

Sveučilište u Zagrebu, Medicinski fakultet/University of Zagreb, School of Medicine
Poslijediplomski studij Biomedicina i zdravstvo/PhD Program Biomedicine and Health Sciences
Dan doktorata 2012/PhD Day 2012
Sažeci istraživanja i prijedlozi istraživanja/Research Abstracts and Research Proposals

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Health Sciences**

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Research Abstracts and Research Proposals**



MEDICINSKA NAKLADA
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PREDGOVOR

ZAŠTO DAN DOKTORATA?

Dani doktorata se već niz godina organiziraju na pojedinim sveučilištima u Europi.

Riječ je o smotri rezultata istraživanja ili tek planova istraživanja polaznika doktorskih studija. Ponekad kao uzori sudjeluju i oni koji su već uspješno doktorirali. To je prigoda da se doktorandi međusobno upoznaju sa svojim istraživanjima, da podjele dobra i loša iskustva, da na njima uče i, ono najpoželjnije, da ostvare međusobnu suradnju u istraživanjima.

Pozvana predavanja svjetski uspješnih znanstvenika trebala bi ih dodatno motivirati i ohrabriti da nastave na tom nimalo laganom putu znanstvenih istraživanja.

Svi doktorandi imaju prigodu i obavezu u obliku postera prikazati rezultate ili tek planove svojih istraživanja.

Odabrana usmena izlaganja radova trebala bi isto tako biti dodatna motivacija koja će jačati duh natjecanja, ali i duh kompetitivne suradnje tako karakterističan za današnja znanstvena istraživanja.

Prisutnost mentora i gostiju dodatno bi trebala stimulirati doktorande na još intenzivnija i inventivnija istraživanja.

Primjedbe kolega, nastavnika ili gostiju treba prihvatiti kao dodatni poticaj, a nikako kao neprijateljstvo jer jedno od bitnih obilježja znanosti je organizirani skepticizam. Najviše možemo naučiti od kritičara.

Danom doktorata vidjet će se pojedinačni doprinosi, ali i cjelovitija slika istraživačke djelatnosti polaznika II. i III. godine dokorskog studija Biomedicina I zdravstvo. To je djelomičan presjek znanstvene djelatnosti Medicinskog fakulteta, ali i ustanova iz zemlje i inozemstva s kojima surađujemo. Raduje da imamo sve više doktorata s dvoje mentora, doktorata u tzv. „*skandinavskom stilu*“ kao i doktorata na engleskom jeziku.

Konačno, Dan doktorata kao smotra naše istraživačke aktivnosti izvrstan je primjer za ocjenu kvalitete studija, ali istodobno i odlična škola prenosivih vještina (*transferable skills*) korisna i onim doktorandima koji se neće profesionalno baviti istraživačkim radom. To su, među ostalim, mogućnosti kritičke sinteze velikog broja podataka, kritičan odnos prema istraživanjima drugih, komunikacijske vještine i sposobnosti da se rezultati sažeto a jasno prikažu u obliku postera ili usmenog izlaganja.

Doktorski studij se od svog osnutka, priznajemo uz određena lutanja karakteristična za cijelu Europu, pokušao prilagoditi Europskim standardima. Odluka europskih ministara da to bude „treći ciklus“ visokog obrazovanja zatekla je pojedine fakultete i sveučilišta nedovoljno pripremljene. Zbog toga je naš studij bio zapravo organizator prvih Europskih konferencija o harmonizaciji doktorskih studija u Europi 2004. i 2005. godine (prije poznate EUA konferencije u Salzburgu 2005.) iz čega je nastala velika europska udruga doktorskih studija ORPHEUS sa sjedištem u nas, koja danas okuplja preko 80 zdravstvenih fakulteta iz gotovo svih Europskih zemalja s godišnjim rasom članstva od 10-14 posto, a u novije vrijeme i s odobravanjem Europske komisije, Europske znanstvene zaklade (ESF) i drugih.

Dan doktorata na doktorskom studiju Biomedicina i zdravstvo je prva takva manifestacija u nas pa se nadamo da će dobra iskustva prihvatiti i drugi doktorski studiji. Zahvaljujući, za naše prilike, visokim kriterijima i inzistiranju na njihovom provođenju današnji doktorandi tijekom studija izrade 5 i više publikacija indeksiranih u *Current Contents*, od toga su najčešće prvi autori u dvije, a najviši čimbenici utjecaja časopisa u kojima se najvažniji od tih radova objavljuju su viši od 2. Do sada je oko 50 polaznika steklo znanstvena zvanja, a više njih su danas nastavnici našeg Fakulteta. Doktorski studij jednostavno ispunjava svoju funkciju stvaranja budućih lidera naše medicine. Mogli bi reći da su ove brojke pokazatelji „kulturne promjene” u shvaćanju doktorata znanosti u nas.

Polaznici su u najvećem broju liječnici kliničari koji se samo dijelom vremena bave istraživanjima, najčešće uz bolesničku postelju. Prema početnim istraživanjima od prijave teme do doktorata u prošlosti bi prošlo više od 8 godina. Danas je taj prosjek smanjen na oko 6 godina, no još uvijek smatramo da je predugačak. Posljedice toga vidimo i na ovogodišnjoj smotri - samo jedna trećina učesnika prikazuje rezultate svojih istraživanja, a ostali samo planove. To je upravo odraz „*part-time*” studiranja i ukazuje na kasan početak istraživanja. Najveća nam je želja da Danom doktorata to promijenimo.

Trajanje izrade doktorata je još uvijek vrlo dugo, a medijan čimbenika odjeka časopisa u kojima se radovi objavljuju mogao bi biti povoljniji. Nadamo se da će Dan doktorata i u tome postupno potaknuti promjene.

Zbog navedenih obilježja Vijeće Fakulteta je donijelo odluku da Dan doktorata bude obavezan za sve doktorande na doktorskom studiju i da se doktorandima boduje s 4 ECTS odnosno 25 sati rada u nastavi.

Nedoumice oko doktorskih studija su još uvijek prisutne, stoga smo na ovogodišnji Dan doktorata pozvali i stručnjake koji će nam govoriti o praksi i standardima doktorata u Europi. Istoj temi posvetili smo i okrugli stol sa domaćim i stranim učesnicima. U tradiciji drugih sveučilišta Roland Jonsson će, kao vodeći imunolog, posebno održati predavanje o svojim istraživanjima.

Nadam se da ćemo na greškama i uspjesima ove godine u narednim godinama biti sve bolji i bolji.

Zdravko Lacković
Voditelj studija

PREFACE

WHY PhD DAY?

PhD Days have traditionally been organized by various universities throughout Europe.

PhD Day is an event showcasing research results and research proposals of PhD students with occasional participation from PhD graduates acting as role models. This is an opportunity for PhD students to exchange research ideas amongst themselves, to share and learn from each other's positive and negative experiences and, most importantly, to promote and facilitate cooperation in research.

Invited lectures of the world renowned scientists should serve as additional motivation and encourage the PhD students to continue on their often arduous path of scientific research.

All PhD students have the opportunity and the obligation to present their research or research proposals in the form of posters.

Selected oral presentations of our candidates should further strengthen the spirit of competitive cooperation as the main feature of interaction of scientists today.

Likewise, the presence of mentors and guests should encourage doctoral students to engage in even more intense and inventive research.

Comments of colleagues, teachers and guest should be received as additional incentives, since organized scepticism is one of the essential features of science. We can learn the most from our critics.

PhD Day will show individual scientific contributions, but will also paint a comprehensive picture of research activity of the PhD program in Biomedicine and Health Sciences. It is meant to serve as a partial cross-section of the scientific activities of the School of Medicine as well as fellow domestic and foreign institutions. We are pleased to have an increasing number of doctorates with two mentors, the so-called „Scandinavian style“ theses, as well as theses in English.

Finally, PhD Day as a public display of our research activities is an excellent opportunity for the quality assessment of the program, but also a great training ground for transferable skills useful even to students who will not be engaged in future research work. These include synthesis of a large amount of critical data, a critical attitude towards the research of others, communication skills and ability to summarize the results clearly in the form of posters or oral presentations.

Our PhD program has strived to adapt to European standards, certain degree of past meandering typical for all of Europe notwithstanding. The decision of the European Ministers to make the PhD programmes the „third cycle“ of higher education found some colleges and universities ill-prepared. This was the main reason why our PhD program organized the first and second European Conference on Harmonisation of PhD Programs in Europe in 2004 and 2005, even before the well-known EUA conference in Salzburg, in 2005. These conferences helped establish ORPHEUS, a large European organization of doctoral studies with headquarters here, at the Zagreb School of Medicine. Since then, ORPHEUS has grown and now consists of over 80 medical faculties from almost every European country with an annual rate of membership growth of 10 - 14 percent. It is viewed favourably by the European Commission, European Science Foundation (ESF) and others.

PhD Day of the PhD program in Biomedicine and Health Sciences is the first such event in Croatia, and we hope our positive experiences will be acknowledged by other doctoral programs. Thanks to the strict implementation of the high criteria for selection of candidates, our PhD students today publish 5 or more publications indexed in Current Contents, and two of those usually as first authors. The most significant of these papers are published in journals with impact factors higher than 2. So far, about 50 of our students progressed to scientific degrees, a number of them going on to becoming professors at our School. Our PhD program effectively fulfils its purpose to create future leaders in medicine. One could say that these figures are indicators of „cultural change” in the understanding of doctorates in Croatia. Our students are mostly medical doctors - clinicians who can do their research only part time, more often than not by the patient’s bedside. According to initial investigations, the past duration of the PhD study, from the application to the program to the finished thesis, was more than 8 years. Today, that average has dropped to about 6 years, which we still consider too long. The consequences can be seen at this year’s PhD Day - only a third of participants have presented results of their research, the others showing their research proposals only. It is a reflection of the part-time nature of the study and indicates their late start of research. We sincerely hope that PhD Day will help us change that and further shorten the study duration.

Admittedly, the study duration is still very long and the median impact factor of published papers could be higher. We hope that the PhD Day would help promote a gradual change in these aspects as well.

Due to all this, the Faculty Council decided to make the PhD Day an obligatory part of the program awarding 4 ECTS or 25 lesson hours to each PhD student.

Since the standards of PhD programs are still a concern, this PhD Day will feature expert lectures on practices and standards of PhD programs in Europe. The roundtable panel consisting of domestic and foreign participants will be dedicated to the same topic. In keeping with the traditions of other universities, we have invited Roland Jonsson, a leading immunologist, to hold a special lecture on his research.

In conclusion, I hope that our mistakes and successes from this year will only make us better in the years to come.

Zdravko Lackovic
Head of studies

PROGRAM DANA DOKTORATA

Medicinski fakultet Sveučilišta u Zagrebu

Doktorski studij: Biomedicina i zdravstvo

Dan doktorata, 25. svibnja 2012.

(Dvorana Čačković)

08:30 - 09:30	Razgledavanje postera
09:30 - 10:00	Otvaranje Dana doktorata
10:00 - 11:00	Doktorati u Europi: Europski standardi i primjer izvrsnosti (predsjedaju Ana Borovečki i Zdravko Lacković) <ul style="list-style-type: none">• Jadwiga Mirecka (Krakow): ORPEHUS-AMSE-WFME European Standards for PhD in Biomedicine and Health Sciences.• Robert Harris (Stockholm): <i>PhD program at Karolinska Institutet - problem of evaluation</i>
11:00 - 11:20	Stanka za kavu
11:20 - 11:50	Research excellence lecture (predsjeda Drago Batinić) <ul style="list-style-type: none">• Roland Jonsson* (Bergen): The complexity of Sjögren's syndrome: novel aspects on pathogenesis
11:50 - 12:30	Posteri odabrani za usmeno izlaganje (predsjedaju Ante Tvrdeić i Marko Jakopović) <ul style="list-style-type: none">• Marijana Škledar: Association between brain-derived neurotrophic factor val66met and obesity in children• Barbara Barun: Serologic markers of sleep disorders in patients with multiple sclerosis• Katja Dumić: Congenital adrenal hyperplasia due to 21-hydroxylase deficiency in Croatia - clinical and molecular data• Iva Pejnović Franelić: Affinity of first year university students towards gambling and betting
12:30 - 13:30	Ručak i razgledavanje postera
13:30 - 15:30	Okrugli stol: Ustroj i razvoj doktorskih studija u Hrvatskoj i Europi <p>U dvorani: Coordinators: Zdravko Lacković and Robert Likić (Medicinski fakultet), Sudionici: Ignac Lovrek (Sveučilište u Zagrebu, FER i Odbor</p>

za doktorske studije), Roland Jonsson (University of Bergen), Jadwiga Mirecka (Jagiellonian University Medical College, Krakow)

Web-cast: Michael Mulvany (University of Aarhus), Ingeborg van der Ploeg (Karolinska Institutet, Stockholm)

15:30 - 17:30

Organizirani obilazak postera s diskusijom (potpisivanje indeksa)

****Roland Jonsson** (Bergen, Norway) Broegelmann Chair in Immunology, Director of Bergen Research School in Inflammation (BRSI), Deputy Head (research) of the Gade Institute, Consultant Haukeland University Hospital. Member of the ORPHUES Executive Committee. Grant reviewer and Advisory Committees for the European Commission. Editor-in-Chief of Scandinavian Journal of Immunology. One of the world leading immunologists (ISI - H-index = 40; > 6300 citations: worldwide no. 3 in Sjögren's syndrome and no.1 in sialadenitis research according to biomedexperts.com). Elected member of the Norwegian Academy of Science and Letters.*

PHD DAY PROGRAM

University of Zagreb School of Medicine
PhD Program: Biomedicine and Health Sciences
PhD Day, 25th May 2012.
(Cackovic Hall)

- 08:30 - 09:30** **Poster Walk**
- 09:30 - 10:00** **PhD Day Opening**
- 10:00 - 11:00** **PhD Programs in Europe: European Standards and Example of Excellence** (Chairs: Ana Borovecki and Zdravko Lackovic)
- Jadwiga Mirecka (Krakow): ORPEHUS-AMSE-WFME European Standards for PhD in Biomedicine and Health Sciences.
 - Robert Harris (Stockholm): *PhD program at Karolinska Institutet - problem of evaluation*
- 11:00 - 11:20** **Coffee Break**
- 11:20 - 11:50** **Research Excellence Lecture** (Chair: Drago Batinic)
- Roland Jonsson* (Bergen): The complexity of Sjögren's syndrome: novel aspects on pathogenesis
- 11:50 - 12:30** **Selected Oral Presentations** (Chairs: Ante Tvrdeic and Marko Jakopovic)
- Marijana Skledar: Association between brain-derived neurotrophic factor val66met and obesity in children
 - Barbara Barun: Serologic markers of sleep disorders in patients with multiple sclerosis
 - Katja Domic: Congenital adrenal hyperplasia due to 21-hydroxylase deficiency in Croatia - clinical and molecular data
 - Iva Pejnovic Franelic: Affinity of first year university students towards gambling and betting
- 12:30 - 13:30** **Lunch Break / Poster Viewing**
- 13:30 - 15:30** **Round Table: Organization and Development of PhD Programs in Croatia and Europe**

In the Hall: Coordinators: Zdravko Lackovic and Robert Likic (School of Medicine), Participants: Ignac Lovrek (University of Zagreb), Roland Jons-

son (University of Bergen), Jadwiga Mirecka (Jagiellonian University Medical College, Krakow)

Web-cast: Michael Mulvany (University of Aarhus), Ingeborg van der Ploeg (Karolinska Institutet, Stockholm)

15:30 - 17:30

Organized Poster Sessions with Discussion (signing of indexes)

***Roland Jonsson** (Bergen, Norway) Broegelmann Chair in Immunology, Director of Bergen Research School in Inflammation (BRSI), Deputy Head (research) of the Gade Institute, Consultant Haukeland University Hospital. Member of the ORPHUES Executive Committee. Grant reviewer and Advisory Committees for the European Commission. Editor-in-Chief of *Scandinavian Journal of Immunology*. One of the world leading immunologists (ISI - H-index = 40; > 6300 citations: worldwide no. 3 in Sjögren's syndrome and no.1 in sialadenitis research according to biomedexperts.com). Elected member of the Norwegian Academy of Science and Letters.

I.

**RESEARCH
ABSTRACTS**

INCIDENCE AND OUTCOME OF VENTILATOR-ASSOCIATED PNEUMONIA IN PERCUTANEOUS TRACHEOTOMISED PATIENTS

Part of Thesis: Correlation Between Percutaneous Tracheotomy and Ventilator-Associated Pneumonia in Surgical and Neurosurgical Patients

PhD candidate: Tihana Magdić Turković, MD

Mentor: Professor Mladen Perić, MD, PhD

Affiliation: University Hospital „Sestre Milosrdnice“

INTRODUCTION: Ventilator-associated pneumonia (VAP) is the most common infection in Intensive Care Units. The most studies reported that tracheotomy was risk factor for VAP, but no studies have compared VAP incidence after tracheotomy with VAP incidence before tracheotomy. Aims of this study was to show that most VAPs occur before a performing a tracheotomy and to compare mortality between percutaneous tracheotomised VAP patients and intubated VAP patients.

PATIENTS AND METHODS: The study was conducted in 15-bed surgical and neurosurgical Intensive Care Unit of Department of Anesthesiology and Intensive Care at University Hospital „Sestre Milosrdnice“ in Zagreb. All patients requiring mechanical ventilation longer than 48 hours during ICU stay were eligible for the study. VAP was defined as type of nosocomial pneumonia occurring more than 48 hours after patients have been intubated and received mechanical ventilation. The clinical diagnosis of VAP was defined by Modified Clinical Pulmonary Infection Score.

RESULTS: These are preliminary results of prospective 3-years study (involve period from October 2009 until February 2012). During the study period, 3535 patients were admitted to our ICU. 312 (8.8%) of these patients received intubation and mechanical ventilation longer than 48 hours. Among all patients mechanical ventilated for longer than 48 hours VAP occur in 94 (30.1%) patients. Incidence of VAP among percutaneous tracheotomised patients was 51%, but most of these VAPs occur before performing a tracheotomy (83,7% versus 16,3%).

Also the mortality was significantly lower in percutaneous tracheotomised VAP patients (regardless VAP occurred before or after performing a tracheotomy) than in intubated VAP patients (26,5% versus 56,3%).

DISCUSSION: The incidence of ventilator-associated pneumonia reported in literature varies widely from 10 to 65%. In our study we found an incidence of VAP among percutaneous tracheotomised patients of 51%, but 83,7% of these developed VAP before tracheotomy, so the tracheotomy can't be risk factor for these VAPs. Incidence of VAP after tracheotomy among percutaneous tracheotomised patients according our results is 16,3%, what is very low.

Also according to comparison between mortality among percutaneous tracheotomised VAP patients and intubated VAP patients tracheotomy actually contributes to better survival.

MeSH/Keywords: pneumonia, tracheotomy

REINKE'S CRYSTALS IN MEN WITH CRYPTORCHIDISM

Part of a thesis: Reinke's crystals in men with cryptorchidism

PhD candidate: Viviana Kozina, M.Ed.Biol.

Mentor: Prof. Dr. Davor Jezek, M.D., Ph.D.

Affiliations: University of Zagreb, School of Medicine, Department of Histology and Embryology

INTRODUCTION: Leydig cells are situated within the interstitium of human testis. Depending on their position, they are classified as peritubular or perivascular cells. Both produce Reinke's crystals (RC). The hypothesis of this research is that in the patients with cryptorchidism number of RC is increased in the whole organ. Since the damaged function Leydig cells tends to offset with mechanisms such as hyperplasia and hypertrophy, the assumption is that their number in the tissue unit volume is increased too. The aim of this research is to investigate the characteristics of Reinke's crystals in men (age 20 to 30) with cryptorchidism.

MATERIALS AND METHODS: 20 biopsies from infertile patients diagnosed with cryptorchidism and six biopsies from men with regular spermatogenesis (20-30 years.) were used. Sections of the testis tissue were stained with haematoxylin and eosin and stained with a modified Masson's method. Specimens were observed by bright field, confocal and transmission electron microscopy (TEM). The number of Reinke's crystals in investigated groups was determined applying stereological methods.

RESULTS: In both groups, Reinke's crystals were noted within the cytoplasm and nuclei of Leydig cells. Some "free" crystals were found within the interstitial space, outside Leydig cells. Confocal microscopy proved to be very useful in the assessment of the shape and 3D reconstruction of the crystal. TEM analysis confirmed a hexagonal form of the crystal, while crystallographic data on sections of 70-300 nm thickness provided a better insight into the organization of the crystal lattice. Stereological analysis revealed a significant increase in the number of crystals in cryptorchid testes when compared with controls.

DISCUSSION: Increased number of crystals in cryptorchid specimens leads to the assumption that the prolonged exposure to higher (abdominal) temperature might stimulate enzymes involved in the synthesis of the proteins of the crystal. Because of the higher number of Reinke's crystals, this group of patients was selected for the purpose of studying the crystal's morphology and number. Clear visualization of Reinke's crystals plays an important role in the diagnosis of tumors of Leydig cells as they are pathognomonic for these type of neoplasms.

