation, chorion- allantois fusion, blastocyst activation and early trophoblast development. It is activated by binding a Wnt- protein ligand, such as Wnt 5a, to a transmembrane Frizzled family receptor that, once activated, passes the signal to cytoplasmic protein Dishavelled (Dsh) that enables the accumulation of beta-catenin in canonical Wnt pathway by disrupting the destruction the complex that would degrade beta-catenin otherwise. Beta-catenin then enters the nucleus and, as a transcriptional co-activator, forms a complex that binds to the promoters of target genes. On the other hand, our knowledge is modest about Hedgehog (Hh), another evolutionary conserved signaling pathway crucial for early pregnancy and we still know a little concerning their interactions. Hh signaling pathway is activated by 3 ligands; Indian- Ihh, Sonic- Shh and Desert-Dhh that bind to its transmembrane receptor Patched (PTCH) and inhibit it, allowing membrane protein Smothened (SMO) to accumulate and activate transcription factors GLI (glioma- associated oncogene) that activate targeted genes. Suppressor- of- fused (Sufu) is an intracellular inhibitor of both Hh/GLI1 and Wnt/ beta-catenin pathway and is required for the integration of the two pathways by binding directly to GLI and beta-catenin. Intrauterine growth restriction (IUGR) is defined as a failure of the fetus to achieve his intrinsic growth potential due to the fetal, placental or maternal reasons, with potential overlap among these entities. The common definition of IUGR refers to a weight below 10th percentile for gestational age. It is a common diagnosis in obstetrics and carries an increased risk of perinatal mortality and morbidity.

Hypothesis: In IUGR placentas we expect reduced expression of Wnt5a and beta-catenin and increased expression of Sufu. We expect that promoter of the tumor suppressor gene Sufu will be hypomethylated in IUGR placentas compared to placentas from physiologic pregnancies.

Aims: General aim is to investigate changes in Sufu, Wnt5a and beta-catenin protein expression in IUGR placentas and compare results with physiologic pregnancies placentas. Specific aims: examine the expression level of Wnt5a and beta-catenin proteins, positive regulators of the Wnt signaling pathway; examine the expression level of Sufu protein, negative regulator of the Hh signaling pathway; analyze DNA methylation pattern of Sufu gene promoter; determine the connection between DNA methylation of Sufu gene promoter and its protein expression; compare Wnt5a, beta-catenin and Sufu expression in both IUGR and physiologic pregnancy placentas.

Materials and methods: Formalin-fixed paraffin-embedded samples of 30 placentas from pregnancies with IUGR and 20 placentas form physiologic pregnancies (controls) will be used for this study. Expression of Wnt5a, beta-catenin and Sufu proteins will be examined using immunohistochemistry. DNA methylation pattern of Sufu gene promotor will be analyzed by methylation-specific PCR (MSP).

Expected scientific contribution: Purpose of this study is to elucidate the role of Sufu protein in the pathogenesis of idiopathic IUGR to improve the procedures and results in such pregnancies.

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: intrauterine growth restriction, Wnt signaling pathway, Hh signaling pathway, Wnt5a, beta-catenin, Sufu

Poster code: T-01-05-127

DEVELOPMENTAL REORGANIZATION OF HUMAN AMYGDALOID NUCLEUS

PhD candidate: Damir Mulc
Part of the thesis: Developmental reorganization of human amygdaloid nucleus
Mentor(s): Assoc. Prof. Mario Vukšić, MD PhD
Affiliation: University of Zagreb School of Medicine, Croatian Institute for Brain Research

Introduction: Structural and functional reorganization of neural circuits is one of the key features of the human brain during development. The amygdaloid nuclear complex (lat. corpus amygdaloideum) is a basal ganglion situated in the temporal telencephalic lobe and functionally represents a key component of the limbic system. It is connected to almost all cortical and subcortical structures playing a crucial role in processing of complex patterns of social behavior, particularly in fear learning. Nevertheless, studies on its prenatal development are very scarce in humans. It has been observed that the corpus amygdaloideum is very early developed in the human brain. The basolateral part of this structure develops from the pallium, while its centromedial part is formed from the subpallium. These different developmental origins also confirm different functional roles of distinct amygdaloid nuclei in later life. In our previous work we have demonstrated a human specific transient modular organization of this structure during midfetal period (Nikolic & Kostović, Anat Embryol 1986).

Hypothesis: Key neurogenetic events during human amygdala development, from its transient modular organization during early fetal period through reorganizational processes during perinatal period, which actually represent neurobiological basis for later appearance of its important functional roles in emotional processing, can be successfully followed by comparative application of histological, MRI and gene expression parameters, and possible temporal and spatial differences in neurogenetic events could explain functional differences present among single amygdaloid nuclei.

Aims: The aim of this doctoral thesis is to investigate prenatal development of the human amygdaloid complex, with special emphasis on modular organization as well as on reorganization of the major cellular and extracellular components using immunohistochemistry, in situ hybridization, transcriptional and in vitro MRI analysis.

Materials and methods: To analyze the expression patterns of different developmentally regulated proteins in relation to synaptic, laminar, neuronal, glial and extracellular matrix development, histological analysis will be performed on postmortem human brains, ranging from 10 postconceptional weeks to 6.5 years. The obtained results will be compared with in vitro MRI brain data of 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. Nature 2011). For the selected candidate genes we will perform in situ hybridization and immunohistochemistry in order to elucidate in which cells are these genes expressed.

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

Acknowledgments: This PhD thesis has been supported by Croatian Science foundation Nr. 3739

MeSH/Keywords: human fetal brain development, transient organization, structural reorganization, basal ganglia

Poster code: T-01-08-007

THE DISTRIBUTION OF DEMYELINATING LESIONS WITHIN THE CEREBRAL WHITE MATTER SEGMENTS AND THEIR CORELLATION 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 corellation 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 spacial 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 oftenly 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 impairements. 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 analysed more precisley using segmentation according to Von Monakow (I-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). The first goal of this study is to precisley describe volume and spatial distribution of primary demyelinating lesions in MS patients within the segments of WM. Afterwards, we will try to correlate this segmental lesion distribution with the clinical presentation of the disease, which will be quantified by the expanded disability status scale (EDSS).

Hypothesis: The volume of primary demyelinating lesions in certain segments of white matter correlates with the clinical status of patients with multiple sclerosis with validated EDSS disability scale.

Aims: The aim of this doctoral thesis is to correlate the volume of white matter lesions with the clinical condition of the patient using EDSS scale. The specific aims are : 1. Correlation of white matter lesion volume with the clinical condition of the patient using EDSS by sex, age and disease duration, 2. Calculate the white matter lesions volume limit values that will be associated with more severe clinical symptoms, 3. Evaluate the multivariate linkage of white matter lesions by sex and age with the more severe clinical status and inability of patients.

Materials and methods: This study will have a correlation retrospective – prospective character of MR brain examination involving 100 patients with MS. The inclusion factors are based on the Barkhof-Tintore Criteria for MS from the MAGNIS 2016 audit. 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.

Expected scientific contribution: 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, white matter segments, demyelinating lesions
Poster code: T-01-08-030

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. Emilija Juretić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Decrease in mortality of very preterm newborns is not accompanied by decrease in short and long-term lifelong morbidity. Many survive with brain damage, and neurodevelopmental disorders are of primary interest. Quality of life descriptors move from „handicap" towards „functional ability". Research in neuroprotective measures yielded inconsistent results. Goal of this study is to determine whether bovine lactoferrin (bLF) oral supplementation in very preterm infants would result in better short term neurological outcome. Secondary outcomes will be differences in incidence of severe complications and morbidity attributive to this population.

Hypothesis: Lactoferrin demonstrates neuroprotective and positive nueromaturational effect in preterm infants. Oral bLF supplementation will result in significant difference in short-term neurological outcome between treated and control group of infants.

Aims: Main objective of this study is to decrease the rate of neurological deviation of very preterm infants at term-corrected age by oral bLF supplementation. Neurotrophic and neuroprotective role of bLF have been well established in animal models. Primary aims are to detect rates of neurological abnormalities using standardized assessment tool during the intervention and at term corrected age. Using electroencephalography (EEG), we would show differences in incidence and severity of cerebral electrical activity abnormalities. Ultrasonographic markers of structural brain damage will also be evaluated. Secondary aims are to demonstrate incidence of short-term morbidity such as late-onset sepsis and necrotizing enterocolitis between groups.

Materials and methods: This is a prospective, randomized, double blind, placebo-controlled study encopassing preterm infants of $\leq$ 32 postmenstrual age at birth. Bovine lactoferrin will be given per oral route to 50 infants and placebo to other 50. Subjects will be further stratified into two groups according to postmenstrual age (PMA): 24-28 weeks and 28-32 weeks of PMA. Disproportional stratification will ensure equal number of participants in each group and allow for interim analysis during research. After admitting a patient to NICU and thorough explanation, an informed consent will be obtained from parents. Educated nurse will create computer generated list and prepare 200 mg of bovine lactoferrin or the same dose of placebo (sucrose) diluted in sterile water or milk. Intervention will begin as soon as initial stabilisation is provided and the attending neonatologist estimates it is safe to begin oral feedings (obligatory within the first 72 hours). Preparation will be given via NGT/OGT twice daily. All groups will recieve the same combination of probiotics as well. Supplementation will stop after completed 35 weeks of PMA. Neurologic assessment (Premie-Neuro) will be provided in two weeks intervals. First assessment will be done as soon as possible, once the condition of the infant allows manipulation. Results will be categorized as normal, questionable and abnormal. At term corrected age, HINE neurologic evaluation will be used. EEG recording will follow the same schedule: first within the first week of life to exclude existing maturational lag, and sequentially in 2-3 weeks intervals. Findings will be grouped as normal, mildly, moderately and severely abnormal. Brain ultrasonography will be conducted according to adopted protocol. Exclusion criteria are congenital malformations of gastrointestinal tract, brain malformations, malformation syndromes and recognized neurogenetic syndromes. „Mixed effect models" statistical analysis will be used.

Expected scientific contribution: First of all to determine whether bovine lactoferrin oral supplementation would yield a positive neurotrophic and neuromaturational effect on a representative cohort of very preterm infants. Relevant progress, compared to previous studies would be the effect of bLF on early neurological outcome. These results could contribute to a comprehensive neuroprotective strategy in very preterm newborns, improving their neurobehavioural outcome and successfull social integration.

MeSH/Keywords: preterm newborn, lactoferrin, neurological outcome

Poster code: T-01-08-041

STUDY OF REORGANIZATIONAL PROCESSES IN HUMAN FETAL CINGULATE GYRUS

PhD candidate: Mihaela Bobić
Part of the thesis: Study of reorganizational processes in human fetal cingulate gyrus
Mentor(s): Professor Nataša Jovanov-Milošević, DVM PhD
Affiliation: Croatian Institute for Brain Research, School of Medicine, University of Zagreb

Introduction: Cingulate gyrus, a region connecting archicortex and neocortex situated at the medial limbus of the human fetal telencephalic wall, shows different transitory lamination pattern in comparison to lateral cortical regions. Marginal zone of the fetal cingulate gyrus is more voluminous while the subplate zone is narrower than in the dorsolateral telencephalic wall. ECM is one of the main constituents of these transient zones.

Hypothesis: Organisation of ECM of the fetal cingulate gyrus differs from the one in dorsal and lateral regions of the brain and is responsible for characteristic patterns of lamination, synaptogenesis and neurogenesis, fiber orientation, and their ingrowth, as well as different vulnerability and reorganizational answer after lesion.

Aims: The doctoral thesis aims to investigate developmental reorganization of the main cellular (neurons and growing axons) and extracellular components of the human fetal cingulate gyrus correlating immunohistochemistry, SCOPE-in situ hybridization, transcriptome analysis as well as in situ and in vivo MRI analysis.

Materials and methods: Analysis will be performed on post-mortem human brain tissue, obtained during regular autopsies of fetuses after spontaneous or medically indicated abortions. Immunohistochemical labeling will be applied to analyze the expression patterns of neural, axonal, glial and ECM molecules. We will analyze the publicly available gene expression database (Kang et al., Nature 2011) to investigate the relation between structural changes and spatiotemporal expression of genes specifically expressed in the cingulate gyrus. In-situ hybridization and immunohistochemical methods will be performed for the selected candidate genes. We will correlate these results with MRI findings regarding the signal intensity, thickness, surface and volume of transient zones of the cingulate gyrus.

Expected scientific contribution: This research will determine the most significant factors and the most vulnerable period in the development of the human fetal cingulate gyrus. The results will serve as a ground for future research of impairments and disturbances in this region, all for better diagnostics and therapy of children at neurorisk.

Acknowledgments: This PhD thesis has been supported by Croatian Science Foundation (projects No. 7379 and DOK-2015-10-3939a), HIMRICO-Adris and UNIZG-BM0054 projects.

MeSH/Keywords: cingulum bundle, marginal zone, subplate zone, extracellular matrix

Poster code: T-01-08-079

THE ROLE OF SUBPLATE NEURONS AND INTERSTITIAL NEURONS IN THE PATHOGENESIS OF EPILEPSY

PhD candidate: Petra Nimac Kozina

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

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

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

Introduction: White matter interstitial neurons (WMIC) are a large, but insufficiently explored group of neurons located beneath the cerebral cortex, between the bundles of white matter. Exact role of WMIC in the functioning of the cerebral cortex has not been proven yet. Interestingly, in many neurological and psychiatric disorders the number, distribution and density of WMIC is altered. Epilepsy is one of disorders where alterations of WMIC have been observed. In the brains of people with epilepsy, it is often possible to find an increased number of WMIC. According to classical authors, these neurons are cortical neurons that did not migrate to the correct position during development. Although there is no single evidence that increased number of WMIC in the white matter is the consequence of neuronal migration disorders, with the exception of clearly defined cortical malformations due to similar disorders (e.g. focal cortical dysplasia), the prevailing opinion to date is that those are residual and aberrantly located cortical neurons. In the proposed study, we will analyse molecular phenotype and developmental origin of WMIC in the areas relevant for the pathogenesis of epilepsy.

Hypothesis: The hypothesis of this research is that increased number of neurons in the white matter of patients with epilepsy is the result of disturbances in the apoptotic elimination of subplate neurons during development rather than migration disorders of cortical neurons.

Aims: The general aim of this research is to determine the molecular profile of neurons within the white matter during development (i.e. subplate neurons) and in the adulthood (i.e. white matter interstitial neurons) and differences in the composition of white matter interstitial neurons in normal healthy individuals and epileptic patients. Specific aims are: a) to determine the molecular profile and classify subplate neurons in three regions during development; b) to determine the molecular profile and classify white matter interstitial neurons in three regions of adult human brain; c) to determine which classes of subplate neurons survive into adulthood; d) to compare white matter neurons in adult brain between healthy controls and patients with epilepsy; and e) to determine the distribution and differences of different GABA A receptor subunits in white matter interstitial neurons between healthy controls and patients with epilepsy.

Materials and methods: The proposed research will be conducted using post-mortem samples of normal fetal and adult human brain which are part of versatile Zagreb Neuroembryological collection. All analysed samples will be without known neurological or psychiatric disorders and without history of substance abuse or long-term use of psychoactive medications. Analysis of the epileptic samples will be conducted on the post-operative tissue samples collected during indicated neurosurgical procedures for treatment of pharmacoresistant epilepsy. The samples are part of the University of Zagreb School of Medicine Department of Pathology and Clinical Hospital Centre Zagreb Department of Pathology. All used samples were collected with the prior ethical approval of IRB. We will analyse three fetal brains per stage (15 – 18 PCW; 24 – 28 PCW and 35 PCW – Newborn) and five adult brains (age 30 – 60 years). We will also analyse 10 adult samples from patients with epilepsy (age 30 – 60 years). To elucidate molecular profile of neurons we will use classical histological methods (e.g. Nissl and Golgi), immunocytochemistry, *in-situ* hybridization and RNAscope for different biochemical markers.

Expected scientific contribution: The expected scientific contribution is better understanding of developmental origin of white matter interstitial neurons and developmental fate of different subplate neuron classes. Furthermore, we will elucidate the role of these neuronal populations in the pathogenesis of epilepsy. We will gather data on location and composition of different GABA A units within defined neuronal population.

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

Poster code: T-01-08-089

APLICATION ACCURACY OF RONNA ROBOTIC SYSTEM FOR STEREOTACTIC NEUROSURGERY

PhD candidate: Domagoj Dlaka
Part of the thesis: Aplication accuracy of RONNA robotic system for 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. Today we are witnessing great efforts in robotic systems construction. The RONNA robotic system was developed through cooperation between the Faculty of Mechanical Engineering and Naval Architecture, University of Zagreb and the University Hospital Dubrava. It is intended for stereotactic neuronavigation procedures in neurosurgery and is successfully applied for brain biopsies.

Hypothesis: Accuracy of RONNA robotic system in stereotactic neurosurgery is equal to other robotic systems described in the literature and equal to accuracy of stereotactic procedures performed with Leksell stereotactic frame.

Aims: To investigate application accuracy of RONNA robotic system in stereotactic neurosurgery in in vitro experiments on phantoms and also in in vivo biopsies on patients.

Materials and methods: Study will be performed on phantoms with accuracy measurement of robotic system RONNA in comparison to stereotactic Leksell frame and neuronavigation with Medtronic Stealth Station. A prospective study of robotic biopsies with RONNA robotic system and stereotactic biopsies with Leksell stereotactic frame will be performed. Measurements will be performed through analysis of preoperative MSCT and postoperative MSCT an MRI images with complete study of all relevant accuracy data such as entry point error, target point error, trajectory traits, patohistological diagnosis accuracy, and also all clinical relevant data such as patient data, tumor size and histology, intraoperative and postoperative complications and outcome.

Expected scientific contribution: Receiving information about application accuracy of robotic system RONNA in stereotactic neurosurgery, such study is not yet performed since the robotic system itself is original product of our research, development and construction. Results of the prospective study that will beside the information about robotic system accuracy give comprehensive information about stereotactic Leksell frame biopsies accuracy.

MeSH/Keywords: brain biopsy, computer assisted surgery, frameless stereotaxy, robotic neurosurgery, RONNA G3

Poster code: T-01-08-090

PREDICTION OF PERIVENTRICULAR LEUKOMALACIA AND INTRACRANIAL HAEMORRHAGE IN PRETERM INFANTS USING CEREBRAL NEAR-INFRARED SPECTROSCOPY

PhD candidate: Tomislav Ćaleta

Part of the thesis: The use of cerebral near-infrared spectroscopy in predicting perinatal brain damage

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

Affiliation: University of Zagreb School of Medicine

Introduction: In Europe, about 25,000 children are born prematurely every year, their mortality reaches 20%, and among the survivors around 25% have some form of neurodevelopmental disorder, including cerebral palsy. Brain damage in premature infants is most commonly seen as peri-intraventricular bleeding (P- / IVH) and periventricular leucomalacia. According to current research, there is an opinion that hemodynamic instability has an important role in their etiology. NIRS is a non-invasive method for estimating cerebral hemodynamics that can be continuously used in babies with very low birth weight. NIRS, measures regional saturation (rScO2) corresponding to the saturation of oxygen hemoglobin in mixed arterial-capillary-venous tissue vasculature. NIRS-measured cerebral oxygenation correlates with cerebral blood.

Hypothesis: Regional cerebral tissue oxygen saturation (rScO2) and fractional tissue oxygen extraction (FTOE) during the first three days of life differ in premature born babies that develop intracranial bleeding and / or periventricular leukomalacia in relation to those in which the above mentioned brain damages do not occur.

Aims: Analyze the impact of rScO2 and FTOE during the first three days of life on the formation of periventricular leukomalacia and intracranial bleeding. Investigate whether there are different trend patterns of rScO2 and FTOE between the PVL and the intraventricular / parenchyma bleeding (IVH, HPI) groups.

Materials and methods: The study will include premature born babies that were born ≤32 weeks gestation and were admitted at the Department of Pediatrics, University Hospital Centre Zagreb, in the first day of life. Patients with chromosomopathies, congenital anomalies, hemodynamically significant ductus arteriosus, IVH grade 3 and 4 at the time of admission, will be excluded from the study. The following perinatal and neonatal variables will be extracted from medical records: demographic (age, sex...), prenatal (complications during pregnancy), perinatal (gestation age, birth weight, Apgar score...), during treatment (SNAP-II and SNAPPE-II scores, blood pressure...). Head ultrasound will be performed within 24 hours upon admission, on the 3rd, 5th and 7th day of stay and then once weekly to the corrected gestational age of 40 weeks. Brain MRI will be performed on two occasions: first recording after stabilization of the clinical condition of the patient to corrected gestational age of 32 weeks and the second recording at the term age (corrected gestational age of 40 weeks). Immediately upon admission, each patient will begin continuous monitoring of rScO2 using near infrared spectroscopy. FTOE will be determined by the equation: FTOE = (tcSao2 - rScO2) / tcSao2. According to the US and MR brain findings, we will classify the patients into three groups: 1. normal finding, 2. Brain white matter damage and 3. intraventricular / intraparenchymal bleeding. Patient Classification will be made after recording the second brain MRI at the term corrected age. Then we will examine whether rScO2 and FTOE values differ between the groups.

Expected scientific contribution: The predictive value of cerebral oxygenation measured with NIRS in the first three days of life on the formation of periventricular leukomalacia and intracranial bleeding. Early detection of high-risk patients would allow timely application of targeted therapy to prevent complications and improve patient outcome.

MeSH/Keywords: Near Infrared Spectroscopy, premature infants, intracranial haemorrhage, periventricular leukomalacia.

Poster code: T-01-08-096

ANALYSIS OF TRANS-SYNAPTIC SPREAD AND AGGREGATION OF TAU PROTEIN AFTER THE INTRACEREBRAL INJECTION OF TAU OLIGOMERS INTO THE RAT ENTORHINAL CORTEX

PhD candidate: Lea Langer Horvat

Part of the thesis: Analysis of trans-synaptic spread and aggregation of tau protein after the intracerebral injection of tau oligomers into the rat entorhinal cortex

Mentor(s): Professor Goran Šimić, MD PhD

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

Introduction: Sporadic Alzheimer's disease (AD) is the most common secondary tauopathy characterized by progressive loss of cognitive functions and behavioral impairment. The two major pathological hallmarks of AD are senile plaques composed of amyloid-β protein and neurofibrillary tangles composed of tau protein. 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. Recent studies on transgenic animal models of AD provide initial evidence that pathogenic protein aggregates could propagate from neuron to neuron, and then self-replicate by templated misfolding of monomeric tau proteins thus igniting further spreading.

Hypothesis: Within the time period of 12 months, the intracerebral injection of tau oligomers into the rat entorhinal cortex will induce aggregation and trans-synaptic spread of pathological tau proteins from the site of injection to the entorhinal projecting cortical regions and these changes will match with animals' cognitive impairment.

Aims: The goal of this study is a critical appraisal of the pathogenic tau protein spread hypothesis of neurodegeneration in wild-type Wistar rats. We will inject a single dose of soluble oligomeric form of human tau protein (4 μg in a total volume of 4 ìl) into the entorhinal cortex to assess: 1) whether such an intervention will cause a neuron-to-neuron physical spread of tau oligomers and tau aggregates, and 2) will those changes be associated with expected cognitive impairment.

Materials and methods: The study will include 3-4 months old male Wistar rats (n=108) divided into three groups. Rats from the first experimental group will be stereotaxically injected with tau oligomers into the entorhinal cortex, the second group will be injected with preformed tau fibrils and the third, control group, with phosphate-buffered saline. Animals will be tested and analyzed 3 days (6 rats per group), 3 months, 6 months and 12 months post-injection (10 rats per group). The following cognitive and behavioral test will be used: open field test, passive-avoidance learning task, T-maze task, novel object recognition task and object-location memory task. To document neurofibrillary changes Bielschowsky silver staining, Gallyas-Braak silver impregnation, Nissl and Thioflavin S staining will be used. To specifically detect tau protein changes in brain sections, we will use immunohistochemistry using the following anti-tau antibodies: T22, TOMA-1, AT8, HT7, CP27, and MC1. Tentative amyloid changes will be assessed using anti-amyloid antibodies. Proteins isolated from the entorhinal cortex and hippocampus will be analyzed by immunoblotting using anti-tau antibodies HT7, AT8, T22 and Tau5. For quantitative measurement of Aβ levels, the adequate enzyme-linked immunosorbent assay (ELISA) will be used.

Expected scientific contribution: We anticipate to better understand mechanisms of spreading of the pathological changes in AD brain and to develop a model that will mimic early events underlying those changes in human disease. The ultimate goal would be to define possible new therapeutic targets and interventions aimed at blocking tau protein oligomerization, aggregation, and spread.

Acknowledgments: This work is funded by the Croatian Science Foundation HRZZ (IP-2014-09-9730) and by the European Union through the European Regional Development Fund, Operational Programme Competitiveness and Cohesion, grant agreement no. KK.01.1.1.01.0007, CoRE- Neuro.

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

Poster code: T-01-08-117

PHD THESIS PROPOSALS

Clinical Medical Sciences

EFFECTS OF VITAL PARAMETERS OPTIMISATION WITH MULTIMODAL MONITORING IN CAROTID THROMBENDARTERECTOMY IN GENERAL ANESTHESIA ON PERIOPERATIVE COMPLICATIONS AND COGNITIVE FUNCTIONS

PhD candidate: Tina Tomić Mahečić

Part of the thesis: Effects of vital parameters optimisation with multimodal monitoring in carotid thrombendarterectomy in general anesthesia on perioperative complications and cognitive functions

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

Affiliation: University of Zagreb School of Medicine

Introduction: Carotid thrombendarterectomy (CEA) is a surgical procedure performed to reduce the incidence of embolic and thrombotic stroke. Although only a preventive procedure, CEA carries the risk of perioperative complications. Perioperative risk needs to be maintained within the limits of up to 3% for asymptomatic and up to 5% for symptomatic patients. In addition to clinically manifest focal neurological deficits, subclinical cognitive deficits are potentially unwanted CEA outcomes. We designed a multidisciplinary (study) research, with preoperative and postoperative neurological testing, new battery of cognitive testing and multimodal monitoring during anesthesiological procedures, to potentially decrease perioperative risks and complications.

Hypothesis: Previous studies did not reach a consensus on the influence of the type of anesthesiological procedure and monitoring of perioperative complications and cognitive outcomes. The maintenance of optimal vital parameters during ACI vascular clamp increases a good cognitive outcome. We assume that multimodal monitoring techniques and a better selection of cognitive tests will allow a more accurate assessment of the effect.

Aims: We will estimate the effect of perioperative multimodal monitoring as TCD (Transcranial Doppler), NIRS (near infrared spectroscopy) and EEG (electroencephalogram) in patients for CEA on a supposed decrease of perioperative complications and improvement of cognitive functions. We will compare the patients with symptomatic and asymptomatic stenosis, and we will also evaluate cognitive functions and their dynamics depending on the time that has passed from neurological symptoms.

Materials and methods: The prospective study will analyse 80 consecutive patients with asymptomatic and symptomatic (TIA in anamnesis) stenosis of the internal carotid artery in which carotid thrombendarterectomy is indicated in general anaesthesia. Cognitive tests will be analysed for all patients preoperatively, first and eighth day postoperatively and after 8 weeks. BHI (breath holding index) will be measured preoperatively and postoperatively. The first group of patients (n = 40) will have expanded intraoperative monitoring involving TCD (Transcranial Doppler), NIRS (Near Infrared Spectroscopy) and EEG, the control group (n = 40) will have only standard anaesthetic monitoring.

Expected scientific contribution: We assumed that with multimodal non-invasive monitoring (TCD, NIRS, EEG) we will reduce the chance of potential new neurological deficits and positively affect the results of our battery of cognitive tests. A scientific contribution will be to clarify the causal relationships of all parameters measured perioperatively with the occurrence of complications and with the results of cognitive function tests.

MeSH/Keywords: CEA ACI, cognitive tests, BHI, NIRS,TCD

Poster code: T-02-01-019

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 post-operative pain management in total knee arthroplasty

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. LIA technique is a simple surgeon administration technique, which provides efficient analgesia in early postoperative stage in TKA. Research data support the use of LIA for postoperative analgesia, but does not support administration through the wound catheter for post-operative analgesia. Sufentanil sublingual tablet system, is a preprogramed, noninvasive, patient activated bedside system that enables patients to manage moderate to severe acute pain in a hospital setting.

Hypothesis: SSTR systems are better in management of postoperative pain in patients undergoing total knee arthroplasty then local infiltration with local anesthetics, and are more suitable for analgesia in post-operative period. Postoperative morphine consumption is higher in patients using LIA for analgesia after total knee arthroplasty then in SSTR system group.

Aims: GENERAL AIM: Comparison of pain management with SSTR system and local infiltration in patients undergoing total knee arthroplasty. SPECIFIC AIMS: Assessment of postoperative pain in patients after knee arthroplasty managed by SSTR system or local infiltration analgesia, measured by total relief pain score and NRS postoperative pain score; Morphine consumption in first postoperative 72 hours; Achievement of rehabilitation goals (first 3 days); Readiness for discharge; Patient satisfaction (third day); Nurse satisfaction/workload; Comparison of complications and adverse effects

Materials and methods: After approval of Ethics committee, and consent of the patients, this prospective intervention clinical study will include 100 of total patients randomly divided in two groups, undergoing elective surgery for total knee arthroplasty, blindly randomized and will be conducted in University Clinical Center of Kosova, Prishtina. Study will consist 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: 50 patients will use SSTR system for postoperative analgesia and 50 patients will use LIA. Both groups will undergo record of the data about the NRS pain scores, total pain relief score, duration of hospitalization, rehabilitation, questionnaire about easy of care for nurse, patient satisfaction questionnaire, complication and adverse effects, all other medication use for management of pain will be recorded in first 72h. 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. Database with all parameters will be created in SPSS. The differences in proportions of qualitative variables between groups will be tested with X2-analysis or Fisher exact test, while Mann Whitney U test and Kruskal Wallis test for testing the difference between quantitative variables when distribution is not normal and Student t-test or ANOVA test when distribution is normal. The level $P<0.05$ will consider as the cut-off value for significance.

Expected scientific contribution: Database with all parameters will be created in SPSS. The differences in proportions of qualitative variables between groups will be tested with X2-analysis or Fisher exact test, while Mann Whitney U test and Kruskal Wallis test for testing the difference between quantitative variables when distribution is not normal and Student t-test or ANOVA test when distribution is normal. The level $P<0.05$ will consider as the cut-off value for significance.

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

Poster code: T-02-01-101

EFFECTS OF CONTINUOUS INTRAVENOUS VANCOMYCIN INFUSION ON SERUM DRUG CONCENTRATION AND HEALTH RELATED OUTCOMES IN INTENSIVE CARE UNIT PATIENTS

PhD candidate: Vedran Lokošek

Part of the thesis: Effects of continuous intravenous vancomycin infusion on serum drug concentration and health related outcomes in intensive care unit patients

Mentor(s): Assist. 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: Target therapeutic drug concentration achievement proportional difference among two groups of patients at the time of first serum concentration measurement. Specific: AUC/MIC >400 achievement proportional difference; total daily doses difference; total daily costs difference; difference in number of therapy adjustments; difference in total number of days spent on therapy; difference in total number of days spent in ICU; 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. Continuous infusion dosing modification: In case of supratherapeutic concentrations (>30mg/l): infusion pause for 4 hours + 25% daily dose reduction In case of subtherapeutic concentration (<20mg/l): 500mg bolus + 25% daily dose increment. 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 results will contribute to significantly better vancomycin dosing and consequently to a reduction in incidence of side effects and improvement in patient outcomes.

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

Poster code: T-02-01-125

THE ROLE OF EPSTEIN-BARR VIRUS IN ETIOPATHOGENESIS, CLINICAL COURSE AND PROGNOSIS IN PATIENTS WITH PRIMARY T-CELL CUTANEOUS LYMPHOMA

PhD candidate: Mikela Petković

Part of the thesis: The role of Epstein-Barr virus in etiopathogenesis, clinical course and prognosis in patients with primary T-cell cutaneous lymphoma

Mentor(s): Assoc. Prof. Romana Čeović, MD PhD

Affiliation: University of Zagreb School of Medicine

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

Hypothesis: CTCL patients with EBV positive tumorous skin lesions will have faster disease progression, worse clinical course and higher incidence in the development of secondary primary malignant tumors.

Aims: The aims of this study are: to determine the prevalence of EBV infection and the prevalence of second primary malignant tumors in patients with CTCL. We will try to determine does the EBV infection cause faster progression of the disease or development of secondary primary malignant tumor. The specific aim of this study is to try to define the group of patients with CTCL who would have a higher risk for faster progression of disease or higher risk for develpoment of second primary malignant tumor.

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

Expected scientific contribution: The role of EBV infection in the pathogenesis of MF is still unknown. Epidemiological data on the occurrence of other primary malignant diseases in patients with CTCL in the Republic of Croatia are unknown. This paper will attempt to determine whether EBV infection is affecting faster CTCL progression and possible association with the occurrence of second primary malignancies in patients with CTCL.

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

Poster code: T-02-02-092

COMPARISON OF INTRACERVICAL AND INTRAVAGINAL APPLICATION OF PROSTAGLANDIN E2 FOR INDUCTION OF LABOUR IN TERM PREGNANCIES WITH UNFAVOURABLE CERVIX

PhD candidate: Katja Vince

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

Mentor(s): Assoc. Prof. Ratko Matijević, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Induction of labour is defined as initiation of labour before its spontaneous onset and is performed in up to one in four deliveries in developed countries. One of the most often used formulations for labour induction in women with unfavourable cervix is prostaglandin E2 (PGE2, Dinoprostone), which has proven to be a safe and efficient formulation. Dinoprostone can be applied in the form of an intracervical or intravaginal gel and studies are still indecisive which form of application is more efficient and safe.

Hypothesis: Intravaginal application of PGE2 for labour induction leads to a shorter time interval from beginning of labour induction to delivery compared with intracervical application of PGE2 in term pregnant women with unfavourable cervix.

Aims: The aim of this study is to compare intracervical and intravaginal application of PGE2 for labour induction in term pregnant women with unfavourable cervix.

Materials and methods: This prospective randomised trial will include pregnant women with term pregnancies, indication for labour induction and an unfavourable cervix. These women will be randomised into two groups: one group will be induced using an intracervical PGE2 formulation, the other using an intravaginal PGE2 formulation. Main outcome will be existence of a difference of 4 hours or more from beginning of labour induction to delivery between the two studied groups. Existence of difference in perinatal outcomes between two studied groups will also be investigated. Furthermore, all pregnant women will undergo clinical examination and cervical length measurement using transvaginal sonography prior to labour induction; with the aim of assessing which method is better at predicting labour induction successfulness.

Expected scientific contribution: This study will reveal new findings about the efficiency of PGE2 formulataions as well as their impact on the course of labour and labour outcome.

MeSH/Keywords: Labour, Induced; Dinoprostone; Cervical Ripening; ultrasonography

Poster code: T-02-05-068

TWO DIMENSIONAL TRANSVAGINAL ULTRASOUND WITH CONTRAST IN PATIENTS WITH DEEP PELVIC ENDOMETRIOSIS

PhD candidate: Marija Gregov

Part of the thesis: Two dimensional transvaginal ultrasound with contrast in estimation of the width and depth of infiltration of the rectocygoid artery in patients with deep pelvic endometriosis

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

Affiliation: University of Zagreb School of Medicine

Introduction: Endometriosis is a clinical entity characterized by the presence of normal endometrial mucosa abnormally implanted in locations other than the uterine cavity. It can be either endopelvic or extrapelvicdepending on the location of endometrial tissue implantation. Endometriosis of the intestine is found in 8-12% patients with endometriosis. The infiltration depth of the intestinal wall by endometriosis strongly correlates with the extent of bowel circumference involement. Transvaginal ultrasound has been shown as a highly sensitive diagnostic tool in the estimation of serose and muscular layer infiltration,but for submucose and mucous layer it appears to be less sensitive.

Hypothesis: Two dimensional transvaginal ultrasound with contrast is a reliable method for estimating the width and depth of infiltration of rectosigmoid epithelium in patients with deep pelvic endometriosis.

Aims: The aim of this study is to determine the ultrasound criteria for assessing the depth of the intestinal wall infiltration by layers and the width of the endometriotic change. Using contrast we will determine the distance of endometriotic changes in the intestine from the anocutaneous boundary

Materials and methods: This is a prospective study wich will include patients with deep pelvic endometriosis and suspected intestinal infiltration who will be treated in the Department of Obstetric and Gynecology University of Zagreb, School of Medicine. Transvaginal ultrasound with aqua as a contrast agent (transrectally applied 300 ml of aquae using a catheter) will be performed in all patients before the operation, after two days of specific prepartion (liqiud diet, enema, oral salt and liquid lactulose intake). Aqua as a contrast agent will disrupt the hose walls and allow us better shew of the intestinal lume. In cases where the endometriosis infiltrates the intestinal mucus and when the size of the change is greater than 3 cm, the intestinal lumen is reduced by 50% it is necessary to perform a radical surgical operation with segmental resection of the intestine. In a patient with a smaller local finding and without the destruction of the lumbar region, it is possible to do nodulectomy or „shaving" of the intestine.

Expected scientific contribution: Using two dimensional transvaginal ultrasound with contrast 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. It is very important for making athe right decision about the extent of the operation for each individual patient

MeSH/Keywords: endometriosis, rectosigmoid colon, transvaginal ultrasound with contrast

Poster code: T-02-05-069

TUMOUR FREE DISTANCE IN MYOMETRIUM AS PROGNOSTIC INDICATOR IN ENDOMETRIAL CARCINOMA

PhD candidate: Ivan Babić

Part of the thesis: Tumour free distance in myometrium as prognostic indicator in endometrial carcinoma

Mentor(s): Professor Ante Ćorušić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Myometrial invasion (MI) with tumor has been shown as a significant prognostic indicator and expressed as a percentage (<or> 50%) is used in the FIGO (International Federation of Gynecology and Obstetrics) classification and is calculated by determining the depth of the myometrial invasion (DOI) and the total thickness of myometrium. Due to the variability in DOI estimation, there is attemption to find a prognostic factor that would have the lower variability.

Hypothesis: Tumour free distance (TFD) is better prognostic factor than DOI and MI according to treatment outcome and five year survival.

Aims: The aim of this study is to determine the TFD cut off value as an indicator of survival, recurrence and spread of tumors in the lymph nodes and to compare the value of TFD with other pathohistological and clinical prognostic factors.

Materials and methods: This is retrospective study. It will include patients with endometrial cancer which were hospitalized and treated at the Department of gynecological oncology and the Department of gynecological surgery of University Hospital Center Zagreb in a five year period (2011 to 2015). In this study we will include only patients who were surgicaly treated (hysterectomy with billateral salpingo-oophorectomy +/- pelvic lymphadenectomy). The outer tumor free myometral part is defined by the distance from the point of the deepest invasion to the serosal surface (tumor free distance TFD). On patohystological slides we will measure TFD, and that will be done in the Department for pathology and cytology of University Hospital Center Zagreb.

Expected scientific contribution: This work together with other so far published works in this area will contribute to a better understanding of the biological behavior of the uterine body cancer and enable better treatment and how to better predict disease outcome.

MeSH/Keywords: myometrium, depth of invasion, TFD, DOI

Poster code: T-02-05-091

COMPARISON OF PREDICTIVE VALUE OF QSOFA, SOFA SCORE AND SIRS CRITERIA FOR MORTALITY OF ADULT PATIENTS WITH COMMUNITY-ACQUIRED BACTERIEMIA

PhD candidate: Marija Kusulja

Part of the thesis: Comparison of predictive value of qSOFA, SOFA score and SIRS criteria for mortality of adult patients with community-acquired bacteriemia

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

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

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 medicinal 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 whether the new criteria may help improve the survival of patients with sepsis.

Hypothesis: The new sepsis criteria: qSOFA and SOFA score, have greater predictive value for mortality during hospitalization of adult patients with community-acquired bacteremia in comparison to the old, SIRS criteria.

Aims: The goal of this dissertation is to compare qSOFA, SOFA score and SIRS criteria as predictors of 28-day mortality and mortality during hospital stay.

Materials and methods: The subjects of this study will be adult patients hospitalized in University hospital for infectious diseases „Dr Fran Mihaljević", Zagreb, with community-acquired bacteremia. The data on patients hospitalized from 2007 to 2016 will be acquired retrospectively from medical documentation. The main outcomes will be 28-day mortality and mortality during hospital stay, while secondary outcomes will be severity of illness during hospital stay (assessed through the need for hospitalization in the intensive care unit and utilization of intensive medical treatment), as well as development of septic shock

Expected scientific contribution: This study is expected to contribute to the assessment and validation of the new sepsis criteria published in 2016, as well as to compare the value of old and new sepsis criteria in predicting mortality in different age groups.

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

Poster code: T-02-07-029

DISTRIBUTION OF ROTAVIRUS GENOTYPES IN CROATIA

PhD candidate: Maja Vrdoljak

Part of the thesis: Epidemiology of rotavirus gastroenteritis in Republic of Croatia

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

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

Introduction: Rotavirus is a major cause of acute gastroenteritis in children less than 5 years of age worldwide, with more than 140 million episodes a year. There are numerous genotypes of Rotavirus whose distribution changes depending on geographic area, season or age of the patient. There are two rotavirus vaccines which are highly effective in reducing the burden of rotavirus gastroenteritis. However, they are not implemented in the national immunisation programme in Croatia. Data on circulating genotypes in one Croatian region (Central Croatia) are shown only in one study. However, there are no data on geographic, seasonal or age distribution of rotavirus genotypes in Croatia, which are necessary before introduction of rotavirus vaccines in national immunization programme.

Hypothesis: There are differences in geographic, seasonal and age distribution as well as in the severity of disease between rotavirus genotypes in children younger than 5 years in Republic of Croatia.

Aims: The aim of the study is to assess circulating rotavirus genotypes in Croatia causing acute gastroenteritis in children younger than 5 years, to analyse possible differences in their geographic, seasonal and age distribution and to estimate disease severity using Vesikari and Clark scoring systems.

Materials and methods: Children aged less than 5 years with acute gastroenteritis, treated in three Croatian hospitals (University Hospital for Infectious Diseases Dr. Fran Mihaljevic, Zagreb, University Clinical Center Split and County Hospital Cakovec) will be included in the study during the two years period. Demographic, clinical and laboratory data will be collected and immunocromatographic analysis of the stool samples for Rotavirus and Adenovirus will be performed, as well as stool culture. Rotavirus positive samples will be further analysed (polymerase chain reaction and sequencing) to identify genotype.

Expected scientific contribution: Insight on molecular epidemiology of rotavirus acute gastroenteritis, which is of great importance for determining the need for implementation of rotavirus vaccines into national immunisation programme.

Acknowledgments: none

MeSH/Keywords: Rotavirus, gastroenteritis, genotype

Poster code: T-02-07-051

INCIDENCE AND PREVALENCE OF SYPHILIS IN HIV-INFECTED PATIENTS IN CROATIA

PhD candidate: Vanja Romih Pintar

Part of the thesis: Incidence and prevalence of syphilis in HIV-infected patients in Croatia

Mentor(s): Assoc. Prof. Davorka Lukas, MD PhD

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

Introduction: Syphilis is a significant growing problem for people infected with HIV (human immunodeficiency virus). In Croatia, all HIV infected patients are treated at the University Hospital for Infectious Diseases (UHID) in Zagreb. HIV and Treponema pallidum co-infection has important public health implications, and having past or current Treponema pallidum infection is associated with an increased risk of acquiring and transmitting HIV infection. Monitoring prevalence and incidence of syphilis in HIV infected patients has not been analyzed so far in Croatia and is of great importance for a better understanding of areas of public health.

Hypothesis: The incidence of syphilis in people infected with HIV has increased in the period from 2010 to 2017.

Aims: To evaluate incidence and prevalence of syphilis in HIV infected men who have sex with other men (MSM) based on positive treponemal (TPHA) and nontreponemal tests (RPR). To investigate factors related to the syphilis infection and reinfection during the period from 2009 to 2017.

Materials and methods: This is a cohort study. We will use routinely collected data from the existing electronic database available at our Clinic. The research will include HIV-infected men over the age of 18, who practice sexual relations with other men and are in outpatient care in the Republic of Croatia in the period from 2009 to 2017. Patients who were previously treated for HIV infection outside the Republic of Croatia or were treated in the HIV/AIDS Infectious Diseases Clinic „Dr. Fran Mihaljević" for less than a year will be excluded from the study. The patient characteristics will be presented with frequencies (for the category variables) or the median with the first and third quartiles for the continuous variables. The incidence will be calculated by the Poisson method. Longitudinal analysis will use Poisson's generalized estimating equation. P-value variables <0.20 will be included in the multivariate analysis. In the subjects with basic negative tests in which the number of new infections is analyzed, we will also use survival analysis.

Expected scientific contribution: This research should contribute to a better understanding of the potential risk factors for increased incidence of syphilis in HIV-infected patients. The results of the research could give contribution for targeted preventive public health measures and reflect in clinical practice trough re-evaluation of need for routine serological monitoring and frequency of follow-up. Data analysis from the study will complement the worldwide data on incidence and prevalence of syphilis and improve surveillance, treatment and prevention of the disease.

MeSH/Keywords: HIV, Syphilis, Croatia, Incidence

Poster code: T-02-07-074

COMPARISON OF IMMUNOGLOBULIN G GLYCOSLYLATION IN INFLUENZA AND BACTERIAL PNEUMONIA PATIENTS

PhD candidate: Marina Kljaković-Gašpić Batinjan

Part of the thesis: Comparison of Immunoglobulin G Glycoslylation in Influenza and Bacterial Pneumonia Patients

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, including influenza, are among the most common infectious diseases in the general population. Their treatment consumes > 70% of the total prescribed antimicrobial drugs. However, the etiology of respiratory tract infections is difficult to confirm, especially at the time of diagnosis. Despite numerous currently available microbiological and laboratory diagnostic tools, each of currently used methods has its limitations regarding sensitivity and specificity. Therefore there is a need for translational studies to develop new biomarkers in order to improve treatment and outcome of acute respiratory infections. Glycosylation is a form of co-translational and post-translational modification which refers to the enzymatic process that attaches glycans to proteins, lipids, or other organic molecules. Glycosylation is necessary for the normal functioning of proteins. Immunoglobulin G (IgG) is an example of a glycoprotein whose function is regulated by glycosylation. Changes in glycosylation profiles of IgG were observed in various diseases including diabetes, malignant, autoimmune, inflammatory, neurodegenerative and infectious diseases. Population studies have shown that among individuals there are large differences in glycosylation profiles and these differences are related to the severity of certain diseases as well as response to therapy.

Hypothesis: Immunoglobulin G glycosylation in influenza patients differs from immunoglobulin G glycosylation in bacterial pneumonia patients.

Aims: To compare the glycosylation profiles of immunoglobulin G in influenza and bacterial pneumonia patients. Specific aims are: (1) to determine whether the immunoglobulin G glycosylation profile can be used as a diagnostic biomarker in distinguishing viral from bacterial infection; and (2) to analyze the dynamics of glycosylation profile of immunoglobulin G during disease with aim to determine prognostic value of immunoglobulin G glycosylation in predicting severity of disease and treatment outcome in influenza and bacterial pneumonia patients.

Materials and methods: The study will include 110 adult patients hospitalized due to influenza and 110 adult patients hospitalized due to bacterial pneumonia. Demographic, epidemiological, clinical, laboratory and other data regarding severity and outcome will be collected. On first, seventh and twenty-eighth day following admission, the glycosylation profiles of serum immunoglobulin G in all the patients plus 110 control subjects will be determined by chromatography. The differences in glycosylation among the groups will be determined by variance analysis (ANOVA).

Expected scientific contribution: The glycosylation profile of immunoglobulin G could serve as a diagnostic biomarker for differentiating between viral and bacterial infections and a prognostic biomarker for predicting disease severity and outcome in influenza and bacterial pneumonia patients.

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

Poster code: T-02-07-126

VITAMIN C, VITAMIN B1, HYDROCORTISONE AND 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): Assist. Prof. Slobodan Mihaljević, MD PhD, Assoc. Prof. Robert Likić, MD PhD

Affiliation: Department of Anesthesiology, reanimatology and intensive medicine, General Hospital Sisak, Croatia

Introduction: Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection. 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 (Sepsis-related) 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.

Hypothesis: The intravenous administration of vitamin C, vitamin B1 and hydrocortisone results in a significant reduction in the SOFA clinical score and reduces mortality in patients with septic shock.

Aims: Main research aims: To investigate the effects of vitamin C, vitamin B1 and hydrocortisone on the clinical course and on the outcome of patients with septic shock. Specific research aims: To analyze the effect of intravenous administration of vitamin C, vitamin B1 and hydrocortisone on biochemical parameters (procalcitonin, C-reactive protein, creatinine, bilirubin, lactat-dehydrogenase), the duration of vasopressor therapy, mechanical ventilation, treatment in the intensive care unit and the survival and mortality of patients.