MeSH / Keywords: human testis, Reinke's crystals, cryptorchidism, stereology

LONG TERM CONSEQUENCES AFTER GESTATIONAL DIABETES MELLITUS

Part of a Thesis: The Influence of Previous Gestational Diabetes Mellitus on Long Term Health of Mother and Child

PhD candidate: Josip Juras, MD

Mentor: Professor Marina Ivanišević, MD, PhD

Affiliations: University of Zagreb School of Medicine, University Hospital Centre - Zagreb, Department of Obstetrics and Gynecology

INTRODUCTION: Prior gestational diabetes (GDM) is a risk factor for later development of pathological glucose metabolism and the incidence of later development of diabetes mellitus (DM) is higher in women with prior gestational diabetes. Apart from pathological glucose metabolism, more often pathological levels of cholesterol, triglycerides and blood pressure are found, that along with greater waist circumference makes the base of metabolic syndrome.

MATERIALS AND METHODS: This research included women with previous gestational diabetes according to WHO criteria from 1999. Detailed anthropometry and biochemical laboratory tests of mother were performed 6 months (n=23), 1 (n=56), 2 (n=59) and 5 (n=23) years after pregnancy (4 groups). Insulin, C-peptide, adipocytokines and inflammatory factors were measured in serum. We used HOMA 2 calculator for calculation of insulin resistance (IR) and IDF criteria for the diagnosis of metabolic syndrome.

RESULTS: There was no difference in age (H=6,418; P=0,093) and BMI between aforementioned groups (H=0,792; P=0,851). Women in the 5 years after GDM group had highest insulin resistance (H=17,007; P<0,001). This group had the highest frequency of impaired glucose tolerance and DM type 2. We found small positive correlation coefficient between IR and years from delivery to examination ($r_p=0,287$; P<0,001). The frequency of metabolic syndrome was 43% in general, and was positively correlated to the years passed from GDM. Women with metabolic syndrome had lower concentration of adiponectin and higher concentrations of leptine, PAI-1, C-peptide, glucose in plasma along with higher IR. The IR ($r_p=0,406$; P<0,001) and BMI ($r_p=0,574$; P<0,001) were both positively correlated to metabolic syndrome. Women with metabolic syndrome had higher concentrations of triglycerides, LDL and HbA_{1c} and lower HDL. These women had higher waist to hip ratio.

DISCUSSION: We can claim that these expectedly changed concentrations are correlated to anthropometrical findings of mother, having influence on the development of long term consequences to health. Women with previous GDM have adverse long term consequences such as higher frequency of impaired glucose tolerance and DM type 2, metabolic syndrome and pathological findings of adipocytokines and lipids. All these findings align women with previous GDM in the group with great risk of cardiovascular diseases.

MeSH / Keywords: gestational diabetes mellitus, diabetes mellitus, metabolic syndrome, insulin resistance, adipocytokines

DETECTION OF THE RESIDUAL DISEASE IN MULTIPLE MYELOMA PATIENTS

Part of a Thesis: new serum test for multiple myeloma patients

PhD candidate: Josip Batinić, MD.

Mentor: Professor Damir Nemet, MD, PhD.

Affiliations: University of Zagreb, School of Medicine, University Hospital Center Zagreb

INTRODUCTION: Although diagnostics and monitoring of patients with multiple myeloma are very well defined, the significance of some laboratory tests is still unclarified. The aims of this research are to determine and reexamine the value of the diagnostics and monitoring methods - especially of immunofixation, concentration of free light chains and immunophenotypization - further more, to introduce and examine the value of the method of quantitative identification of heavy chains of monoclonal immunoglobulins of the class IgA, IgG, IgM in serum (Hevylite) as a new method and, finally, to determine the interrelations between standard and more recent methods.

MATERIALS AND METHODS: The research is going to be carried out on two groups of patients suffering from multiple myeloma. In the first group we intend to determine the value of the new test and multiparameter flow cytometry immunophenotyping in staging of the disease. In the second group we intend to monitor the patients undergoing therapy using the mentioned methods in order to establish their oscillations during therapy thus determining their potential to predict response and early relaps, and possibly their prognostic value. Analysis will be performed at the Department of Laboratory Diagnostics at the University Hospital Center Zagreb, disease assesment by standard procedures: serum protein electrophoresis, immunofixation of serum, serum free light chain levels and their ratio, quantitative immunoglobulines, with addition of Hevylite test; tumor mass will be assessed by bone marrow analysis: morphology, multiparameter flow cytometry immunophenotyping (CD38, CD138, CD45, CD117, CD56, CD28), cytogenetics and FISH analysis.

RESULTS: We performed Hevylite analysis on 40 patients in the first group and preliminary results show that the new test corellates well with standard tests and that has at least the same sensitivity to detect monoclonal immunoglobulines. Especially interesting were results for patients who have normal values of immunoglobulines by standard tests, but abnormal results by Hevylite test; true value of these findings remains to be investigated.

DISCUSSION: We believe that, by combining the above mentioned methods, we will be able to understand the disease better, define different disease stages more precisely, which could result in new prognostic factors and different treatment strategies.

Acknowledgments: I would like to thank: Professor Danica Matišić and her staff, Professor Drago Batinić and his staff from the Department of Laboratory Diagnostics at the University Hospital Center Zagreb who perform serum and bone marrow analysis; The Binding Site Group Ltd. for donating the Hevylite assays; doctors and nurses at the Division of Hematology, Department of Internal Medicine, University Hospital Center Zagreb. MeSH /

Keywords: multiple myeloma, minimal residual disease, immunoglobuline heavy chain, immunophenotyping.

PLASMACYTOID DENDRITIC CELLS, EFFECTOR T-LYMPHOCYTES AND INFLAMMATORY CYTOKINES IN GRAFT-VERSUS-HOST DISEASE

Part of a PhD thesis „Plasmacytoid Dendritic Cells, Effector T-Lymphocytes And Inflammatory Cytokines In Graft-Versus-Host-Disease“

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INTRODUCTION: Graft-versus-host disease (GvHD) is the most frequent complication of the allogeneic stem cell transplantation (allo-SCT). Acute GvHD is usually observed within the first 100 days after allo-SCT and the chronic GvHD (cGVHD) normally occurs after day 100. In the pathophysiology of GvHD, myeloid dendritic cells were held responsible for the antigen-presenting phase and CD4+ Th1 cells for the effector phase of GvHD. Recently, populations of plasmacytoid dendritic cells (pDC) and CD4+ Th17 cells were found to be important in autoimmune diseases. These populations haven't been studied in GvHD yet and the aim of this research was to evaluate the role of pDC in the antigen-presenting and Th17 in the effector phase of GvHD.

MATERIALS AND METHODS: Blood samples were taken at day 100 after allo-SCT. Number and function of pDC and Th17 as well as the concentration of serum interleukins was analyzed in 79 patients in order to evaluate their relation to development and grade of aGvHD and cGVHD.

RESULTS: We observed a significant decrease of pDC in the blood of patients with grade 2-4 GvHD as compared to patients with grade 0-1 aGvHD. We also observed a trend of decrease of pDC in the blood of patients who later on developed cGVHD in comparison to patients who did not although it did not reach statistical difference. Moreover, we observed that Th17 cells were significantly decreased in the blood of patients with 2-4 aGvHD when compared to patients with grade 0-1 aGVHD. We also saw a significant decrease in the number of Th17 cells in patients who developed extensive cGVHD after day 100 compared to patients without or with limited cGVHD. Finally, we found eight cytokines to be significantly correlated with the risk of development of cGVHD.

DISCUSSION: This study provides evidence for a role of both pDC and Th17- mediated responses and a potential new pathophysiological link between PDCs and Th17 in human acute and chronic GvHD. Better understanding of the pathophysiology of GvHD might lead to the finding of specific molecules in GvHD and pave the way for new targeted therapies in preventing and controlling GvHD.

Keywords: allo-SCT, GvHD, plasmacytoid dendritic cells, Th17 subpopulation

SUPERSONIC SHEAR IMAGING ELASTOGRAPHY FOR CHARACTERIZATION OF LIVER TUMORS - PRELIMINARY FINDINGS

Part of a Thesis: Characterization of liver tumors using quantitative sonoelastography

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INTRODUCTION: Supersonic shear imaging (SSI) is a new quantitative elastography technique for measuring tissue stiffness. Studies with other elastography-based imaging methods have demonstrated some differences in stiffness with respect to tumor etiology. The aim of this study was to investigate the possibility of using SSI to differentiate between various liver tumors.

MATERIALS AND METHODS: Supersonic shear imaging (SSI) is a new quantitative elastography technique for measuring tissue stiffness. Studies with other elastography-based imaging methods have demonstrated some differences in stiffness with respect to tumor etiology. The aim of this study was to investigate the possibility of using SSI to differentiate between various liver tumors.

RESULTS: Three groups of liver tumors were investigated: 1) hemangiomas, 2) primary malignant hepatic tumors and 3) metastases. After obtaining good quality B-mode ultrasound images the stiffness was measured five times for each tumor, and the mean value was taken for the analysis. The nature of the tumors was defined through a standard diagnostic workup according to current guidelines, including contrast enhanced multi-slice CT, MRI and/or cytology/histology, as applicable. Mean tumour stiffness for each group was calculated as well as cut-off values between the groups.

DISCUSSION: In this preliminary study it was possible to differentiate between the hemangiomas and malignant liver tumors by using SSI. According to zhis preliminary results the assesment of tumor stiffness by this quantitative elastography method might potentially reduce the need for additional diagnostic workup for liver hemangiomas. Differentiation between the primary malignantant liver tumors and metastases was not possible with SSI.

MeSH / Keywords: supersonic shear imaging, sonoelastography, liver tumours

POSITIVE CORRELATION BETWEEN INTERSTITIAL FIBROSIS AND LEFT ATRIAL REMODELING IN PATIENTS WITH MITRAL REGURGITATION AND ATRIAL FIBRILLATION

Part of a Thesis: The Effect of the Left Atrial Myocardial Histologic Changes on Reverse Structural Remodeling in Patients Surgically Treated for Mitral Regurgitation

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Mentor: Associate Professor Hrvoje Gašparović, MD, PhD, FETCS

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INTRODUCTION: Mitral regurgitation (MR) and atrial fibrillation (AF) lead to left atrial (LA) enlargement. MR generally progresses insidiously, because the heart compensates for increasing regurgitant volume by left-atrial enlargement. AF induces a series of structural changes that result in left atrial remodeling. Fibrosis is the hallmark of histological changes. The purpose of this study is to determine the correlation between left atrial histological changes and its structural remodeling in patients with MR and AF.

MATERIALS AND METHODS: The study consisted of 20 elective consecutive MR and permanent AF cardiac surgery patients. From all consented patients a sample of the left atrial myocardium was obtained intraoperatively. The samples were stained with hematoxylin and eosin, Mallory's trichrome and immunohistochemically using an anti-Bak antibody (DAKO Corp. A/S, Glostrup, Denmark) and quantified for fibrosis, cardiomyocyte hypertrophy, cardiomyocytolysis and apoptosis using the semiquantitative model (0-3=absent-mild-moderate-severe). Intraoperative transesophageal echocardiography was used to evaluate for the LA volume (Vmin, Vmax) and area (Amin, Amax). The data are presented as mean values \pm standard deviation.

RESULTS: The average LA volume, area, and the expression of interstitial fibrosis were as following: Vmin $81,3 \pm 60,9$ cm², Vmax $118,2 \pm 74,7$ cm², Amin $20,5 \pm 10,8$ cm², Amax $26,9 \pm 12,1$ cm², fibrosis $2,1 \pm 0,8$. We observed a positive correlation between the expression of interstitial fibrosis and LA structural remodeling (Vmin $r=0,537$ $p=0,039$, Vmax $r=0,516$ $p=0,049$, Amin $r=0,561$ $p=0,029$, Amax $r=0,528$ $p=0,043$). Cardiomyocytolysis, apoptosis and cardiomyocyte hypertrophy had a positive correlation as well, but not significant. A negative correlation between the left ventricular ejection fraction and the degree of cardiomyocytolysis ($r=-0,549$ $p=0,034$) was also observed.

DISCUSSION: Our results show that MR and AF lead to LA remodeling expressed as structural and ultrastructural changes. As expected remodeling severity influences the expression of interstitial fibrosis. Although yet we have not found statistically significant prediction on the immediate post-operative LA function we expect such results after a 6-12 month of follow up.

Acknowledgements: I would like to acknowledge the continued assistance of all the staff at the Department of Pathology and Cytology, University Hospital Centre Zagreb. Also, I would like to thank Ms. Ankica Ajduković the director of A & B d.o.o. for her grant to the research.

Keywords: mitral valvular disease, mitral regurgitation, left atrial remodeling, left atrial reverse remodeling, atrial fibrillation.

CORRELATION OF ECP (EOSINOPHIL CATIONIC PROTEIN) WITH SEVERITY OF CAROTID ATHEROSCLEROSIS - PRELIMINARY RESULTS

Part of a Thesis: Correlation of ECP (eosinophil cationic protein) with severity of carotid atherosclerosis and all-cause mortality in patients on hemodialysis

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Mentor(s): Prof. Velimir Božikov MD PhD, Prof. Željko Romić MPharm. PhD

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INTRODUCTION: Prevalence of cardiovascular diseases in hemodialysis patients is high. It is considered that the cause for accelerated atherosclerosis lies in the „new“ risk factors like hsCRP, homocysteine, lipoprotein (a), inflammation and oxidative stress. Eosinophil cationic protein (ECP) is zinc containing inflammation protein which is normally found in the eosinophil granules. According to the recent studies concentration of ECP is proportional to the growth of atherosclerotic plaque in the coronary vessels, and shows that ECP is a biomarker of coronary atherosclerosis. The aim of this study is to evaluate a possible correlation between serum ECP levels and carotid intima-media thickness and plaque score in patients on hemodialysis.

MATERIALS AND METHODS: One hundred hemodialysis patients (52% males, mean age 67+/-14) and 30 healthy subjects matched were included in the study. Serum eosinophil cationic protein levels were measured using highly specific and sensitive fluoro-enzyme-immunochemistry method (FEIA) (UniCap, Phadia, Uppsala, Sweden). Carotid intima-media thickness and plaque score were measured by high-resolution B-mode ultrasonography.

RESULTS: Compared to control subjects, HD patients had significantly increased carotid intima-media thickness and plaque score ($p < 0,007$). In HD patients a significant correlation was found between serum ECP and plaque score ($r = 0,57$, $p < 0,001$).

DISCUSSION: Our findings suggest that eosinophil cationic protein could be associated with the severity of carotid atherosclerosis in hemodialysis patients. Furthermore, it may indicate that ECP is important to the formation of atherosclerosis in HD patients.

Acknowledgements: The author acknowledges financial support from Jadran Galenski Laboratorij d.o.o.

MeSH / Keywords: eosinophil cationic protein, carotid atherosclerosis, hemodialysis, intima-media thickness

OPERATIVE MORTALITY IN RUPTURED ABDOMINAL AORTIC ANEURYSMS - DEVELOPMENT OF A PREDICTION MODEL

Part of a Thesis: Data Mining in Development of Prediction Model for Operative Mortality in Ruptured Abdominal Aortic Aneurysms

PhD candidate: Tomislav Meštrović, MD, MrSc

Mentor(s): Associate Professor Mladen Petrunić, MD, PhD; Associate Professor Zdenko Sonicki, MD, PhD

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INTRODUCTION: Abdominal aortic aneurysm rupture (rAAA) is associated with high operative mortality (50%). It is therefore important to recognize the factors that influence the operative mortality and to develop the prediction model. Existing prediction models, predominantly based on statistical methods, are either too complex or of limited accuracy. By combining data mining with statistical methods, a model of better predictive characteristics and applicability in surgical practice, is expected to be developed.

MATERIALS AND METHODS: Variables related to operative mortality will be obtained by retrospective analysis of medical records of patients operated for the rAAA between 1996. and 2010. in University Hospital Centre Zagreb. 100-150 records are expected to be harvested. Data mining and statistical methods will be used for identification of factors associated with the operative mortality and for developing prediction model. Predictive characteristics of the obtained model will be evaluated and compared with the existing ones.

RESULTS: To date, 18 published articles that define and/or review prognostic scoring models for rAAA have been scrutinized and accepted as references. Data from medical records of 21 operated patients were harvested and analysed using statistical methods as well as data mining methods. Out of 21 analysed patients, 9 died (42.86%), all of them requiring postoperative haemodialysis. In contrast, all patients with normal renal function survived, and the difference was statistically significant on univariate analysis (Chi Square, $p < 0.05$). A decision tree generated by J48 data mining algorithm enabled further classification of patients with transient renal insufficiency according to their ASA-status, with area under receiver operating characteristic curve of 0.86, which is comparable to, or better than in previously reported models.

DISCUSSION: Preliminary results are promising in terms of generating a novel prediction model for operative mortality of rAAA. Further harvesting and analysis of medical records will be done, by combining multivariate statistical analyses with data mining techniques, to generate a definitive prognostic model. Apart from improving prediction of operative mortality in rAAA, results of this study could contribute to methodology of medical data analysis in situations with multiple complex predictive factors.

MeSH / Keywords: prediction, model, rupture, aneurysm, abdominal, aorta.

ARTIFICIAL DISC IN SURGICAL TREATMENT OF THE SINGLE LEVEL CERVICAL DISC DISEASE

Part of a Thesis: Arthroplasty will show better results comparing clinical and radiologic outcome then ACDF in surgical treatment of the single level cervical disc disease

PhD Candidate: Marjan Rožanković, MD

Menthor: Professor Miroslav Vukić, MD, PhD

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INTRODUCTION: Anterior cervical discectomy and fusion (ACDF) is still the gold standard for surgical treatment of cervical spine degenerative disease. However, results of many studies suggest that it may cause degenerative changes at levels immediately above and below the fusion, known as adjacent segment degenerative disease (ADD). Cervical arthroplasty has recently been introduced as an alternative to standard procedure of ACDF. Its advantages include maintenance of normal spinal motion following anterior cervical discectomy and avoidance of the abnormal kinematic stresses with adjacent segment degeneration. Furthermore, it showed decreased surgical morbidity, decreased complications from postoperative immobilization and an earlier return to previous level of function.

MATERIALS AND METHODS: Seventy patients with single-level cervical disc disease producing radiculopathy were divided into two groups to undergo ACDF or arthroplasty. All patients were evaluated with pre- and postoperative serial radiographic studies and clinically, using The Neck Disability Index (NDI), Visual Analog Scale (VAS) and neurological status at 3, 6, 12 and 24 months, respectively.

RESULTS: The results of our study indicate that cervical arthroplasty using Discover Artificial Cervical Disc provides favorable clinical and radiological outcomes in follow up period of 24 months. There has been significant improvement in clinical parameters, VAS and NDI, at 3, 6, 12 and 24 months in arthroplasty group comparing to control group. Dynamics of changes in VAS and NDI scores are highly significant among both groups ($P < 0,001$). When this dynamics is compared between groups, there were significant differences regarding artificial disk and ACDF groups among postoperative neck VAS levels: ACDF group had higher VAS scores in all repeated measures (ANOVA for repeated measures, $F=2,78$, $P=0,034$)

DISCUSSION: Our study shows, for the first time, the efficacy of Discover Cervical Disc arthroplasty in clinical and radiographic parameters after a two year follow-up. There was statistically significant improvement in the VAS score and NDI postoperatively in all patients underwent arthroplasty and in comparison to the ACDF. The Discover artificial cervical disc replacement offers favorable outcome compared to ACDF for a single-level cervical disc disease at short- and long-term follow-up.

MeSH / Keywords: ACDF, cervical disc disease, arthroplasty, Discover artificial cervical disc.

BLEEDING RISK ASSESSMENT USING MULTIPLE ELECTRODE AGGREGOMETRY IN PATIENTS FOLLOWING CORONARY ARTERY BYPASS SURGERY: A PROSPECTIVE STUDY

Part of a Thesis: Perioperative Bleeding Risk Assessment Using Impedance Aggregometry And Thromboelastometry In Patients Following Cardiac Surgery

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Mentor: Bojan Biočina, M.D. , PhD

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INTRODUCTION: Individual variability in the response to antiplatelet therapy (APT), frequently administered preoperatively, has been established by various platelet function assays and could reflect bleeding tendency after coronary artery bypass surgery (CABG). Our hypothesis is that multiple electrode whole-blood aggregometry (MEA) can identify patients at risk for excessive bleeding.

MATERIALS AND METHODS: We enrolled 211 patients (155 male and 56 female) undergoing isolated CABG in a prospective observational study. Patients were divided into 4 groups with respect to their preoperative APT management. MEA, using the ASPI and the ADP test, was performed prior to surgery. The primary endpoint was chest tube output and the secondary endpoint was perioperative packed red blood cell concentrate (PRBC) administration. Patients were characterized as bleeders if their 24 hour chest tube output exceeded the 75th percentile of distribution.

RESULTS: Significant differences in both the ASPI ($p < 0.001$) and ADP test values ($p = 0.038$) were observed among APT groups. The proportion of patients characterized as bleeders differed significantly among the groups with respect to preoperatively administered APT ($p = 0.039$). Significant correlations between the ASPI test ($r = -0.170$, $p = 0.014$) and ADP test ($r = -0.206$, $p = 0.003$) with 24 hour chest tube output were found. The receiver operating curve revealed an ASPI test value of < 20 area under curve units (AUC) (AUC 0.603, $p = 0.023$) and an ADP test < 73 AUC (AUC 0.611, $p = 0.009$) as a „bleeder” determinant. 161 patients (76.3%) received PRBC. Comparison of the ASPI test values between patients with respect to PRBC administration revealed lower values in the ASPI test in a group of patients transfused with PRBC (mean, 27.88 vs. 40.32 AUC, $p = 0.002$).

DISCUSSION: Our study showed that MEA is a useful method of predicting CABG patients with excessive postoperative bleeding. Prediction of excessive bleeding along with haemostatic interventions on the basis of MEA should be evaluated in the context of the clinical outcome.

MeSH / Keywords: Blood loss; Multiple electrode aggregometry; Platelet aggregation inhibitors; Coronary artery bypass surgery

ANALYSIS OF ANTIMICROBIAL DRUG USE AND MICROBIOLOGY FINDINGS AS A METHOD FOR SURVEILLANCE OF HOSPITAL INFECTION PREVALENCE IN A CLINICAL HOSPITAL - PRELIMINARY RESULTS

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INTRODUCTION: Healthcare associated infections (HAI) are infections that patient acquire during the course of treatment for other conditions within a healthcare setting. Each year in the European Union 4 million patients are affected with this form of infection and approximately 37 000 of them die as a direct result of the infection. Most common HAI affect urinary tract 30%, respiratory tract 21%, surgical site infections 15%, sepsis 6.5%, and other 27.5%.

MATERIAL AND METHODS: A retrospective study was performed from 1.7.-13.7.2010. in the University Hospital Centre Zagreb. All patients admitted to the hospital before 08:00 a.m. were included. For each patient a standard form was filled created by the European Centre for Disease prevention and Control (ECDC). An active infection was defined according to ECDC standards. Descriptive statistical, Pearson's chi-square test, Fischer's test were used in the statistical analysis.

RESULTS: In this study 1157 patients were included. HAI were identified in 33.55% of patients. Urinary tract infection was the most common infection (33.72%), than respiratory tract infection (20.35%), sepsis (12.79%), surgical site infection (11.63%), and other 21.51%. There were no differences in gender. Intern medicine was the department with most indentified HAI. 38.3% of patients were older than 60 years old. Surgery since admission had 47.4% of patients. According to McCabe score, 55.1% of patients had non-fatal disease, 41.1% had ultimately fatal disease, and only 3.8% had rapidly fatal disease. Peripheral vascular catheter had 67.4% of patients, central vascular catheter 28.5%, and urinary catheter 35.1%. Mechanical ventilation had 6.8% of patients. Antimicrobial drugs were given parenterally 82.7% and orally 17.3%. Microbiological swabs were taken in 33.1% case. Microbiological findings were positive in 60.33%. Most common isolated microorganism was Escherichia coli (15.56%). 85% of antimicrobial drugs were given in accordance with the guidelines of the University Hospital Centre Zagreb. The most frequently used antimicrobial drugs were cefazolin and ciprofloxacin.

DISCUSSION: These are preliminary results and further statistical analysis is required to assess the surveillance of hospital infection prevalence with antimicrobial drug use and microbiological findings, and their sensitivity and specificity.

Acknowledgments: I would like to thank Professor Smilja Kalenić and Dr. Zrinka Bošnjak for their great support, advice and help.

MeSH / Keywords: Healthcare associated infection, Hospital infection, Infection control, Surveillance

SEROTYPE DISTRIBUTION AND ANTIMICROBIAL RESISTANCE OF INVASIVE PNEUMOCOCCAL ISOLATES IN CHILDREN < 14 YEARS OF AGE IN CROATIA, 2005-2009

Part of thesis: Invasive pneumococci – serotype distribution and antimicrobial resistance in Croatia

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INTRODUCTION: Streptococcus pneumoniae is a leading cause of bacterial pneumonia, meningitis, and sepsis in children worldwide. The aim of this study was to assess serotype distribution and antibiotic resistance in pneumococci causing invasive infections in children <14 years of age in Croatia from 2005 to 2009.

MATERIALS AND METHODS: Invasive pneumococcal strains were collected through the microbiological laboratory network organised by the Croatian Committee for Antimicrobial Resistance Surveillance. Capsular typing was performed by the Quellung reaction (Statens Serum Institut, Copenhagen). In vitro susceptibility testing was performed by disc diffusion method according to CLSI guidelines. In strains with reduced susceptibility to penicillin (as detected by oxacillin screen disk), MIC for penicillin was determined (E-test, Biomerieux, France).

RESULTS: A total of 192 invasive pneumococcal isolates (186 from blood and 10 from CSF) were isolated in children < 14 years of age between 2005 and 2009. The most prevalent serotypes were 14 (45 isolates), 18C (26 isolates), 6B and 23F (24 isolates each) comprising 62% of all invasive pneumococcal isolates. Dominance of these serotypes was best seen in children 12- <60 months of age. Non-susceptibility to penicillin was 22% and isolates mostly belonged to serotypes 14 and 19A. Resistance to macrolides was 35% and isolates mostly belonged to serotypes 14, 19A and 6B.

CONCLUSIONS: Incidence of invasive pneumococci varies with child`s age and is the highest in children 12- <60 months. Non-susceptibility to penicillin and resistance to macrolides was mostly associated with serotypes 14 and 19A. Serotype 14 is covered by all the available vaccines whereas serotype 19A is covered by 13-valent vaccine only.

MeSH / Keywords: Streptococcus pneumoniae, invasive pneumococcal disease, serotyping, antimicrobial resistance, vaccine

EFFICACY OF LOW DOSE PAMIDRONATE IN PREVENTION OF BONE LOSS AFTER RENAL TRANSPLANTATION

Part of a Thesis: Efficacy Of Pamidronate In Prevention Of Bone Loss After Renal Transplantation

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INTRODUCTION: Bone loss in kidney transplant recipients presents a major clinical problem since it adds on already present bone osteodystrophy. Additional bone loss is mostly caused by immunosuppressive therapy (corticosteroids) which follows transplantation. It has been shown that bone loss is most significant during the first 12 months after renal transplantation (RT).

Bisphosphonates are medicines that reduce bone loss and are effective in treatment of diseases that affect bone metabolism. There are some studies reporting that bisphosphonates attenuate bone loss in kidney transplant recipients. However, no consensus has been reached on dosage, initiation and duration of the treatment.

In this study our aim is to test the effectiveness of low dose pamidronate (in order to limit adverse effects such as adynamic bone disease and nephrotoxicity) in bone loss prevention during the first year after renal transplantation.

MATERIALS AND METHODS: In this study two groups of renal transplanted patients were followed for 12 months. First group received dose of 30mg of pamidronate (n=17) after normalisation of kidney graft function (mean 34.4 days) and at three months after RT. Second group (n=14) was not treated with pamidronate. We measured bone density for all patients at lumbar spine, femur and distal radius. Bone density was measured by dual-energy X-ray absorptiometry (DXA) at RT, 6 and 12 months after RT. Biochemical determinations were performed at regular intervals during follow up, at RT, three, six and twelve months after RT.

RESULTS: Pamidronate significantly reduced bone loss at lumbar spine and femur. Both groups were comparable at the beginning of the study regarding age, weight, sex and initial bone density. During the follow up, according to creatinine levels graft function was comparable in both groups without noticeable bisphosphonate nephrotoxicity. Twelve months after RT all patients had functioning renal grafts. PTH levels were comparable in both groups during the whole study.

DISCUSSION: Low dose of pamidronate in early post renal transplantation period prevents bone loss at lumbar spine and femur without adverse effect on renal function.

Acknowledgements: I would like to thank to professor Zvonimir Mareković for thorough help during my work on this thesis.

MeSH / Keywords: renal transplantation, bisphosphonates, densitometry, corticosteroids.

SEROLOGIC MARKERS OF SLEEP DISORDERS IN PATIENTS WITH MULTIPLE SCLEROSIS

Part of Thesis: Serum Inflammatory Factors as Markers of Daily Somnolence/ Fatigue in Patients with Multiple Sclerosis

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INTRODUCTION: Multiple sclerosis (MS) is inflammatory disorder of central nervous system characterized with cytokine misbalance in favor of pro-inflammatory, somnogenic cytokines. 25-62% MS patients have sleep disorders which is correlated with fatigue, the most common symptom of MS. Objective of this study is to determine the level of IL-12, IL-17, leptin, MCP-1, TGF β , VEGF, E-selectin, VCAM, BDNF, TNFR1, GM-CSF, INF - gamma, IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, TNF- alpha in MS patients and healthy controls and their association with clinical parameters.

MATERIALS AND METHODS: Serum inflammatory markers were measured by Luminex in 56 patients and 32 healthy controls. Clinical parameters assessed included sleep quality and sleepiness (Polysomnography, Multiple Sleep Latency Test, Epworth Sleepiness Scale, and Pittsburgh Sleep Quality Index), fatigue (Fatigue Severity Scale Multidimensional Fatigue Inventory (MFI), quality of life (Medical Outcomes Study Short Form-36), pain (Pain visual analog scale) and depression (Center for Epidemiological Studies-Depression Scale). Multinomial logistic regression was used to assess group differences. Pair t-tests were used for pre- port analysis.

RESULTS: MS patients with sleep disorders (MS-SD, N=39) had significantly higher levels of leptin (p=0.045) and soluble E-selectin (p=0.075) and lower levels of IL-1 β (p=0.036), IL-4 (p=0.043), IL-5 (p=0.043), and IL-8 (p=0.035) comparing to MS patients without sleep disorders (MS-NSD, N=17) and healthy controls (HC, N=32). MS-NSD had lower levels of GM-CSF (p=0.039), IL-4 (p=0.036), IL-5 (p=0.041); IL-8 (p=0.053); VCAM (p=0.068) comparing to HC.

DISCUSSION: Results obtain from this study might help to untangle complex pathophysiological interplay between immune disruption of MS and sleep disorders. Elevated leptin and E-selectin levels observed only in MS patients with sleep disorders implies their importance as markers of sleep disorders per se. Given the proinflammatory propensity of leptin, treatment of sleep disorders might alleviate symptoms of multiple sclerosis which is to be proven in future prospective studies. Furthermore, lower level of anti-inflammatory and anti-somnogenic markers (IL-8, IL-5, IL-4) in MS-NSD than in HC group points that besides immunological misbalance other factors have important role in development of sleep disorders in MS. Finally, distinctive markers from this study might help future research in selection from plethora of putative markers.

Acknowledgments: I would like to thank to Professor Vesna Brinar for her support throughout the whole period of this research and also to the both of my mentors for enormous patientce and understanding.

MeSH / Keywords: multiple sclerosis, sleep, fatigue, obstructive sleep apnea, cytokines

EVALUATION OF NON-MOTOR SYMPTOMS AND EXECUTIVE FUNCTIONING IN PATIENTS WITH CERVICAL DYSTONIA

Part of a Thesis: Implication of Local, Intramuscular Application of Botulinum Toxin on Executive Functions and Psychiatric Comorbidities in Patients with Cervical Dystonia

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Mentor: Professor Maja Relja, MD, PhD

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INTRODUCTION: Cervical dystonia (CD) belongs to group of primary focal dystonias, which by definition do not have underlying neurodegeneration and are unaccompanied by other neurologic abnormalities. Researchers conducted so far were mainly focused on predominant motor symptoms of dystonia while accompanied non-motor disorders were often being disregarded. In addition, studies examining cognition in CD patients are missing. Botulinum toxin (BTX) application represents first line treatment for CD. BTX cleaves SNAP-25 protein required for vesicle docking and fusion with the plasma membrane, thus preventing acetylcholine release into the synaptic cleft. It was thought that BTX only has local, peripheral effect. However, several neurophysiological studies provided evidence for central effect of BTX due to retrograde axonal transport. Relationship between central effects of BTX and various nonmotor features of dystonia remains unrevealed.

MATERIALS AND METHODS: Up to now, our investigation included 29 patients with CD and 36 healthy controls (age and education matched). None of the CD patients were previously exposed to anticholinergic medications. All participants were psychiatrically assessed with validated scales: Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI), Starkstein Apathy Scale (AS), Fatigue Severity Scale (FSS) and Pittsburgh Sleep Quality Index (PSQI). Executive functions were examined with computerized COGTEST battery set of 5 tests examining several cognitive domains: 'Auditory Number Sequencing', 'Spatial Working Memory', 'Strategic Target Detection', 'Continuous Performance Test - Flanker version' and 'Tower of London'. Global cognitive functioning was assessed with MMSE.

RESULTS: Our preliminary results showed significantly higher level of depression, anxiety and fatigue in patients with CD ($p < 0,05$). There was no statistical significance in sleep quality and apathy ($p > 0,05$) between CD patients and healthy controls. Control group scored better on verbal memory testing, spatial memory testing, cognitive flexibility and showed more sustained attention but without statistical significance. Reasoning and planning were statistically worse in CD patients in study conducted so far.

CONCLUSION: Psychiatric comorbidities are common in CD patients although etiology is unknown. Mild executive dysfunction, although statistically insignificant was also observed in CD patients. Investigation of possible impact of BTX application on nonmotor symptoms requires longer follow-up period with larger number of patients.

Acknowledgements: I would like to thank my mentor professor Maja Relja for support, guidance and inappreciable advices through every step of this study.

MeSH / Keywords: Cervical dystonia, botulinum toxin, cognition, nonmotor symptoms

EPCAM AND CONNEXIN 43 - ADDITIONAL PROGNOSTIC MARKERS FOR PROSTATE CANCER

Part of a Thesis: Prognostic Value of EpCAM and Connexin 43 Expression in Prostate Cancer

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Mentors: Davor Tomas, MD, PhD and Assistant professor Borislav Spajić, MD, PhD

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INTRODUCTION: The epithelial cell adhesion molecule (EpCAM) is a transmembrane glycoprotein that was originally identified as a marker for carcinoma and connexins (Cxs) are transmembrane proteins that build cell-to-cell channels in gap junctions. Several studies investigated the role of connexin43 (Cx43) in different carcinomas and EpCAM expression in prostate carcinoma, none investigated prognostic role of Cx43 in prostate cancer and none of them confirmed prognostic role of EpCAM. The aim of study was to investigate EpCAM and Cx43 expression and their relationship with established prognostic features in prostate carcinoma.

MATERIALS AND METHODS: The study included a cohort of 102 patients treated with radical prostatectomy for clinically localized prostate carcinoma. Immunohistochemistry was performed to evaluate the EpCAM and Cx43 expression in prostate cancer and non-neoplastic prostate tissue.

RESULTS: EpCAM expression was higher in prostate carcinoma ($P < 0.001$). EpCAM expression in prostate cancer was associated with established features indicative of worse prognosis (preoperative ($P = 0.009$) and postoperative ($P = 0.004$) Gleason score, follow-up time ($P < 0.001$)). Patients with higher preoperative and postoperative Gleason score and short follow-up time had tumors with higher expression of EpCAM. Negative correlation of follow-up time and EpCAM expression indicated that tumors in patients with biochemical recurrence (BCR) harbored higher EpCAM expression. Expression of EpCAM was higher in patients with BCR ($P < 0.001$). Tumors in T3 stage showed significantly higher EpCAM expression compared with T2 tumors ($P = 0.002$). Univariate ($P < 0.001$) and multivariate ($P < 0.001$) analyses showed that EpCAM expression was a significant predictor of shorter biochemical recurrence free-survival.

Cx43 expression in prostate cancer was associated with follow-up time ($P < 0.001$) and preoperative PSA ($P < 0.007$). Patients with lower Cx43 expressions in tumors have shorter follow-up time, shorter disease-free survival and higher preoperative PSA. Tumors with positive surgical margins ($P < 0.001$) showed lower Cx43. In univariate ($P < 0.001$) and multivariate ($P = 0.014$) analyses, decreased Cx43 expression was found to be a significant predictor of BCR free-survival.

CONCLUSION: Results confirmed high level of EpCAM expression and decreased Cx43 expression in prostate cancer supporting their potential role in prostatic cancer progression. EpCAM and Cx43 could serve as additional prognostic markers for the recognition of patients with an increased risk of disease recurrence that need introduction of secondary therapy.

Keywords: connexin 43, EpCAM, prognosis, biochemical recurrence free-survival

GLYPICAN-3 EXPRESSION IN HEPATOCELLULAR CARCINOMA IN PATIENTS WITH LIVER TRANSPLANTATION

Part of a Thesis: Expression of Glypican-3, Beta-catenin and CD34 in Hepatocellular Carcinoma in Patients with Orthotopic Liver Transplantation

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Mentor: Research Assistant Ana Borovečki, MD, PhD

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INTRODUCTION: The aim of this study is to investigate glypican (GPC)-3 immunohistochemical expression characteristics (positivity, localization, intensity) in association with clinicopathological data. Also to determine prognostic impact of GPC-3 expression, clinical and pathological characteristics, especially in the patients group meeting and group exceeding Milan criteria.

MATERIALS AND METHODS: Between 1998 and 2011, 71 patients with primary hepatocellular carcinoma (HCC) have undergone liver transplantation. Pathological tumor characteristics- histological type, differentiation grade and microvascular invasion, were analysed on the 4 μ m thick paraffin-embedded blocks sections. For the analysis of GPC-3 expression Tissue Microarray paraffin blocks with the Tissue-Tek Qiuck-Ray System (Sakura, Netherlands) were constructed (in each patient 3 mm tissue cores, two HCC and one adjacent non-cancerous tissue). The primary monoclonal anti-GPC3 antibody (clone 1G12, dilution 1:50, Cell Marque, Rocklin, USA) and the Kit EnVision FLEX, High pH (Dako Autostainer / Autostainer plus) were used. Cooking in the PTlink at 97°C for 20 minutes was performed as the antigen retrieval method. The GPC-3 staining in $\geq 30\%$ of tumor cells was considered as positive expression.

RESULTS: GPC-3 positive expression was found in 56% (40/71) of HCC. In mostly specimens 96% (68/71) the protein expression was localized in the cytoplasm, in 4% (3/71) circumferential membranous pattern was observed and in all specimens with strong intensity. GPC-3 positive expression was more frequently observed in tumors with present microvascular invasion ($p=0.008$), but no prognostic significance was found during the mean follow-up period of 589 days. There was neither correlation between GPC-3 expression and any of the analysed clinicopathological features nor prognostic significance. Also no difference was found in the overall survival between the patients groups meeting and exceeding Milan criteria.

DISCUSSION: More frequently observed GPC-3 positive expression in tumors with present microvascular invasion may indicate worse clinical behavior. According to this study results and literature data the correlation between GPC-3 expression, analysed clinicopathological characteristics and prognosis in HCC has not yet been clarified and further investigations are needed.

Keywords: glypican-3, hepatocellular carcinoma, liver transplantation

HUMAN EPIDERMAL GROWTH FACTOR RECEPTORS 1 AND 2 (EGFR/HER1 AND HER-2/NEU) STATUS IN INVASIVE APOCRINE CARCINOMA OF THE BREAST

Part of a Thesis: EGFR and HER-2/neu expression in apocrine carcinoma of the breast

PhD candidate: Semir Vranić, M.D.

Mentor(s): Professor Zoran Gatalica, M.D., D.Sc.

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INTRODUCTION: Apocrine carcinomas of the breast represent a rare subtype, constituting less than 5% of all breast cancers. We studied EGFR and HER-2/neu in apocrine breast carcinomas meeting strict morphologic and immunophenotypic criteria with regards to both protein expression and gene copy number.

MATERIALS AND METHODS: Fifty-five breast carcinomas morphologically diagnosed as apocrine, were evaluated for the steroid receptor expression profile characteristic of normal apocrine epithelium [androgen receptor-positive/estrogen receptor-negative/progesterone receptor-negative], and for the expression of EGFR and Her-2/neu proteins, and the copy number ratios of the genes EGFR/CEP7 and HER-2/CEP17. An extended cohort composed of 72 invasive ductal carcinomas of no-special-type were used for further exploration of CEP17 polysomy (defined as three or more CEP17 signals).

RESULTS: Pure apocrine carcinomas (ER-, PR-, AR+) (38 cases, 69%) are either HER-2 overexpressing breast carcinomas (52%) or triple-negative (ER-, PR-, Her-2-) breast carcinomas (48%). Apocrine-like carcinomas (ER+/-, PR+/-, AR+/-) (17 cases, 31%) belong predominantly to the luminal phenotype (76%). Pure apocrine carcinomas show consistent over-expression of either EGFR or Her-2/neu. EGFR gene amplification was observed in two pure apocrine carcinomas and one apocrine-like carcinoma. CEP7 polysomy (defined as three or more CEP7 signals) was seen in 61% pure apocrine carcinomas and 27% of apocrine-like carcinomas a showed a weak positive correlation with EGFR protein expression. HER-2/neu gene amplification is the primary mechanism of Her-2/neu activation and is found in 52% of all apocrine carcinomas. CEP17 polysomy was observed in 10 pure apocrine carcinomas (32%) and 8 apocrine-like carcinomas (50%). CEP17 polysomy may be seen without HER-2/neu gene amplification as confirmed on extended cohort of invasive ductal carcinomas. Increased CEP17 signals led to discordant interpretation of HER-2/neu gene status in 12 out of 33 cases (36%) harboring more than 6 copies of the HER-2/neu gene.

DISCUSSION: Breast carcinomas with apocrine differentiation are heterogeneous in molecular terms. EGFR and HER-2/neu play important roles in the pathogenesis of apocrine carcinomas and these findings may have significant therapeutic implications. CEP17 polysomy is a common finding in invasive mammary carcinoma and may lead to discordant interpretation of HER-2/neu gene amplification in a significant proportion of the cases.

Acknowledgements: Dr. Semir Vranić was a research fellow at the Creighton University School of Medicine (Omaha, Nebraska, United States of America) and had been supported by the UICC American Cancer Society Beginning Investigators Fellowship (ASCB) award (ACS/08/004) funded by the American Cancer Society (ACS).