Materials and methods: Materials and methods: The study has been planned as a prospective, randomized single-blind study. The study will be conducted by the Department of Anaesthesiology, Reanimatology and Intensive Care at the Zagreb University Hospital Centre. The total number of patients wil be divided in two groups: 30 patients in the experimental group and 30 in the control group. The experimental group patients will receive standard therapy and vitamin C (1,5 g every 6 hours), vitamin B1 (200 mg every 6 hours), and hydrocortisone (50 mg every 12 hours). The control group will receive only standard therapy. The drugs will be administered from the start of the diagnosis for up to 4 days and will be followed-up until the final discharge from the ICU. In order to calculate the SOFA score, the following parameters will be used for each subgroup: PaO2/FiO2 ratio, platelets, mean arterial pressure, bilirubin, creatinine, Glasgow coma score, need for vasopressor therapy and diuresis. After ICU discharge, we will calculate the duration of vasopressor therapy, mechanical ventilation, treatment in the intensive care unit and finally the survival and mortality of patients.

Expected scientific contribution: By using cost effective, readily accessible and safe treatment, this prospective clinical study in our patient population will yield results that will greatly contribute to the current research and the knowledge of new pharmacological possibilities in the treatment of septic shock.

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

Poster code: T-02-08-013

REMOVAL OF CYTOKINES IN SEPTIC PATIENTS WITH ACUTE KIDNEY INJURY BY CONTINUOUS REPLACEMENT RENAL THERAPY

PhD candidate: Stella Davila Šarić

Part of the thesis: Removal of cytokines in septic patients with acute kidney injury by continuous replacement renal therapy

Mentor(s): Professor Drago Batinić, MD PhD, Vedran Premužić, PhD, research associate

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

Introduction: Sepsis is a well-known risk factor for acute kidney injury, and almost half of all patients with acute kidney injury are septic. Septic patients have a strong association with acute kidney injury and mortality. Local or systemic release and production of inflammatory cytokines, such as tumor necrosis factor alpha (TNF-á), interleukin 2, 6, and 8, stimulate the formation of sepsis and participate in the pathogenesis of septic shock and acute kidney injury. The onset of acute kidney injury is a predictor of more frequent mortality, has a significant effect on the occurrence of multiple organ failure and is associated with prolonged stay in intensive care units. Considering the connection between sepsis and acute kidney injury, an increased amount of circulating cytokines that lead to septic shock and increased mortality in these patients, the goal of study is to determine the effect of various methods of continuous replacement kidney treatment on the removal of inflammatory cytokines, renal function recovery and overall survival.

Hypothesis: Continuous venovenous hemodialysis is more effective in the removal of proinflammatory cytokines in septic patients with acute kidney injury and leads to better recovery of renal function and total survival compared to continuous veno-venous hemofiltration.

Aims: The general goal of this prospective, non-interventional and open study is to determine the effect of various methods of continuous replacement kidney treatment on the removal of inflammatory cytokines, renal function recovery and overall survival. The specific objective of this study is to evaluate when to start a continuously compensated kidney treatment, to estimate diuresis as an additional marker in the assessment of renal function and overall mortality.

Materials and methods: This study will involve 160 voluntary patients admitted to the Intensive Care Unite of surgical patients after surgery. Patients will be randomised in two groups. Patients will be treated either with CVVH or CVVDHF method of renal replacement therapy. Laboratory testing before and after renal replacement therapy. Proinflamatory cytokines (tumor necrosis factor alpha - TNF-α), interleukin 2, 6, and 8) in serum and in urin of patients will be tested using flow cytometry analysis method before and after renal replacement therapy using Luminex 100/200 and FlexMAP 3D. In order to test all the objectives of this study, the plan is to monitor the clinical course of patients during hospitalization and total survival for a period of one year.

Expected scientific contribution: The results of this study will show how much the kidneys are involved in the removal of proinflammatory cytokines, specifically how held diuresis helps in the removal of cytokines in septic patients with acute kidney injury.

Acknowledgments: Departament of anesthesiology, reanimatology and intensive medicine and Departament of nephrology, arterial hypertension, dialysis and transplantation, University hospital Centre Zagreb

MeSH/Keywords: Sepsis, Septic shock, Cytokines, Acute kidney injury, Biomarkers, Renal replacement therapy, Renal recovery, Mortality

Poster code: T-02-08-015

SERUM ACTIVIN A LEVEL IN PATIENTS DIAGNOSED WITH SQUAMOUS CELL LUNG CANCER

PhD candidate: Lela Bitar
Part of the thesis: Serum activin A level in patients diagnosed with squamous cell lung cancer
Mentor(s): Assoc. Prof. Marko Jakopović, MD PhD
Affiliation: University of Zagreb School of Medicine

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

Hypothesis: The serum activin A level is a marker of the extent of the disease and disease progression in patients with squamous cell lung cancer.

Aims: The aim of this study is to determine whether there is a difference between serum activin A level in patients with squamous cell lung cancer and control group and whether there is a difference in serum activin A levels in patients diagnosed in early stages of squamous cell lung cancer and in the advanced stages. Also, to ascertain whether serum activin A levels are correlated with disease progression and progression free survival in the first line of treatment. We will investigate if there is a correlation between serum activin A level and sex, age and smoking status of the patients.

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

Expected scientific contribution: The serum level of activin A is expected to be used as a novel biomarker to estimate the extent of the disease and progression in squamous cell lung cancer patients.

MeSH/Keywords: lung cancer, non small cell lung cancer, squamous cell lung cancer, activin A

Poster code: T-02-09-022

EXPRESSION OF HISTONE DEACETYLASES AND TNFα IN COLONIC MUCOSA IN PATIENTS WITH ULCERATIVE COLITIS

PhD candidate: Lidija Prka

Part of the thesis: Expression of histone deacetylases and TNFá in colonic mucosa in patients with ulcerative colitis

Mentor(s): Assist. Prof. Marko Banić, MD PhD, Professor Vesna Eraković Haber, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Eucariotic cells organize their genetic material (DNA) around histones creating chromatin. Generally, relaxation of chromatin structure allows and condensation denies access to transcriptional factors. Best known mechanisms of chromatin modifications are methylation of DNA and acetylation, phosphorylation and methylation of histone proteins. Most important enzymes involved in chromatin modifications are histone acetylases (HAT) and histone deacetylases (HDAC) – acetylation leads to relaxation and deacetylation leads generally to condensation of chromatin. Till now, 18 different HDACs have been discovered. These epigenetic modifications are considered to be a mechanism by which different environmental factors could influence disease development and progression and are of great interest in research of chronic conditions, like rheumatological diseases and inflammatory bowel disease (IBD). However, most data we have on HDAC expression and their possible role in IBD come from animal studies and models of experimental colitis which is the reason we want to further investigate HDAC expression in IBD patients.

Hypothesis: The expression of HDAC2, HDAC5, HDAC6 and HDAC9 in colonic mucosa of patients with ulcerative colitis (UC) is different comparing to healthy controls and there is a correlation between expression of each HDAC and concentration of tumor necrosis factor alpha (TNFá).

Aims: Our main goal is to investigate the expression of HDAC2, HDAC5, HDAC6 and HDAC9 in colonic mucosa of UC patients compared to healthy controls and to correlate results with concentration of TNFá in colonic mucosa of UC patients. Secondary aims are to identify cells where HDAC expression is detected and to investigate weather different expression of HDACs correlates with clinical and/or endoscopic activity of UC.

Materials and methods: We are planning to include 30 patients and 15 healthy controls. Apart from demographic informations and medical history, we will take blood samles to determine erythrocyte sedimentation rate, complete blood count, renal and liver function, C-reactive protein, and stool samples to determine fecal calprotectin and microbiological analysis. Colonoscopy will be performed according to standard protocol and biopsy samples will be taken. Expression of HDAC2, HDAC5, HDAC6 and HDAC9 genes will be determined with real-time quantitative polymerase chain reaction (qRT-PCR) and normalized to expression of referent gene glyceraldehyd-3-phosphate dehydrogenase (GAPDH). TNFá concentration will be determined with enzyme-linked immunosorbent assay (ELISA). Results will be analyzed with standard statistical tests. P values less than 0.05 will be considered statistically significant.

Expected scientific contribution: Only scarce data exist on expression of HDACs in colonic mucosa of IBD patients. We believe that results of this research will contribute to the knowledge and better understanding of pathophysiology of IBD.

MeSH/Keywords: histone deacetylase; colitis, ulcerative; tumor necrosis factor alpha, inflammatory bowel disease

Poster code: T-02-09-026

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: The hematopoietic „niche" is a special anatomic-functional unit essential for homeostasis of hematopoietic stem cells, for quiescence, mobilization, self-renewal and differentiation. It is composed of numerous cells of mesenchymal origin and extracellular matrix. Hematopoietic niche cells by the cytokine secretion and the activation of intracellular signal pathways alter the cell cycle and thus determine the fate of hematopoietic stem cells.

Hypothesis: Osteogenic macrophages play a significant role in the microenvironment and maintenance of the malignant Philadelphia-negative myeloproliferative neoplasm clone. Depletion of osteogenic macrophages with consequent reduced expression of Oncostatin M promotes proliferation and mobilization of malignant cell clones. Increased expression of HSP27 and decreased expression of osteonectin (a product of the SPARC gene) are also involved in maintaining the malignant cell. Autophagy as a self-degradation process of cellular molecules and organelles promotes the proliferation and immortality of the malignant cell.

Aims: - to demonstrate that depletion of osteogenic macrophages, consequently decreased expression of Oncostatin M, as well as decreased expression of osteonectin (SPARC gene product) and increased expression of HSP27 participate in the maintenance and mobilization of malignant Philadelphia-negative myeloproliferative neoplasm cell; based on the expression of specific antigens by immunohistochemistry methods (F4/80, CD169) to investigate the correlation between changes in antigen expression and indirectly the number of osteogenic macrophages with the development of Philadelphia-negative myeloproliferative neoplasms; to investigate the molecular interaction of osteogenic macrophages with hematopoietic cells (by immunohistochemical determination of Oncostatin M concentrations); based on immunohistochemistry and gene expression methods to quantify LC3 molecules and Beclin1 as autophagy markers; to determine the influence of the SPARC and HSPB1/HSP27 genes on the maintenance and mobilization of the malignant cell clone; to determine correlation of all findings of immunohistochemistry and gene expression on clinical parameters and laboratory findings.

Materials and methods: Respondents in this study will be patients suffering from Philadelphia-negative myeloproliferativne neoplasm (primary and secondary myelofibrosis). All participants will be examined for the clonic nature of the disease by determining JAK2, MPL and CALR mutations. The study will be conducted on patients treated in the Hematology and Coagulation Department of the UH „Sveti Duh" and the Hematology Department of UH Dubrava. The examination will be prospective in the period from November 2017 and will include newly diagnosed patients. The control group will be patients whose blood cells and bone marrow tissue are sampled in the assessment of the presence of malignant lymphoma and no pathological findings have been found. The expected cohort was 30 subjects in the examined and 10 in the control group. The material to be analyzed in this study will be bone marrow tissue obtained in bone marrow biopsy and blood and serum taken during hospitalization or outpatient monitoring. Bone marrow will be immunohistochemically determined by binding antibodies to specific macrophage antigens (F4/80, CD169) to determine the number of macrophages. Bone marrow tissue will be immunocytochemically tested for Oncostatin M, osteonectin and HSP27 concentrations. Immunohistochemistry will also quantify LC3 and Beclin1 molecules as autophagy markers. Bone marrow samples will be measured by the PCR (chain reaction polymerase) method to express the HSPB1/HSP27, SPARC and LC3 genes. In conclusion the correlation of all findings of immunohistochemistry and gene expression with parameters of clinical image and laboratory findings will be made.

Expected scientific contribution: Enhancing ethiopathogenesis and new therapeutic perspectives of Philadelphia-negative myeloproliferative neoplasms.

MeSH/Keywords: osteogenic macrophages, autophagy, Oncostatin M, osteonectin, chronic Ph- myeloproliferativne neoplasm

Poster code: T-02-09-028

DOES STATIN PRETREATEMENT IN ACUTE CORONARY SYNDROMES IMPROVE OUTCOMES?

PhD candidate: Saša Pavasović

Part of the thesis: Influence of high dose statins within 24 hours of acute coronary syndrome on clinical outcome and platelet reactivity

Mentor(s): Academician Davor Miličić

Affiliation: University of Zagreb School of Medicine, Department of cardiovascular diseases, UHC Zagreb

Introduction: Coronary arterial disease is one of the most important public health issues of today. Cardiovascular diseases represent the leading cause of death in the world today. It is estimated that in 2015 26,5% of deaths are attributable to cardiovascular diseases, while 15.5% of deaths were due to coronary artery diseases. Statins decrease endogenous cholesterol synthesis and increase the expression of LDL receptors and removal of lipoproteins from circulation. Other than their effect on cholesterol lowering, statins display some pleiotropic effect not related to their cholesterol lowering properties. They affect inflammation, immune response, oxidative balance, improve endothelial function and display possible inhibitory effect on hemostatic pathways.

Hypothesis: Administration of high dose statins within 24h after symptoms onset in acute coronary syndromes improves clinical outcomes and has a synergistic effect with antithrombotic drugs in reducing platelet reactivity.

Aims: To determine if: - high dose statins administered within 24h of an acute coronary syndrome affect in-hospital mortality, 3 months mortality or 6 months mortality. - the proposed effect of statins depends on type of an acute coronary syndrome, sex, age, renal function, time after symptom onset, successful PCI or chosen anti-platelet therapy - 80mg of atorvastatin combined with aspirin and ticagrelor affects platelet reactivity.

Materials and methods: This observational study will include 20 000 patients with acute coronary syndrome (ACS) included in the international survey of ACS (ISACS-TC). Patients will be divided into 2 groups dependent on if they received statin therapy within 24h of ACS or later, after which the effect of this therapy on survival will be analyzed. This study will also include a subgroup of 150 patients included in said registry and in the Study of platelet reactivity. This subgroup will be analyzed to determine if statins combined with acetylsalicylic acid and ticagrelor affects platelet reactivity. Power analysis was performed in advance for both the main study and the sub-study. The required sample size for the survival analysis is 1051 patients total with the alpha error at 0.05 and power at 0.90, while the sample size for the sub-study of platelet reactivity is 120 patients total with the alpha error at 0.05 and power level at 0.90.

Expected scientific contribution: Should the hypothesis be confirmed, administration of high dose statins within 24 hours of the onset of symptoms of an acute coronary syndrome could significantly advance the strategy of treatment of acute coronary syndromes. The investigation of platelet reactivity on a limited sample size could additionally illuminate possible impact of high dose statins on platelet reactivity in patients with acute coronary syndromes treated with a combination of acetylsalicylic acid and ticagrelor.

Acknowledgments: The platelet reactivity sub-study was funded by the Croatian Science Foundation.

MeSH/Keywords: acute coronary syndrome, outcomes, statins, platelet reactivity, survival

Poster code: T-02-09-048

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.

Hypothesis: 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.

Aims: 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 ÷2-test, multivariate logistic regression, and ANOVA.

Expected scientific contribution: This research would further contribute to the detection of genes associated with asthma, with emphasis on atopic asthma, and help to identify the risk factors for the development of severe asthma.

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

Poster code: T-02-09-056

EFFECT OF ECHOCARDIOGRAPHIC OPTIMIZATION OF BIVENTRICULAR ELECTROSTIMULATOR

PhD candidate: Marija Brestovac

Part of the thesis: Advantage of echocardiographic optimization of biventricular electrostimulator on outcomes of cardiac resynchronization therapy

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

Affiliation: University of Zagreb School of Medicine

Introduction: Cardiac resynchronization therapy (CRT) is the treatment in cases of heart failure in patients that have left bundle branch block that results in synchronized myocardial contraction and increased left ventricular (LV) function and better outcomes. According to guidelines and recommendations for implantation of biventricular electrostimulator (BE) and follow-ups there is still a significant number of patients without clinical and echocardiographic (ECHO) response to CRT. There are two ways of optimizing BE stimulation, according to QRS width and ECHO parameters of dyssynchrony, which is not a standard method, although it is known that the atrioventricular synchrony can only be ensured by optimization of transmitral doppler flow.

Hypothesis: Echocardiographic optimization of stimulation of the myocardium after implantation of biventricular electrostimulator results in a greater number of patients with better left ventricular function recovery and a more significant reduction in endsystolic volume compared to the electrocardiographic method of stimulation optimization.

Aims: The aim of this study is to determine if ECHO stimulation immediately after CRT implantation will result in better LV function compared to standard method.

Materials and methods: Patients with LV dyssynchrony and implanted BE will be included in this study. In the first group BE will be optimized by echocardiography immediately after implantation and in three consecutive checkups along with measures of LV function indicators. In the control group BE will be optimized by standard method in the operating room immediately after implantation.

Expected scientific contribution: If echocardiographic optimization of stimulation of the myocardium after implantation of biventricular electrostimulator results in a greater number of patients with better left ventricular function recovery and a more significant reduction in endsystolic volume compared to the electrocardiographic method of stimulation optimization then these results can be applied in the further treatment and follow up of patients with implanted biventricular electrostimulator in order to reduce the number of patients with inadequate response to resynchronization therapy.

MeSH/Keywords: CRT optimization, cardiac resynchronization therapy, echocardiographic optimization of CRT device

Poster code: T-02-09-059

HEPATITIS E VIRUS INFECTION AFTER LIVER TRANSPLANTATION

PhD candidate: Petra Dinjar Kujundžić
Part of the thesis: Hepatitis E virus infection after liver transplantation
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) infection is an enterically transmitted infection and generally self-limiting disease. However, in solid organ transplant recipients has been recognized as a cause of acute and more importantly chronic hepatitis with rapid progression to cirrhosis. Epidemiological data and clinical burden of HEV infection among solid organ transplant population are still insufficiently addressed

Hypothesis: Liver transplantation (LT) increases the risk of developing HEV infection.

Aims: This study aims to determine the prevalence and incidence of HEV infection before and after LT, and to identify risk factors for HEV infection and it's clinical burden after LT.

Materials and methods: The study consists of two cohorts; cross-sectional (600 patients) and prospective (200 patients). The subjects will be screened for HEV infection before and every 6 months after LT for one year. Blood samples will be analysed for anti-HEV IgG, using an enzyme immune assay and confirmed by Western blot, and for HEV RNA using qRT-PCR followed by sequence analysis of the positive samples. Clinical, biochemical and on indication histological data will be monitored, and all participants will complete a socio-demographic risk factor assessment questionnaire.

Expected scientific contribution: This study will determine epidemiological data of HEV infection in populations with end-stage liver disease and after liver transplantation. Given the scarcity of HEV data among immunocompromised population in this part of Europe, this study will provide the scope of the issue, also establishing the clinical modalities and associated risk factors of HEV infection among risk populations.

Acknowledgments: I would like to thank my mentors asst. prof. Anna Mrzljak and prof. Adriana Vince all their hard work on this project.

MeSH/Keywords: Hepatitis E virus, Seroepidemiologic Studies, Chronic infection, Risk Factors, Croatia, Transplant Recipients, Liver Transplantation

Poster code: T-02-09-073

INFLUENCE OF CONTINUOUS POSITIVE AIRWAY PRESSURE IN PATIENTS WITH OBSTRUCTIVE SLEEP APNEA ON PLASMINOGEN ACTIVATOR INHIBITOR-1 VALUE

PhD candidate: Tea Blažević

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

Mentor(s): Assist. Prof. Edvard Galić, MD PhD

Affiliation: University of Zagreb School of Medicine, Clinical Hospital Sveti Duh, Psychiatric Clinic Vrapče

Introduction: Obstructive sleep apnea (OSA) is a disorder characterized by sleep disruptions due to a complete (apnea) or partial (hypopnea) collapse of the upper respiratory tract, leading to hypoxemia and hypercapnia. Polysomnography is the gold standard in OSA diagnosis. Apart from changing lifestyle habits, the mainstream therapy is the use of continuous positive air pressure (CPAP) for at least 4-6 hours per night. Earlier studies have found that OSA independently increases the risk for cardiovascular events and mortality which are reduced after long term treatment with CPAP. It is known that in most cases acute myocardial infarction (MI) is an atherothrombotic event, accompanied by decreased fibrinolytic activity. According to some studies the thrombogenic tendency can be explained due to elevated values of plasminogen activator inhibitor-1 (PAI-1) which is the main physiological inhibitor of the fibrinolytic system. In patients with OSA, elevated PAI-1 values were found correlating with the severity of OSA. Several, but not all, studies have shown a decrease in PAI-1 value after CPAP therapy.

Hypothesis: The use of continuous positive airway pressure therapy in patients with obstructive sleep apnea leads to reduction of the value of the plasminogen activator-1 inhibitor.

Aims: 1. To determine the influence of therapy with CPAP in patients with diagnosed severe OSA on PAI-1 value 2. To determine the influence of therapy with CPAP in patients with diagnosed severe OSA on NT-proBNP value 3. To determine the influence of therapy with CPAP in patients with diagnosed severe OSA on nocturnal arterial pressure value 4. To determine the influence of therapy with CPAP in patients with diagnosed severe OSA on heart rate variability and QTc interval

Materials and methods: This is a prospective cohort study. Participants will be selected after performing an overnight polysomnography, with confirmed OSA diagnosis (AHI?30). Data will be collected at two time points - before CPAP therapy is introduced and after 6 months of CPAP use for at least 4 hours per night. Blood samples will be taken for the analysis of laboratory parameters including full blood cell count, C-reactive protein, urea, creatinin, potassium, sodium, fasting glucose, HbA1c, complete lipidogram and coagulogram, NT-proBNP, PAI-1 (ELISA). Participants will be provided with 24-hour holter electrocardiogram, 24-hour blood pressure holter and echocardiography. After 6 months the same measurements will be repeated. The study will not include patients under the age of 18, pregnant women, those with AHI <30, severe chronic obstructive pulmonary disease, severe chronic renal insufficiency, atrial fibrillation, with acute heart failure, cerebrovascular insult transitory ischemic attack or acute coronary syndrome in the last 6 months, those taking anticoagulant therapy, and known psychiatric disorders that could affect compliance. The required number of participants established by the t-test with the 80% strength test and the significance level of 0.05 is 35 with the expected decrease in PAI-1 by 25% after 6 months of CPAP therapy considering its initial value. A sufficient number of participants is expected to be collected in the 4-6-month period.

Expected scientific contribution: OSA influence on the cardiovascular system is still insufficiently researched, particularly the effect of therapy with CPAP on cardiovascular risk reduction. One of the reasons is that, in many cases, studies have been conducted on a small number of subjects with different inclusion and exclusion criteria, for a short period of time and with inconclusive results. This research will hopefully give some new information about the effect of therapy with CPAP in patients with severe OSA on the fibrinolytic system. The importance of early diagnosis and adequate therapeutic approach lies in the fact of possible reduction of cardiovascular risk, morbidity and ultimately mortality.

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

Poster code: T-02-09-075

EFFICACY AND SAFETY OF INDIVIDUALIZED P2Y12 RECEPTOR ANTAGONISTS TREATMENT IN PATIENTS AFTER ACUTE MYOCARDIAL INFARCTION WITH ST-SEGMENT ELEVATION

PhD candidate: Hrvoje Jurin

Part of the thesis: 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

Mentor(s): Academician Davor Miličić

Affiliation: University of Zagreb School of Medicine

Introduction: Acute coronary syndrome with ST-segment elevation (STEMI) represents the most serious manifestation of coronary artery disease. Besides prompt coronary artery recanalization, most oftenly achieved by percutaneous intervention, antiagreggational therapy represents the cornerstone of medical treatment. Although current guidelines still redommend universal dosing of such drugs, several studies have shown that inadequate response to antithrombotic drugs in terms of insufficient thrombocyte inhibition leads to worse clinical outcomes. In this study we aim to analyze the effect of individualized P2Y12 receptor antagonists dose tailoring, depending on in vitro platelet activity measurement, on clinical outcomes of patients with STEMI.

Hypothesis: Individualized P2Y12 receptor antagonist treatment, based on in vitro thrombocyte activity measurement tests, can improve clinical outcomes of patients with STEMI.