MeSH / Keywords: Breast Carcinoma - Special Types - Apocrine Carcinoma - EGFR - HER-2/neu - CEP17 polysomy - Gene Amplification

THE EFFECT OF EPINEUROTOMY ON THE MEDIAN NERVE VOLUME AFTER THE CARPAL TUNNEL RELEASE

Part of a Thesis: The Effect of Epineurotomy on the Median Nerve Volume after the Carpal Tunnel Release

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Mentor: Professor Ranko Bilić, MD, PhD

Affiliation: County General Hospital- Požega

INTRODUCTION: The carpal tunnel syndrome is the most common canalicular syndrome in humans and compressive neuropathy of the median nerve within the tunnel is a common cause of hand disability. Surgical release of the tunnel reduces pressure, allows for restoration of the intraneuronal blood flow and physiological function, and is treatment of choice in persistent/progressive cases. Long-lasting nerve compression may result in fibrotic changes generating further mechanical pressure and narrowing of the nerve. In such cases, longitudinal epineurotomy of the nerve has been suggested as an option that could convey a greater pressure release, a more prominent nerve volume recovery and better outcomes. The aim of this study was to evaluate the effects of epineurotomy on the post-surgical median nerve volume and clinical outcomes in the carpal tunnel syndrome patients with a prominent nerve narrowing.

MATERIALS AND METHODS: Prospective, randomized, double-blind controlled trial. Patients (n=50) were randomized (1:1) to open-field surgical carpal tunnel release followed by a longitudinal epineurotomy of the nerve (Test), or to open-field release without epineurotomy (Control).

RESULTS: In both groups, the nerve volume was higher 90 days post-surgery than before the surgery. With adjustment for the pre-surgery value, age, sex and whether the affected hand was a dominant one, the increase was somewhat higher in the Test group (mean 9.9 mm³ in the ITT and 10.5 mm³ in the PP set, p<0.001 respectively) than in the Control group (7.2 mm³ in both ITT and PP sets, p=0.002). At 180 days, the volume increased further, and the adjusted difference vs. the pre-surgery value was somewhat lower in the Test (mean 13.3 and 13.7 mm³ for ITT and PP data, respectively, p<0.001) than in the Control group (mean 14.6 and 14.7 mm³ for ITT and PP data, respectively, p<0.001). No relevant electrophysiological or clinical difference between groups was observed. The subjective pain reduction was slightly more prominent in the Control group at 180 days.

DISCUSSION: Even in selected carpal tunnel syndrome patients, longitudinal epineurotomy confers no benefit regarding the nerve volume or clinical outcomes over a simple carpal tunnel release.

MeSH / Keywords: carpal tunnel syndrome, median nerve, epineurotomy.

CONGENITAL ADRENAL HYPERPLASIA DUE TO 21-HYDROXYLASE DEFICIENCY IN CROATIA - CLINICAL AND MOLECULAR DATA

Part of a Thesis: Clinical, Biochemical and Molecular Characteristics of Patients With Congenital Adrenal Hyperplasia in Croatia

PhD candidate: Katja Dumić, MD

Mentors: Professor Ingeborg Barišić, MD, PhD and Professor Zorana Grubić, PhD

Affiliations: Children's University Hospital Zagreb, Department of Pediatrics, Division of Clinical Genetics; Clinical Hospital Centre Zagreb, Department of Laboratory Medicine, Tissue Typing Centre; Mount Sinai School of Medicine, Department of Pediatrics, Steroid Disorder Division, New York, USA

INTRODUCTION: Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency (21-OHD) is autosomal recessive disorder caused by mutation of CYP21 gene. Aim of this study was to analyze genotype-phenotype correlation and potential ethnic-specific distribution of CYP21 gene mutations in Croatian patients with CAH.

MATERIALS AND METHODS: Molecular analysis of the CYP21 gene was performed in 93 patients (186 alleles) and 194 family members (388 alleles). Allele-specific PCR was used to detect point mutations while high-resolution genotyping (Southern blotting and sequencing) was performed to detect deletions and conversions.

RESULTS: Clinical evaluation and genotyping was performed in 93 (male/female=30/60) patients with 21-OHD. Salt-wasting (SW) form of disease was found in 65 patients (male/female=21/44), while 28 patients (male/female=12/16) had simple virilizing (SV) CAH. Compound heterozygosity was found in 59, while 32 patients were homozygous. Mutation frequency distribution was as follows: In2 35.5%, deletions/conversions 19.4%, Ex8₃₅₆ 15.1%, Ex4 11.3%, Ex8₃₁₈ 5.4%, Ex3 2.1%, Ex6_{cluster} 1.6%, Ex10₄₂₆ 1%, Ex7 1% and Ex10₄₈₃ 0.5%. Mutations were categorized into 4 groups (null, A, B, and C) according to their residual enzymatic activity. Overall positive genotype-phenotype correlation was observed in 89% of patients. Intra-familial phenotypic variability between siblings was observed in 2 different families (SW form in one sibling and SV form in the other). Genotyping of family members revealed 7 new patients with SV CAH. Two families were consanguineous while in two families fathers were found not to be biological fathers.

DISCUSSION: Male to female ratio was 33/60 implying that males die unrecognized or are diagnosed later in life. These data stress the need for introduction of newborn screening for CAH in Croatia. Second most common point mutation found in our population was Ex8₃₅₆ (rarely found in other European populations, but very common in Asians). Genotyping of patients from neighbouring countries should reveal whether this mutation is an ethnic-specific mutation for Slavic population. Partial genotype-phenotype correlation was observed, as well as intra-familial phenotypic variability. This data alert that clinicians/genetic counselors should be very careful in predicting phenotype upon genotype, especially in preconceptional counseling and prenatal diagnosis of CAH. Database of patients with CAH in Croatia was established.

Acknowledgements: I would like to express sincere gratitude to my teachers and mentors: Maria I. New, Ingeborg Barišić and Zorana Grubić whose help, stimulating suggestions and encouragement helped me in all the time of research. Special thanks to Dr. Tony Yuen who gave me important guidance during my first steps in molecular genetics.

Keywords: congenital adrenal hyperplasia, 21-hydroxylase deficiency, CYP21 gene, mutation, genotype-phenotype correlation

EARLY PREDICTORS OF NEURODEVELOPMENTAL OUTCOME IN CHILDREN WITH CONGENITAL CYTOMEGALOVIRUS INFECTION

Part of a Thesis: Early Symptoms of Congenital CMV Infection Predict Neurodevelopmental Outcome

PhD candidate: Ivana Đaković, MD, research fellow

Mentor: Full professor Vlatka Mejaški Bošnjak, MD, PhD

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

INTRODUCTION: Congenital cytomegalovirus infection (CCMVI) is the most common vertically transmitted infectious disease. Infected children are often asymptomatic, but infection can also cause severe neurodevelopmental disorders such as hearing and visual impairments, cerebral palsy, mental retardation, epilepsy or autism.

MATERIALS AND METHODS: The study is planned to describe 95 children with proven CCMVI in order to determine correlation between neurodevelopmental outcome and first symptom or group of symptoms of infection. Until now 54 children with clinical, laboratory, neurophysiological and neuroimaging perinatal examination was included in study, of which 31 was re-examined at the age more then 24 months.

RESULTS: More than half of examined children were born from mothers first pregnancy, 75% children were term born. At least one of the symptoms of CCMVI was present in newborn period in 39/54 children, mostly isolated jaundice. Microcephaly, intrauterine growth retardation, neonatal sepsis syndrome or hepatitis were also observed, often combined. However, CCMVI was diagnosed in newborn period only in 13 patients. In others hepatitis (13), prolonged jaundice (3), neuroimaging findings (15) and psychomotor delay (7) were most often guide marks. Of the 31 re-examined children 8 developed cerebral palsy, 10 had minor neurological dysfunction, while others didn't have larger motor disorder. Eleven children had mild and only one child severe vision impairment, seven had hearing impairment (4 with severe damage). Epilepsy occurred in 8 children and all of them were permanently treated. Cognitively impaired were 13 of them (mild 7, moderate 2, severe 4), while 18 had inappropriate speech development.

DISCUSSION: From this preliminary data on relatively small sample it can be concluded that isolated jaundice as symptom of CCMVI is not a predictor of adverse psychomotor outcome in infected children. Other symptoms or their combinations have yet to be statistically validated when data for all children are collected. Determination of most reliable predictors is important for setting early suspicion on CCMVI, early diagnosing and predicting neurodevelopmental outcome. Thus, it would enable early habilitation and deciding upon potentially harmful antiviral therapy.

Acknowledgements: I would like to thank all of the team of Department of Neuropediatrics, Children's Hospital Zagreb, as well as Ministry of Science, Education and Sports for financial support of project on which I am employed „Neurodevelopmental outcome of children with intrauterine growth retardation and/or hypoxia“, project manager Prof Vlatka Mejaški Bošnjak, MD, PhD.

MeSH / Keywords: congenital cytomegalovirus infection, symptoms, neurodevelopmental outcome

BIOMARKERS OF SYSTEMIC AND LUNG INFLAMMATION IN CHILDHOOD ASTHMA

Part of a Thesis: Biomarkers of Systemic and Lung Inflammation in Controlled and Uncontrolled Childhood Asthma

PhD Candidate: Marta Navratil MD

Mentors: Mirjana Turkalj, MD, PhD, Assistant Professor Davor Plavec, MD, PhD

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INTRODUCTION: Previous studies support a hypothesis of persistent systemic inflammation in asthma in parallel with local inflammation. Asthma control and severity is mostly monitored by symptoms and lung function measurements. However, this does not measure airway inflammation directly. Although several clinical markers of asthma have been evaluated in many previous studies, an ideal measure of asthma activity has not been determined.

AIM: To compare different biomarkers of inflammation in children with controlled and uncontrolled asthma and to investigate their value in evaluation of asthma control.

MATERIALS AND METHODS: 62 consecutive asthmatic children (age 11 ± 3.3 years, 32 girls) with controlled ([C], n=19) and uncontrolled asthma ([NC], n=43) were studied. Measured lung function and inflammatory biomarkers included: spirometry, exhaled NO (F_{eNO}), high-sensitivity C-reactive protein (hs-CRP), peripheral blood white blood cells (WBC) counts and differentials.

RESULTS: Hs-CRP was significantly higher in uncontrolled than in controlled asthma (hs-CRP, median [IQR], mg/L; 0.56 [0.60] vs 0.25 [0.34], $P=0.008$). Discriminant analysis (backward stepwise) depicted hs-CRP and lymphocytes (as Z-score for absolute count) as significant discriminative factors for asthma control ($F=8.319$, $P=0.0007$) with 82.3% diagnostic accuracy. Divided into quartiles hs-CRP showed the significant inverse association with F_{eNO} ($F=7.359$, $P=0.003$, ANOVA) with no significant difference for asthma control ($F=1.032$, $P=0.386$). Post-hoc analysis revealed that F_{eNO} values were significantly lower in the third and the fourth quartile of hs-CRP in comparison to the first and the second one ($P<0.05$ for all).

DISCUSSION: In asthmatic children with uncontrolled asthma serum hs-CRP was increased compared to children with controlled asthma. Although F_{eNO} values were also increased (insignificantly) and inversely correlated with hs-CRP they were probably reflecting different etiology underlying the loss of control. The role of peripheral blood biomarkers in asthmatics is still poorly investigated so new studies are required.

Acknowledgements: The results presented were obtained in the scope of a scientific project 277-0000000-3436, entitled Comparing effects of two ways of driving asthma therapy in children 12-18 years, carried out with support from the Ministry of Science, Education and Sports of the Republic of Croatia. We would also like to thank to Srebrnjak Children's Hospital for financial support and contribution to this study.

MeSH / Keywords: asthma, biomarkers, c reactive protein, child, inflammation

THE ROLE OF CYP2D6 AND ABCB1 GENOTYPES IN PATIENTS USING LONG-ACTING INJECTABLE RISPERIDONE

Part of a Thesis: CYP2D6 and ABCB1 Polymorphisms in Patients Using Long-Acting Injectable Risperidone

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Mentor: Associate Professor Nada Božina, MD, PhD

Affiliations: University of Zagreb School of Medicine, University Hospital Centre - Zagreb

INTRODUCTION: An increased risk of adverse effects and discontinuation of risperidone therapy is observed in patients with schizophrenia. Risperidone is metabolized to its active metabolite, 9-hydroxyrisperidone, mainly by the cytochrome P450 enzymes CYP2D6. Its antipsychotic effect is assumed to be related to the active moiety, that is, the sum of risperidone and 9-hydroxyrisperidone. Both risperidone and 9-hydroxyrisperidone are substrates of P-glycoprotein (MDR1). The aim of this study is to investigate the role of genetic variations of CYP2D6 and ABCB1 (MDR1) on serum concentrations of risperidone and 9-hydroxyrisperidone in patients using long-acting injectable risperidone.

MATERIALS AND METHODS: Thirty-five patients with schizophrenia treated with 25-50 mg long-acting injectable risperidone were genotyped, and their serum steady-state concentrations of risperidone and 9-hydroxyrisperidone were measured on 5th and 13th day following risperidone injection. Pharmacogenetic analysis were performed for polymorphisms of CYP2D6 (*3, *4, *5, *6 and (*)1xN) and ABCB1 (G2677T/A, C3435T). The CYP2D6 genotyping was performed by long-distance PCR (for duplications and allele *5), while real-time PCR analysis by Taqman assays was used for genotyping CYP2D6 (for alleles *3,*4,*6), ABCB1 G2677T/A and C3435T variants. Serum concentrations of risperidone and 9-hydroxyrisperidone were measured by high-performance liquid chromatography (HPLC).

RESULTS: The distribution of genotypes CYP2D6 wt/wt, wt/mut, and mut/mut was 16, 16 and 3 respectively; for ABCB1 2677 G/G, G/T, T/T was 13, 14 and 8; for ABCB1 3435 C/C, C/T and T/T was 9, 15 and 11, respectively. The total serum concentration range of risperidone on 5th day following injection was 43.2-273.4 nmol/L, and on 13th day following injection was 9.8-47.1 nmol/L. Prevalence of low activity CYP2D6 alleles predisposing to slower metabolism was significantly higher in the group of schizophrenic patients comparing to the healthy population. Concentrations and dose corrected concentrations of risperidone and 9-hydroxyrisperidone exhibited large interindividual differences. Moreover several patients belonging to extensive CYP2D6 phenotype had on 13th day concentration of risperidone 0 nmol/L, meaning they were underdosed and have high risk for the relapse of disease symptoms.

DISCUSSION: Our preliminary results pointed to unacceptable interindividual differences in long-acting injectable risperidone concentrations that can lead to therapy failure or adverse reactions.

Acknowledgements: I would like to thank Professor Alma Mihaljević-Peleš and Maja Živković, MD from the Department of Psychiatry, University Hospital Centre - Zagreb

MeSH / Keywords: pharmacogenetics, CYP2D6, ABCB1, risperidone, schizophrenia

POLYMORPHISMS IN 17Q12-21 ARE ASSOCIATED WITH ASTHMA EXACERBATION AND LUNG FUNCTION IN ASTHMATIC CHILDREN

Part of a Thesis: Associations between Chromosome 17q12-21 Gene Variants and Asthma in Childhood

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INTRODUCTION: More than 15 studies associated genetic variants in 17q21 region with asthma. We aimed to investigate whether amongst Croatian asthmatic children, genetic variants in this region are associated with asthma severity and exacerbation.

MATERIALS AND METHODS: We recruited 423 children aged 6-18 years with physician-diagnosed asthma. Information on hospital admission with asthma exacerbations was retrieved from medical records. Data on wheeze frequency and environmental tobacco smoke (ETS) exposure was collected using validated questionnaire. Lung function (FEV₁%predicted) was measured using spirometry. We analyzed 35 haplotype-tagging SNPs in 17q21.

RESULTS: We found significant associations between 4 SNPs and hospital admissions (rs12150079, rs7212938, rs2290400, rs8067378). For example, G allele homozygotes in rs12150079 were at higher risk of being admitted to hospital than carriers of A allele (aOR 1.85, 95%CI 1.26-2.72, p=0.002); this SNP was also associated with current wheezing. Six SNPs were associated with lung function (rs9635726, rs921651, rs9900538, rs3169572, rs4795403, rs471692). In addition, we observed significant interaction between 3 SNPs (rs12603332, rs8067378, rs9303277) and in utero ETS exposure in relation to lung function (p<0.04), in that amongst children of mothers who smoked during pregnancy, major allele homozygotes had lower FEV₁%predicted than minor allele carriers, but amongst non-exposed children there was no difference in lung function between different genotype groups.

DISCUSSION: Among Croatian schoolchildren with asthma variants in 17q12-21 region are associated with disease severity. We have identified a significant interaction between genetic variants and in utero tobacco smoke exposure, with lung function being diminished in specific genotypes, but only amongst children exposed to ETS.

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MeSH / Keywords: Asthma, Child, Croatia, 17q, Polymorphism, Single Nucleotide, Smoking

THORACIC ULTRASOUND IN PATIENTS WITH MALIGNANT PLEURAL EFFUSION

Part of a Thesis: Ultrasound diagnosis of pleural effusion is properly positioned in clinical practice

PhD candidate: Nevenka Piskač Živković, MD

Mentor: Professor Neven Tudorić, MD, PhD

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INTRODUCTION: Malignant pleural effusion is often difficult to distinguish from lymphocytic effusion of other, especially tuberculous, etiology. Medical thoracoscopy, VATS (Video Assisted Thoracic Surgery) and pleural biopsy are often necessary for final diagnosis. In contrast, thoracic ultrasound (US) does not have confirmed specificity due to fact that different pathological conditions could have similar US characteristics. The aim of this study was to evaluate usefulness of combining different diagnostic procedures in differentiation malignant pleural effusions from effusions of other etiology.

MATERIALS AND METHODS: Data from 79 patients with confirmed malignant or tuberculous pleural effusion were retrospectively analyzed. We compared their thoracic US findings, pleural effusion cytology, and macroscopic appearance of the pleural effusion at the initial thoracentesis.

RESULTS: In 45 patients pleural effusion was septated due to lymphocytic and fibrinous depositions (US finding). In 37 of these patients (82%), the effusion was tuberculous while in remaining 8 patients (18%) pleural effusions were malignant ($p < 0.01$; Yates). In 88% of patients with malignant septated pleural effusion the fluid was hemorrhagic or sanguinolent. In fact, only one patient with malignant effusion (non-Hodgkin lymphoma) had macroscopically clearly yellow effusion.

DISCUSSION: We concluded that non-hemorrhagic (non-sanguinolent), lymphocytic and septated pleural effusion did not have malignant etiology. The only exception was pleural effusion from a patient with malignant lymphoma. The cited results confirmed thoracic US as useful supplemental diagnostic procedure in patients with pleural effusion.

MeSH/Keywords: ultrasound, malignant pleural effusion

LONG - TERM FOLLOW - UP OF PATIENTS ON SILDENAFIL FROM CROATIAN PULMONARY HYPERTENSION REGISTRY

Part of thesis: Efficacy And Safety Of Long-Term Treatment With Sildenafil In Patient With Pulmonary Hypertension

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Mentor: Proffesor Miroslav Samaržija, MD, PhD

Affilitations: University of Zagreb School of Medicine, University Hospital Centre-Zagreb, University Hospital for Lung Diseases 'Jordanovac', Zagreb, Croatia

INTRODUCTION: Sildenafil is found to improve exercise capacity and haemodynamics in selected patients with pulmonary hypertension. We present data from patients with severe pulmonary hypertension, following their 6 - minute walking distance, right ventricle pressure measured by cardiac ultrasound, Borg dyspnea score and NYHA class.

MATERIALS AND METHODS: Over 8 - year period we commenced 40 patients on sildenafil 25 - 50 mg tds, NYHA class II-IV, with various etiology of pulmonary hypertension (30 patients with PAH; which included 19 patients with IPAH, 5 with connective tissue disease, 5 with congenital heart disease, 1 with PH associated with anorexigen intake; 8 patients with CTEPH, 1 patient with PH due to COPD and 1 patient with PH due to hematologic malignancy) were followed for mean od 17.3 months (range, 1 to 72 months). Long - term efficacy was assesed by 6 - min walking distance (6MWD), NYHA class, Borg dyspnea score, and right ventricle pressure measeured by cardiac ultrasound. Clinical events were monitored to asses time to disease progression.

RESULTS: Righ ventricle pressure was reduced from 97.45 mmHg before treatment to 90.5 mmHg after 12 months of treatment ($p < 0.05$). 6MWD increased from 316 m to 375m after 3 month, then further to 384m at 6 months and further to 407m after 12 months of treatment($p < 0.05$). Eleven patients (79% of all failures) had failure of treatment after 3 months, additional two after 6 months and one more after 12 months of treatment. Therapy is still ongoing in 26 patients, and in 19 patient is longer than 12 months. In those patients with prolonged benefit, mean duration of treatment is 33.2 months (range 12 to 72 months). In patients with prolonged benefit we noticed improvements in Borgy dyspnea score and NYHA class.

DISCUSSION: Long - term use of sildenafil in patients with severe pulmonary hypertension of various etiologies is associated with a sustained improvement in exercise capacity and right ventricle pressure. Most of the treatment failures were noticed after first three months of treatment.

MeSH / Keywords: pulmonary hypertension, sildenafil, right ventricle pressure.

PROGNOSTIC VALUE OF CARDIAC BIOMARKERS AND ECHOCARDIOGRAPHY IN PATIENTS WITH ACUTE PULMONARY EMBOLISM

Part of a Thesis: Echocardiography and plasma levels of cardiac troponins and natriuretic peptides are markers of right ventricular dysfunction in pulmonary embolism

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Affiliations: University of Zagreb School of Medicine, University Hospital „Sestre milosrdnice“ Zagreb

INTRODUCTION: Right ventricular dysfunction (RVD) is central hemodynamic event in acute pulmonary embolism (APE), and represents independent prognostic factor of adverse event. According to recent studies, echocardiography and cardiac biomarkers (cardiac troponins and natriuretic peptides) are useful tools in assessment of RVD. The aim of study was to evaluate prognostic value of echocardiography and cardiac biomarkers associated with early adverse outcome of APE patients.

MATERIALS AND METHODS: This present study was a single-center prospective study of APE patients conducted from September 2006 to November 2008 at Intensive Care Unit (ICU) in University Hospital Sisters of Mercy in Zagreb, Croatia. The study population included 104 patients. The diagnosis of APE was confirmed in all patients using spiral computed tomography. Patients were, according to European society of cardiology guidelines, divided into three severity groups: high-risk (n=33; 31.7%), intermediate-risk (n=51; 49.1%) and low-risk (n=20; 19.2%). Brain-type natriuretic peptide (BNP), N-terminal proBNP and cardiac troponin T (cTnT) were measured at admission and 12, 24 and 72 hours after admission. Echocardiography was performed within 24 hours after admission. Main outcome measure was in-hospital death. Differences in survival were analyzed with the Kaplan-Meier method, the results were compared with the use of log-rank test. A value of $p < 0.05$ was taken as level of statistical significance. The association between risk factors and death was examined by means of univariate analysis.

RESULTS: Out of 104 patients 19 (18,7%) patients died, none of whom was in low risk group ($p < 0,001$). Echocardiographic findings of RVD was associated with higher mortality, especially tricuspid regurgitation ($p = 0,027$). Initial BNP levels correlated better with outcome than the severity of APE ($p = 0,003$) unlike N-terminal proBNP levels ($p = 0.082$). Serum concentrations of cTnT at admission showed good correlation with disease severity notably in high risk patients ($p < 0,001$) and was associated with higher mortality ($p = 0,038$).

DISCUSSION: Our results indicate that plasma levels of BNP and cTnT and echocardiographic signs of RVD are associated with higher mortality in APE patients and therefore could play role in therapeutic strategy. Multivariate logistic regression analysis will be performed to estimate factors independently associated with death.

Keywords: acute pulmonary embolism, right ventricular dysfunction, brain-type natriuretic peptide, cardiac troponin T, echocardiography

THE EFFECT OF PENTADECAPEPTIDE BPC 157 ON HEALING OF RECTOVAGINAL FISTULAS IN RATS

Part of Thesis: The Effect of Pentadecapeptide BPC 157 on Healing of Rectovaginal Fistulas in Rats

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INTRODUCTION: It is known that rectovaginal fistulas are great medical therapeutic problem because in many cases they require the formation of bipolar anus praeter or Hartmann operation and the establishment of intestine continuity in the second act. Given that pentadecapeptide BPC 157 helps healing of gastrocutaneous (Skorjanec et al, Dig Dis Sci (2009) 54:46-56), duodenocutaneous (Skorjanec et al, DDW 2010) and colocutaneous fistulas (Klicek et al, J Pharmacol Sci 108, 7 - 17 (2008)). Momentary there is no adequate pharmacological therapy for rectovaginal fistula. In this study we investigated the effect of pentadecapeptide BPC 157 on the rectovaginal fistula healing.

MATERIALS AND METHODS: For the model of rectovaginal fistula, we used female Wistar rats. In deep anesthesia on the rats, we made a longitudinal incision on the rear wall of the vagina and anterior rectal wall in the length of 5 mm and formed rectovaginal fistula using single-layer extension suture technique. Pentadecapeptide BPC157 (Diagen d.o.o., Ljubljana, Slovenia) dissolved in saline solution was applied in daily doses of 10 μ g/kg / ng/kg i.p. during the entire duration of the experiment (1, 3, 5, 10, 14 and 21 days). The control group animals got daily equivalent amount of 0.9% NaCl-solution i.p. (5 mL/kg). During the sacrifice we measured the volume required for leakage of fluid through fistula (mL H₂O), evaluated the macroscopic differences in rectovaginal fistula healing (diameter of the fistula (mm, rectal and vaginal side). Microscopical evaluation was also performed.

RESULTS: In all pentadecapeptide BPC 157 treated rats, we noticed throughout the experiment a significant increase in pressure needed to leakage of fluid through fistula compared to control animals (14th day - 5,3 +/- 0,3 mL H₂O vrs 1,1 +/- 0,4 ml H₂O). We also noted a significant reduction in diameter of the fistulas after the third postoperative day in BPC 157 groups, which spreads throughout the experiment (complete closure of fistulas during 14 days), while the diameter of fistulas in the control group showed less tendency of closing and are also present even after 21th postoperative day (21th day - 0 +/- 0 (BPC157 - rectal side), 0 +/- 0 (BPC 157 - vaginal side) vrs 3,1 +/- 0,6 (con. rectal side), 3,3 +/- 0,8 (con. vaginal side).

DISCUSSION: Considering the results obtained, we suggest that pentadecapeptide BPC 157 could be an adequate pharmacological solution to the problem of rectovaginal fistulas healing.

MeSH / Keywords: pentadecapeptide BPC 157, rectovaginal fistula

PENTADECAPEPTIDE BPC 157 AS A THERAPY FOR CORROSIVE MUCOSAL LESIONS IN RATS

Part of a Thesis: The Effect Of Pentadecapeptide BPC 157 In The Therapy Of Caustic Lesions Of The Tongue, Esophagus, Stomach And Duodenum Induced By 96% Alcohol In Rats

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INTRODUCTION: Corrosive mucosal lesions are very important medical and socioeconomical problem in general. It has usually been seen as an accidental intake of a corrosive substance (children) or in an attempts of committing suicide (adults). Experimental researches of caustic injuries on animal models are rare and inconclusive. Ingestion of a chemical agent (absolute alcohol) was used for model of A.Robert's cytoprotection. A safe stable gastric pentadecapeptide BPC 157(GEPPPG-KPADDAGLV, MW 1419, stable in human gastric juice, LD1 not achieved) may be the novel mediator of Robert's cytoprotection, and thereby, may counteract all corrosive mucosal injuries. Successful in inflammatory bowel disease trials, it counteracts esophagitis, sphincters failure, gastrointestinal ulcer, skin ulcer, and fistulas in rats.

MATERIALS AND METHODS: Corrosive mucosal injuries in anesthetized rats were induced by applying 1ml of 96% alcohol at the tongue, whilst BPC 157 (10 ↔g, 10 ng/kg) or saline (5ml/kg) were given intraperitoneally immediately after. Tongue, esophagus, stomach and duodenal mucosal defects, lower esophageal sphincter (LES) and pyloric sphincter (PS) pressure were evaluated at the time period of 30 sec, 1, 5, 15, 30, 60, 120 minutes, and 24 hours

PRELIMINARY RESULTS: Control. Mucosal defects after 30 sec: tongue (lesions area, mm², 52.0±2.8), esophagus (110.5±43.1), stomach (125.0±84.9) and duodenum (9.0±1.41). Mucosal defects after 24 h showed progression: 24.7±21.3 (tongue), 174.6±111.4 (esophagus), 271.3±76.9 (stomach), 30.9±48.7 (duodenum). Sphincters pressures remained continuously low i.e., 46.2±11.3 cm H₂O (LES 24 h); 34.0±13.4 cm H₂O (PS 24 h). BPC 157 ug-regimen initially exhibited smaller mucosal defects on tongue (18.5±9.2), esophagus (8.5±6.4), stomach (3.0±4.2) and duodenum (4.5±4.9) after 30 sec. Mucosal defects remained significantly lower than control during 24 hours (7.1±10.0 (tongue), 18.3±32.9 (esophagus), 61.3±65.5 (stomach), 4.7±3.9 (duodenum)) . Sphincters pressures were restored i.e., 58.8±11.6 cm H₂O (LES 24 h); 45,1±10.0 cm H₂O (PS 24 h). BPC 157 ng-regimen shows the same effectiveness (vs. control p<0.05)).

DISCUSSION: BPC 157 could be used as a therapy for corrosive mucosal lesions.

Acknowledgements: I would like to thank both of my mentors for making this experiment possible

MeSH / Keywords: pentadecapeptide BPC 157; corrosive mucosal injuries

THE EFFECT OF PENTADECAPEPTIDE BPC 157 ON THE COLOVESICAL FISTULA HEALING AT THE RAT MODEL

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INTRODUCTION: Enterovesical fistula present an important clinical problem caused by diverticulosis, IBD, colorectal cancer . The pentadecapeptide BPC 157 has already shown the effectiveness in healing gastrocutaneous and colocutaneous fistulas. We suggest it as a possible therapy of colovesical fistulas as a model of internal fistulas. In our study we will research its effectiveness in healing of colovesical fistulas.

MATERIALS AND METHODS:75 male Wistar albino rats will be randomly assigned into five groups. Colovesical fistulas will be created at 5 cm from anocutaneous borderline, with diameter of 3 mm. Control groups will received drinking water, saline i.p(5 mL/kg). Therapy groups will received pentadecapeptide BPC 157 i.p. (10 \rightarrow g/kg, 10 ng / kg) and perorally (10 \rightarrow g/kg) .

RESULTS: At the end of each experimental period (7 ,14, 28 days) the animals will be sacrificed and the biomechanical, functional, macroscopic and microscopic assesment will performed. Our preliminary results shows that BPC 157 has positive effect on the healing of all three components of internal fistulas: colonic defect, bladder defect and on the fistulous canal.

DISCUSSION: Fistulas occure as a problem of non contemporaneously healing different tissue defects, that do not heal spontaneously. Internal fistulas are more complex than external fistulas. According to this, results could present a new possible pathway in therapy of colovesical fistulas as a model of internal fistulas.

MeSH/ Keywords : bpc 157, Wistar rat, fistula

PENTADECAPEPTIDE BPC 157: COUNTERACTING EFFECT ON INTRAMUSCULAR APPLICATION OF SUCCINYLCOLINE IN RATS

Part of a Thesis: Pentadecapeptide BPC 157: Counteracting Effect on Intramuscular Application of Succinylcholine in Rats

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INTRODUCTION: Pentadecapeptide BPC 157 improves muscle healing (even after complete transection or crush) and has neuroprotective properties. Thereby, we tested BPC 157 counteracting effect on intramuscular (IM) application of succinylcholine.

MATERIALS AND METHODS: Succinylcholine (200 \leftrightarrow g/kg) was given into the right quadriceps muscle of the rats, and we assessed local paralytic effect in injected muscle, immediate leg contracture (also presented long after systemic muscle disability has abated), initial agitation before muscle disability, countless muscle twitches before complete muscle tonus loss, motionless laying, and thereafter, violent screaming upon light touch, and muscle fibers decrease, and edema in injected and non-injected quadriceps muscle and diaphragm at 1, 3, 5, and 7 days after IM succinylcholine. BPC 157 was given at 30 min before succinylcholine (10 \leftrightarrow g, 10ng/kg intraperitoneally) or through 24 h per-orally (10 \leftrightarrow g/kg in drinking water) until the succinylcholine application or immediately after succinylcholine (10 \leftrightarrow g/kg intraperitoneally).

RESULTS: BPC 157 completely eliminated the local succinylcholine effect (leg contracture) and markedly attenuated or eliminated behavioral agitation, muscle twitches, motionless laying. No violent screaming upon light touch appeared in IM succinylcholine-rats. BPC 157 counteracted muscle fibers decrease, and edema that otherwise appeared in injected and non-injected quadriceps muscle and diaphragm.

DISCUSSION: Since improving muscle healing after muscle transection, crush injury and systemic corticosteroids application and demonstrating significant neuroprotective capabilities, BPC 157 was tested to counteract succinylcholine muscle disability in rats. The rats that had been treated with BPC 157 (micrograms and nanograms dose, intraperitoneal and peroral regimen) exhibited a markedly postponed and mitigated course, IM succinylcholine-disability less pronounced, much shorter duration, and quick rescue without resting painful reaction or agitation, no cyanosis and urination, less defecation. The BPC 157 application immediately after IM succinylcholine was able to produce an alike beneficial effect. The injected leg contracture was absent in all BPC 157 rats, either leg contracture did not appear in rats that received BPC 157 before succinylcholine or leg contracture disappeared in those succinylcholine rats that received BPC 157 thereafter.

MeSH / Keywords: Antagonist; Pentadecapeptide BPC 157; Succinylcholine

EFFECTS OF PPAR γ , APOE, ACE AND AT1R GENE VARIANTS ON DEVELOPMENT OF METABOLIC SYNDROME

Part of a Thesis: Effect of Gene Interactions and Environment Factors on the Incidence of Metabolic Syndrome

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INTRODUCTION: Metabolic syndrome (MS) is a multifactorial complex disorder that encompasses several metabolic diseases such as hypertension, abdominal obesity, dyslipidaemia, insulin resistance that markedly increase the risk of type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD). It is well known that MS has a complex pathogenesis in which interactions are involved between certain susceptibility genes and several environmental exposures. The contribution of genetic factors to the development of MS has been widely recognized, but the identity of the genes has not yet been fully clarified. Aim of this study was to investigate the possible role of gene polymorphisms of PPAR γ (Pro12Ala), ApoE (E2, E3, E4), ACE (I/D) and AT1R (1166A>C) in MS.

MATERIALS AND METHODS: A total of 400 individuals were studied including 281 patients with MS and 119 subjects without MS criteria. Measurements included: fasting glucose in blood, total cholesterol, triglycerides, LDL-C, HDL-C, CRP, blood pressure (BP), waist circumferences and measurements of weight and height for body mass index (BMI) calculations. Genotyping was performed for ApoE (E2, E3, and E4) by Real-time PCR, for PPAR γ (Pro12Ala) and AT1R (A1166C) by PCR-RFLP, for ACE (I/D) by PCR based method.

RESULTS: Patients had significantly more high blood pressure, significantly higher BMI and waist circumferences and significantly higher levels of triglycerides, CRP and glucose and lower levels of HDL. We found significant association of AT1R variants and the development of MS ($p=0.03$), with the C allele being associated with lower risk. We found association of waist circumference with: ACE variants ($p=0.03$), with D allele carrying the higher risk, and AT1R with A as the risk allele ($p=0.05$). APOE4 variant was associated with the waist circumference ($p=0.05$) and the levels of HDL ($p=0.03$), and ACE genotype was associated with glucose levels ($p=0.02$), with II genotype carrying the lowest risk.

DISCUSSION: The results suggest that gene variants of AT1R, ACE and ApoE could be susceptibility factors of obesity, glucose intolerance and lipid status contributing to the development of metabolic syndrome.

Acknowledgements: This study was supported by grant of Croatian Ministry of Science, Education and Sports as part of Project No. 108-1080134-0136 „Functional genomics and proteomics of risk factors for atherosclerosis“.

MeSH / Keywords: Metabolic Syndrome X, Genetic Polymorphisms, PPAR gamma, Apolipoproteins E, Angiotensin Converting Enzyme, Angiotensin Type 1 Receptor

ASSOCIATION BETWEEN BRAIN-DERIVED NEUROTROPHIC FACTOR VAL66MET AND OBESITY IN CHILDREN

Distribution of the brain derived neurotrophic factor (BDNF) genotypes and serum level of N-glycans as predictors of overweight in children

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Mentors : Fran Borovečki, MD, PhD, assistant professor¹, Nela Pivac, DVM, PhD, senior scientist²

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INTRODUCTION: Obesity in children and adolescents is a worldwide health problem, characterized by various somatic, psychosocial and psychiatric complications, and is often associated with adult obesity and related complications. Brain-derived neurotrophic factor (BDNF) is a neurotrophin with important roles in feeding behavior, food intake regulation, energy metabolism and weight control. A common polymorphism of the BDNF genotype (Val66Met) has been associated with various forms of eating disorders, alterations in body mass index (BMI) values and obesity in adult populations.

MATERIALS AND METHODS: The aim of this study was to determine the association between the gene variants of the BDNF Val66Met polymorphism and obesity in 96 healthy Caucasian children of the same ethnic background of Croatian origin, subdivided according to the National BMI percentile criteria, but without any form of eating disorders. The DNA was isolated from blood samples (4 ml) using the „salting-out” method. Genotyping of the BDNF Val66Met was carried out using the TaqMan-based allele-specific polymerase chain reaction assay in the ABI Prism 7300 Sequencing Detection System apparatus. Participants were categorized into two groups: a normal weight group (including underweight and normal weight group), and an obese group (including overweight and obese group). Chi-square test was used to test the differences in the frequency of the BDNF genotypes in studied groups.

RESULTS: The frequency of the Met/Met, Met/Val and Val/Val genotypes, Met and Val alleles, and Met carriers (the combined Met/Met and Met/Val genotypes versus the homozygous Val/Val genotype) differed significantly between underweight, normal weight, overweight and obese children. This significant difference was induced by the presence of one or two Met alleles in obese group of children.

DISCUSSION: These results showed for the first time the significant association between the presence of one or two Met alleles and obesity in ethnically homogenous groups of healthy Caucasian children. These data confirmed the major role of BDNF in energy metabolism, food regulation and BMI.

MeSH/Keywords: Body mass index, Brain-derived neurotrophic factor (BDNF Val66Met), Caucasians, Healthy children, Obesity

P-GLYCOPROTEIN AND PI3K/AKT AND MAPK ACTIVATED SIGNALING PATHWAY MOLECULES IN ACUTE MYELOID LEUKEMIA BLASTS

Part of thesis: P-Glycoprotein and activated signaling pathways molecules PI3K/Akt and MAPK in acute myeloid leukemia blasts

PhD candidate: Sanja Perković, MSc

Mentor: Professor Drago Batinić, MD, PhD

Affiliation: University Hospital Centre Zagreb

INTRODUCTION: Acute myeloid leukemia (AML) is a heterogeneous group of clonal hematopoietic stem cell disorders characterized by uncontrolled proliferation, survival supremacy, differentiation block and apoptosis avoidance mechanisms. Signaling pathways that regulate essential cellular processes are often constitutively activated in AML making their own contribution to leukemic clone growth and predominance. P-Glycoprotein (P-gp) is a transmembrane pump that is responsible for multidrug resistance mechanism and in its activated form is associated with worse prognosis in patients with AML. The aim of the study was to determine if there is association between P-gp expression and activity, activated molecules of PI3K/Akt and MAPK signaling pathways and other adverse prognostic factors in AML (immunophenotype, cytogenetic aberrations and molecular mutations).

MATERIALS AND METHODS: One hundred de novo diagnosed AML samples were tested for P-gp function using Rhodamine123 fluorescent dye and Verapamil as an inhibitor of P-gp function. Mrk16 monoclonal antibody was used for P-gp expression assessment. Activated signaling pathways were detected by fixation, permeabilization and staining samples with antibodies specific for phosphorylated Akt, ERK1/2 and p38 molecules. All data was collected and analyzed on BD FACSCalibur flow cytometer and CellQuest Pro Software. MedCalc was used for statistical analysis of association between categorical variables by Fischer's exact test and correlation analysis for continuous variables.

RESULTS: There was a strong correlation between P-gp expression and activity ($P < 0.0001$, $r = 0.71$), as well as between Akt- and p38-phosphorylation ($P < 0.0001$, $r = 0.48$) in AML blasts. P-gp activity ($P < 0.000000001$) and expression ($P = 0.005$), but not Akt/ERK/p38-phosphorylation were strongly associated with CD34 expression. In addition, P-gp activity also showed significant correlation with the age of patients ($P = 0.03$, $r = 0.22$), whereas correlation with the Akt-phosphorylation and cytogenetics was of borderline significance ($P = 0.07$ and $P = 0.06$, respectively). With regard to prognostic FLT3-ITD-positivity, it was more frequent among CD34-negative (i.e. monocytic) AML forms with no significant association with P-gp activity and/or expression.

DISCUSSION: Although the correlation between P-gp activity and phosphorylation status of Akt molecule was of borderline significance, P-gp activity was strongly associated with known adverse prognostic factors in AML. Phosphorylated p38 accompanied with Akt phosphorylation could indicate crosstalk between two signaling cascades in AML. Further analysis of signaling pathways in defined subgroups of AML is needed to elucidate their role in leukemia maintenance and prognosis.

Acknowledgements: This study was supported in part by grant from Croatian Ministry of Science, Education and Sports, number 214-1081347-0355 (PI Drago Batinić).

MeSH / Keywords: acute myeloid leukemia, P-Glycoprotein, signal transduction pathway, FLT3, flow cytometry.

ARTERIAL HYPERTENSION IN RHEUMATOID ARTHRITIS AND OSTEOARTHRITIS, A COMPARATIVE CROSS-SECTIONAL STUDY

Part of a Thesis: Prevalence of arterial hypertension is higher in patients with rheumatoid arthritis than in patients with osteoarthritis and general population

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Affiliations: Division of Clinical Immunology and Rheumatology, Department of Internal Medicine, Dubrava University Hospital, University of Zagreb School of Medicine, Zagreb, Croatia, coordinative centre of the study.

INTRODUCTION: The increased prevalence of hypertension (HT) in rheumatoid arthritis (RA) and osteoarthritis (OA) patients was reported. Chronic inflammation might contribute to

high prevalence of HT in RA. In this study we compare the prevalence of HT in RA and OA patients, exposed to high and low grade of chronic inflammation respectively to assess association of chronic inflammation and HT.