Aims: General aim: To explore whether one can, based on the results of thrombocyte activity measurement tests, individualize dosing of P2Y12 receptor antagonists in order to improve clinical outcomes of patients with STEMI. Specific aims: 1. To explore whether individualized dose tailoring of P2Y12 receptor antagonists in patients with observed high on-treatment platelet reactivity can achieve better thrombocyte inhibition in comparison to patients treated with fixed dose of ticagrelor; 2. To compare aggregometry results of patients treated with ticagrelor to the ones treated with tailored dose of P2Y12 receptor antagonists; 3. To investigate whether a strategy of individualized dose tailoring of P2Y12 receptor antagonists improves the safety of dual antiplatelet therapy in comparison to fixed dose of ticagrelor.

Materials and methods: The study will include at least 140 adult patients hospitalized due to STEMI and treated with percutaneous coronary intervention (PCI) and standard dose of dual antiplatelet therapy (DAPT). 10-24 hours after PCI a blood sample will be taken and the level of thrombocytic activity (TA) will be measured using Multiplate® ADP test. Optimal level of TA will be set to 19-46 U according to international consensus. After hospital discharge all the patients will be treated using acetylsalcylic acid (ASA) and ticagrelor for one month. After one month period the second TA measurement will take place and the patients will be randomized in two groups – first group (control group) who will continue the "standard" treatment using ASA and ticagrelor for one year, and a second group (study group) who will continue DAPT using clopidogrel or ticagrelor based on aggregometry test results as follows: 1) patients with high on-treatment TA using standard clopidogrel dose will continue study using double dose of clopidogrel, 2) patients with high on-treatment TA using double dose of clopidogrel will continue study using standard dose ticagrelor, 3) patients with normal (19-46 U) and low (<19) TA will continue study using standard dose clopidogrel. During clinical follow-up of 12 months patients will have 7 clinical visits. Study primary objective will be major adverse cardiac and cerebrovascular event (MACCE) defined as the composit of myocardial re-infarction, ischemic cerebrovascular insult, sudden death and the need for revascularization. Secondary study objective will be 30-day and 1-year myocardial infarction, in-stent thrombosis, ischemic cerebrovascular insult, need for revascularization, minor and major bleeding event, cardiovasular death, all-cause death.

Expected scientific contribution: Based on the fact that the cornerstone of modern medical treatment is in fact an individualized patient approach, we assume that the future of antiplatelet treatment of patients with acute coronary syndromes is in individualized dose tailoring of such drugs. We expect that the results of this study will show that the optimization of antiagreggational effect of antithrombotic drugs by means of tailoring P2Y12 receptor antagonists dose, based on Multiplate® device derived measurements, improves clinical outcomes in patients with STEMI.

MeSH/Keywords: Platelet aggregation inhibitors, acute coronary syndrome, ST elevation myocardial infarction, P2Y12 receptor antagonists, platelet activity, clinical outcome

Poster code: T-02-09-088

ASSOCIATION OF SERUM ENDOTHELIAL LIPASE WITH LEVELS AND FUNCTIONAL CHARACTERISTICS OF HDL PARTICLES AND ENDOTHELIAL DYSFUNCTION IN PATIENTS WITH METABOLIC SYNDROME

PhD candidate: Iva Klobučar

Part of the thesis: Association of serum endothelial lipase with levels and 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 Medicine, Zagreb, Croatia

Introduction: HDL particles induce NO synthesis in vascular endothelial cells, inhibit LDL particles oxidation and reduce adhesion molecules expression on endothelial cells, resulting in vasodilation and anti-inflammatory effect. Endothelial lipase is an enzyme which degrades HDL particles and its concentration is increased in metabolic syndrome. Endothelial dysfunction is a state of impaired vasodilation potential and presents the first and still reversible step to atherosclerosis.

Hypothesis: Increased serum concentration of endothelial lipase in the patients with metabolic syndrome is associated with lower HDL particle levels, resulting in the development of endothelial dysfunction. Endothelial lipase concentration could be a measure of the occurrence and severity of early, subclinical stages of atherosclerosis.

Aims: The aim of this study is to describe relation between the serum levels of endothelial lipase, serum levels of HDL particles and the stage of endothelial dysfunction of the brachial artery (established indicator of subclinical atherosclerosis), in healthy volunteers and patients with metabolic syndrome.

Materials and methods: This observational, cross-sectional study in the University Hospital Centre Sisters of Charity, involves 130 individuals - 65 healthy volunteers and 65 patients with metabolic syndrome. Criteria for the diagnosis of metabolic syndrome are defined by A Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity from 2009. Exclusion criteria are any acute disease or chronic disease worsening within 6 months prior to the inclusion in the study, severe renal insufficiency and liver cirrhosis, malignant and autoimmune diseases. Patient history will be recorded and physical examination performed. A total of 24 ml of venous blood will be taken from each patient, centrifuged and serum stored at the temperature of -80°C until the laboratory analysis in the Institute of Molecular Biology and Biochemistry of the Medical University of Graz, Austria. Beside standard laboratory biochemistry tests, concentration of endothelial lipase and lipoprotein particles will be measured from the serum samples. Endothelial dysfunction will be evaluated using ultrasound measurement of brachial artery diameter changes after stimulation of endogenous NO production (FMD - flow-mediated dilation) and after application of nitroglycerin (NMD - nitroglycerin-mediated dilation). Brachial artery diameter changes will be measured automatically and continuously using software FloWave.US v. 0.2.0 (Coolbaugh CL, Vanderbilt University Institute of Imaging Science, Nashville, Tennessee, USA), in accordance with currently valid guidelines. All data will be manually imported in the electronic database in Microsoft Excel 2010 (Microsoft, Redmond, Washington, USA) and statistically analysed using IBM SPSS Statistics Version 25 (IBM Corporation, Armonk, New York, USA). Study is a part of scientific project Endothelial lipase, HDL and endothelial dysfunction, supported by Austrian Science Foundation (FWF; grant P 27166-B23). Research is conducted in cooperation with the Institute of Molecular Biology and Biochemistry, Medical University of Graz, Austria (Agreement on cooperation between the University of Zagreb and Medical University of Graz, 21 February 2005. Principal investigators: Prof. Saša Frank (Graz) and Prof. Vesna Degoricija).

Expected scientific contribution: Expected scientific contribution of the study is discovering further information about endothelial lipase function and elucidating its role in the development of atherosclerosis in humans. Endothelial lipase serum concentration will be evaluated as a novel indicator of the occurrence and severity of subclinical atherosclerosis.

Acknowledgments: -

MeSH/Keywords: endothelial lipase, HDL, endothelial dysfunction

Poster code: T-02-09-093

GENETIC BACKGROUND OF INNATE IMMUNITY IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND LUNG CANCER

PhD candidate: Irena Sokolović

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

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

Affiliation: University of Zagreb School of Medicine

Introduction: Chronic obstructive pulmonary disease (COPD) and lung cancer result from an interaction of genetic base and environmental factors. There might be overlapping pathogenetic mechanism that promotes development of both diseases. Some studies revealed potential genetic loci important for lung function and development of inflammatory and malignant lung disease. This research will examine the correlation between structural changes in genes responsible for regulation of inflammatory response and COPD and lung cancer development.

Hypothesis: Toll-like receptor (TLR) polymorphisms are associated with increased chance of developing COPD and lung cancer by modulating activation and regulation of inflammatory microenvironment.

Aims: The frequency of different SNPs (single nucleotide polymorphisms) of genes encoding TLRs will be studied. We will investigate the correlation of the analyzed polymorphisms and clinical characteristics of the examinees. Aim of this research is to gain insight into the functionality of analyzed genes and determine their possible role as genetic markers of disease risk.

Materials and methods: Subjects are divided into three groups: COPD only, COPD and lung cancer and lung cancer only. Standard diagnostic criteria were applied to all subjects. Peripheral blood samples are collected at Clinical Center for Pulmonary Diseases Jordanovac, University Hospital Centre Zagreb. After the salting out procedure for extracting DNA from patients leukocytes, genomic DNA analysis will be conducted. Specific TaqMan probes are used for the polymerase chain reaction (PCR) to determine the presence and frequency of particular genetic variations. ELISA (enzyme-linked immunosorbent assay) is used to determine concentration of cytokines in patients serum and compare those values with the genetic background. The National Center for Biotechnology Information database was used for selection of polymorphisms involved in encoding TLRs. We analysed data that referred to association of genes of interest with impaired immune response regulation and cancer development. We will design case-control study and test association between disease status and SNPs. A logistic regression will be constructed to determine the correlation between SNPs and categorized groups. SNP's impact on the risk of disease development will be expressed by odds ratio (OR).

Expected scientific contribution: The presence and distribution of genetic variations could be an independent predictor of lung disease phenotype. Their identification would lead to improved risk prediction, better understanding of disease mechanisms and enable future specific treatment.

MeSH/Keywords: Chronic Obstructive Pulmonary Disease, Lung Neoplasms, Single Nucleotide Polymorphism, Innate Immunity

Poster code: T-02-09-097

ANALYSIS OF FACTORS RELATED TO IN-HOSPITAL AND ONE-YEAR MORTALITY IN PATIENTS WHO SUFFERED FROM MYOCARDIAL INFARCTION

PhD candidate: Igor Tagasovski

Part of the thesis: Analysis of factors related to in-hospital and one-year mortality in patients who suffered from myocardial infarction

Mentor(s): Academician Davor Miličić

Affiliation: University of Zagreb School of Medicine; Department of Cardiovascular Diseases, University Hospital Centre Zagreb

Introduction: Acute myocardial infarction remains the leading cause of morbidity and mortality worldwide. It occurs when myocardial ischemia, a diminished blood supply to the heart, exceeds a critical threshold and overwhelms myocardial cellular repair mechanisms designed to maintain normal operating function and homeostasis. Ischemia at this critical threshold level for an extended period results in irreversible myocardial cell damage or death. Acute coronary syndrome (ACS) refers to any group of clinical symptoms compatible with acute myocardial ischemia and includes unstable angina (UA), non–ST-segment elevation myocardial infarction (NSTEMI), and ST-segment elevation myocardial infarction (STEMI). Despite modern therapies, acute myocardial infarction is still associated with significant in-hospital and long-term mortality. Therefore, studies on a role of various factors that could be causative associated with worse clinical outcomes are still on-going. In the proposed investigation about 300 patients from the Croatian branch of the ISACS-TC Registry with the seat at the University of Bologna will be analysed regarding factors potentially relevant for increased in-hospital mortality, e.g. advanced age, female gender, higher creatinine levels, bundle branch block in the ECG, and LAD as the infarction-related artery in females who undergo primary percutaneous coronary intervention. Beside fore mentioned factors, other potentially relevant determinants of the clinical outcomes will be included, in order get enough parameters to confirm or exclude the hypothesis extracted from the preliminary investigation, by use of the multivariate analysis. In that context, results of the study could become clinically relevant, thus emphasizing a need for a more comprehensive in hospital and post discharge treatment ad follow up in patients in whom predictors of increased in-hospital and one-year mortality have been found.

Hypothesis: Female gender, advanced age, increased creatinine levels, bundle branch block in the ECG and the LAD as the infarction related artery in females who undergo primary percutaneous coronary interventions are important predictors of increased in-hospital and one-year mortality in patients treated from acute myocardial infarction. The hypothesis is based on our previous investigation on a part of the entire population of cca 3000 patients included in the Croatian Branch of the ISACS-TC Registry.

Aims: AIM: To identify relevant predictors of worse clinical outcomes in patients who suffered from the acute myocardial infarction; PURPOSE: To improve in-hospital treatment strategies and follow up in patients at higher risk of death after acute myocardial infarction

Materials and methods: Patients: cca 3000 patients included in the ISACS-TC Registry – Croatian Branch. Registry: ISACS-TC is one of the world's largest registries of patients who suffered acute coronary syndromes, with a seat at the University of Bologna. Croatia i.e. Department of Cardiovascular Diseases, University Hospital Centre Zagreb, has been participating within the Registry since January 2013. At the moment there are more than 2.500 Croatian patients who were consecutively included within the Registry. Methodology: Hypothesis will be tested by use of multivariate analysis clinical and laboratory parameters that could be independent risk factors for increased in-hospital and one-year mortalty, as well as some other clinical and laboratory outcomes, e.g: reinfarction, hospitalization for cardiac reason, heart failure, NT-proBNP at discharge and after the 1st year.

Expected scientific contribution: Acute myocardial infarction is still one of the leading causes of death worldwide. There fore it is still in a focus of scientific interest, in particular regarding potential independent determinants of the worse outcomes, that should be identified and treated/corrected over time, in order to reduce mortality and other major adverse cardiac events. Proposed investigation could be a relevant contribution to the risk stratification in patients who suffered from the acute myocardial infarction.

MeSH/Keywords: Myocardial infarction, Predictors of worse outcomes, In-hospital and One-year post-infarction mortality

Poster code: T-02-09-099

KNOWLEDGE AND SELF-EFFICACY AS PREDICTIVE FACTORS OF TYPE 2 DIABETES MELLITUS

PhD candidate: Miroslav Ćaćić
Part of the thesis: Knowledge and self-efficacy as predictive factors of type 2 diabetes mellitus
Mentor(s): Assoc. Prof. 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. Assessment of the patient's level of knowledge of diabetes is an important step in adapting group education programs to achieve better 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.

Hypothesis: The level of knowledge and self-efficacy correlates positively with indicators of good regulation of diabetes and a better quality of life.

Aims: General aim: The main purpose of this research is to show that better knowledge of diabetes and better self-efficacy lead to better regulation of diabetes and a better quality of life. Specific aims: validation of the DKQ-24 questionnaire as a specific instrument for assessing the level of knowledge of diabetic patients; Validation of the DSMES questionnaire as a specific instrument to assess the patient's ability to comply with recommended treatment measures and to take care of their own health; Determine the correlation between the duration of the disease with the level of knowledge and self-efficacy; Determining the role of marital status, working status and degree of education at the level of knowledge and self-efficacy; Determining differences and similarities in knowledge and self-efficacy at the gender level; Correlation of knowledge and self-efficacy with objective indicators of the regulation of diabetes mellitus (HbA1c, lipidogram, creatinine, ast, alt); Correlation of knowledge levels and self-efficacy with the quality of life (WHOQoL-BREF questionnaire).

Materials and methods: This study will be carried out at the Department for Endocrinology, Diabetes and Metabolism Disorders in Clinical Hospital Center Sestre milosrdnice (KBC SM). The examination will be conducted as a cross-sectional study on patients with type 2 DM recruited from the clinic and department of the Institute. Study will include all patients, who are examined in our Clinic from 01.01.2018. until reaching predicted number of subjects. Other criteria for inclusion will be meeting the criteria for diagnosis of DM type 2 according to World Health Organization (WHO) guidelines (plasma glucose concentration $\geq$7 mmol / l or glucose concentration in plasma for 2 hours after loading with glucose $\geq$11.1 mmol / L), whose antidiabetic therapy was not corrected at least 3 months before being included in the study and acceptence to participate in the study. Exlusion criteria will be severe mental disorders (psychotic and bipolar affective disorder). The research will be carried out according to the principles of the Helsinki Declaration with the approval of the competent Ethics Commissions. A total of 250 patients are expected to be included in this study.

Expected scientific contribution: The results should show that better knowledge and greater self-efficacy lead to better regulation of diabetes and a better quality of life. The results should show predictive significance of the degree of education, gender, working and marital status on knowledge and self-efficacy. Our research will show that there are differences in the level of knowledge and self-efficacy among groups of patients who differ in disease duration.

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

Poster code: T-02-09-118

THE EVALUATION OF THE RIGHT VENTRICULAR FUNCTION IN PATIENTS WITH HIGH DEGREE ATRIOVENTRICULAR BLOCK WITH IMPLANTED PACEMAKERS

PhD candidate: Edita Pllana-Pruthi

Part of the thesis: The evaluation of the right ventricular function in patients with high degree atrioventricular block with implanted pacemakers

Mentor(s): Assist. Prof. Šime Manola, MD PhD, Assist. Prof. Dardan Kocinaj, MD PhD

Affiliation: University of Zagreb School of Medicine, KBC „Sestre milosrdnice – Klinika za bolesti srca i krvnih zila", University of Prishtina School of Medicine, University Clinical Centre of Kosova – Clinic of Cardiology

Introduction: There are different kinds of heart conditions that imply the need for implanting heart artificial pacemakers. A permanent pacemaker is an electrical device that provides electrical stimuli to the heart, when one is inappropriately absent or slow. Absolute indications for implanting a permanent pacemaker maker are: Symptomatic Sick sinus syndrome or Sinus Node Dysfunction (SND), which incudes, sinus bradycardia, Sino atrial exit block, Type I and Type II, and sinus pause/ arrest, chronotropic incompetence. Also, tachycardia-bradycardia syndrome, atrial fibrillation with sinus node dysfunction, symptomatic atrioventricular block or second and third degree heart block, Bradycardia exacerbating prolonged QT syndrome and cardiac resynchronization (CRT) therapy with biventricular pacing. The criteria about what kind of pacemaker should be used in certain patients is based on the pacing ECS guidelines.

Hypothesis: We think that the drop of RV function in patients after receiving a VVI pacemaker will be greater than the drop of RV function in patients after receiving DDD pacemakers. The incidence of bradyarrhythmias in Kosovo is yet to be defined.Also the mode of treatment of bradyarrhythmias in Kosovo is yet to be defined.

Aims: GENERAL AIM: To define the incidence of each rhythm mentioned in the introduction section. To track the left ventricle and most importantly to track the right ventricle performance before and after pacemaker implantation. SPECIFIC AIMS: To evaluate the implementation of the ESC criteria for the pacemakers' indications and implantation in Kosovo. To evaluate how the specific kind of pacemakers impact the right ventricular function.

Materials and methods: This will be a prospective observational study. This study will include altogether 100 patients. The patients will be divided in two groups. The patients with VVI pacemakers or one chamber pacemakers and patients with DDD pacemakers or dual chamber pacemakers. Patients with third degree block, regardless its persistency, who will be receiving DDDs and patients with third degree block regardless of its persistency who will be receiving VVI. The patients will all be white Caucasian with similar comorbidities and gender. ECG is a must for every patient.For evaluating the left and right ventricle, we will be using the 2D ultrasound technique. Complete set of standardized views: PLAX (dimensions of aorta, left atrium, interventricular septum, posterior wall, right ventricle, diastolic and systolic left ventricle diameter, ejection fraction EF and shortening fraction FS), parasternal RV inflow, PSAX, apical 4-chamber (with right ventricle–focused apical 4-chamber) with standardized dimension measurement. TAPSE. Pulsed i color coded TDI of right ventricle (TDI of tricuspid annulus).Tricuspid valve regurgitation (vena contracta radius, PISA at adequate Nyquist limit) Transtricuspid E/e. RVOT acceleration time (PW Doppler). Right ventricular pressure estimation (TR PG +RAP). Inferior vena cava diameter (with respiratory tracing). We will follow our patient for one year.

Expected scientific contribution: Because there is a great lack of funds, there is not much research in the field of medicine nor any other field, done in Kosovo. So, with our project we tend to bring some new insights on bradyarrhythmias in Kosovo. And on the international scale we tend to strengthen the data on the impact of pacemakers on left ventricle and complications that come with it, and most importantly, to add data on the impact of pacemakers on the right ventricle since this data is vague.

MeSH/Keywords: Echocardiography, pacemaker, right ventricle

Poster code: T-02-09-146

THE ROLE OF HEDGEHOG SIGNALING PATHWAY IN DEVELOPMENT OF CALCIFIC AORTIC VALVE STENOSIS

PhD candidate: Josip Varvodić

Part of the thesis: The role of Hedgehog 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: Several studies have revealed an important role of Hedgehog signaling pathway in various age-related and cardiovascular diseases. Among others, this pathway has also an active and well-established role in patohistogenesis of human atherosclerotic lesions. Due to the overlapping risk factors and histopathological features of atherosclerosis and calcific aortic stenosis (CAVS) it is reasonable to expected that various components of Hedgehog signaling pathway are also actively involved in the development and progression of CAVS.

Hypothesis: We except higher expression of individual morphogens/ligands (DHH, IHH, SHH), receptors/co-receptors (PTCH1/2), as well as transcriptional (GLI1/2/3) and other regulatory factors (SCUBE 2) of Hedgehog signaling pathway in peripheral blood and/or stenotic aortic valve tissue samples of CAVS patients versus their expression in peripheral blood of voluntarily healthy subjects and AVI patients as well as AVI and pathologically unaltered control aortic valve tissue samples.

Aims: The aims of the proposed study are determination of mRNA and protein expression pattern of: (a) ligands/morphogens (DHH, IHH, SHH); (b) receptors/co-receptors (PTCH1, PTCH2, SMO); and (c) transcriptional and other regulatory factors (GLI1/2/3, SUFU, HHIP, SCUBE1/2/3) of Hedgehog signaling pathway in CAVS and control aortic valve tissue; (d) determination of biomarker potential of SCUBE1, -2 and -3 in peripheral blood of CAVS patients, and (e) comparison of obtained gene/protein expression values with clinical and demographic data of CAVS patients.

Materials and methods: Study will encompass healthy voluntary subjects (n = 100), adult patients undergoing valve replacement for severe calcific aortic valve stenosis (n= 100) and aortic valve insufficiency (AVI, n = 50) as well as pathologically unaltered aortic valve tissue samples obtained from heart transplant recipients (n= 15-25) and autopsy procedure (n= 15 - 25). Part of each surgically excised aortic valve will be embedded in FFPE tissue blocks for immunohistochemical (DHH, SHH, IHH, PTCH1, PTCH2, SMO, GLI1/2/3, SUFU, HHIP, and SCUBE1, -2 and -3) analysis while the other part will be used for protein and RNA isolation for qRT-PCR and western - blot analysis. Furthermore, ELISA analysis will also be used for protein (SCUBE1, -2 and -3) analysis of plasma samples obtained from healthy voluntary subjects and preoperative and postoperative (3-6 months) peripheral blood of CAVS and AVI patients. Statistical analysis will be performed with GraphPad Prism statistical software. In brief: normal variable distribution will be checked by Kolmogorov-Smirnov/Shapiro Wilks test. The association between demographic and clinical data with CAVS disease will be determined using the student T test or ÷2/Fisher exact analysis while the comparison between the groups will be performed using independent Student T test, one-way ANOVA or Mann-Whitney U and Kruskal-Wallis test, correspondingly. Association of gene/protein expression data with CAVS disease will be determined by the logistic regression analysis and the interrelationship between quantitative gene/protein expression data and individual clinical parameters of CAVS patients will be established by Pearson correlation coefficient and linear regression analysis. Significance will be set at P (two tailed) < 0.05. Bonferroni's correction for multiple comparison will also be applied where appropriate.

Expected scientific contribution: Determination of currently unknown biomarker potential and expressional pattern of individual morphogens and mediators of Hedgehog signaling pathways in peripheral blood and aortic valve tissue of CAVS patients compared to control and AVI tissue samples will further enhance our understanding of biological pathways responsible for etiology and clinical manifestation of CAVS

MeSH/Keywords: aortic valve stenosis, aortic valve insufficiency, Hedgehog proteins, aortic valve

Poster code: T-02-10-025

INTERDEPENDENCE OF INTRACRANIAL AND LUMBAR CEREBROSPINAL FLUID PRESSURE IN PATIENTS WITH RUPTURED INTRACRANIAL ANEURYSMS

PhD candidate: Ivan Pašalić

Part of the thesis: Interdependence of intracranial 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: Intracranial space consists of brain parenchyma (87%), cerebrospinal fluid (CSF) (9%) and blood (4%). According to the classic hypothesis, CSF is produced in the choroid plexus (localized within the brain ventricular system) and resorbed at the dural venous system sites. It has been widely accepted that CSF circulates from the both lateral ventricles, across the third and the fourth ventricle, towards the Magendie and Luschka foramina and the cerebellomedullary cistern, before finally ending in the basal cisterns and the subarachnoidal space. However, many modern studies have shown completely different mechanisms of CSF physiology and hydrodynamics. According to the new hypothesis of CSF circulation, production and resorption of CSF takes places alongside the whole ventricular system, while the direction of circulation depends on the arterial pressure. Hypertensive hydrocephalus is one of the most common complications of spontaneous subarachnoid hemorrhage, occuring in 20% of all patients with ruptured intracranial aneurysms.

Hypothesis: Higher craniospinal CSF pressure gradient in patients with ruptured intracranial aneurysms is associated with a higher chance of developing hypertensive hydrocephalus and a consequentially higher necessity for a ventriculoperitoneal shunt (VP shunt) placement.