MATERIALS AND METHODS: The consecutive 627 RA and 352 OA patients entered this cross-sectional multicentric study. The HT was defined as a systolic blood pressure (BP) ≥ 140 and/or diastolic BP ≥ 90 mmHg or current use of any antihypertensive drug. Overweight/obesity and the elderly were defined as body mass index (BMI) ≥ 25 and the age ≥ 65 yrs respectively.

RESULTS: : Prevalence of HT was higher in OA than in RA (73,3% and 59,5% respectively). In both groups the prevalence of HT was higher in the elderly and overweight (OA:83,9% and 75,7%; RA:75,5% and 67,3%; respectively) than in the younger and patients with BMI <25 and similar to HT prevalence in Croatian population. In RA there was an association with inflammatory markers which disappeared in multivariate analysis. Multivariate analysis (logistic regression) of HT predictors in OA, RA and OA-RA difference showed age and BMI present in all three models.

DISCUSSION: A robust association of age and BMI with HT prevalence in both RA and OA and with their differences was shown. HT prevalence in RA depended on population to which patients belong, and for Croatia is similar to general population.

Acknowledgements: This work was supported by grant by Ministry of science, education and sport Republic of Croatia (198-1081874-0183 to Jadranka Morović-Vergles).

MeSH / Keywords: Rheumatoid arthritis, Osteoarthritis, Arterial hypertension

BIOLOGICAL PROPERTIES OF HUMAN MESENCHYMAL STEM CELLS EXPANDED *IN VITRO* IN MEDIA SUPPLEMENTED WITH HUMAN PLATELET LYSATE

Part of a Thesis: Biological Properties Of Mesenchymal Stem Cells Expanded In Vitro In The Presence Of Human Platelet Lysate

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INTRODUCTION: Human mesenchymal stem cells (MSCs) are nonhematopoietic multipotent cells that express regenerative and immunomodulatory properties *in vitro* and appear immunoprivileged what gives them considerable therapeutic potential. So far, MSCs have been clinically applied to facilitate engraftment in hematopoietic stem cell transplantation and to treat acute graft versus host disease (aGVHD). Because of very low incidence in the bone marrow (BM) aspirates MSCs need to be expanded *in vitro*. Recently human platelet lysate (PL) has been evaluated as fetal bovine serum substitute in MSCs *in vitro* expansion.

MATERIALS AND METHODS: Mononuclear cells (MNC) were isolated from the BM of 11 healthy individuals by Ficoll density gradient centrifugation and plated at 160000 MNC/cm² in media supplemented with 10%PL. After reaching 80% confluence, cells were harvested by trypsinization and replated at 1000 MSCs/cm² until reaching passage 5 (P5). At each P biological properties of cultured cells were evaluated. The number of cells was determined by light microscopy techniques. The MSCs clonogenic potential was determined by colony forming unit-fibroblast (CFU-F) assays and characteristic immunophenotype by flow cytometry. Multipotency was assessed by inducing MSCs into adipogenesis and osteogenesis *in vitro* (n=6). Immunomodulatory properties of cultured MSCs were evaluated by adding 10-40000 MSCs to mixed lymphocyte reaction (MLR) (n=1). Karyotype analysis was performed to validate chromosomal stability of expanded cells (n=6).

RESULTS: Cell growth analysis showed that, by the P5, median time needed for one MSC population doubling (PD) was 36hr. Cultured cells were devoid of hematopoietic markers already in P1, and more than 96% of cells expressed CD90, CD73 and CD105. Cells cultured in the presence of 10%PL differentiated into adipocytes and osteoblasts. In MLR experiment, MSCs displayed immunomodulatory effect. Analysed metaphases displayed normal karyotype with exception of one trisomy 8 detected in P4, but not in P5. Since such karyotype anomalies are not stable and sustainable they do not have selective advantage and are not of pathological nature.

DISCUSSION: MSCs cultured in the presence of PL display all expected biological and immunomodulatory properties. This study provides a basis for future evaluation of PL as a valuable FBS substitute in clinical grade MSCs expansion.

MeSH / Keywords: mesenchymal stem cells, platelet lysate, immunomodulation

IN VITRO EFFICACY OF LEVOFLOXACIN AGAINST *CHLAMYDIA TRACHOMATIS* STRAINS

Part of a Thesis: Efficacy of Azithromycin, Doxycycline and Levofloxacin against urogenital *Chlamydia trachomatis* strains in vitro

PhD candidate: Tomislav Meštrović, MD

Mentor: Associate Professor Sunčanica Ljubin-Sternak, MD, PhD

Affiliations: Croatian National Institute of Public Health, Polyclinic „Dr. Zora Profozić“

INTRODUCTION: *Chlamydia trachomatis* is the most common bacterial agent of sexually transmitted diseases associated with a number of symptoms and clinical syndromes. Albeit rare, appearance of resistant chlamydial strains may narrow our treatment options in the future, hence in vitro susceptibility testing to commonly used antibiotics is necessary.

MATERIALS AND METHODS: Susceptibility testing of *C. trachomatis* was performed in 96-well microtiter plates containing McCoy cells. Levofloxacin powder was obtained from Sigma-Aldrich (St. Louis, Missouri, USA). 10 strains isolated from cervical swabs and 10 strains from urethral swabs were tested. Each well was inoculated with 100 μ l of the isolated test strain passaged to yield 5000 to 10000 inclusions. After centrifugation and incubation, 100 μ l of each drug dilution has been added to the appropriate wells, providing a concentration range of 0.008 to 8 μ g/ml. Following 72-hour incubation at 35 °C in 5% CO₂, cultures were fixed and stained with fluorescein-conjugated antibody (Pathfinder®, Bio-Rad Laboratories, France). The minimal inhibitory concentration (MIC) represents the lowest concentration of antibiotic without visible inclusions. The minimal chlamydicidal concentration (MCC) was determined after passage in the absence of antibiotics.

RESULTS: MIC values of cervical strains ranged from 0,125 to 1,0 μ g/ml, and MCC values from 0,25 to 2,0 μ g/ml. MIC₅₀ and MIC₉₀ were both 0,5 μ g/ml, MCC₅₀ was 0,5 μ g/ml and MCC₉₀ 1,0 μ g/ml. MIC values of urethral strains ranged from 0,125 to 1,0 μ g/ml, and MCC values from 0,25 to 1,0 μ g/ml. MIC₅₀ and MIC₉₀ were 0,5 and 1,0 μ g/ml, respectively; MCC₅₀ and MCC₉₀ of urethral strains were also 0,5 and 1,0 μ g/ml, respectively. MCC values were equal to MIC values or 1-2 dilutions higher. MIC₅₀ is equivalent to the median MIC value and MIC₉₀ signifies the MIC value at which at least 90% of the strains are inhibited.

DISCUSSION: Our first results did not reveal antimicrobial resistance in tested clinical isolates, but further testing on a bigger number of samples and comparison with standardly prescribed antimicrobial drugs (azithromycin and doxycycline) is needed. This type of research is conducted for the first time in our country and should provide the basis for future clinical research.

Acknowledgements: This work is part of the project „Research on the etiology, epidemiology, diagnostics and treatment of patients with prostatitis syndrome“ (P.I. Professor V. Škerk) funded by The Croatian Science Foundation.

MeSH / Keywords: antimicrobial susceptibility; *Chlamydia trachomatis*; levofloxacin; minimal inhibitory concentration; minimal chlamydicidal concentration

MOLECULAR PROFILING OF THE UPPER URINARY TRACT TRANSITIONAL CELL CANCERS ASSOCIATED WITH ARISTOLOCHIC ACID NEPHROPATHY

Part of a Thesis: Molecular Profiling of the Upper Urinary Tract Transitional Cell Cancers Associated with Aristolochic Acid Nephropathy

PhD Candidate: Sandra Karanović, MD

Mentors: Professor Bojan Jelaković, MD, PhD, Associate Professor Fran Borovečki MD, PhD

Affiliations: School of Medicine University of Zagreb, Univeristy Hospital Center Zagreb, New York University Langone Medical Center

INTRODUCTION: Approximately 50% of endemic nephropathy(EN) patients develop upper urothelial tract cancers(UUC). Aristolochic acid(AA) was proven as the etiological factor both for EN and UUC. Our goal was to establish patterns of gene expression and post-transcriptional gene regulation mechanisms involved in AA induced carcinogenesis.

MATERIALS AND METHODS: Paired samples of tumors and adjacent normal urothelial tissues of 15 patients from Croatian endemic region were analyzed. miRNA profiling was performed by high-capacity qPCR using Applied Biosystems megaplex RT primer pools for 742 human miRNAs and the microfluidics TaqMan Low Density Arrays. mRNA profiling of the same RNAs was performed using Affymetrix HG U133 Plus 2.0 arrays. Integrative analysis of miRNA:mRNA data was performed using TargetScan target predictions and pathway analyses(Ingenuity, GSEA, GO).

RESULTS: A signature of 138 miRNAs differentially modulated(74 elevated and 64 reduced) and 5438 significantly modulated mRNAs(2021 elevated and 3417 reduced) were identified in tumors vs. unaffected tissue samples. Integrating the miRNA and mRNA data, 1159 predicted, inversely correlated targets of the 45 upregulated miRNAs and 703 predicted, inversely correlated targets of the 44 down-regulated miRNAs were identified. Cancer-related miRNAs(miR-17-92 cluster) were upregulated in UUC while their tumor suppressor targets were reduced. Additionally, the anti-metastatic miR-200 family was elevated in tumors, concurrently with low levels of its targets. Inhibition of migratory process was documented by increased miRNAs targeting cellular movement, migration and invasion(miR-143/145, miR-214, let-7), via repression of promigratory genes. Comprehensive biological interpretation and mining of the integrated miRNA:mRNA data set identified pathways of DNA damage response and DNA repair, mitochondrial transport, bladder cancer, deregulated oncogenic and developmental signaling pathways and remodeling of the chromatin structure. Of note was the downmodulation of TGF β effects, suppression of EMT and migration. Collectively, these findings imply that UUCs are characterized by counterbalance of increased cell proliferation, DNA repair and reduction in effectors of cell-matrix contacts, cell-cell contacts and motility, accompanied by major chromatin remodelling changes.

DISCUSSION: UUC tumorigenesis appears to involve primarily miRNA-mediated process of oncogenic activation of tumor growth and repression of tumor suppressor genes, and chromatin silencing and repression of pro-metastasis programs rendering the non-metastatic nature of these carcinomas.

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MeSH / Keywords: endemic nephropathy, aristolochic acid, urothelial cancer, microRNA

PHOSPHORYLATED HER-2 RECEPTOR AS AN INDICATOR OF BREAST CANCER RESISTANCE TO TRASTUZUMAB

Part of a thesis: Phosphorylated HER-2 receptor as an indicator of breast cancer resistance to trastuzumab

PhD candidate: Snježana Ramić, M.Sc. Molecular Biology

Mentor: Associate Professor Fabijan Knežević, MD, PhD

Affiliation: University Hospital for Tumours, University Hospital Centre „Sisters of charity“, Illica 197, Zagreb, Croatia

INTRODUCTION: Over-expression of epidermal growth factor receptor-2 (HER-2) leads to oncogenic transformation of cells. HER-2 is over-expressed in 15-25% cases of breast carcinomas resulting with a high risk of relapse and poor prognosis of the disease. Patients with HER-2 positive tumours receive trastuzumab (Herceptin®) which is an efficient anti-HER-2 therapy that reduces recurrence and improves survival. Still, some patients develop metastasis during treatment as a result of resistance to trastuzumab. HER-2 receptor can be over-expressed, but not activated. Phosphorylation of HER-2 receptor (pHER-2) is the final step of its activation and results in downstream signalling. Therefore, pHER-2 is a true indicator of receptor activity and function. Variations in HER-2 phosphorylation indicate changes in receptor or its signalling pathway. Lack of expression or weak expression of pHER-2 might determine patients who will be resistant to trastuzumab therapy.

MATERIAL AND METHODS: We measured expression of pHER-2 on 85 samples of HER-2 over-expressing invasive breast carcinomas. Monoclonal antibody against Tyr1248-phosphorylated HER-2 receptor was used for immunohistochemical analysis. Intensity of membrane/cytoplasmic staining was graded as 0, without staining and 1, weak staining (negative); 2, moderate and 3, strong staining (positive). Differences in the pHER-2 expression between trastuzumab-sensitive and resistant tumours was analyzed using Fisher's exact test ($P < 0.05$).

RESULTS: Our preliminary results showed that over-expressed HER-2 is generally in its active/phosphorylated form. We detected 7% of tumours without staining, 32% with weak, 27% with moderate and 34% with strong staining. All patients received trastuzumab, but 22.4% ($n=19$) had disease progression during therapy. Trastuzumab-resistant tumours had weak level of HER-2 phosphorylation. They had 63% cases of negative pHER-2 (nine with weak and three without staining). Trastuzumab-sensitive tumours had only 22% cases of negative pHER-2 (18 with weak and three without staining). The difference in pHER-2 expression between trastuzumab-resistant and sensitive tumours was significant ($P=0.0175$).

DISCUSSION: Although trastuzumab is the most widely used anti-HER-2 therapy, some patients do not benefit from it. pHER-2 expression could have clinical impact on application of trastuzumab therapy. Pre-selection of tumours that are HER-2 positive, but weak pHER-2, can indicate tumours resistant to trastuzumab therapy. Further analysis and methodology optimization must be considered.

Keywords: breast cancer, HER-2, phosphorylation, trastuzumab, drug resistance

EFFECT OF BONE MORPHOGENETIC PROTEINS ON DIFFERENTIATION OF LEUKEMIA NB4 CELL LINE

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INTRODUCTION: Acute promyelocytic leukemia (APL) is characterized by accumulation of abnormal promyelocytes that do not differentiate into normal granulocytes and by a specific chromosome translocation t(15;17). All-trans retinoic acid (ATRA) is used for treatment of APL patients with high rate of complete remission, but with risk of the onset of retinoid resistant condition. Bone morphogenetic proteins (BMPs) are multi-functional growth factors that belong to the transforming growth factor β superfamily. They have an important role in proliferation, differentiation, morphogenesis, and apoptosis of hematopoietic cells. In this research we used in vitro model of APL, NB4 cell line that carries t(15;17) translocation and differentiates in response to ATRA.

MATERIALS AND METHODS: NB4 cells were treated with ATRA alone (1 \leftrightarrow M) or in combination with the various doses of BMP2, BMP4 or BMP6 (from 5 pg/mL to 250 pg/mL). Cell cytopins were stained with May-Grunwald-Giemsa (MGG) and analyzed for cell morphology. Analysis of cell surface myeloid-specific antigens CD11b and CD11c was performed by flow cytometry. RNA extracted from cultured cells were reversely transcribed; cDNA was amplified by quantitative PCR, using TaqMan assays for fusion transcript PML/RARA, several differentiation markers and intracellular molecules involved in promyelocyte proliferation and differentiation.

RESULTS: Treatment of NB4 cells with ATRA induced myeloid differentiation, as evidenced by increase in the expression of CD11b and CD11c markers. Combined treatment with ATRA and BMP2, BMP4 or BMP6 partially blocked cell differentiation seen in response to ATRA alone. MGG staining showed ATRA-induced morphological maturation and differentiation of NB4 cell line. Suppression of differentiation seen in combined treatment with ATRA and BMPs was in concordance with induced expression of proto-oncogene cKit and lower expression of genes associated with NB4 cell line maturation (myeloid-specific transcription factors PU.1 and CEBP, and cyclin D1 degradation enzyme UBE2D3).

DISCUSSION: Our results indicate that BMPs may be involved in the resistance to ATRA treatment. Mechanism of this effect could be mediated by changes in the expression of regulatory molecules affected by BMPs. Further investigation is needed to explore the mechanism of BMPs activity in promyelocytic cells and their possible role in resistance to ATRA seen in APL patients.

Keywords: acute promyelocytic leukemia, cell differentiation, bone morphogenetic proteins, all trans retinoic acid

TRANSLATIONAL RESEARCH ON PAIN AND DURAL INFLAMMATION CAUSED BY INJURY OF PERIPHERAL BRANCHES OF TRIGEMINAL NERVE

Part of a Thesis: Neurogenic Inflammation Of Dura Mater And Pain in the region of head and neck

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INTRODUCTION: In study on experimental animals (rat) we investigated if dural neurogenic inflammation can be evoked by trigeminal neuropathy induced by infraorbital nerve constriction (IoNC). Additionally, peripheral injection of botulinum toxin A (BTX-A) recently approved for migraine treatment was tested in IoNC model.

Clinically several surgical studies showed that decompression of different trigeminal nerve branches is effective in relieving migraine (or migraine like) pain. In one our case study we investigated the effect of surgical decompression in a patient with severe headache and facial palsy caused by high mega jugular bulb (HJB) compressing the facial and trigeminal nerve.

MATERIALS AND METHODS: Rats which developed mechanical allodynia 14 days after the IoNC were injected with BTX-A (3.5 U/kg) into vibrissal pad. Allodynia was tested by von Frey filaments and neurogenic inflammation was measured as colorimetric absorbance of Evans blue - plasma protein complexes. To test the role of axonal transport of BTX-A in sensory neurons an axonal transport blocker colchicine (5 mM, 2 \leftrightarrow l) was injected into the trigeminal ganglion.

In a patient, HJB was diagnosed with computed tomography and magnetic resonance imaging. The patient underwent a surgical exploration of the jugular foramen area with exposure of the facial nerve and removal of the jugular bulb anomaly.

RESULTS: Single unilateral BTX-A injection bilaterally reduced IoNC induced dural neurogenic inflammation, as well as allodynia. Effects of BTX-A on pain and dural neurogenic inflammation in IoNC model were dependent on axonal transport through trigeminal sensory neurons.

In a clinical study immediately after surgery, there was a dramatic drop in the intensity of the headache. Facial nerve palsy resolved completely after 2 months.

DISCUSSION: Present results demonstrate for the first time that the bilateral dural neurogenic inflammation can be evoked by IoNC, and is dependent on neurotransmission in trigeminal nerve. Although the central site of BTX-A action seems the only logical explanation, the precise mechanism requires further elucidation. Clinical part of our research is in line with observation on experimental animals and shows that in some cases migraine (or migraine like) pain can be eliminated by decompression of trigeminal and facial nerve.

Acknowledgements: I would like to express gratitude to prof. Mislav Gjurić MD, PhD for his clinical research contribution. Sources of funding: Croatian Ministry of Science, Education and Sport and Deutscher Akademischer Austausch Dienst. Presented results have been published (Filipović B, Matak I, Bach-Rojecky L, Lacković Z (2012) Central Action of Peripherally Applied Botulinum Toxin Type A on Pain and Dural Protein Extravasation in Rat Model of Trigeminal Neuropathy. PLoS ONE 7(1): e29803. doi:10.1371/journal.pone.0029803; Filipović B, Gjurić M, Hat J, Glunčić I. High mega jugular bulb presenting with facial nerve palsy and severe headache Skull Base. 2010 Nov;20(6):465-8)

MeSH / Keywords: neurogenic inflammation, infraorbital nerve constriction injury, botulinum toxin A, high mega jugular bulb

AXONAL TRANSPORT OF BOTULINUM TOXIN A FROM PERIPHERY TO CNS IN SENSORY AND MOTOR NERVES

Part of a Thesis: Central Antinociceptive Activity of Botulinum Toxin A

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INTRODUCTION: Botulinum toxin A (BTX-A) has been approved for treatment of movement disorders and migraine. Widely assumed peripheral mechanism of action has been questioned by recent studies which demonstrated the axonal transport in facial nerve and within central nerves. Findings in our laboratory suggested a central antinociceptive activity following axonal transport in sciatic nerve.

MATERIALS AND METHODS: To characterize the axonal transport of BTX-A, toxin's enzymatic activity in CNS was assessed using immunofluorescent detection of its cleaved substrate synaptosomal-associated protein 25 (SNAP-25) following injections into the rat whisker pad, hind-paw, intramuscular injection into the gastrocnemius and intraneural injections into the sciatic nerve. Intraneural and intraganglionic colchicine was employed to prevent the axonal transport. To investigate the significance of axonal transport for antinociceptive activity of BTX-A, we assessed the effect of peripheral and intraganglionic injections of low dose BTX-A in orofacial formalin-induced pain in rats.

RESULTS: Following whisker pad BTX-A injection, cleaved SNAP-25 has been observed in medullary dorsal horn. Cleaved SNAP-25 following subcutaneous, intramuscular and intraneural toxin injection in rat hind limbs has been observed in ipsilateral dorsal and ventral horn of corresponding lumbar spinal cord segments. Central SNAP-25 cleavage following BTX-A injection into the sciatic nerve was prevented by colchicine. In ventral horn, BTX-A protease was localized within cholinergic neurons. Facial and intraganglionic injections of botulinum toxin prevented the orofacial pain dependently on axonal transport in trigeminal sensory nerve.

DISCUSSION: Our results suggest that the axonal transport of BTX-A in different sensory and motor nerves commonly occurs after peripheral applications. Axonal transport in sensory neurons followed by central enzymatic activity is involved in botulinum toxin's antinociceptive effects. Possible functional role of axonal transport in motor neurons remains to be examined.