Aims: To evaluate the clinical significance of intracranial and intraspinal CSF pressures as well as craniospinal CSF pressure gradient in patients with a ruptured intracranial aneurysm. To determine whether CSF pressure gradient has an impact on outcomes (development of hypertensive hydrocephalus and overall mortality) in the aforementioned group of patients.

Materials and methods: In a 2-year period, ten adult patients, treated at the Department of neurosurgery (University hospital center Zagreb) for a spontaneous subarachnoid hemorrhage due to a ruptured intracranial aneurysm, will be included. Diagnostic procedure for all patients will consist of patient history, neurological status, assessment by Hunt-Hess scale and an appropriate radiological evaluation (brain MSCT, brain MSCT angiography and/or DSA). All patients will undergo neurosurgical treatment, which according to the modern practice includes clipping of the aneurysm and both external ventricular and external lumbar drainage. Following the surgery, all patients will be monitored in the Neurosurgical Intensive Care Unit. Besides all the standard hemodynamic monitoring, during a four-day period intraventricular and intraspinal pressures will be continuously measured in two different patient positions: lying horizontally and in a head tilted position (at a head-spine angle of thirty deegrees).

Expected scientific contribution: Validation of the new CSF circulation hypothesis in a „real-life" scenario. Potential detection of a new outcome parameter (craniospinal CSF gradient) in patients with subarachnoid hemorrhage due to a ruptured intracranial aneurysm.

MeSH/Keywords: cerebrospinal fluid, craniospinal pressure gradient, hypertensive hydrocephalus, subarachnoid hemorrhage, ruptured intracranial aneurysms.

Poster code: T-02-10-035

INFLUENCE OF TOTAL VOLUME OF CEREBROSPINAL FLUID REDUCTION AND INTRACRANIAL PRESSURE CHANGES ON CLINICAL STATUS OF PATIENTS WITH IDIOPATHIC NORMOTENSIVE HYDROCEPHALUS

PhD candidate: Klara Brgić

Part of the thesis: Influence of total volume of cerebrospinal fluid reduction and intracranial pressure changes on 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 urinary incontinence, dementia, and gait disturbance and is characterized by an increased amount of cerebrospinal fluid intracranially, without reduction of the subarachnoid space. 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.

Hypothesis: In patients with idiopathic normotensive hydrocephalus, significant clinical improvement is achieved after lumbar drainage and evacuation of cerebrospinal fluid, dominantly changing the spinal volume of cerebrospinal fluid, thus affecting intracranial pressure.

Aims: The aim of this research is to examine how changes in the volume of cerebrospinal fluid in patients with idiopathic normotensive hydrocephalus, dominantly in spinal fluid space, change intracranial hydrodynamics and intracranial pressure, thus leading to improvement of the clinical condition of the patient. SPECIFIC AIMS: 1. To examine the impact of lumbar/intracranial pressure changes during 72 h measurement period on the clinical status of the patient.; 2. To examine the correlation of quantitative changes in CSF biomarkers, sampled after external lumbar drain (ELD) placement and than after 36 and 72 h, with changes in patient´s clinical status.

Materials and methods: Patients with clinical and neuroradiological signs of idiopathic normotensive hydrocephalus will be subjected to testing by evacuation of CSF through external lumbar drainage over a 72h period, during which the values of intracranial pressure, corresponding to lumbar pressure in lying position, will be monitored. Prior to external lumbar drainage placement, the subjects will be evaluated (MMSE, Japanease NPH scale) to determine the severity of the clinical symptoms. Each patient will be thoroughly informed about the procedure. They'll have to sign informed consent prior to the procedure and to complete a standard MRI questionnaire. MRI volumetric analysis of CSF spaces of the neuroaxis using standard high-resolution sagittal T1 sequences for the head (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. Additionally, biomarkers from CSF (amyloid, total and phosphorylated tau proteins...) will be quantified in three time points (0, 36 and 72 h) and their relation to change in clinical status will be analyzed.

Expected scientific contribution: According to our hypothesis we expect that there will be no significant change in the volume of cerebrospinal fluid in the cranial region, and that dominant changes will be in the spinal part, suggesting that spinal fluid space is the dominant compensatory space and intracranial pressure depends on the intraspinal volume. This result would be in contrast to the classic hypothesis of secretion, distribution and absorption of CSF, and it supports the new hypothesis that hydrostatic-osmotic pressures between blood, interstitial compartment and CSF regulate CSF volume and ICP. The purpose of this study is better understanding of the pathophysiology of idiopathic normotensive hydrocephalus.

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

Poster code: T-02-10-062

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 Šimić, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) are most frequently prescribed drugs for treatment of hypercholesterolemia and can cause adverse drug reactions (ADRs) like myopathy, rhabdomyolysis and hepatotoxicity. Interactions of rosuvastatin with many different drugs are documented. Rosuvastatin does not undergo extensive biotransformation by P450 (CYP) enzymes, thus pharmacokinetic variability and significant drug interactions depend on variable drug transport by membrane transporter proteins ABC and SLC. Reduced activity of OATP1B1 encoded by SLCO1B1 gene diminishes the intake of rosuvastatin in the liver while reduced activity of ABCG2 facilitates drug intake via enterocyte membranes but makes it difficult to transfer / eliminate drug on the liver-bile barrier.

Hypothesis: In the development of ADRs of rosuvastatin the genetic predisposition of an individual regarding polymorphisms of transport proteins ABCG2, ABCB1 and SLCO1B1 plays an important role in addition to clinical parameters and drug-drug interactions.

Aims: - to assess the role of polymorphic genes ABCG2, ABCB1 and SLCO1B1 as possible predictors of the ADRs of rosuvastatin; -to investigate the incidence of variant allele ABCG2 421C> A, SLCO1B1 521T> C, SLCO1B1 388A> G and ABCB1 3435C> T among patients with and without rosuvastatin induced toxicity; - to evaluate the role of clinical parameters (age, gender, liver function and kidney function, comorbidity and concomitant therapy) in development of ADRs: to assess the importance of interactions of rosuvastatin with other drugs in concomitant therapy; - to evaluate drug-drug-gene interactions

Materials and methods: The study will include 80 patients who have experienced ADRs of rosuvastin and 120 controls without side effects. Written informed consent of patients is required before introduction into the study. The examination will last 2 years. The ADRs will be considered according to Karch - Lasagna criteria. Genomic DNA will be extracted from 3 ml of full blood containing EDTA isolation. Genotyping will be 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).

Expected scientific contribution: The proposed research will contribute to the knowledge of the role of pharmacogenetic predisposition or polymorphic genes ABCG2, ABCB1 and SLCO1B1 in the emergence of undesirable effects of rosuvastatin. Significant scientific contribution will be a cognition of the modifying role of polymorphisms investigated on the interaction of rosuvastatin with drugs in concomitant therapy (drug-drug interactions) resulting in adverse effects. The results could ce used to create rosuvastatin dosing algorithms.

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

Poster code: T-02-13-027

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. Recommendations and guidelines help physicians make ethically challenging decisions regarding end-of-life patients. There are no official guidelines in Croatia. There were no national or international studies conducted in Croatia concerning the experiences and attitudes of medical professionals on end-of-life decisions.

Hypothesis: Medical professionals working in intensive care units in the Republic of Croatia have similar professional and ethical attitudes on treatment of end-of-life patients.

Aims: The aim of this research is to examine professional and ethical attitudes of medical professionals working in intensive care units in the Republic of Croatia on treatment of end-of-life patients.

Materials and methods: A cross-sectional study involving physicians and nurses will be conducted in intensive care units of several university and general hospitals using a questionnaire constructed by Grosek et al. The first part of the research will involve the standardisation of the questionnaire for Croatian population. 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, including withholding and withdrawing of therapy, and instigating ždo-not-resuscitate' orders. It is comprised of four parts including open and closed type questions, and is anonymous. After the standardisation and validation are completed, the questionnaire will be used in the second part of the study. The questionnaire will be handed out personally by the researcher to all medical professionals working in adult intensive care units pertaining to departments of anaesthesiology, internal medicine and neurology. All nurses and specialist doctors working in the ICU will be included, as all doctors working in other departments, but who perform overnight shifts in the aforementioned ICUs. All other medical professionals who work in the ICU or are there on educational basis will be excluded. All of the collected data will be analysed regarding the type of ICU, workplace, level of education, physicians' specialisation, age, sex, and years of work. The data will be analysed using licensed statistical tools.

Expected scientific contribution: In this research the experiences and attitudes of medical professionals on treatment of end-of-life patients in ICUs in the Republic of Croatia (a model for countries in transition) will be examined. A research model which could contribute to evaluation of end-of-life decisions in countries in transition will be evaluated and confirmed. This research could also contribute to the amelioration of medical care quality, and to the making of appropriate guidelines.

Acknowledgments: I would like to thank my mentors for their guidance and patience, and my family for their support.

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

Poster code: T-02-15-084

DONOR LAMELLA THICKNESS INFLUENCE ON VISUAL ACUITY AFTER DESCEMET'S STRIPPING AUTOMATED ENDOTHELIAL KERATOPLASTY (DSAEK)

PhD candidate: Ana Meter

Part of the thesis: Donor lamella thickness influence on visual acuity after Descemet's stripping automated endothelial keratoplasty

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

Affiliation: University of Zagreb School of Medicine

Introduction: Corneal transplantation is a procedure by which a patient's blurred cornea is replaced by a clear donor cornea. With the development of corneal transplantation surgery it is possible to transplant the inner or outer corneal layer. Descemet's stripping automated endothelial keratoplasty (DSAEK) is a procedure where is transplanted the inner corneal layer which consist of endothelium, Descemet's membrane and thin layer of stroma.

Hypothesis: Thinner lamellas in Descemet's stripping automated endothelial keratoplasty (DSAEK) results with better best corrected visual acuity.

Aims: The aim of the study is to prove that the lower thickness of donor lamella in the Descemet's stripping automated endothelial keratoplasty results in better visual acuity.

Materials and methods: Study is going to be conducted on 50 patients with corneal disease. On all patients Descemet's stripping automated endothelial keratoplasty (DSAEK) will be performed. Data on gender, age, diagnosis, visual acuity, obtained by a routine complete ophthalmological examination of patients before and after surgery, and corneal thickness and lamella measured with the front OCT (Ocular coherence tomography) will be collected.

Expected scientific contribution: We expect that our research will confirm that thinner lamellas in DSAEK corneal transplantations will result in better visual acuity and that in the preparation of donor corneas we should try to prepare lamellas as thinnest as possible.

MeSH/Keywords: Descemet's stripping automated endothelial keratoplasty, lamella thickness, visual acuity

Poster code: T-02-18-024

THE RELATION BETWEEN INTRACRANIAL AND INTRAOCULAR PRESSURE IN PATIENTS WITH ACUTE TRAUMATIC AND NON TRAUMATIC INTRACRANIAL INJURIES

PhD candidate: Maja Bakula

Part of the thesis: The relation between intracranial and intraocular pressure in patients with acute traumatic and non traumatic intracranial injuries

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

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

Introduction: There are contradictory data on intracranial (ICP) and intraocular pressure (IOP) correlation in the literature. Since invasive methods of intracranial pressure measurement carry a number of possible complications, there is an interest for a surrogate non-invasive method of monitoring increased intracranial pressure.

Hypothesis: There is no direct correlation between acute changes in intracranial pressure and intraocular pressure.

Aims: Aim of our study is to evaluate if measuring of intraocular pressure can predict acute changes of intracranial pressure.

Materials and methods: The study will be conducted on 29 patients admitted to intensive care units of our hospital due to traumatic or non traumatic brain injury that require continous intracranial pressure monitoring. The intracranial pressure will be measured with external ventricular drain (EVD) and intraocular pressure will be measured with handheld tonometer. IOP and ICP will be measured upon EVD placement and in to consecutive days. Exclusion criteria will be: politraumatized patients, patients with orbital or ocular bulb injury, patients with previous history of glaucoma and patients with corneal disease. All data will be analyzed with adequate statistical methods and correlation between ICP and IOP will be evaluated.

Expected scientific contribution: We believe that our research will provide new perspective about the relationship of acute elevation of intracranial pressure and its impact to intraocular pressure.

MeSH/Keywords: tonometry, intraocular pressure, intracranial pressure, intracranial injury

Poster code: T-02-18-036

EARLY STRUCTURAL AND FUNCTIONAL EYE CHANGES IN PATIENTS WITH DYSTHYROID ORBITOPATHY AND ELEVATED INTRAOCULAR PRESSURE

PhD candidate: Petra Kristina Ivkić

Part of the thesis: Early structural and functional eye changes in patients with dysthyroid orbitopathy and elevated intraocular pressure

Mentor(s): Jelena Juri Mandić, PhD, research associate

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

Introduction: Dysthyroid orbitopathy or Graves orbitopathy (GO) is an organ-specific autoimmune orbital disease associated with autoimmune thyroid disease, in which IgG antibodies bind to TSH receptors causing inflammation of the extraocular muscles (EOM) and infiltration of interstitial tissue, orbital fat tissue and lacrimal gland with inflammatory cells associated with glycosaminoglycan accumulation and retention of fluid. Due to these changes, volumetric and contraction changes occur to the EOM. In the vast majority of patients with GO regardless the stage of the disease, we found elevated intraocular pressure (IOP) values. Ethiopathogenesis of the secondary elevated IOP in GO is not fully clarified. It is generally accepted thesis that increasing the volume of orbital tissue disables normal venous drainage, causing additional congestion of orbital tissue. The elevated IOP at various stages of the newly detected GO can lead to specific changes in the structure of the optical nerve fibers, retina and macula which is different from the damage found in patients with primary elevated IOP.

Hypothesis: Secondary increased intraocular pressure in patients with dysthyroid orbitopathy leads to specific reversible changes of the nerve fibers, retina and macula.

Aims: GENERAL OBJECTIVE: To determinate the specific structural and functional changes of the eye that occur in patients with GO. SPECIFIC OBJECTIVES: 1) To determinate changes in the nerve fibers of the retina, optic nerve, and in the ganglion cell macular complex in patients with a newly discovered GO in different stages of the disease. 2) To determinate is there any influence of elevated IOP on structural changes in retinal nerve fibers and the ganglion cell complex in patients with GO. 3) To determinate the correlation between perimetry and optical coherence tomography in patients with GO and elevated IOP. 4) To determinate is there any reversible structural changes of retinal nerve fibers and ganglion cell complex in patients with GO on two-year follow-up period.

Materials and methods: We will divide respondents into two groups of patients with newly diagnosed GO and different clinical stage of the disease, and one control group of respondents. First group consists of patients with GO and IOP up to 22 mmHg. Second group consists of patients with GO and elevated IOP above 22 mmHg. Third control group consists of patients with glaucoma, without GO, comparable age and sex distribution. The study will not include participants with ophthalmic comorbidities that are not a consequence of the underlying disease which affects on biomechanical changes in eye structures: high myopia (> -5 dpt), high hypermetropia (> +3 dpt), optical disk disease, macular disease, vascular and degenerative retinal disease, blur of optical media, amblyopia, other orbital diseases and patients with neurological disorders who have pathologically changed perimeter findings due to underlying disease, and respondents with clinical activity score from 8 to 10. Optical coherence tomography and recording of the optical nerve fibers, retinal and ganglion cell complex with Octopus field 900 in G2 software with trend analysis will be performed in the period of 2 years initially and every 6 months or, depending of clinical indication, even more frequently.

Expected scientific contribution: Discovering and analysis of specific structural changes in the nerve fibers of the retina, optical nerve and ganglion cell complex will lead to a better understanding of the secondary elevated IOP entity in patients with newly diagnosed GO in different stages of disease and right selection of appropriate treatment modalities for earlier and better health and social rehabilitation.

Acknowledgments: Thanks to my menthor, Jelena Juri Mandić MD PHD, on the infinitely patience and support.

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

Poster code: T-02-18-054

PALPEBRAL FISSURE WIDTH AND TEAR FILM DYSFUNCTION

PhD candidate: Ana Čović
Part of the thesis: Palpebral fissure width and tear film dysfunction
Mentor(s): Assist. Prof. Igor Petriček, MD PhD
Affiliation: University of Zagreb School of Medicine

Introduction: One of the main functions of tear film are its stability and prevention of ocular surface desiccation. The core mechanisms of dry eye are driven by tear hyperosmolarity and tear film instability. One of the causes of tear hyperosmolarity is increased tear evaporation which is directly connected to ocular surface exposed to external conditions and desiccation. Palpebral fissure width is the factor that correlates positively with exposed ocular surface and is well investigated among patients with endocrine exophthalmos. However, the relationship between palpebral fissure height in primary gaze and dry eyes among healthy individuals according to age and sex groups has not been published in any peer-rewieved journal. It is known that increased palpebral fissure width correlates positively with increased tear film evaporation. Since the palpebral fissure width in primary position varies in healthy population, it can be perceived as a risk factor for developing dry eye symptoms and signs.

Hypothesis: Palpebral fissure width in primary eye position is a factor that influences ocular surface exposure to external conditions, affects tear film stability and causes increased tear evaporation. Patients with symptoms of tear film dysfunction will have wider palpebral aperture measured at primary eye position compared to patients without symptoms of tear film dysfunction in the same age and sex group.

Aims: To determine the impact of palpebral fissure height in primary eye position on tear film dysfunction among patients with and without dry eye symptoms, in the same age and sex group.

Materials and methods: Study will include 200 adult patients, divided into two groups-patients with dry eye symptoms and patients without dry eye symptoms. Enrolled in this study will be every adult patient attending the standard ophthalmic examination, who will, after being introduced to the aim of the research, sign the informed consent and give his/her written consent. Patients with ocular trauma, acute inflammation of the eye and other ocular surface diseases, reconstructive and cosmetic eyelid operations, as well as patients who are unable to cooperate would not be included. First a standardised Schein questionnaire will be used in order to determine the severity of dry eye symptoms. Palpebral fissure height will be mesured in all patients in primary position of gaze with a clear plastic ruler held as close to the eye as possible. Central vertical distance between the upper and lower lid margin will be measured. After that, other tests will be preformed that are routinely used for dry eye diagnostics (conjunctival hyperemia, LIPCOF, NIBUT, TBUT).

Expected scientific contribution: To determine at the theoretical level the impact of palpebral fissure width on tear film dysfunction in the same age and sex groups. At the practical level, this research could determine the possibility of differentiation between hyperevaporative and hyposecretory dry eye based on dry eye symptoms and measurement of palpebral fissure height in primary eye position during standard slit lamp examination.

MeSH/Keywords: Palpebral fissure width, Dry eye syndrome, Tear film stability

Poster code: T-02-18-098

VISUAL OUTCOME AFTER IMPLANTATION OF NEW-GENERATION MULTIFOCAL INTRAOCULAR LENSES

PhD candidate: Mateja Končarević

Part of the thesis: Visual outcome after implantation of new-generation multifocal intraocular lenses

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

Affiliation: University of Zagreb School of Medicine

Introduction: Over the past few decades, cataract surgery has greatly improved, primarily because of progress in the surgical procedure itself, but also because of the advancement in the technology of intraocular lenses. With progress improvement of intraocular lens design and the progress of surgical methods, the rate of cataract surgery is increasing. Nowadays, active lifestyle and need for good vision at all working distances leads to an increase in number of patients approaching the surgery in the initial stages of cataract, also in order to address their refractive error. First generations of multifocal lenses, along with providing good vision at all working distances, also exhibited some of negative side-effects, such as decreased contrast sensitivity, visual impairment in poor light (scotopic) conditions, and a impaired vision at intermediate (intermediate) distances. New generation of multifocal intraocular lenses, whose effectiveness will be tested in this study should significantly reduce or even completely eliminate the disadvantages of previous generations of multifocal intraocular lenses. Unlike multifocal lenses, standard monofocal intraocular lenses cannot enable good vision at all distances, but no side-effects are observed as in first generation of multifocal intraocular lenses.

Hypothesis: Implantation of new generation multifocal intraocular lenses is an effective surgical method of correcting refractive error at all distances without significant change in quality of vision compared to monofocal intraocular lenses.

Aims: Try to prove that the implantation of the new generation multifocal intraocular lens provides a high level of visual acuity at all working distances, spectacle independence, negative phenomena and high level of patient satisfaction.

Materials and methods: In the prospective randomized trial, four groups of 50 patients are bilaterally embedded: a) one of the three types of new generation multifocal intraocular lenses (trifocal intraocular lens, intraocular lens with elongated focal point and diffractive intraocular lens with minimal, low addition) and b) monofocal intraocular aspherical lenses (control group). Patients selected for participating in the study will be over 45 years of age, objectively measured hypermetropic distance correction (range +0.50 to +5.00 spherical diopter, +0.25 to +0.75 cylinder diopter) and correction for near vision (minimum +1.00 diopters). Follow up is planned for a period of 2 years after cataract surgery. For objective analysis of visual outcome, uncorrected and best corrected visual acuity for all distances, contrast sensitivity, defocus curve, rate of opacity of posterior capsule, visual disturbance levels under reduced (scotopic) light condition and the subjective level of patiet satisfaction with the result of the surgery will be measured. Results of multifocal intraocular lens groups will be compared with each other and with the results of control group of patients with imlpanted monofocal lenses. Data processing will use STATISTICA 7.1 application program, the classic descriptive statistics for determining the arithmetic mean, range, and standard deviation of the results. In comparative scales, Student tests will be applied. If the distribution of results is not normal, nonparametric tests will be used for comparison.Statistical significance will be evaluated at the level p 0.05, with the 95% boundary of the correspondence.

Expected scientific contribution: In recent literature, the studies are describing efficacy of new generation of multifocal intraocular lenses, but there is no study comparing optical quality of vision between the three major groups of the new generation intraocular lenses mutually and compared with monofocal lenses. The expected scientific contribution of the study is to determine which of the currently available new generation lenses provides the most optimal quantitative and qualitative visibility comparable to the quality of monofocal lens vision.

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

Poster code: T-02-18-136

CORRELATION OF HORMONAL RECEPTOR EXPRESSION AND CLINICAL PARAMETERS IN EXTRAUTERINE LEIOMYOSARCOMA AND LIPOSARCOMA

PhD candidate: Milena Peitl

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

Mentor(s): Assoc. Prof. Fedor Šantek, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Estrogen alpha and beta receptors (ER1α and ER1β), 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 extrauterine leiomyosarcomas (eLMS) and liposarcomas (LS) may prove as fruitful as previous sarcoma (uLMS) research.

Hypothesis: Extrauterine leiomyosarcomas (eLMS) and liposarcomas (LS) may express estrogen alpha and beta receptors (ER1α and ER1β), progesterone (PR) and androgen receptors (AR).

Aims: General goal of this research is to identify expression patterns of hormonal receptors (ER1α, ER1β, PR and AR) in eLMS and LS, while correlating them with specific clinical parameters. Specific goals are: 1) to investigate and determine the frequency of expression of ER1α, ER1β, PR and AR in eLMS and LS; 2) to determine if a difference exists between ER1á, ER1â, PR and AR in eLMS and LS, depending on specific clinical parameters (age, gender, anatomical localisation, tumour size, presence of metastases at the time of diagnosis); 3) to investigate coexpression patterns of investigated receptors.

Materials and methods: This research will be performed on 20 pathohistologicaly verified eLMS and 20 verified LS specimens in the form of adequately prepared paraffin and/or formalin tissue cubes. In total, this research will include 40 conjoined eLMS and LS specimens. Tissue specimens will be 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 2017. Tissue material will be subjected to immunohistochemical validation using targeted monoclonal antibodies for ER1α, ER1β, PR and AR, according to manufacturer instructions. Cut off values for expression of ER1α, ER1β, PR and AR in eLMS and LS will be 1% and 10% of positively coloured cells. ER/PR positive tumour breast tissue will be used as a positive control for ER 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 eLMS and 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 will be gathered from the existing archives.

Expected scientific contribution: Expected scientific contribution is to determine the suspected existence of certain subtypes of eLMS and LS, according to their hormonal status, which may lead to development of targeted and more efficacious hormonal treatment.

MeSH/Keywords: Leiomyosarcoma; Liposarcoma; Estrogen, Receptor alfa; Estrogen, Receptor beta; Receptors, Progesteron; Receptors, Androgen

Poster code: T-02-19-063

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 subtype of 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 and progesterone receptors and HER2 receptors. Because of that, TNBC is not susceptible to endocrine or antiHER2 therapy. Basically, to date TNBC stayed an „orphan" cancer, with neither any usable biomarkers with predictive and prognostic value, nor any specific therapeutic target. The basis of therapeutic approach nowadays is still classic chemotherapy. Previous research has 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 influence on the behavior of this type of tumor.