Acknowledgements: Antibody to cleaved SNAP-25 was a kind gift from Ornella Rossetto (University of Padua, Italy). Sources of funding: Croatian Ministry of Science, Education and Sport and Deutscher Akademischer Austausch Dienst. Presented results have been published or accepted for publication (Matak et al., *Neuroscience* 2011, 186: 201-207; Matak et al., *Neurochem Int* 2012, doi: 10.1016/j.neuint.2012.05.001)

MeSH / Keywords: botulinum toxin A, antinociceptive activity, synaptosomal-associated protein 25, axonal transport

EFFECTS OF LOCAL MICROEJECTION OF 5HT AGONISTS INTO THE PREBÖTZINGER COMPLEX (PBC) AND ADJACENT VENTRAL RESPIRATORY COLUMN (VRC) ON THE CANINE BREATHING PATTERN

Part of a Thesis: The Role Of 5HT1A And 5HT2A Receptors Within The PreBötzingler Complex (pBC) And Ventral Respiratory Column (VRC) In Eliciting Respiratory Responses To Systemic Administration Of Selective Serotonin Agonists

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INTRODUCTION: Biogenic amines have powerful modulatory effects on the respiratory network. Selective serotonin agonists have been recently shown to prevent opioid-induced depression of breathing without diminishing their analgesic effect. It is not known where they produce their effects on respiration. Immunohistochemical identification and electrophysiological studies in vitro suggest the presence of serotonergic (5HT) receptors within the preBötzingler complex. We hypothesized that the serotonin agonists produce their effect on breathing in the preBötzingler complex (pBC).

MATERIALS AND METHODS: Acute non-survival experiments were performed in decerebrate, vagotomized, paralyzed and mechanically ventilated dogs during isocapnic hyperoxia. Multi-barrel micropipettes were used to record respiratory neuronal activity and microinject artificial cerebrospinal fluid (aCSF), glutamate agonist D,L-homocysteic acid (DLH, 20mM), 5HT1a agonist 8-hydroxy-2-(di-n-propylamino) tetraline (8OH-DPAT, 200→M) and 5HT2a agonist 2,5-dimethoxy-4-iodoamphetamine (DOI, 200 →M) unilaterally at multiple sites in 1mm increments (0-7 mm rostral of obex) within the VRC and pBC. Subsequently DOI and 8OH-DPAT were given IV. Ejected volumes were measured via the meniscus of the pipette with a microscope (resolution 2nl). Inspiratory duration (TI) and expiratory duration (TE) were measured from the phrenic neurogram (PNG). The pBC within the ventral respiratory column (VRC) was functionally located via: stereotaxic coordinates, the presence of a mixture of respiratory neuron subtypes and its typical tachypneic response to microinjection of DLH.

RESULTS: Microinjections of 8OH-DPAT and DOI did not produce any significant changes in TI, TE and PPA at any location within VRC. aCSF microinjected into VRC also did not produce any significant changes, while DLH microinjections produced significant changes. In contrast, DOI (235±48 →g/kg) given IV produced 10±5 % increase in TI with no changes in TE and PPA, while 8OH-DPAT (19±2 →/kg) given IV decreased TI and TE by 33±5% and 62±7% with no changes in PPA. (n=13).

DISCUSSION: 5HT1a and 5HT2a agonists (8OH-DPAT and DOI) given IV produced tachypnea, while microinjections of 8OH-DPAT and DOI into the VRC and pBC did not produce any significant alternations of the breathing pattern in dogs. Our data suggests that systemically administered amines are likely to affect sites outside the VRC and pBC, which may indirectly alter breathing via projections to the VRC/pBC.

Acknowledgements: Supported by VA Medical Research Funds and NIH grant GM 059234 funds

MeSH / Keywords: pBC, preBötzingler, 8OH-DPAT, DOI, 5HT1a, 5HT2a, VRC, respiration

ALTERATION OF MEMORY FUNCTIONS AND CHOLINERGIC TRANSMISSION IN THE BRAIN OF THE STREPTOZOTOCIN-INDUCED RAT MODEL OF SPORADIC ALZHEIMER'S DISEASE

Part of a Thesis: Cholinergic system in the brain of the streptozotocin-induced rat model of sporadic Alzheimer's disease

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INTRODUCTION: The etiopathogenesis of sporadic Alzheimer's disease (sAD) is unknown and the onset and development of its pathophysiological hallmarks are difficult for tracking in humans. There is a need for the representative animal sAD model which should be well characterized and validated as a model suitable for novel drug testing. Rats treated intracerebroventricularly with streptozotocin (STZ-icv) have been recently proposed as a non-transgenic sAD model which demonstrates AD-like pathology. We aimed to characterize the STZ-icv dose- and post-treatment time-dependency of cognitive impairment and cholinergic deficit in the brain of the STZ-icv rat model.

MATERIALS AND METHODS: Adult Male Wistar rats were injected bilaterally icv with STZ (0.3, 1 and 3 mg/kg) or vehicle (controls) and sacrificed one week, and one, three, six and nine months after the treatment. Cognitive deficits were measured by Passive Avoidance Test (PA). Acetylcholinesterase (AChE) activity was measured in hippocampus (HPC) and cortex (CTX) by Ellman's method. Protein expression of muscarinic cholinergic receptor M1 in the HPC and CTX was measured by SDS-PAGE electrophoresis, followed by Western blot analysis. Data were analysed by Kruskal-Wallis and Mann-Whitney U test ($p < 0.05$).

RESULTS: STZ-icv rats exhibit significant dose- and time-dependent cognitive deficits in PA test (40-90%). AChE activity in the STZ-icv treated rats was significantly elevated in HPC after one week (22%), one (20%) and nine (32%) months (3 mg/kg) and in PTC after six months (10% /0,3 mg/kg; 28% /1 mg/kg). One and 3 mg STZ dose significantly altered the expression of muscarinic M1 receptors three months after the injection, manifested as increment in CTX (+82,89% and +67,83%) and decrement in HPC (-18,06% and 15,01%), while after 9 months, the expression of M1 receptor in CTX was decreased with all three STZ doses (-22,5%/0.3 mg/kg, -20,39%/1 mg/kg and -18,34%/3 mg/kg).

DISCUSSION: STZ-icv rat model demonstrates long-term cognitive and hippocampal cholinergic deficits which tend to correlate mutually at the highest STZ dose regimen, varying from the acute response followed by normalization and finally progressive decompensation effects.

Acknowledgements: Supported by UKF and MZOS (108-1080003-0020).

MeSH / Keywords: sporadic Alzheimer's disease, intracerebroventricular streptozotocin, memory, cholinergic transmission

EFFECT OF BLOOD SEROTONIN LEVELS ON FOUR MONTHS OLD RAT SKELETON

Part of a Thesis: Blood serotonin levels effect on bone remodeling metabolism

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INTRODUCTION: Serotonin participates in many processes of a living organism. Serotonin has a major role in vasoconstriction, peristaltic bowel movement, blood clotting and has an important role as a neurotransmitter in the central nervous system. Latest discoveries point out serotonin's effect on bone.

MATERIALS AND METHODS: To examine serotonin effect on bone in this experiment we used well described animal model with altered blood serotonin levels. Four months old Wistar-Zagreb 5HT (WZ-5HT) rats of both gender were used. Animals were developed by selective breeding through generations to create two distinct sublimes of animals with high and low blood serotonin levels, respectively. Due to separation of serotonin systems in the body by blood brain barrier, bone structure was investigated at peripheral (distal part of femur) and axial skeleton (lumbar spine). Animals were weight on a digital scale and femur length was measured with a caliper. Bone structure was analyzed using \leftrightarrow CT and DXA methods.

RESULTS: Animals of both genders with high serotonin level had increased weight, femur length and bone tissue volume. In four months old female animals no difference in trabecular bone parameters between high and low blood serotonin level animals were observed on periphery, while in axial skeleton trabecular parameters were significantly better in animals with low serotonin level. Peripheral and axial skeleton bone parameters in males revealed negative effect of high blood serotonin levels on bone.

DISCUSSION: In four months old animals effect of serotonin on development and growth was evident due to difference in weight and bone length. In females serotonin had no effect on femur bone parameters while on female and male axial skeleton and male peripheral skeleton high serotonin levels had negative impact on bone parameters. Our findings point out that serotonin has a distinct effect on trabecular bone properties and could subsequently involve other factors, like estrogen due to different findings in four months old male and female rats.

Acknowledgements: Special thanks to Lipa Čičin-Šain, from Institute „Ruder Bošković“, for collaboration and for providing the animals for this study. Also many thanks to Đurdica Car for help around handling the animals.

MeSH / Keywords: serotonin, bone remodeling, \leftrightarrow CT, trabecular parameters

IS THERE A LINK BETWEEN NURSES' DEDICATION AND PATIENT SAFETY?

Part of a Thesis: Presenteeism and Absenteeism of Health Care Workers and Patient Safety Culture

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Mentor: Professor Jadranka Mustajbegović, MD, PhD

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INTRODUCTION: Working as a nurse often involves great dedication and sacrifices - working night shifts, working overtime and coming to work when sick, the term also known as presenteeism. Previous research showed that in nursing, lower productivity can have serious consequences which can affect health of the caregiver as well as their patients. These consequences include medication errors, needle stick injuries, near errors, and decreased patient satisfaction. The aim of this study was to investigate whether nurses' dedication to their work, coming to work when sick, affects patients' safety

MATERIALS AND METHODS: This is a cross-sectional study which included medical nurses. Data was collected during April and May 2012. The study was conducted in a general hospital in Croatia. This research specifically studies nurses, as they are one of the largest occupational groups in health care and they are crucial care givers in health care. A total of 194 questionnaires were distributed and 150 of them were returned which makes 77.32% response rate. Nurses from non-surgical departments, surgical department, pediatric, obstetrics, psychiatry, anesthesiology and ICU participated in this research. Two questionnaires were used, Stanford Presenteeism Scale short form (SPS-6) and Hospital Survey on Patient Safety Culture.

RESULTS: Mean SPS-6 score was 21,3 (SD=4,58), minimum was 7, maximum 30. Thirty nine nurses (26%) had SPS-6 score 18 or lower. Linear regression was conducted to assess whether the 14 dimensions of the HSOPSC are associated to the SPS-6 score. The model was not statistically significant ($R^2=.047$, $p=0.955$). Binary logistic regression was conducted to assess whether presenteeism in nurses with lower SPS-6 score was associated to patient safety. The model was not statistically significant ($\chi^2(8)=.34$, $p=0.825$).

DISCUSSION: Our results give a different perspective of nurses' presenteeism compared to previous research. Adequate and productive nursing workforce is crucial to the delivery of high-quality, cost-effective health care and our results show that there is no evidence that nurses' presenteeism was associated with decreased patient safety. Creating an environment that supports balance between work and life is not done easily in health care. Greater gains may be realized by improving on-the-job productivity and investing in preventive and early intervention service.

Acknowledgements: This study was supported by Ministry of Science, Education and Sports, project title: „Health at the workplace and healthy working environment“, 108-1080316-0300

MeSH / Keywords: nurse, stanford presenteeism scale, patient safety culture

TOBACCO AND ALCOHOL CONSUMPTION AMONG KOSOVARS

Part of a Thesis: Prevalence of chronic diseases risk factors and specific health determinants in a transitional country – The case of Kosova

PhD candidate: Sanije Hoxha - Gashi MD,

Mentors 1: Professor Emeritus Silvije Vuletić, MD, PhD, Associate Professor Merita Berisha, MD, PhD

Affiliation (s): University of Zagreb School of Medicine, National Institute of Public Health of Kosova

INTRODUCTION: Health behaviors are an important determinant of both current and future health status of the population and are modifiable actions that impact on health in the short and long term. For chronic disease, there are a small number of risk factors common to many diseases (Queensland Health, 2008). The major (modifiable) behavioral risk factors identified in the World Health Report 2002 are: tobacco use, harmful alcohol consumption, unhealthy diet (low fruit and vegetable consumption) and physical inactivity.

MATERIAL AND METHODS: According to the STEPS methodology (www.who.int/chp/steps/) respondents aged 15-64 years old are selected randomly within each sex and 10-year age-group. The total sample size is 6,400 men and women in all regions of Kosova (seven regions). The following assumptions are used for sample size calculation: level of confidence 95%, margin of error 5%, baseline level of risk factors 50%, expected response rate 90% and a design effect of 1.5. From 6,400 people contacted, 6,117 have responded, so the respond rate is 95.5%.

RESULTS: From 6,117 responders 3028 (49.5%) were male and 3,089 (50.5%) were female. Currently 28.4% of responders smoke cigarettes, males more often than females (Males 37.4% vs. Females 19.7%). Daily smokers are 25.6% of responders. Also, men smoke often cigarettes daily than women (Males 35.2% vs. Females 16.3%). Daily smokers smoke from one cigarette per day till (one case) 80 cigarettes. Most of the smokers smoke manufactured cigarettes. The average of manufactured cigarettes smoked per day is for men 23.9 (around one packet) and for women 14.7 cigarettes (less than one packet per day). Lifetime abstainers of alcohol are 75.5% among the males and 93.1% among the females or for both sexes 84.4%. Percentage who is abstainers in the past 12 months is 88.5%, higher among females than among males (Females 95.8% vs. Males 81.0%). Percentage who currently drinks alcohol in the past 30 days is 8.4%, (Males 14.6% vs. females 2.3%).

CONCLUSION/DISCUSSION: Smoking: 1/3 of population is daily smokers, more men than women. Moreover, smokers smoke a lot, on average one packet of cigarettes per day. According to the data of the survey, the percentage of alcohol consumption is not high in Kosova.

Acknowledgements: I would like to thank French Embassy in Kosova for financial support.

MeSH/Keywords: Tobacco, alcohol consumption, Kosovo population

BEGINNINGS OF EUGENICS IN CROATIA: FRAN GUNDRUM'S IDEAS ABOUT THE PROBLEMS OF PROSTITUTION AND CRIMINALITY

Part of a Thesis: Race and Patriotism: Formation of Eugenics in Croatian Medicine and its Public Influence 1859-1945

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Mentor: Stella Fatović-Ferenčić, MD, PhD

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INTRODUCTION: The subject of eugenics in Croatia has not been sufficiently researched. Although some of the Croatian scientists wrote on the subject of ethnical differences in biological terms, it was not until the works of Croatian physicians at the beginning of the 20th century that eugenics was proposed to solve social issues. Here I bring the preliminary results of the analysis of Fran Gundrum's (1856-1919) eugenics oriented to the phenomena of prostitution and criminality.

MATERIALS AND METHODS: These results are based on the archived sources kept at the Croatian Academy of Sciences and Arts, and all Gundrum's publications on prostitution and criminality published between 1905 and 1914.

RESULTS: Gundrum's works were filled with Darwinian ethics, Lamarckism, and eugenics. Regarding the suppression of prostitution, he suggested the establishment of colonies where „incurable“ prostitutes would be exiled and controlled by the institution of medical police with the use of methods such as Bertillonage, dactyloscopy and photography. Concerning criminality, he proposed different measures depending on the type of criminal he was addressing. Sterilization and deportation he proposed to those whom he found to be a particular threat to the society in order to punish them, but also to prevent the continuation of their genetic disposition to progeny. He also proposed the prohibition of marriage as a measure to some other groups such as the mentally ill, idiots, and epileptics.

DISCUSSION: Gundrum's work reflects different layers and attitudes of the period. Most clearly evident is the influence of Lamarckism. Gundrum believed that various public health interventions could change the hereditary constitution of the people, the reason why he accepted Forel's Lamarckian notion of blastophtorie. The most obvious expression of Lamarckian influence is Gundrum's method of deportation of criminals, which was based on his knowledge about the colonization of Australia. Besides the mentioned, he suggested the establishment of medical police based on methods of criminal anthropology used by Cesare Lombroso, although in other aspects he disagreed with him. Building up on such viewpoints, Gundrum definitely pioneered in shifting the focus of medicine from the illness of an individual to the illness of a society.

Acknowledgements: I am most grateful to my mentor for the support and productive discussions on the topic.

MeSH / Keywords: eugenics, prostitution, criminals, Lamarckism, Fran Gundrum

FORMAL MEDICAL ETHICS EDUCATION - NECESSITY OR EXCESSITY

Part of a Thesis: Professional ethics competences questionnaire (PECQ): an assessment tool for evaluation of medical students' professional ethics characteristics and competences

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Mentor: Professor Gordana Pavleković, MD, PhD

Affiliation: University of Zagreb School of Medicine, „Andrija Štampar“ School of Public Health, Zagreb

INTRODUCTION: Professional medical ethics should be the core attribute of every physician. It is of great importance to train medical students, future physicians for professionally ethical behavior. However, formal medical ethics education is only one small part in the complex process of getting ethical knowledge, skills and opinions. This qualitative study investigates what family medicine residents and final year medical students perceive as ethical problems which frequently occur in practice and explores how best to incorporate related competencies into medical training.

MATERIALS AND METHODS: A small-scale qualitative study involving two focus groups with separately 9 doctors and 9 final year medical students in Zagreb, Croatia was conducted. Participants were included through purposeful sampling. Interviews were audio-recorded, transcribed verbatim, and data was analyzed by means of thematic analysis. **RESULTS:** A number of frequent ethical dilemmas were considered - namely, openness and sincerity in discoursing the diagnosis and prognosis with the patient and his family, professional secrecy against the right of patient's family and related society to know the truth, and adequate information providing and patient's awareness in order to give eligible consent. The relative importance of each of these factors varied significantly and was influenced by participants' own beliefs and values. Common opinion revealed unreadiness and incompetence to deal with frequent ethical demands. Suggestions for medical ethics curriculum change were proposed in sense of introducing problem based ethical cases integrated in other subject (gynecology, internal medicine, pediatrics, family medicine) and organizing formal medical ethics lectures into smaller groups where the most frequent and not necessary the most interesting but rare specialist cases would be dealt with. Both groups acknowledged that they would strongly benefit from mentored small group discussion regarding frequent ethical dilemmas.

DISCUSSION: Although our results evidenced that all participants can identify frequent ethical problems and dilemmas residents presented greater frustration by not knowing how to resolve them. However, training in communication skills and some kind of ethical guidelines could mitigate their problem.

Acknowledgements: I would like to thank all focus group participants (medical students and family medicine residents) for sharing their time, experience and opinions in group discussions.

MeSH / Keywords: Medical ethics, medical education

ELEVATED BLOOD PRESSURE IN SCHOOL CHILDREN AND ASSOCIATED RISK FACTORS

Part of a Thesis: Risk factors of Elevated Arterial Blood Pressure among School Children and Adolescents

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Mentor: Professor Vesna Jureša, MD, PhD

Affiliations: University of Zagreb School of Medicine, Andrija Štampar School of Public Health

INTRODUCTION: Elevated blood pressure (BP) in children and adolescents is determined on the normative distribution of BP in healthy children. The aim of this study was to determine prevalence of high normal and elevated BP and to assess personal medical history, family history and overweight as associated risk factors among healthy school children.

MATERIALS AND METHODS: The study population was composed of 965 children (48.7% girls) in 8th grade of primary school in Croatia. Data were obtained from questionnaire and anthropometric measurements. Body mass index (BMI) was calculated as weight/height² (kilograms/square meter) and average value of two BP measurements, performed in a single occasion, for systolic (SBP) and diastolic (DBP), was calculated and used in analysis. Children with BMI \geq 90th percentile were considered as overweight. Children with mean value of SBP and/or DBP \geq 120/80 mmHg or \geq 90th percentile to have high normal BP, and \geq 95th percentile to have elevated BP.

RESULTS: The prevalence of high normal BP was 32.7%, % in girls 28.5 and 36.8% in boys. The prevalence of elevated BP was 6.5%, in girls 7.9% and 5.3% in boys. After first screening 39.2% of children with whether high normal or elevated BP could be considered to be „at risk“. The rate of overweight was higher in boys and girls with high normal and elevated BP than in those with normal BP. Of girls, 3.7% with normal, 19.4% with high normal and 29.7% with elevated BP were considered to be overweight. Of boys, 2.4% with normal, 9.3% with high normal and 30.8% with elevated BP were overweight. The most common associated factor in boys and girls was hypertension in family history, reported by 44.5% girls with normal, 43.3% high normal and 51.4% with elevated BP and 34.1, 35.2, 42.3%, of boys respectively.

DISCUSSION: The associated risk factors observed in this study can be used as independent markers of having elevated BP in school children. Population of children may be followed-up throughout school age resulting in implementation of databases with the purpose of revealing risks, thus making it possible to take useful measures trying to prevent diseases and promote healthy life style.

Acknowledgements: The study was supported by the Ministry of Science, Education and Sports Republic of Croatia, project number 108-1080135-0263.

MeSH / Keywords: children, adolescent, blood pressure, risk factors, school health service

AFFINITY OF FIRST YEAR UNIVERSITY STUDENTS TOWARDS GAMBLING AND BETTING

Part of a Thesis: Affinity of first year university students towards gambling and betting

PhD Candidate: Iva Pejnović Franelić, MD

Mentor(s): Professor Josipa Kern, PhD; Associate Professor Zoran Zoričić, MD, PhD

Affiliation(s): Croatian National Institute of Public Health, Andrija Stampar School of Public Health, University of Zagreb School of Medicine

INTRODUCTION: Gambling and higher education have been linked in some countries far in history. Lotteries served to establish Harvard, Yale, Columbia and Princeton. Harvard meta analysis reported that among college students the average rate of at-risk gambling is 7% and problem gambling is 5% which is higher than the rates among adult population. Despite the growing trend and negative health, psychological, social, financial and personal consequences gambling has only recently been recognized as an important public health problem. Aim was to determine the extent of gambling and betting and share of at-risk and problem gambling and betting among the first year university students, distinction according to faculty belonging to a particular scientific field of study and association with socioeconomic and psychosocial environment.