Hypothesis: Patients with early TNBC and the presence of TIL and AR have a longer overall survival (OS), in comparison to patients with early TNBC which do not have present TIL or AR.

Aims: Main goal of this study is to investigate the prognostic value of the presence of TIL and AR in patients with early TNBC. As specific goals we will investigate the prevalence of TIL, prevalence of AR and prevalence of simultaneously present TIL and AR in this population, TIL and AR relationship to disease free survival (DFS), overall response (OR), and overall survival (OS) five years after the diagnosis. We will investigate the specific prognostic value of TIL and AR, independent of other clinical, sociodemographic, patohistological and therapeutic variables.

Materials and methods: This study will be conducted as a retrospective cohort study in the general population of early TNBC patients, who were diagnosed and treated at the University Hospital for Tumors in the period from January 1, 2009 till December 31, 2012. A consecutive sample was selected, and the inclusion criteria were age (35-75 years), gender (female) and PHD (early TNBC). The required sample size was calculated, predictors (independent variables - TIL and AR) and confounding variables were determined. Primary (OS) and secondary outcomes (DFS, OR) are defined. A retrospective analysis of the medical records of the patients will be performed, and a database will be created, that will contain clinical, sociodemographic, pathohistological and therapeutic parameters for each patient. On standard FFPE pathohistological tissue samples, where a negative reaction to estrogen and progesterone receptors and HER2 was previously determined, the presence of TIL and AR will be analyzed. The TIL will be analyzed according to the given recommendation of International Working Group for the Evaluation of TIL on H - E samples, and the AR by method of immunohistochemistry. The research has been approved by the Ethics Committee of the Sestre milosrdnice University Hospital Center, and will be conducted according to all the Helsinki Declaration rules. Patients' identity will be completely protected and known only to the investigator, during the first data entry in the database, after which each patient will be assigned a password. Collected data will be processed statistically, using the method of multivariate binary logistic regression, Cox regression, and other necessary statistical tests.

Expected scientific contribution: Determination of prognostic and predictive values of TIL and AR has a high clinical value. These are easily measurable and detectable biomarkers, which are analyzed by inexpensive diagnostic methods. TIL and AR are the first biomarkers in an otherwise „orphan" tumor. If their role in predicting response to therapy and survival is confirmed, they can direct therapeutic intensity and access, and may potentially serve as new therapeutic goals for the use of immunotherapy, endocrine therapy, and other therapeutic modalities.

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

Poster code: T-02-19-064

EPIGENETIC MODIFICATIONS OF IL17 GENE IN EARLY INVASIVE BREAST CANCER

PhD candidate: Ljubica Radmilović Varga
Part of the thesis: Epigenetic modifications of IL17 gene in early invasive breast cancer
Mentor(s): Assist. Prof. Natalija Dedić Plavetić, MD PhD
Affiliation: University of Zagreb School of Medicine

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

Hypothesis: The DNA methylation degree measured by pyrosequencing as an indicator for epigenetic modification of the IL17 gene is directly correlated with serum IL-17 concentration in patients with early invasive breast cancer.

Aims: The aim of this study is to investigate the correlations between the methylation IL17 gene levels and IL-17 protein serum concentration in patients with early invasive breast cancer. We will determine and compare the IL17 gene methylation degree with the IL-17 concentration in the serum of women with an early invasive breast cancer (different surrogate biological subtypes) and in the serum of healthy women. Also, we will determine the correlation of the degree of methylation DNA (IL17) isolated from blood of women with early breast cancer with the expression of IL-17 in tumor tissue and with other clinical and pathohistological characteristics. The correlation of the degree of methylation DNA (IL17) isolated from blood of the women with an early breast cancer and two-year disease-free survival will be analyzed.

Materials and methods: Breast cancer paraffin-embedded tissue specimens and blood samples will be obtained from a series of 150 female patients with primary operable invasive breast carcinomas referred to the University Hospital Center Zagreb, between January 2012 and January 2017. As a control group, we will analyze serum samples from 30 healthy women volunteers. The DNA IL17 methylation degree will be measured by pyrosequencing. IL-17 serum levels will be measured by the enzyme-linked immunoadsorbent assay (ELISA) and the IL-17 tissue expression will be determined immunohistochemically. The tumor size, histological type, histological and nuclear grade, estrogen and progesterone receptor status, the involvement of axillary lymph nodes, HER-2 status, and lymphovascular invasion will be obtained for all patients. All of these patients are followed-up prospectively for two years according to routine standard institutional practice with local recurrence, distant metastases or death as primary outcome end-points. According to this information, the data on two-year disease-free survival will be collected.

Expected scientific contribution: The study will provide information on differences in epigenetic modification in women with early invasive breast cancer compared to healthy female population, while contributing to a better understanding of the biological behavior of individual breast cancer subtypes and to clarifying the role of epigenetic changes in IL17 genes and expression of prominent cytokine IL- 17 that can play an important role in breast cancer etiopathogenesis but also affect the clinical course and disease outcome.

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

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

Poster code: T-02-19-066

LOCAL APPLICATION OF RECOMBINANT HUMAN BONE MORPHOGENETIC PROTEIN 6 TO OSTEOPOROTIC RATS PRETREATED SYSTEMICALLY WITH BISPHOSPHONATES

PhD candidate: Petra Jurina

Part of the thesis: Local application of recombinant human bone morphogenetic protein 6 to osteoporotic rats pretreated systemically with bisphosphonates

Mentor(s): Academician Slobodan Vukičević

Affiliation: Laboratory for Mineralized Tissues, Center for Translational and Clinical Research, University of Zagreb School of Medicine

Introduction: Atypical femoral fractures are rare fractures associated with long-term bisphosphonate therapy in osteoporosis patients. They appear most often bilaterally, with high nonunion rate and development of pseudoarthrosis, despite the most advanced osteosynthesis methods, and represent significant disability for the patient. Studies show that synergy of anabolic influence of bone morphogenetic proteins and antiresorptive of bisphosphonates achieves better and faster bone healing with the formation of firmer callus.

Hypothesis: Local application of rhBMP6 at the site of intramedullary fixation of femoral fracture in osteoporotic rat model previously exposed to the systemic administration of bisphosphonates accelerates bone healing and increases bone strength of the callus due to neutralisation of bone resorption mechanisms.

Aims: General aim of research is to show the effect of systemic administration of bisphosphonates and local administration of rhBMP6 to the healing site of the femoral osteotomy in the osteoporotic rat mode. Specific aims of research are to determine the optimal dose and duration of the previous bisphosphonate exposure for further effective local administration of rhBMP6 in the rat autologous blood clot, to determine the optimal dose of rhBMP6 that is required for safe and rapid healing of the bone defect in osteoporotic rat, to monitor the quality of healing fracture and to analyse the quality of the resulting callus in the osteoporotic rat.

Materials and methods: Experimental research will be conducted using animal model of Sprague-Dawley rats, 4 months of age, weighing 250-300g. Rats wil be ovariectomized (OVX), in order to achieve osteoporotic model due to estrogen deficiency. Total number of 100 rats will be used. The study was approved by the Ethics Committee of the Medical School in Zagreb. Research will be divided into experiment A and B. In experiment A the most effective dose and exposure time to alendronate that results in antiresorptive effect will be determined. 6 weeks after ovariectomy, rats will be exposed to alendronate in 0,5 mg/kg, 1 mg/kg and 2 mg/kg dose orally through gastric tube, once a week, through 6 weeks. After 6 weeks, rats will be sacrificed. 40 rats in experiment A will be divided into following groups (N=8 per group): A (sham OVX), B (OVX, without alendronate), C (OVX, 0,5 mg/kg alendronate, D (OVX, 1 mg/kg alendronate), E (OVX, alendronate 2 mg/kg). Bone mineral density (BMD) and bone mineral content (BMC) will be determinated and compared in three measures, before exposure to alendronate, 3 weeks after exposure and before sacrifice. In experiment B the optimal dose of rhBMP6 that is needed for safe and accelerated healing of the femoral fracture previously exposed to alendronate will be determinated. 6 weeks after ovariectomy, groups from B to E will be exposed to alendronate in dose and time according to results of experiment A. After exposure to alendronate, all groups will undergo femoral diaphysial osteotomy, intramedullar fixation (ORIF) and local application of autologous blood coagulum with or without rhBMP6. 60 rats in experiment B will be divided into following groups (N=12 per group): A (ORIF), B (ORIF, alendronate), C (ORIF, alendronate, rhBMP6 10µl), D (ORIF, alendronate, rhBMP6 20 µl), E (ORIF, alendronate, rhBMP6 45µl). After 7 weeks all rats will be sacrificed. Bone healing and quality of the callus will be evaluated with bone markers, radiographs, microCT, biomechanical testing, histology and histomorphometry. On all gathered data statistical analyses will be performed.

Expected scientific contribution: Expected contribution of this research is a better understanding of bone healing processes in the treatment of atypical femoral fractures on a rat model by local application of rhBMP6 to the site of the femoral osteotomy that could result in a faster and more efficient bone healing due to neutralising effect of bisphosphonates on the catabolism of the BMP.

MeSH/Keywords: BMP-6, bisphosphonates, atypical femoral fractures, osteoporosis

Poster code: T-02-20-043

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 biochemical changes in patients with trochanteric region fracture fixation with DHS versus PFN

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 can trigger systemic inflammatory reaction syndrome and are related to patient outcome.

Hypothesis: Inflammatory, soft tissue damage and fibrinolytic markers will significantly increase after both procedures. DHS and PFN will show different levels of invasivity in different variables.

Aims: The aim of the study is to compare the perioperative changes of IL-6, CRP, ESR, Procalcitonin, CK, and D-Dimer between two defined groups.

Materials and methods: This study is designed as prospective, cohort study which will be undertaken in University Clinical Center of Kosovo. 50 patients (25 for each group) with trochanteric region fractures AO/OTA 31.A1–31.A2 treated with DHS or PFN based on AO principles will be included. After the approval of ethics board committee, patients will be informed about the study and their consent will be obtained. The blood samples will be taken from the patients prior to surgery and 1 hour, 24 hours, day 2, day 3 after surgery. The analyses will be performed in a licensed laboratory in Kosovo with validated methods but some of the blood markers will be analyzed in a licensed laboratory in Zagreb. For statistical analysis will be used Student's t-test, chi-square test, and multivariate logistic regression. The significance level will be p-value <0.05.

Expected scientific contribution: Almost all the studies performed until now that measured the levels of markers described above showed that these factors increase after surgery with osteosynthesis in proximal femoral fractures. But some of these studies that evaluated these Inflammatory, soft tissue damage and fibrinolytic markers changes after treatment with DHS and PFN, separately or together, show contradictory results whether which triggers more them. This will be the first study to compare DHS and PFN "head to head", with multiple Inflammatory, soft tissue damage and fibrinolytic markers in same time. We believe that the results will help scientific community and ultimately practicing surgeons to choose the best treatment option for their patients.

MeSH/Keywords: Hip fracture, IL-6, ESR, CRP, d-dimer, CK, body temperature, procalcitonin, femoral nailing, DHS, comparative study

Poster code: T-02-20-103

PROGNOSTIC SIGNIFICANCE OF TISSUE PROTEOMIC PROFILING IN PRIMARY SQUAMOUS CELL OROPHARYNGEAL CARCINOMA

PhD candidate: Boris Ivkić

Part of the thesis: Prognostic significance of tissue proteomic profiling in primary squamous cell oropharyngeal carcinoma

Mentor(s): Professor Vladimir Bedeković, MD PhD, Assoc. Prof. Lovorka Grgurević, MD PhD

Affiliation: 1. Department of otorhinolaryngology and head and neck surgery, Clinical Hospital Centre „Sestre milosrdnice", 2. Department of Anatomy, University of Zagreb School of Medicine, Division of Proteomics, Centre for Translational and Clinical Research

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

Hypothesis: Proteomic profile of primary squamous cell oropharyngeal carcinoma correlates with prognostic factors and disease survival rate.

Aims: The main goal would be an idea for studying the correlation between proteomic biomarkers and disease prognosis in patients with oropharyngeal cancer. The aim of this research is to make a proteomic profile of tumorous tissue and see the correlation between disease specific survival rate of these patients. In our research, we will compare the proteomic profiles of tissue samples in patients with primary oropharyngeal squamous cell carcinoma with pathohistological parameters such as size of tumor (T), regional metastasis (N), distant metastasis (M) and pathohistological grade of tumor (G) of stage III and IV of the disease.

Materials and methods: By researching proteomic profiles of 40 tissue samples of patients with primary oropharyngeal squamous cell carcinoma we will follow the expression of proteomic tissue biomarkers. Ten palatinal mucosa tissue samples of patients who were not diagnosed with squamous cell carcinoma will be used as a control sample. Patients will be followed through a period of five years postoperatively, or, till the date of the patients death due to primary diagnosis. After the follow-up period, we will search for the connection between proteomic profile of tumorous tissue and disease specific survival, which will hopefully help us to expand our knowledge in proteomics and biomarkers of oropharyngeal squamous cell carcinomas.

Expected scientific contribution: We expect to show the expression of known tumor biomarkers and hopefully to discover some new potencial proteins which can be of significant importance in prognosis of patients with oropharyngeal carcinoma. Also, we will try to contribute for the better understanding of the protein biomarkers behavior in oropharyngeal squamous cell carcinoma tissue.

Acknowledgments: Thanks to my menthors, Professors Vladimir Bedeković and Lovorka Grgurević, on the infinitely patience and support.

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

Poster code: T-02-21-055

CARTILAGE REGENERATION IN THE SHEEP NASAL SEPTUM

PhD candidate: Ivan Raguž

Part of the thesis: Cartilage Regeneration in the Sheep Nasal Septum

Mentor(s): Assist. Prof. Alan Ivković, MD PhD, Professor Drago Prgomet, MD PhD

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

Introduction: Rhinoplasty frequently includes harvesting of nasal septum cartilage for graft production. Unfortunately, the amount of septal cartilage available for surgery is often inadequate, particularly in secondary rhinoplasty. This research will attempt to determine whether nasal septal cartilage can regenerate after its submucosal resection in the sheep since neocartilage formation has not heretofore been described in this animal model. Neural ridge derivated cells are recognized during their embryonic development due to their multipotency and plasticity as well as their capacity to develop into different types of cells and tissues. These cells are in the focus when researching regenerative tissue abilities because of their partial preservation of these properties and in adulthood. As the nasal septum is derived from the neural ridge, few researches have already been focused on its regenerative potential but their results are inconsistent when taking into consideration maturity of the animal models.

Hypothesis: After submucosal resection of nasal cartilage of the sheep, initial cartilage regeneration can be find in form of cartilaginous matrix in the space created after the surgical procedure, which is located between the corresponding layers of the perichondrium.

Aims: GENERAL OBJECTIVE: To prove the initial formation of a new cartilage in the area of the defect that arose after the resection of piece of quadrangular cartilage from the nasal septum of the sheep. SPECIFIC OBJECTIVES: 1) To investigate the quality of new cartilage through histological, immunohistochemical and biochemical methods. 2) To evaluate the possibility of hypothetical cartilage regeneration with regard to the perichondrium quality. 3) To compare new cartilage with normal cartilage.

Materials and methods: By dorsoventral incision, submucosal resection will be performed on 30 sheep followed by reapproximation of the perichondrium. After 6 weeks or 6 months, the sheep will be sacrificed, septi will be fixed, sectioned, and examined histologically and immunohistochemically. After rinsing in PBS, the histological analysis samples will be fixed in 4% paraformaldehyde or 10% formalin. The tissue will fit into the paraffin and cut with a rotational microtome on the 5 ìm thick cuts. The following staining will be used: hematoxylin eosin, Safranin O / fast green, Goldener's modification Masson, Picrosirius Red. For immunohistochemical staining, antibodies to collagen I, II, agrekan and integrin alpha will be used. 11. Proteoglycan content will be determined by the method of dimethylmethylene blue (DMMB) binding. The lyophilized samples will be decomposed by papain mixed with a DMMB solution and an absorption at 525 nm will be read immediately to the retrophotometer. The obtained values will be compared with standard curves.

Expected scientific contribution: After regeneration of cartilage of the nasal septum on the animal model of an adult rabbit has been proven, such evidence on the animal model of the sheep, would indicate a strong possibility of such regeneration in the human nasal septum. Such regeneration process in the future could be enhanced by the insertion of a cytokine carrier that stimulates ability of septal perichondrium to regenerate and repair the cartilage tissue.

MeSH/Keywords: nasal septum, animal model, cartilage regeneration, rhinoplasty

Poster code: T-02-21-144

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: University Hospital Dubrava, University of Zagreb School of Medicine

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 has been shown to have an independent detrimental prognostic effect in the survival of these patients. The promalignant role of thrombin in the propagation of malignant tumors is still not fully clarified. The immunohistochemical (IHC) presence of thrombin has not yet been investigated in healthy pancreatic tissue or PDAC. PAR-1 (whose agonist is thrombin) is detected in the pancreatic adenocarcinoma. It was not found in the healthy pancreatic epithelium. There is no available data on the relationship between thrombin, PAR, TB/EMT and PDAC.

Hypothesis: Positive IHC expression of thrombin and PAR-1 in PDAC is associated with areas of TB, usually of higher grade, and with elevated N-cadherin IHC expression and negative E-cadherin IHC expression.

Aims: To investigate grade of tumor budding, 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 general data (age and sex of the patient, tumor size, vascular invasion, perineural infiltration, presence of positive regional lymph nodes and number of positive regional lymph nodes) and association of grade of TB with general data.

Materials and methods: Retrospective research (cross-sectional study) at the Department of Pathology and Cytology of University Hospital Dubrava (UHD), which will include archive material of the department and general data of 80 patients operated in UHD due to PDAC in 2007-2017 period. TB grade will be determined on IHC stained (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, from the area of TB at the invasive front, from the peripheral/stromal/mesenchymal part of the tumor and from the surrounding healthy pancreatic tissue) to produce IHC stained slides (thrombin, PAR -1, E- and N-cadherin). The results are analyzed with light microscope.

Expected scientific contribution: TB might have a higher prognostic value than some standard prognostic factors, such as the TNM stage. If the association between thrombin and PAR-1 expression in PDAC tissue could be demonstrated, then these parameters could be additional prognostic factors. Better understanding of EMT, in terms of association with thrombin and PAR, could lead to improved oncologic therapeutic approach (inhibition of PAR signaling and thrombin effect).

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

Poster code: T-02-23-053

SERUM CALPROTECTIN AS AN EARLY BIOMARKER FOR BACTERIAL URINARY TRACT INFECTIONS IN CHILDREN YOUNGER THAN THREE YEARS OF AGE

PhD candidate: Mirta Lamot

Part of the thesis: Serum calprotectin as an early biomarker for bacterial urinary tract infections in children younger than three years of age

Mentor(s): Assoc. Prof. Miroslav Harjaček, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Despite a ubiquitous use of various clinical and laboratory markers such as leukocyte count (WBC), C-reactive protein (CRP) and procalcitonin (PCT), febrile illnesses in children can still cause a major diagnostic challenge for physicians caring for children. This challenge is even more pronounced in era when expanding number of studies show long-lasting effect of increased antibiotic exposure in preschool children on autoimmune phenotype development later in life. The reliable and promptly available biomarker of infections requiring antibiotic could therefore certainly save children from unnecessary antibiotic exposure. Over the last few years a large body of evidence has shown the important role and abundant presence of S100 proteins during inflammation, especially in chronic diseases such are inflammatory bowel disease and juvenile idiopathic arthritis in which measurement of fecal and serum calprotectin (S100A8/S100A9 protein complex), respectively, correlates with disease activity considerably better than clinical and other laboratory findings. More interestingly, a recent study has shown the serum calprotectin is more specific and sensitive in prediction of neonatal sepsis than traditionally employed markers such as WBC and CRP. However, to best of our knowledge no study has investigated the possible role of serum calprotectin in differentiating viral and bacterial cause of febrile illnesses in children younger than three years of age. Although in most cases febrile illnesses in this group are caused by a self-limiting viral infection, it is estimated 5-7% of infants presenting with fever would have a urinary tract infection (UTI). The reference standard for diagnosis of UTI is urine culture which is time consuming and often leads to engagement of antibiotic treatment while pending for results. Therefore, serum calprotectin could have substantial added value in the diagnosis of UTI in febrile children resulting in opting out unnecessary antibiotic treatments.

Hypothesis: Serum calprotectin is a sensitive and specific marker for acute bacterial urinary tract infection in children.

Aims: The primary aim is to correlate concentration of serum calprotectin in febrile children younger than 3 years of age with results of reference standard for UTI. Secondary aims are to correlate serum calprotectin concentration and inflammatory markers (WBC, CRP, PCT), isolated bacteria type, fever duration, outcomes of UTI (i.e. atypical course, urosepsis or vesicoureteral reflux).

Materials and methods: Total of one hundred consecutive patients aged 0-36 months coming to Pediatric Emergency Department of Clinical Hospital Center Sestre milosrdnice and Children's Hospital Zagreb with fever lasting less than 72h and positive urinalysis (i.e. leukocyte esterase and/or nitrates) will be enrolled in the study. Exclusion criteria will be history of chronic inflammatory disease, ongoing use of antibiotics and clinical, radiological or microbiological diagnosis of bacterial infection from another source. Patients will be divided in two groups, cases and controls, depending on the results of urine culture analysis. Serum calprotectin will be measured by a commercial ELISA assay (Buhlmann MRP8/14, Switzerland). Statistical analysis will be performed using the statistical software GraphPad Prism version 7 for Mac OS X (La Jolla California, USA). Using a nominal significance level of á=0.05 and a power test of 80%, if the concentration of serum calprotectin in one group is below 1.5 ìg/mL and an expected increase of the concentration in other group is more than 50%, minimal sample size is 56 patients (i.e. 28 cases and 28 controls).

Expected scientific contribution: This study will show if serum calprotectin concentration significantly differs in febrile children with proven bacterial UTI. These results could aid in avoiding unnecessary diagnostic procedures and therapeutic interventions. Since calprotectin has some distinguished tasks in propagation of local inflammation it could also be an attractive candidate for the therapy of acute inflammatory disease.

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

Poster code: T-02-24-128

RELATIONSHIP OF TROPONIN T AND I IN URINE AND PLASMA IN INFANTS AFTER SURGICAL REPAIR OF VENTRICULAR SEPTAL DEFECT (VSD)

PhD candidate: Matija Bakoš

Part of the thesis: Determination of troponin T and I correlation in urine and plasma in neonates and infants after cardiac surgery

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

Affiliation: University of Zagreb School of Medicine

Introduction: Congenital heart defects are one of the most common causes of death in the perinatal and early neonatal period and are also anomalies that are transmitted as chronic diseases in adulthood. The prevalence of congenital heart disease in Croatia ranges from 7 to 8 in 1000 live births, and among all congenital heart defects, ventricular septal defect (VSD) is the most frequent (range of 25-30%). About 35,000 - 40,000 newborns, or 50-70 children with VSD, are born in Croatia annually. After cardio surgery, cardiac myocytic lesion, and thus the release of cardio selective enzymes, occur. Cardio selective enzymes that are determined in University Hospital Center Zagreb are troponin T and NT-proBNP. These cardiac troponins are highly specific markers for the diagnosis of myocardial damage, and their elevated blood concentration is a sign of cardiac damage. Troponin T is a larger molecule than troponin I, its molecular weight is 43 kDa, while the mass of troponin I molecule is about 22.5 kDa. As the molecules become smaller, there is a greater likelihood of their filtration through the glomerular membrane in the kidneys and the possibility of troponin or part of the troponin molecule being detected in the urine. There are only few studies wich show the presence of troponin in the urine.

Hypothesis: Cardiac surgery in infants is associated with an increase in the value of troponin T and I in the urine.

Aims: To investigate the dynamics of troponin T and I levels in the urine after cardiopulmonary surgery in infants. Determine troponin T and I levels in plasma and urine in infants before, immediately after and in days after cardiac surgery. Determine whether there is an interconnection between the duration of the operation and the increase in troponin T and I levels in the urine. Determine whether there is an interconnection between troponin T and I levels in urine and ejection fraction. To ascertain whether there is an interconnection between levels of troponin T and I in urine and estimated glomerular filtration rate. To determine whether specific drug treatment influence the change in levels of troponin T and I in the urine.

Materials and methods: The subjects will be infants after cardio surgical closure of the ventricular septal defect. From the residual blood and from the morning urine (2ml) we will determine troponin T and troponin I. Around 200 measurement of both troponin T and I will be performed. Furthermore, we will value troponin T and I from plasma and urine in six measurements on the: a) day of admission in hospital, usually the day before cardiac surgery, b) admission into the Pediatric Intensive Care Unit, c) first postoperative day, d) third postoperative day, e) fifth postoperative day, f) tenth postoperative day. Complete heart echocardiography will be performed several times. In all cases P values <0.05 will be considered statistically significant. Based on the power analysis and the required total number of infants, the estimated duration of the study is 2 years.

Expected scientific contribution: The scientific contribution is a better understanding of the biochemistry of troponin T and I molecule secretion. An interconnection between the duration of the heart muscle intervention and the effect of drugs in the early and late postoperative period with the values of troponin in the urine will be determined. Determined association, weight of cardiac damage and other conditions (eg myocarditis) could be monitored by measuring the concentration of troponin molecules in the urine.

MeSH/Keywords: ventricular septal defect troponin T troponin I

Poster code: T-02-24-132

ACTIVIN A IN DIAGNOSTICS AND MONITORING OF LIVER FIBROSIS IN CHILDREN

PhD candidate: Branka Runtić Jurić
Part of the thesis: Activin A in Diagnostics and Monitoring of Liver Fibrosis in Children
Mentor(s): Assoc. Prof. Jurica Vuković, MD PhD
Affiliation: University of Zagreb School of Medicine

Introduction: While liver biopsy is a golden standard for diagnostics and monitoring of liver fibrosis, it creates significant risk for the patient. Therefore, it would be beneficial to substitute it with reliable non-invasive test(s). Although some promising results have been achieved using biomarkers and transient elastography, reliable substitute for liver biopsy is yet to be found. This study aims to examine the possibility of using Activin A as one such substitute.

Hypothesis: Activin A can be used, both independently and in combination with transient elastography, as an early indicator of liver fibrosis in children.

Aims: General aim of this study is to analyse the usability of Activin A as an early indicator, both independent and in combination with transient elastography, of liver fibrosis in children. Specific aims are the following: (1) analyse correlation between Activin A levels and the level of fibrosis, as established on the basis of liver biopsy, (2) analyse correlation between transient elastography, the level of fibrosis, as established on the basis of liver biopsy, (3) analyse whether combination of analysis of Activin A levels and transient elastography demonstrates higher reliability of assessing liver fibrosis, as established on the basis of liver biopsy.

Materials and methods: We shall conduct this cohort prospective multicentre study on approximately 60 patients aged between 2 months and 18 years. Study will be limited to patients who are diagnosed with or suspected of having a liver disease, and who are required to undergo liver biopsy for purposes of assessing the effects of the disease of diagnosing it in the first place. Patients from University Hospital Centre Zagreb, Sisters of Charity Hospital and Clinic for Children's' Diseases Zagreb shall be included in this study. After obtaining informed consent, blood samples shall be taken from patients prior to histopathological evaluation (liver biopsy). Moreover, transient elastography shall be performed within one month from liver biopsy. Taking into account progress of the liver disease and/or assessing treatment outcomes, aforementioned analysis might be repeated. Data will be processed and statistically analysed.

Expected scientific contribution: We aim to find correlation between Activin A levels, transient elastography's results and the level of fibrosis, which would indicate possibility of reducing number of liver biopsies on paediatric patients

MeSH/Keywords: children; liver fibrosis; noninvasive markers; Activin A; transient elastography

Poster code: T-02-24-140

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 of 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 an insight in both lesion morphology and vascularisation patterns. The full potential of breast-MRI and possible indications for use are still a subject of clinical research. Sonoelastography is a novel ultrasound method displaying tissue elasticity, and shear-wave elastography (SWE) can quantify the lesion stiffness by measurement of multiple elasticity indicators. The results of a conventional second-look US and second-look US with SWE lesion characterisation will be compared to investigate if SWE can affect the decision of lesion biopsy. Correlation between MRI characteristics and SWE stiffness will be determined for lesions detected after surgical treatment of breast cancer.

Hypothesis: „Second-look" ultrasound with shear-wave elastography assessment of breast lesions detected on magnetic resonance imaging of the breast after the surgical treatment of breast carcinoma may change the decision of need to biopsy the detected lesion, based on the measurement of the lesion stiffness, which can further increase specificity and sensitivity of „second-look" ultrasound in detection of breast carcinoma recurrence, especially in MRI-non-specific lesions.

Aims: To compare MRI lesion classification with second-look B-mode ultrasound classification and SWE ultrasound classification for postoperative breast lesions (according to BIRADS system). This will help determine the value of SWE in decision to perform a biopsy of the lesion. To compare sonoelastographic values of breast lesions with histological findings, and determine values characteristic for carcinoma recurrences and values for benign lesions. To determine if MRI suspicious mass-lesions appear stiffer on SWE. To determine if there is a correlation between MRI characteristics and SWE stiffness of non-mass lesions and non-specific masses

Materials and methods: All patients that underwent postoperative breast MRI and second-look US with SWE between Jan 1st 2011 and Dec 12th 2018 will be included in the study. All lesions will be categorized using American College of Radiology Breast Imaging-Reporting and Data System (BIRADS). In BIRADS 2 category, diagnosis will be made on imaging findings only, since it denotes normal imaging findings and any invasive procedure would be unethical. SWE exam will be performed using state-of-art US scanner (Aixplorer, Supersonic Imagine, Aix en Provence, France) and measurements of mean, maximal and minimal stiffnes of the stiffest part of the lesion will be taken using region of interest 2 mm in diameter, measured in kilopascals (kPa). Stiffness ratio between the lesion and breast fat tissue will also be noted. In case of suspicious breast lesions, a core-biopsy will be performed using 14 gauge biopsy needle, under local anesthesia, to obtain histological findings. Patients with positive findings of carcinoma recurrence will be referred to a plastic surgeon and oncologist for further treatment. An informed consent will be obtained for every patient. Appropriate statistical tests will be used to seek differences between lesions with positive and negative histological findings.

Expected scientific contribution: This study will hopefully contribute to increase the corpus of knowledge in field of breast carcinoma recurrence imaging. It is expected that SWE will prove useful in second-look US after breast MRI and that it will reduce the amount of underestimation of breast lesions.

MeSH/Keywords: breast MRI, breast US, shear-wave elastography, breast carcinoma recurrence

Poster code: T-02-25-039

MORPHOLOGICAL AND QUANTITATIVE MAGNETIC RESONANCE EVALUATION OF HEALING OF OSTEOCHONDRAL DEFECTS AFTER TREATMENT WITH CELLULAR AND TISSUE THERAPY

PhD candidate: Rudolf Vukojević

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

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

Affiliation: University of Zagreb School of Medicine

Introduction: Articular cartilage is known to have limited healing potential, so the development of a full-thickness articular cartilage defect will cause joint dysfunction and with time develop into osteoarthritis. 7T MRI provides excellent visualization of the repair tissue and its relationship to adjacent native articular cartilage and bone. A sheep animal model was designed to test the possibility of extending the indications for tissue grafts to more challenging conditions such as the „kissing lesions" that include cartilage damage on both articulating joint surfaces. MRI modalities were used to evaluate the osteochondral repair ex vivo.

Hypothesis: Our hypothesis is that healing of „kissing lesions" is showing better results when treated with N-TEC (tissue therapy) than N-CAM (cellular therapy) treated lesions.

Aims: The aim is to use this non-invasive powerful diagnostic tool to evaluate and compare the healing of osteochondral defects after treatment with tissue therapy with that of a cellular therapy.

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

Expected scientific contribution: To extend the range of potential clinical indications for tissue grafts to more challenging conditions such as the „kissing lesions" (so far untreatable preosteoarthritic lesions) that include cartilage damage on both articulating joint surfaces. To show better result in healing kissing lesion when treated with tissue grafts in comparation to treated with cellular terapy.

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

MeSH/Keywords: MRI, dGEMRIC, kissing lesions, cartilage tissue engineering

Poster code: T-02-25-065

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

Mentor(s): Assoc. Prof. Mislav Jelić, 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 extensor mechanism of the knee consists of quadriceps femoris muscle with its tendon, the patellofemoral joint, the patellar ligament (tendon), and the tibial tubercle. The ultimate goal of this complex 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. The malalignment of the extensor mechanism of the knee is routinely demonstrated with radiological imaging, namely computerized tomography (CT), and, rarely, magnetic resonance imaging (MR). 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, however, the femoral trochlea does not have the shape of a pit, and thus the bottom cannot be reliably 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.

Hypothesis: The measurement of the TT-TIM distance is a simple and reliable new measuring method for evaluation of the malalignment of the extensor mechanism of the knee on the axial CT-images.

Aims: To test a new measuring method for evaluation of the malalignment of the extensor mechanism of the knee on the axial CT-images.

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 from 1.7.2013. to 1.1.2016. The methods were compared regarding differences due to knee instability, the correlation, either mutual, or with age, sex, laterality, and longitudinal rotational knee angles, and interrater agreement. Lastly, the threshold of the new method was proposed.

Expected scientific contribution: To improve the overall radiological diagnostics of patellofemoral instability.

MeSH/Keywords: Radiology, Knee, Patellofemoral instability, Computed tomography, TT-TG distance

Poster code: T-02-25-083

EFFICIENCY OF ULTRASOUND-GUIDED RADIOLOGICAL INTERVENTION METHODS IN THE TREATMENT OF THE ROTATOR CUFF CALCIFIC TENDINITIS

PhD candidate: Andro Matković

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

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

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

Introduction: Rotator cuff calcific tendinitis (RCCT) is a disease caused by the deposition of hydroxyapatite crystals in the rotator cuff tendons. It is characterized by recurrent episodes of pain and markedly reduced function of the affected shoulder. One of the minimally invasive treatment methods indicated in the acute and resorptive stage of the disease, is the ultrasound-guided percutaneous irrigation with saline augmented with a corticosteroid injection. The success of this therapy was differently evaluated - from a credible to controversial. For this reason, scientists suggest possible substitutions for corticosteroid injection such as hyaluronic acid or platelet rich plasma (PRP). The issue of PRP in the treatment of injuries and diseases of the muscles and tendons of rotator cuff is currently not fully clarified, but some results are suggesting the use of PRP in treating the condition of the rotator cuff muscle tendon and state the need for further research justifying the study of the PRP role in the treatment of RCCT. Given the unequal results of the treatment using ultrasound guided percutaneous irrigation augmented with corticosteroids and the idea of exploring possible alternatives to corticosteroids, among else by using PRP, we believe that research and comparison of the three different methods is of scientific and clinical significance.

Hypothesis: The therapy of rotator cuff calcific tendinitis using platelet rich plasma, and especially using ultrasound-guided percutaneous lavage augmented with platelet rich plasma is more efficient than the presently used method using ultrasound guided percutaneous lavage augmented with corticosteroid application.

Aims: The general aim of this research is to compare the effectiveness of three different ultrasound guided interventional methods in the treatment of rotator cuff clacific tendinitis. The specific aims are: 1. to identify the short-term effects of of three different ultrasound guided interventional methods in the treatment of rotator cuff calcific tendinitis; 2. to identify the mid-term effects of of three different ultrasound guided interventional methods in the treatment of rotator cuff calcific tendinitis; 3. to identify the long-term effects of of three different ultrasound guided interventional methods in the treatment of rotator cuff calcific tendinitis; 4. to determine the time of symptom disappearance in patients after applying an individual intervention; 5. to record eventual complications for each intervention.

Materials and methods: The total sample consists of 90 patients randomly assigned into three equal groups without knowledge in which group they belong. An x-ray image and ultrasound exam will be obtained to determine size and Gartner index of deposit. Each group will be subjected to a different treatment - one group to ultrasound-guided percutaneous irrigation with saline heated at 37° C augmented with corticosteroid injection, another group to ultrasound-guided percutaneous irrigation with saline heated at 37° C augmented with PRP and a third to ultrasound guided injection of 4ml PRP into the calcification region. On treatment day the pain level will be measured by visual analog scale and shoulder function with QuickDASH and SPADI questionnaires. Follow-ups are 3 and 12 weeks and 6 months after the treatment. The sample size was determined using power test. Descriptive statistics will be calculated and distribution normality will be tested. ANOVA will be used to test the differences between groups and changes after treatment with ANOVA for repeated measurements.

Expected scientific contribution: A significant scientific contribution of this study is that a randomized, controlled clinical trial will investigate the role of PRP in RCCT treatment as it will determine and clarify the present knowledge about RCCT treatment. This will contribute to the overall scientific and clinical knowledge about RCCT treatment. This research also opens up the space for new research that could lead to a scientifically based consensus on clinical practice pertaining to RCCT.

MeSH/Keywords: Rotator cuff; Calcific tendenitis; Interventional musculoskeletal procedures; ultrasound; Platelet-rich plasma

Poster code: T-02-25-086

SCREENING OF ABDOMINAL AORTIC ANEURYSMS IN KOSOVO, UNIVERSITY CLINICAL CENTER OF KOSOVO

PhD candidate: Fjolla Hyseni

Part of the thesis: Screening of Abdominal Aortic Aneurysms in Kosovo,University Clinical Center of Kosovo,Pristina

Mentor(s): Assist. Prof. Gordana Ivanac, MD PhD, Assist. Prof. Serbeze Kabashi-Mucaj, MD PhD

Affiliation: University Clinical Center of Kosovo, University of Zagreb School of Medicine

Introduction: Abdominal aortic aneurysm (AAA), a progressive expansion and weakening of the abdominal aortic wall, is a common and potentially lethal vascular disease. AAA is more prevalent in men over the age of 65. However, it affects both adult men and women of all ages and ethnic backgrounds. The overall prevalence of AAA in men >50 years is 3.9–7.2?% (6–7?% in smokers, 2?% in non-smokers) whereas in women the overall prevalence is 1.0-1.3?% (0.8-2.0?% in smokers, 0.03–0.6?% in non-smokers). Significant risk factors for the development of AAA include advanced age, male sex, smoking, and family history of AAA. Abdominal aortic aneurysms are often undiagnosed because large proportions are asymptomatic until the development of rupture, which is generally acute and often fatal. Most AAAs are very often diagnosed incidentally during imaging studies performed for other diseases. While the prognosis of abdominal aortic aneurysm (AAA) is poor, ultrasound imaging is an accurate and reliable test for detecting AAA before rupture. Screening for abdominal aortic aneurysms (AAA) is currently recommended by several vascular societies. In countries where it has been introduced the prevalence of AAA differed greatly and was mainly related to cigarette smoking. The screening programs also had an enormous impact on the decrease of AAA ruptures and reduced mortality rate.

Hypothesis: It is expected that screening for AAA in patients undergoing ultrasound examination of abdominal aorta, abdominal aortic aneurysms will be present in the Kosovo's population especially in men over 60 years of age and will be influenced by modified factors such as smoking.

Aims: The aim of this study is to determine the prevalence of AAA in men and women of different age groups from 60 years and older, the relationship of selected risk factors with the presence of AAA and the Impact of improvement to the health care system in organizing national ultrasound screening programs in Kosovo related to AAA diagnosis and treatment.

Materials and methods: Prospective study of opportunistic hospital-based patients in University Clinical Center of Kosovo – Clinic of Radiology – Department of Ultrasound Imaging –UCCK. The study will be conducted from (July 2018-December 2019) of men and women 60 years and older. Additionally; each subject will have to fill out the questionnaire with demographic data, smoking habits, existing comorbidities and familial occurrence of AAA. A questionnaire will be filled-out during the visit. In Clinical Center of Kosovo – Clinic of Radiology - Department of Ultrasound Imaging –UCCK I will examine five patients per/day which fulfill the inclusion criteria. Emergency cases will be excluded from the study. It is expected that within one and a half year of this study about 2000 patients will meet the criteria to perform ultrasound examination. The baseline examination will include a single ultrasound scan of the abdomen that will be performed to assess the aorta from the renal arteries to the bifurcation and the diameter of the aorta will be measured at its widest point. The cutoff value for determining an aortic aneurysm will be at a diameter of ≥30?mm. Ultrasound unite at UCCK- will be acquired on unite PHILIPS HD7 with probe of C5-2 active array for the measurement of abdominal aorta in maximal perpendicular AP diameter (outer-outer diameter).

Expected scientific contribution: Results from this study will contribute in obtaining data regarding prevalence of abdominal aortic aneurysms among men and women 60 years and older detected in Kosovo and risk factors that are related to AAA. Results might also induce changes in public health care policy regarding AAA national screening programmes that are related to AAA.

MeSH/Keywords: Abdominal aortic aneurysm (AAA), ultrasound, screening

Poster code: T-02-25-142

ROLE OF MULTIPARAMETRIC MAGNETIC RESONANCE IMAGING OF THE BREAST IN PREDICTING OUTCOME OF LESIONS OF UNCERTAIN MALIGNANT POTENTIAL (B3) DIAGNOSED AT CORE NEEDLE BIOPSY

PhD candidate: Vedran Tantegl

Part of the thesis: Role of multiparametric magnetic resonance imaging of the breast in predicting outcome of lesions of uncertain malignant potential (B3) diagnosed at core needle biopsy

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

Affiliation: University of Zagreb School of Medicine

Introduction: Female breast carcinoma is the most common malignant disease among women in Croatia, and is the leading cause of death from malignant diseases. Detection of malignant breast lesions by core needle biopsy (CNB) or vacuum-assisted biopsy is the gold standard for palpable or radiologic suspicious lesions which provides histopathologic information and assists treatment planning. The smallest number of breast lesions is classified as B3 (borderline lesions), and those are lesions of uncertain malignant potential, mainly histopathologically benign lesions that show heterogeneity or may be associated with malignancy. Despite efforts to determine whether a B3 lesion can be correlated with clinical and radiologic characteristics and thus reduce the high percentage of benign lesions on surgical excision, it is unclear what radiologic characteristics, other than microcalcifications, favor malignancy.

Hypothesis: Multiparametric MRI analysis can predict the final histologic diagnosis of B3 lesions diagnosed by CNB.

Aims: 1) To analyze the accuracy and clinical value of ultrasound core needle biopsy of lesions of uncertain malignant potential 2) To compare the magnetic rasonance (MR) imaging characteristics of lesions of uncertain malignant potential with the final findings of histopathologic analysis of surgically biopsied lesions. 3) To determine if water diffusion, kinetic analysis and/or MR spectroscopy can predict which of the B3 lesions diagnosed by CNB will show malignancy at the final histological analysis. 4) To determine if there is correlation between water diffusion, kinetic analysis and/or MR spectroscopy of B3 lesions and whether multiparametric MRI analysis can be used to differentiate between different types of B3 lesions.

Materials and methods: Prospective study will include 100 female patients with a B3 lesion diagnosed by CNB who had breast MRI exam made. Besides standard MRI protocol, additional imaging sequences were done: diffusion weighted imaging and MR spectroscopy. Multiparametric magnetic resonance characteristics (apparent diffusion coefficient, dynamic curves, spectroscopy) will be compared to final histopathologic findings. Patients will be advised either surgical biopsy or radiologic imaging follow-up every six months. Surgical biopsy will provide final histologic diagnosis of the lesion, it will be qualified as malignant or benign. Lesion will be qualified as benign if it hasn't changed in size or morphology within two years. All data will be saved in table form and parametric or non-parametric statistics will be done according to data distribution.

Expected scientific contribution: There are no guidelines for the management of B3 lesions. In most institutions a multidisciplinary team must make a decision whether to use follow-up imaging or to surgically remove the lesion, which is expensive, potentially dangerous and unpleasant for the patient. The acquired results should elucidate whether multiparametric magnetic resonance characteristics can predict the final histologic diagnosis of lesions of uncertain malignant potential diagnosed at core needle biopsy. That would reduce the number of unnecessary surgical biopsies of benign lesions, as well as assist prompt treatment of malignant lesions.

MeSH/Keywords: B3 lesions, breast MRI, apparent diffusion coefficient, dynamic curves, spectroscopy

Poster code: T-02-25-145

METABOLIC SYNDROME CAUSES MORE SEVERE CLINICAL PRESENTATION AND OUTCOME OF STROKE

PhD candidate: Ivana Kern

Part of the thesis: Metabolic syndrome causes more severe clinical presentation and outcome of stroke

Mentor(s): Assoc. Prof. Zdravka Poljaković, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Metabolic syndrome is defined as a group of interrelated cardiovascular risk factors (hyperglycemia, central obesity, atherogenic dyslipidemia, hypertension) which share generating pathophysiological mechanisms and mediators. Metabolic syndrome is rapidly increasing health problem worldwide which could be explained by the rise of obesity prevalence. The revised National Cholesterol Education Program- Adult Treatment Panel III (NCEP- ATP III) definition is most widely accepted and cited in the literature because it provides usable approach for the diagnosis of metabolic syndrome. According to the NCEP- ATP III and afterwards published International Diabetes Federation (IDF) diagnostic criteria, one of the metabolic syndrome constitutive parameter is central obesity. This research is conducted because of leaking evidence on influence of certain diagnostic parameters of metabolic syndrome on severity and outcome of stroke and the need to perform endovascular procedures on cerebral arteries.

Hypothesis: Metabolic syndrome causes more severe clinical presentation and outcome of stroke

Aims: The aims of this research are: 1) to investigate interrelation between certain diagnostic parameters of metabolic syndrome with clinical severity and outcome of acute stroke; 2) to investigate influence of metabolic syndrome on appearance of overall clinical complications during acute stroke hospitalization; and 3) to investigate influence of metabolic syndrome on outcome and recanalization degree of cerebral arteries after applied endovascular procedures.

Materials and methods: This cross sectional study analyses 100 patients hospitalized in the Stroke unite of Department of neurology in University Hospital Centre Zagreb with acute ischemic stroke treated either by reperfusion technics (thrombolysis, endovascular- thrombectomy) or conservatively. Inclusion criteria is acute ischemic stroke clinically presented with acute neurologic deficit with/without positive neuroimaging of brain (computed tomography-CT). Patients are divided into two groups with or without metabolic syndrome and subdivided into two subgroups with/without severe stroke (severe stroke is defined by National Institute of Health Stroke scale NIHSS>12). The patients are analyzed by baseline demographic data, cardiovascular risk factors, anthropometric data, severity parameters of stroke, applied methods of treating stroke and outcome measures during 12 months follow up.

Expected scientific contribution: Expected scientific contribution of this research is to clarify connection between certain diagnostic parameters of metabolic syndrome and severity of acute ischemic stroke and to identify predictors of more severe functional outcome in patients with acute ischemic stroke. To investigate how presence of metabolic syndrome influences reperfusion of cerebral arteries thus striving to ensure more adequate prevention measurement and active treatments in order to avoid most severe functional outcomes after ischemic stroke in patients with metabolic syndrome.

MeSH/Keywords: metabolic syndrome, ischemic stroke, anthropometry, obesity, thrombolysis, thrombectomy

Poster code: T-02-30-050

ASSESSMENT OF THE EFFECT OF AEROBIC EXERCISE ON COGNITIVE FUNCTIONS AND CEREBRAL CIRCULATION IN PERSONS WITH SUBCORTICAL VASCULAR COGNITIVE IMPAIRMENT, NO DEMENTIA

PhD candidate: Petra Črnac Žuna

Part of the thesis: Assessment of 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: Sveti Duh University Hospital, University Hospital Centre Zagreb

Introduction: Vascular cognitive impairment refers to the loss of cognitive function due to cerebrovascular disease, mostly manifesting as the supcortical form, with executive cognitive dysfunction. Subcortical vascular cognitive impairment without dementia (scVCI-ND) does not cause significant difficulties in the performance of activities of daily living, but has a high risk of progression to dementia. Physical inactivity, obesity and vascular risk factors are associated with accelerated cognitive decline and dementia in the elderly. So far, systematic reviews and meta-analyses have shown a tendency of improvement in cognitive tests results after aerobic exercise, but mostly non-significant, possibly due to a short intervention period or a short follow-up. Sensitive biomarkers are needed to detect early changes in brain function, even before significant changes in the results of cognitive tests or brain structures. Functional transcranial Doppler Sonography (fTCD) enables measurement and monitoring of blood flow velocity and cerebral perfusion changes caused by specific neural activation.

Hypothesis: There are significant differences in the assessed cognitive functions and brain circulation parameters measured by transcranial Doppler between the experimental and the active control group of patients with scVCI-ND.

Aims: The aim of this study is to evaluate the effect of additional structured aerobic exercise on cognitive functions and cerebral circulation parameters measured by fTCD compared to standard treatment protocol in subjects with scVCI-ND.

Materials and methods: The study is planned as a randomized controlled trial. A consecutive sample of patients with scVCI-ND will be recruited through the outpatient cognitive neurology clinic and will consist of 35 patients in the experimental and 35 patients in the active control group. Initial assessment includes physical examination, self-assessment of physical activity for the elderly (PASE), neurological examination, neurocognitive testing (computerized cognitive testing, Rey Auditory Verbal Learning Test (RAVLT), Rey–Osterrieth complex figure test (ROCF), „digit-symbol" test, „Trail Making Test B" (TMT B), incongruent Stroop Test); Neuropsychiatric Inventory (NPI), Physical Ability Test (6-Minute Walk, 30-seconds Chair Stand Test), Instrumental Activities of Daily Living Scale (Lawton Brody IADL), Quality of life questionnaire; fTCD assessment of cerebral vasoreactivity and blood flow velocity with a 20-minute paradigm including breath-holding test and cognitive tasks (verbal fluency test, incongruent Stroop test, TMTB). In both groups, standard measures of modification of vascular risk factors will be conducted, including two additional educational lessons lasting 60 minutes, during a 6 month period. The experimental group will be performing additional structured aerobic activity of moderate intensity twice a week for 60 minutes during a 6 month period under supervision. Exercise intensity will be set at 40% of the age-related target heart rate, with a gradual increase to 70-80% of the age-related target heart rate over the first 12 weeks. After completion of the six-month period, the initial assessment will be repeated, followed by a six-month follow-up period during which both groups receive only standard treatment. After the end of the follow-up period, the initial assessment will be repeated.

Expected scientific contribution: The theoretical contribution of this study will be a better understanding of the mechanism of the effect of aerobic exercise on cognitive functions, while the clinical purpose would be the possibility to use fTCD in the validation of early aerobic exercise induced effect and the possibility to use structured aerobic exercise in the prevention of progression of vascular cognitive impairment.

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

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

Poster code: T-02-30-114

PHD THESIS PROPOSALS

Public Health and Health Care

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: Data quality assessment of implementation 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, Department of medical statistics, epidemiology and medical informatics and Croatian Institute of Public Health

Introduction: The National Program of Breast Cancer Early Detection in the Republic of Croatia has been conducted since 2006 using mammography 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 and effectiveness of the screening program is subject to continuously evaluation. The quality indicators ensure monitoring implementation and data collection quality. Performance indicators reflect the provision and the quality of activities constituting the screening process. Acceptable and desirable values have been set for them. 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. In the screening program evaluation, after the completion of each cycle, is necessary to calculate the number of women with interval cancer by linking with the Cancer Registry. The aim of interval cancer monitoring is twofold because radiological review of interval cancer serves quality assurance and education. For the purpose of evaluating the screening program, the monitoring of interval cancer as a surrogate indicator allows the calculation of parameters for early evaluation of the screening program effect. It is known that interval cancer may have different characteristic in comparison to the others screening detected cancers and can be more aggressive, exhibit higher histological grade, larger tumour size and larger TNM stage. The risk factors for their development have not been fully explored.

Hypothesis: A positive anamnesis of breast cancer in the blood relatives is a significant predictor of the interval cancer in the female population of the Republic of Croatia.

Aims: General aim: analysis of screening based on the program implementation indicators and analysis of interval cancers as an effect of program implementation. Specific aims: determining the correlation between positive breast cancer anamnesis in the blood relatives and interval breast cancer occurrence through the development of the logistic predictive model and comparison of interval cancers with other screening detected cancers.

Materials and methods: Materials: 1. Data from the application of the National Program for early detection of breast cancer in the Republic of Croatia. Available are invitational surveys, mammography readings based on BI-RADS classification, and cancer diagnosis from the received "Onco-type Form" and "Notification of malignant neoplasm Form". 2. Data from the Cancer Registry for women of the age group covered by the program. Methods: After accessing the screening program application data, without insight into the woman's identity, using an id, systematic analysis of screening based on epidemiological indicators of implementation will be performed and expressed as appropriate proportions. Analysis of interval cancers will be performed by linking with the Cancer Registry Data. In the development of the model for predicting interval cancer, univariate and multivariate logistic regression will be used.

Expected scientific contribution: The expected scientific contribution of the proposed research is the assessment of the quality of implementation and the impact of the national program screening, and obtaining an interval cancer prediction model for the female population in Croatia.

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

Poster code: T-03-01-016

RISK FACTORS FOR ALCOHOL ABUSE AMONG SIXTEEN-YEAR-OLDS IN CROATIA

PhD candidate: Diana Jovičić Burić
Part of the thesis: Risk Factors for Alcohol Abuse Among Sixteen-Year-Olds In Croatia
Mentor(s): Assist. Prof. Sanja Musić Milanović, MD PhD
Affiliation: Croatian Institute of Public Health

Introduction: Alcohol consumption is one of the leading public health problems. According to the World Health Organization, worldwide 3.3 million deaths every year result from harmful use of alcohol, this represent 5.9 % of all deaths. Alcohol consumption is one of the the leading causes for disease and death in Europe. Despite the known harmful effects of alcohol consumption it still remains an acceptable form of behavior. Particularly sensitive period to start drinking alcohol is adolescence. In this period many developmental changes occur, from psycho-physical to social and neurological.

Hypothesis: Sixteen-year-olds in Croatia who have the perception that most or all of their friends get drunk have at least 3 times higher risk for tendency to develop alcohol dependence and those who have taken sedatives prescribed by the doctor or have the perception that most or all of their friends drink alcohol or they have a perception that their close person drinks to much have that risk at least 1.5 times higher. Sixteen-year-olds in Croatia who have the perception that people risk harming themselves if they drink alcohol or have a perception of stronger parental control or are satisfied with their relationship with mother, or have better school achievement, have at least 30% lower risk for tendency to develop alcohol dependence.

Aims: The aim of this study is to explore and evaluate, based on socio-ecological approach, the influence of individual and environmental risk factors on the intensity and frequency of alcohol consumption in sixteen-year-olds in Croatia.

Materials and methods: A cross-sectional survey will be conducted in March 2019. using the ESPAD (European School Survey Project on Alcohol and Other Drugs) questionnaire, on a sample of first and second grade secondary school students in Croatia. For the selection of the respondents it will be used a random stratified sample. Sampling unit is a class within a particular type of secondary education program. The analysis will include minimum 2500 students who turn sixteen years in the year of the research. The analysis of isolated individual and environmental factors will be carried out for variables of the frequency and intensity of alcohol consumption (drinking in the last 12 months, in the last 30 days, drinking five or more drinks in the last 30 days, the amount of alcohol consumed on the last day they drank). The subgroup of sixteen-year-olds that have tendency to develop alcohol dependence will be differentiated by criteria: drinking in the last 12 months and drinking in the last 30 days, drinking five or more drinks in a row in the last 30 days and the amount of alcohol consumed the last day they were drinking (drinking 30 grams of pure alcohol or more, which is equivalent to three standard drinks, with one standard drink having 10 grams of pure alcohol). Data analysis will be performed by computer program for statistical analysis SPSS ver 23.0 (ID: 729038). Depending on the type of data distribution, parametric or alternative nonparametric analytical procedures will be performed. For continuous variables preliminary analysis of the normalization of distribution by the Kolmogorov-Smirnov test will be performed. Data will be analyzed by multivariate logistic regression models. Results will be analyzed at á=0,05.

Expected scientific contribution: Obtained results will serve for early identification of sixteen-year-olds proned to alcohol addiction and as guidelines for development of public health interventions.

Acknowledgments: I would like to thank my mentor and colleagues from CIPH for their help in conducting this research.

MeSH/Keywords: alcohol drinking, adolescents, risk factors, cross-sectional studies

Poster code: T-03-01-020

USE OF CROATIAN CENTRAL HEALTH INFORMATION SYSTEM AS A DECISION SUPPORT TOOL – MODEL OF BENZODIAZEPINE TREATMENT MANAGEMENT

PhD candidate: Katarina Gvozdanović

Part of the thesis: Possible use of Croatian central health information system (CEZIH) as a decision support tool – model of benzodiazepine treatment management

Mentor(s): Assist. Prof. Aleksandar Džakula, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Croatian central health information system (CEZIH) is a national healthcare integration platform which contains different functionalities and large amount of administrative and clinical data. At present, data contained in CEZIH is not used for the systematic drug-related decision-making. For the purposes of assessing CEZIHs' potential as a decision support tool for medicines, the system will be tested on the model of regulation of therapy with benzodiazepines. Literature and anecdotal data indicate that benzodiazepines are often misused, however, the extent of misuse, prescribing pattern and prescribers and users characteristics have not been established for Croatia due to lack of data. Based on CEZIH data we will describe the benzodiazepine use pattern in real life and share this information with relevant stakeholders in drug treatment decision process as well as with prescribers to understand their knowledge and attitude to CEZIH and to test whether it can be used to make/enforce regulatory decisions.

Hypothesis: Based on the available functionalities, structure, type, quantity and quality of data, CEZIH can be used as a decision support system, implementation channel and tool for measuring the effectiveness of regulatory decisions for the purpose of achieving evidence-based healthcare decisions in Croatia.

Aims: To examine the possibility of reaching and implementing drug related regulatory decisions based on data and functionalities available in CEZIH

Materials and methods: Research will be done in three phases. Analysis of benzodiazepine use in real life will be used as a model. Descriptive statistics, interview and surveys will be main research methods. The first phase of the research will be focused on determining the pattern and trends in benzodiazepine use by utilizing available data from CEZIH. Prescriptions (and dispensation) data for any benzodiazepine during the defined time period will be used as a main dataset for further analysis. Obtained use pattern data will be compared with the applicable guidelines/product information of the benzodiazepines approved by HALMED to detect deviations from the desirable clinical behaviour. In the second phase of the study, interviews will be conducted with key stakeholders in the health system that may affect the clinical practice of benzodiazepines. The focus of the interview will be on the critical deviations established during the first phase of the survey, and the interviews will examine their attitudes and knowledge on the possibilities of using CEZIH in decision-making processes. The third phase of the research will be carried out using interviews and surveys on the chosen sample of prescribers, family medicine physicians, whose purpose is to determine their knowledge and attitudes about the possibility of using CEZIH as a channel for regulatory intervention. Data collected during all three phases of the research will be used to evaluate the quality of data in CEZIH.

Expected scientific contribution: This study will be the first comprehensive scientific evaluation of data quality and technical solutions of CEZIH with regards to drug usage data for the purpose of support to decision-makers and implementation of regulatory measures in practice.

MeSH/Keywords: Croatian central health information system, health information system, regulatory decision making, evidence-based regulatory decisions, benzodiazepine treatment, quality of data

Poster code: T-03-01-104

EPIDEMIOLOGICAL CHARACTERISTICS OF SUICIDE AND AN ELECTRONIC INTERVENTION OF SUICIDE PREVENTION AMONG INTERNET USERS IN THE REPUBLIC OF CROATIA

PhD candidate: Vanja Pajić

Part of the thesis: Epidemiological Characteristics of Suicide and an Electronic Intervention of Suicide Prevention 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: Suicide is one of the leading causes of death from injury in Croatia and the world. Screening of persons at risk of suicide remains a difficult goal because of the low level of sensitivity and specificity of the available identification procedures and low basic rates of such behavior in the population. Internet search data can serve as an indicator for suicide risk, and it would be worthwile to explore the value of keywords that would allow for a screening of people at risk of suicide, as well as enable preventive actions to reduce suicide incidence. This paper will show the epidemiological characteristics of suicide in Croatia using data from the Register of Suicides of Croatia. We will investigate the relationship of internet search information pointing to the risk factors of suicidal behavior and suicide, and by using the data obtained to propose a method of screening vulnerable individuals using the online tools.

Hypothesis: There is a pattern of Internet keyword search that is positively associated with the incidence of suicides in the population of the Republic of Croatia

Aims: GENERAL OBJECTIVES OF RESEARCH: Describe the occurrence of suicide in the Republic of Croatia; Research the feasibility of an Internet intervention for suicide prevention among Internet users at increased risk of suicide. SPECIFIC OBJECTIVES OF RESEARCH: - Investigate and describe the occurrence of suicides committed in Croatia from 2012 to 2018 using data available in the Croatian Commited Suicides Registry; - Identify the risk factors of suicide by using data in the Croatian Commited Suicides Registry by performing a literature review; - Estimate the predictive value of keywords for predicting suicides; - Design and build a screening model for Internet users at risk of suicide; - Assess the feasibility of an intervention campaign using selected key concepts at the level of Croatia and in the county with the highest coefficient of correlation between data on Internet search and data from the Croatian suicide register.

Materials and methods: The material that will be used in this research is data resulting from Google's Internet search engine results, obtained with Google Trends. Data on suicides will be downloaded from the database of the Croatian Institute of Public Health for the period 2012-2018. Aggregated data for the specified period and geographic area will be used. We will show age-standardized rates of mortality by gender, age, degree of education, marital status, place of residence and manner of suicide. Based on a literature review and analysis of the epidemiological characteristics of suicide by using the Croatian Commited Suicides Registry, we will select the terms that indicate the presence of some of the risk factors of suicide. The terms will then be analyzed using the Google Trends tool, investigating the relationship of key terms with suicide incidence from the Croatian Commited Suicides Registry. The terms used in Internet search that show the highest coefficient of correlation in the analysis will be selected for the screening of people with suicidal risk factors. A probationer will be carried out through a Google AdWords campaign. The relationship between internet search data and suicide incidence will be determined by analyzing the correlation between selected terms as the number and time range function and the suicide incidence of suicide records for the selection of indicators.

Expected scientific contribution: Description of epidemiological characteristics of suicide in Croatia, given the specificity and cultural conditionality of online behavior. A screening model of internet users at risk of suicide by using keywords related to suicide. Feasibility analysis of suicide prevention interventions for internet users. This dissertation will result in a framework program for the Internet suicide prevention.

MeSH/Keywords: mental disorders, behavioral disorders, suicide, epidemiology, digital epidemiology, infodemology, public health, public health information technology

Poster code: T-03-02-034

IDENTIFICATION OF PRIORITIES IN NURSING INTERVENTIONS FOR PREVENTION AND TREATMENT OF HYPERTENSION

PhD candidate: Goranka Rafaj

Part of the thesis: Identification of priorities in nursing interventions for prevention and treatment of hypertension

Mentor(s): Assist. Prof. Aleksandar Džakula, MD PhD

Affiliation: University of Zagreb School of Medicine

Introduction: Hypertension is a significant, globally present public health problem from the aspect of morbidity, mortality and economic burden. It is the leading and most significant modifying risk factor for the development of cardiovascular diseases, stroke, kidney diseases, peripheral vascular diseases and retinopathy.By strengthening the role of nurses in the control and prevention of hypertension, it is possible to exert a significant influence on the reduction of risk factors, the control of taking prescribed therapy and monitoring of persons under risk of developing hypertension as well as patients suffering from it.

Hypothesis: The association of hypertension with demographic, behavioral and socioeconomic determinants of health, and the unused potential of the healthcare system for implementation of nursing interventions, both demand a greater engagement of medical nurses and identification of types and priorities in nursing interventions. The potential for nursing interventions in the prevention and management of hypertension is impacted by political, economic, sociocultural, technological and scientific factors.

Aims: The aim of this study is to identify the needs, potential and criteria for the development of interventions that could be implemented by medical nurses in the prevention and management of hypertension.

Materials and methods: For the purpose of determining critical factors in defining the needs, possibilities, priorities of nursing interventions in the prevention and treatment of hypertension, the data on the population, medical staff and interventions shall be analyzed. The research shall include data and published results of studies related to the Croatian Health Survey, library data about the primary and secondary prevention of cardiovascular diseases, the EH UH 1 study, the EH UH 2 study, data of the Croatian Institute of Public Health, Croatian Health Insurance Fund, the Act on Nursing, The Act on Patients Rights Protection and data available from government agencies.The research shall be conducted in three phases according to the objectives. In the first phase, research and analysis of publicly available data, published papers and additional analyses of studies conducted in the past 15 years that are related to the prevention of cardiovascular diseases and, specifically, hypertension are to be carried out. In the second phase, data from the EH UH 2 as well as data available from the Croatian Institute of Public Health and the Croatian Health Insurance Fund regarding health resources shall be used for the purpose of determining the frequency and differences of behavioral risks, variable health behavior and the connection between hypertension and demographic, behavioral and socio-economical determinants that are significant for the implementation of nursing interventions. Variables to be used are related to subjects with normal and increased arterial pressure. Results shall be analyzed by means of descriptive statistics and measures of association. The third phase shall involve the development of content in the form of guidelines based on the obtained results and new insights, which may be used in the Evidence Based Decision Making and Policy Documents.

Expected scientific contribution: Significant frequency of arterial hypertension in the Croatian population suggests the need for research into the ability of nurses to support evidence based practice. This study is expected to contribute to the development of a model of nursing interventions based on identification of critical points and evaluation of resources, with possible generalization to similar populations worldwide, and also to the development of guidelines for nursing interventions for prevention and management of arterial hypertension.

MeSH/Keywords: hypertension, nursing intervention, management of hypertension

Poster code: T-03-02-109

RATIONALITY ASSESSMENT OF THE OUTPATIENT PRESCRIPTION OF BENZODIAZEPINES IN THE REPUBLIC OF CROATIA

PhD candidate: Marija Delaš Aždajić

Part of the thesis: Rationality assessment of the outpatient prescription of benzodiazepines in the Republic of Croatia

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

Affiliation: University of Zagreb School of Medicine

Introduction: It is commonly known that the habit of prescribing pharmacotherapy is a subject to individual factors such as age, gender, cultural background, patient needs and requirements, the influence of pharmaceutical industry and many others. According to data on the frequency of prescribing drugs available from the literature, psycho-pharmacological drugs are on the second place, immediately after cardiovascular drugs, with a continuous annual increase of 11%. Similar trend is also present in the Republic of Croatia, where the total costs of the healthcare system for psycho-pharmacological drugs increased by 20.1 % in the period between 2001 and 2010. Benzodiazepines belong to the second generation of anxiolytics, which, by their appearance in the 1960s, provided wide neuropsychiatric application. By acting on the benzodiazepine receptors, these drugs produce anxiolytic, hypnotic, miorelaxant and anticonvulsant effects. Although benzodiazepines have very high efficacy and utility, the irrational use of these drugs may result with deleterious consequences for the patient, especially in the long-term use. Development of tolerance, loss of therapeutic effect and addiction development are the most common side effect. Additionally, abrupt interruption of benzodiazepine occurs as a rebound phenomenon in the form of anxiety or insomnia. Unfortunately, there is a tendency of increased long-term use of benzodiazepine in the Republic of Croatia, partly due to a tradition of prescribing and partly due to a rapid drug action and good efficacy with very few initial side effects. Accounting for 86.9 % of prescribed psychofarmaceuticals utilization in Croatia, utilization of benzodiazepines requires revision and the development of respective guidelines as a measure of rationalization. Although there is a noticeable ongoing effort to reduce the frequency of prescribing medicines by reducing financial resources, additional rationalization is necessary. Rational pharmacotherapy means the correct prescribing of the correct dose to the right patient in the predicted time period with the best possible effect and the lowest possible cost for the patient and the society.

Hypothesis: The rationality of outpatient prescription of benzodiazepines in the Republic of Croatia is in line with the existing guidelines.

Aims: The purpose of this study is to determine rationality in the prescription of benzodiazepines in the Republic of Croatia for years 2015 and 2016.

Materials and methods: The epidemiological methods of descriptive and analytical observation will be used to analyze the available database provided by Croatian Health Insurance Fund, the only insurance company in the Republic of Croatia that organizes and carries out national compulsory health insurance. The insurance company is computer networked with all the primary health care departments which, on the basis of recommendations from the psychiatric specialist, may prescribe benzodiazepines. The database that will be used for analysis, enables the insight into sex and age structure of the patients using electronic prescription to collect their drug (benzodiazepine) in the pharmacy, as well as additional regional or inter-regional differences in the frequency of prescribing benzodiazepines and the difference in diagnosis based on which of these medicines were prescribed. This database will provide the information about prescription frequency of benzodiazepines via outpatient utilization, retrospectively for years 2015 and 2016. Correlations for diagnosis, sex, age and duration of therapy will be assessed. Rationality assessment of the benzodiazepines prescription will also be done using appropriate statistical methods and tests.

Expected scientific contribution: This research will show the pharmacoepidemiological situation and provide information about the current trend of benzodiazepines prescription in the Republic of Croatia. In this way relevant data will be available, providing the base for further health quality improvement and pharamacoeconomical benefit of the society and the country. Furthermore, the data will contribute internationally to develop more accurate recommendations, since inappropriate utilization of benzodiazepines is recognized as a significant public health problem in most developed countries.

MeSH/Keywords: benzodiazepines, pharmacoeconomics, pharmacoepidemiology, drug utilization, Republic of Croatia

Poster code: T-03-02-119

ATTITUDES OF GENERAL POPULATION, STUDENTS OF MEDICINE AND NURSES ABOUT NURSING AS A PROFESSION

PhD candidate: Sanda Franković

Part of the thesis: Attitudes of general population, students of medicine and nurses about nursing as a profession

Mentor(s): Professor Miroslav Mastilica, MD PhD

Affiliation: University of Zagreb School of Medicine, School of Nursing Mlinarska

Introduction: Nursing encompasses autonomous and collaborative care of individuals of all ages, families, groups and communities, sick or well and in all settings. Nursing includes the promotion of health, prevention of illness, and the care of ill, disabled and dying people. Advocacy, promotion of a safe environment, research, participation in shaping health policy and in patient and health systems management, and education are also key nursing roles. Modern understanding of nursing specifies nursing as a professional discipline which is equally based in science as it is in practical skills based on research (evidence) of its own practice. Goal of this thesis is to determine to what level are certain elements of nursing profession developed in Croatia and to what extent its autonomy and professional status is recognized by the tested groups. Three group will be tested: general public, sixth year students of medicine and employed nurses.

Hypothesis: Opinions of the public, medical students and nurses on nursing as a profession differentiate depending on the level of education and demographic characteristics.

Aims: To determine difference in opinions of medical students, nurses and the public about nursing as a profession depending on demographic characteristics (gender, experience, place of employment, level of education). To explore socio-demographic predictors of positive opinion. To determine in what measure does observed population perceives autonomy of nurses.

Materials and methods: Research will use questionnaire about attitudes concerning nursing (Nursing image questionnaire), seventh version (NIQ-7), created by Toth and associates. Èukljek made a linguistic and cultural adaptation of questionnaire so it can be used on a Croatian population. Research will use a questionnaire about demographic characteristics of examinee. A separate questionnaire will be constructed to test perception of nurse's autonomy. After a construction of a questionnaire it will be used in a pilot test on a sample of 100 subjects. Subjects will be students of the final year of School of Medicine in Zagreb (150 subjects); nurses employed in University Hospital Centre Zagreb, University Hospital Centre "Sestre Milosrdnice", Clinical Hospital "Sveti Duh", Clinical Hospital "Merkur", Health centres Zagreb Centre, East and West (600 subjects), and citizen members of Neighbourhood Councils and members of Council of Local Boards of Zagreb (300 subjects). Gathered data will be processed with descriptive and inferential statistical methods. Analysis of the normal distribution will be done using Kolmogorov-Smirnovljev test, parametric tests will be used in case of normal distribution, otherwise non-parametric tests will be used. Variance analysis (Kruskal-Wallis test) will be used to test significance of differences between the variables values between three groups. For the analysis of socio-demographic prediction of positive stance logic regression will be used. Level of significance will be á=0,05.

Expected scientific contribution: Research should determine predictors of positive and negative attitude towards nursing as a profession. By enlarging already existing knowledge about nursing profession as a multidimensional concept opens a new field of research in our scientific community. Attitudes of medical students are a significant indicator of readiness to accept nurses as future associates and understanding of their role. Attitudes of the public can indicate on level of trust and readiness to accept advice and adopt recommended behaviour. Attitudes of nurses are indicators of professional socialization and commitment of development and advancement of nursing care.

MeSH/Keywords: attitudes, nursing, profession, general population, students of medicine

Poster code: T-03-02-120

Dan doktorata 2018 / PhD Day 2019
Knjiga sažetaka / Abstract Book

Izdavač / Publisher
Sveučilište u Zagrebu, Medicinski fakultet / University of Zagreb, School of Medicine

Suizdavač / Co-publisher
Medicinska naklada, Zagreb

Oblikovanje naslovnice / Cover design
Petra Križan, Dizajncafe d.o.o.

Naklada / Edition
300 primjeraka / copies

Tisak / Print
Medicinska naklada, 2018