MATERIALS AND METHODS: Sample (900 male, 1093 female) from the scientific project „Characteristics, trends and determinants of addictive behavior in youth“, 2009. Data were analyzed by multivariate logistic regression models by gender.

RESULTS: 55.5% of the first year university students in Zagreb gamble and bet. 9.5% of students at-risk and problem gamblers. Different scientific fields of study have not shown to be the risk factor for at-risk and problem gambling and betting. Male gender was the strongest associated factor for at-risk and problem gambling and betting. Male students whose environment gambles, who smoke, drink at risk, involved in a sports club and risky driving have more chance to engage in at-risk and problem gambling. Those male students who receive scholarship have less chance for at-risk and problem gambling. Girls who have low self-esteem, use drugs and those involved in church/religious community and volunteer work have more chance for at-risk and problem gambling.

DISCUSSION: Most of the sociodemographic factors were shown not to have significant effect on at risk and problem gambling and betting. We can conclude that all students should be included in health promotion and universal prevention programs with special attention to youths involved in sports, to those who smoke, are involved in risky driving and drinking, to girls who use drugs, show low self-esteem, are involved in church/religious community and volunteer work.

Acknowledgements: Special thanks to colleagues from CNIPH and colleagues from Public health institute „Dr. Andrija Štampar“ for help with the work.

MeSH/Keywords: gambling and betting, students, scientific fields of study

EXPERIENCE AND KNOWLEDGE ABOUT PATIENT INFORMED CONSENT: A FIELD SURVEY ON A REPRESENTATIVE SAMPLE IN THE REPUBLIC OF CROATIA

Part of a Thesis: Informed Consent for Diagnostic and Therapeutic Procedures in the Hospital System in the Republic of Croatia

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Mentor: Assistant Professor Ana Borovečki, MD, PhD

Affiliations: University of Zagreb School of Medicine Andrija Štampar School of Public Health. University Hospital Merkur, Zagreb

INTRODUCTION: Informed consent is the process by which a patient expresses acceptance or refusal of a proposed medical procedure on the basis of information provided by a health worker about the medical procedure and the possible complications. So far, in transition countries in central and southeastern Europe, as well as the Republic of Croatia, there has been no research in which would be investigated the knowledge of citizens about informed consent, using a representative sample. That is why, the aim of this research was to investigate the experience and assess the knowledge of the Croatian citizens about informed consent.

MATERIALS AND METHODS: A survey was conducted using an independently created questionnaire on a nationally representative sample of 1023 adult subjects. The subjects were divided into two groups: those who had been and those who had been not in hospital for treatment in the past 5 years.

RESULTS: Of the total number of subjects one quarter (25%) had been treated in hospital. Sixty-one per cent of all subjects stated that they were partially acquainted with patients' rights. For some of the hospitalized subjects average level of information received in hospital from health workers represented complete information on their health, procedures and risks. Fifteen per cent of the hospitalized subjects did not know or could not remember if they had to sign consent for medical procedures. Of the subjects who had not been hospitalized, 37% were not acquainted with the term „informed consent“ and two thirds (67%) would not give consent for a procedure if they were not completely informed about the illness, the procedure, risks, complications and other methods of treatment

DISCUSSION: The knowledge of patients' rights and informed consent of the Croatian citizens is incomplete. This requires additional engagement in promoting patients' rights and education of citizens about the process of informed consent, both by the health system and by non-governmental organizations. It is necessary, as required by the law, to define the criteria for patients complete information procedures on the basis of which patients could make adequate and informed decisions.

Acknowledgements: I would like to thank Milan Milošević, MD, PhD, Assistant Professor Sanja Babić-Bosanac, PhD and Professor Jadranka Mustajbegović, MD, PhD for contribution as co-authors in paper.

MeSH/ Keywords: informed consent, Republic of Croatia, survey, knowledge, patients' rights

II.

RESEARCH PROPOSALS

EFFECT OF HEMODILUTION ON RENAL TUBULAR FUNCTION AFTER EXPOSURE TO EXTRACORPOREAL CIRCULATION IN CORRELATION WITH AGE AND SEX IN PATIENTS

Part of a Thesis: Effect of hemodilution on renal tubular function after exposure to extracorporeal circulation in correlation with age and sex in patients

PhD candidate: Mirabel Mažar, MD

Menthor: Professor Vesna Vegar Brozović, MD, PhD

Affiliation: Clinical Hospital Center Zagreb

INTRODUCTION: Renal dysfunction following cardiopulmonary bypass (CPB, extracorporeal circulation) is well recognized. The pathophysiology is multifactorial. A theoretical reduction in the incidence and severity of postoperative renal impairment has been proposed by advocating the various reno-protective strategies. Thus far, no single strategy has demonstrated conclusively its ability to prevent renal injury after CPB. Relation between lowest hematocrits and renal function after exposure of CPB may enable more effective renal protective strategies.

HYPOTHESIS: The aim is to follow the influence of hemodilution upon damage of renal tubuli by means of early and delicate indicators. The hypothesis is that moderate hemodilution which is caused by diluting blood by the so called „priming“ liquid of the CPB has an important role in reducing damage of renal tubuli due to its positive effects upon physiology of kidneys. The research will also try to show possible age and sex differences in relation to harmful effect as well as harmful effect of too much hemodilution.

GENERAL AIM: The aim of this scientific research can be described in three ways: (1) To determine initial values of the indicator of kidney damage in urine and serum before the beginning of CPB; (2) To determine the value of the indicator of kidney damage in urine and serum after the end of CPB depending on temporal dynamics (5 and 48 hours after the activity of harmful effects); (3) To compare the values of the indicators of kidney damage depending on the level of hemodilution, age and sex, and to correlate these values among the groups of subjects

SUBJECTS : The subjects will be patients who are subjected to heart operations by means of usage of the system of CPB. The subjects will be divided into several groups. Depending on an average decline of hematocrit during CPB, the subjects will be divided into three groups. The first group will consist of the subjects with the decline of hematocrit up to 15% of the initial values, the second group from 16 to 30% and the third group with more than 30%.

According to age, the subjects will be divided into four groups. With regard to the length of CPB, the subjects will be divided into the groups with longer (more than 90 minutes) and shorter duration (less than 90 minutes) of CPB. The subjects will be also grouped according to sex.

METHODS: The aim of this research is to determine the contents of urea and creatinine in serum and activity of NGAL and the contents of A1M in urine. The first samples of urine and serum will be taken before an operation. These samples make the basic value without the influence of CPB. The second samples of urine and serum will be taken 5 hours after the operation and the third samples will be taken 48 hours after the operation and they will give the values after CPB.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: Scientific contribution of this research would be in trying to determine the range of hematocrit which would protect kidney function during CPB. The research would determine the level of hematocrit at which the least damage of renal tubuli happens caused by the activity of CPB. The research would determine a direction of the range of aimed hematocrit that should be aimed at during the beginning of CPB. The research will also try to show possible differences depending on age and sex.

Keywords: kidney, renal tubular damage, cardiopulmonary bypass, cardiac surgery, hemodilution, renal protection

THE EFFECTS OF CSF ON THE CLINICAL PRESENTATION OF SPINAL ANESTHESIA ARE INFLUENCED BY THE CHANGE IN SPECIFIC DENSITY OF THE LOCAL ANESTHETIC SOLUTION

Part of the Thesis: The Influence of Cerebrospinal Fluid Volume and Length of the Lumbo-sacral Segment of the Spine on Spinal Anesthesia Characteristics During Administration of Levobupivacaine and Sufentanyl in a 1,3% and 5,2% Glucose Solution

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Mentor: Ino Husedžinović MD, PhD, Full Prof.

Affiliations: University Hospital "Dubrava", Zagreb, Croatia

INTRODUCTION:

The clinical presentation of spinal anesthesia (SA) is determined by the distribution of the local anesthetic solution (LA) in the subarachnoid space. Even though the choice of LA and technique of block fixation can yield a selective SA, a major drawback is still a high lack of predictability in the clinical presentation of SA. The cerebrospinal fluid volume (CSF) in the lumbo-sacral spine segment is considered to be the most significant individual factor contributing to the clinical presentation of SA.

HYPOTHESIS: By increasing the specific density of the LA solution, there is a decrease in the correlation coefficient between lumbo-sacral CSF volume and sensoric block height and an increase in the negative correlation between lumbo-sacral segment length in relation to the same parameters.

AIMS: To determine the relationship and predictive values of CSF volume and lumbo-sacral spinal segment length on SA characteristics, when administering LA solutions of different specific densities.

MATERIALS AND METHODS: The trial will include a total of 44 patients, who will be divided into two groups. Group I shall receive the LA in a 1,3% glucose solution, while Group II will receive a 5,2% glucose solution of LA. The clinical presentation of SA is defined by the height of the sensoric component and the intensity of the motor component of the subarachnoid block. Loss of temperature sensation is tested by using ice packs with the patient reporting no temperature discrimination. Motor component intensity is determined according to the modified Bromage scale. The measurement of lumbo-sacral spinal segment length and CSF volume from the cranial edge of the Th12-L1 intervertebral disc to the end of the dural sac will be determined by using magnetic resonance imaging on an MR 1,5T Magnetom Avanto, Siemens.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH:

The proposed trial shall determine whether an increase in the specific density of a local anesthetic solution can reduce the variability in the clinical presentation of spinal anesthesia, by altering the LA solution behaviour relative to the CSF volume of the lumbo-sacral spinal segment, thereby influencing the sensoric component height, motor component intensity and block regression time.

MeSH/Keywords: anesthetic techniques, spinal anesthesia; local anesthetics, levobupivacaine; cerebrospinal fluid volume

LIFE STRESS AND HUMAN PAPILLOMA VIRUS INFECTION

Part of a Thesis: Influence of Stress on Clinical Manifestation of Human Papilloma Virus Infection

PhD candidate: Hrvoje Cvitanović, MD

Mentor: Associate Professor Liborija Lugović Mihić, MD, PhD

Affiliations: General Hospital Karlovac

INTRODUCTION: The cause and course of various diseases can not be explained only by the action of biological factors, but it is important to also consider psychological and social elements. Life stress plays a major role in etiology of various dermatoses including skin infections. Relationship between life stress and the manifestation of human papillomas virus (HPV) suggests that life stress affects the immune status of patients and consequently clinical manifestations of HPV infection.

HYPOTHESIS: The hypothesis is that there are differences in clinical manifestation of HPV infection (genital and extragenital) with respect to the degree of life stress measured by the Recent life changes questionnaire (RLCQ) and maladaptive reaction in Brief COPE test. **AIMS:** The aim is to examine the impact of life stress events on clinical manifestations of HPV infection according localization, number of changes, duration of persistence, age and gender. **MATERIALS AND METHODS:** The study will include 180 patients. Subjects will be patients who treated for HPV infection in the Department of Dermatology and Venereology, General Hospital Karlovac. Subjects will be divided into two groups. The first group will have clinically evident HPV infection and high degree of stress measured by the RLCQ tests and maladaptive reaction in Brief COPE test, while the control group patients will have clinical HPV infection and negative degree of life stress measured by RLCQ.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: The study could explain the connection between life stress events, coping with life stress and clinical manifestation of HPV infection. Evidence of relationships between life stress and HPV infection could be important in clinical practice, especially in the treatment of resistant forms of HPV infections where psychological elements could be one of the causes of persistence of infection.

Acknowledgements: I would like to thank management of General Hospital Karlovac on support.

MeSH / Keywords: Papilloma Infections, Life Stress

HUMAN PAPILOMA VIRUS - POSSIBLE COFACTOR IN DEVELOPMENT OF NONMELANOMA SKIN CANCER

Part of a Thesis: The Influence of Human Papillomavirus on Nonmelanoma Skin Cancer Etiology

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INTRODUCTION: Cutaneous HPV types have been established and associated with precancerous lesions, in situ carcinomas and NMSC in numerous studies, but the actual importance of these viruses in the etiology of these lesions still remains unknown. Whereas HPV-DNA has been detected in normal skin and in premalignant lesions „hit and run“ mechanism, which no longer requires the viral agent but the activity of HPV oncoproteins, has been suggested. Recent studies are also trying to establish association between UV radiation and organism's immune response to HPV.

HYPOTHESIS: There is an increased prevalence of HPV DNA in precancerous lesions (actinic keratoses- AK) and in situ carcinomas (Mb. Bowen), as well as in basal cell carcinomas localized on permanently sun-exposed skin (face), and basal and squamous cell carcinomas in geographic areas with increased insolation.

AIMS: To establish the role of HPV in development of NMSC by determining its prevalence in AK, Mb. Bowen, SCC and BCC. To investigate the prevalence and differences between various HPV types according to tumor localization (face/trunk) and patients' geographic distribution (two geographic regions with different insolation- cities of Zagreb and Rijeka).

MATERIALS AND METHODS: HPV DNA will be detected from paraffin-embedded biopsy materials of AK, Mb. Bowen, SCC and BCC by reverse hybridization assay. Part of the E1 region of the β -papillomavirus genome will be amplified by PCR. Genotyping of 25 skin HPV types (5, 8, 9, 12, 14, 15, 17, 19, 20, 21, 22, 23, 24, 25, 36, 37, 38, 47, 49, 75, 76, 80, 92, 93 i 96) will be carried out. Identification of high-risk HPV types 16 i 18 will also be performed. To explore differences in sun intensity (dependent of geographical latitude), city-specific data on the erythemal UV index will be obtained and median values will be calculated for cities Zagreb and Rijeka.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: To confirm „hit and run“ theory and support a role for HPV infection in skin carcinogenesis as a co-factor in association with UV. To provide a possibility of different therapy approach in treating skin cancers (local immunomodulatory treatment and possible vaccination).

MeSH / Keywords: HPV, basal cell carcinoma, squamous cell carcinoma, actinic keratosis, carcinoma in situ, ultraviolet rays

THE CLINICAL PATTERN VARIATIONS OF TINEA CAPITIS ASSOCIATED WITH NEW AND ATYPICAL PATHOGENS

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INTRODUCTION: Significant changes in epidemiology, aetiology and in the clinical pattern of fungal scalp infections caused by *Microsporum* spp. have been observed which are usually moderately inflammatory, but, most recently, cases of severe kerion-like tinea capitis have been also registered. In the last decade, up to 70 cases of kerion-like deep seated mycosis due to *Microsporum* spp. were verified by culture in the Reference Laboratory for Dermatological Mycology in the University Department of Dermatology and Venereology, Zagreb University School of Medicine, instead of *Trichophyton* spp. which has traditionally been the expected pathogen in those cases.

HYPOTHESIS: The same fungal species might evoke different clinical pattern. Thus, interspecies polymorphism within *Microsporum canis* isolates might have been responsible for this phenomenon thus, not the same genotype of *Microsporum canis* species might have been the reason for either non-inflammatory (superficial) or inflammatory (deep seated) clinical pattern of tinea capitis.

AIMS: To identify the patients with deep seated tinea capitis caused by *Microsporum canis*, to study the genotypic variability within isolates of *Microsporum canis* from those patients and to compare these results with the isolates of *Microsporum canis* from the patients with the superficial tinea capitis.

MATERIALS AND METHODS: Sixty strains of *Microsporum canis* from patients with both superficial and deep-seated tinea capitis will be isolated and identified to species using standard and advanced mycological procedure techniques. Molecular identification of the positive isolates will be then assessed by the amplification of the Internal Transcribed Spacer (ITS) regions of fungal ribosomal DNA (rDNA) followed by the sequencing of the amplification products. The PCR primers ITS1 and ITS4 which amplify the variable ITS1 and ITS2 sequences will be used. Only the patients with superficial tinea capitis due to *Microsporum canis* will represent the control group.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED REASEARCH: To try to understand more clearly the pathogenicity of the fungi causing tinea capitis and to try to contribute to the scientific explanation why the same fungal species might evoke completely different clinical pattern. An attempt of the scientifically founded modification of the treatment regimen, of tinea capitis due to resistant pathogens, such as *Microsporum canis*.

EFFECT OF MICROENVIRONMENT PH ON BIOAVAILABILITY OF RISEDRONATE

Part of a Thesis: Effect of Microenvironment pH on Bioavailability of Risedronate

PhD candidate: Ana Lebo, MD, MSc

Mentor: Vladimir Trkulja, MD, PhD

Affiliations: Department of Pharmacology, Zagreb University School of Medicine, Croatia, PLIVA-Croatia Ltd., Zagreb, Croatia

INTRODUCTION: Risedronate is a pyridinyl-bisphosphonate that binds to hydroxyapatite and inhibits bone resorption. Despite the low mean oral bioavailability of the tablet (< 1%) risedronate is administered orally. As other bisphosphonates, it causes local irritation of the upper gastrointestinal mucosa. Risedronate is not metabolized systemically and is excreted primarily through kidneys. Renal clearance is not concentration-dependent. Thus it represents a good model drug to investigate the role of microenvironment pH on its absorption. Whether pH-change has clinical significance is presently not fully understood. Additionally, pharmacokinetic differences due to ethnicity have not been studied. The present research aims at elucidating the factors that affect risedronate's absorption and bioavailability.

HYPOTHESIS: A change of microenvironment pH plays a role in risedronate's bioavailability. **AIMS:** 1) To assess the pH vs other factors affecting risedronate's bioavailability. 2) To explore age- or gender-dependent differences in bioavailability 3) To assess ethnic disparities in risedronate's plasma concentrations 4) To evaluate adverse events (AEs) and 5) To determine clinical significance of these findings.

MATERIALS AND METHODS: We will analyze a set of data from approximately 150 healthy subjects who participated in 3 randomized, single-dose crossover bioavailability/bioequivalence (BA/BE) studies with risedronate 35 mg tablets, paralleled by analysis of in vitro dissolution profiles and physico-chemical parameters. We will perform statistical analyses to achieve the aims above, focusing on differences that can be attributed to pH-change. AEs will be analyzed in relation to pharmacokinetic and other parameters (disintegration data). Meta-analysis of risedronate plasma concentrations will be done relative to physiologic (creatinine clearance) and demographic parameters (ethnicity, age, BMI, gender). The trials were approved by ethics committees.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: Osteoporosis is of great public health importance owing to increased longevity and increasing prevalence. Bisphosphonates remain to be the mainstay of both prevention and treatment of osteoporosis. This multidisciplinary research includes industry and academia view on influence of physico-chemical properties on bioavailability and its clinical significance. The results will contribute to a better understanding of interplay between physico-chemical, physiological and clinical factors relating to bisphosphonates. A strong focus is put on implications for patients' safety and quality of life.

Acknowledgements: I wish to thank PLIVA-Croatia Ltd. for their support and interest in this project and for the shared discussions.

MeSH/Keywords: bioavailability, bisphosphonates, pH, risedronate, adverse events

OSTEOCALCIN AND TYPE 2 DIABETES

Part of a Thesis: „Osteocalcin and type 2 diabetes mellitus“

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Mentor: Assistant Professor Ivana Pavlić-Renar, MD, PhD

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INTRODUCTION: Recent studies and pharmacological experiments in mice suggest that osteocalcin, a bone-specific protein secreted by the osteoblasts, might act as a hormone affecting insulin production and sensitivity, glucose utilization and energy expenditure.

Blood osteocalcin levels were shown to be significantly lower in diabetics than in non-diabetic controls and inversely related to fat mass and blood glucose.

Most clinical studies investigating possible metabolic effects (or associations) with osteocalcin levels, did not distinguish total (TOC) and noncarboxylated osteocalcin (ucOC), the suggested active form of osteocalcin in mice, which has fewer than three carboxylated residues, and has a lower affinity for bone. Improved glycemic control appears to increase TOC but does not necessarily have the same effect on ucOC.

Additional studies with ucOC as an outcome are needed to clarify the effects of improved glycemic control on this marker.

HYPOTHESIS: Undercarboxylated osteocalcin (ucOC) level is associated with changes in level of glucose control assessed by HbA1c; i.e. undercarboxylated osteocalcin (ucOC) is involved in glucose metabolism not related to bone turnover.

AIMS: The general aim of the study is to assess association of undercarboxylated osteocalcin (ucOC) and changes in HbA1c as a marker of glucose regulation in type 2 diabetes.

Specific aims are to assess the relationship of serum total osteocalcin (TOC) and ucOC level with markers of blood lipid metabolism (serum cholesterol- total, HDL and LDL and triglycerides), anthropometric measurements (body mass index and waist/hip ratio) and markers of bone turnover (Ca and phosphate in plasma and daily urine, bone isoenzyme of ALP, crosslaps teopeptide).

MATERIALS AND METHODS: In 50 consecutive poorly controlled type 2 diabetic patients, ucOC and TOC will be measured before and after three month of improved glucose control defined as at least 1% decrease in HbA1c, by life style change.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: The results of this thesis are expected to give contribution in revealing longitudinal association on undercarboxylated osteocalcin and parameters of diabetes control.

Acknowledgements: DAAD(Deutscher Akademische Austausch Dienst) and University of Prishtina for financial support.

MeSH / Keywords: osteocalcin, underdecarboxylated osteocalcin, type 2 diabetes, HbA1c.

PTH ASSAY IN EARLY POSTOPERATIVE DIAGNOSIS OF HYPOCALCAEMIA

Part of a Thesis: Predictive Value Of PTH Assay In Early Postoperative Diagnosis Of Hypocalcaemia

PhD candidate: Renata Curić Radivojević, MD

Mentor: Professor Drago Prgomet, MD, PhD

Affiliation(s): University of Zagreb School of Medicine, University Hospital Centre - Zagreb

INTRODUCTION: Hypocalcaemia is the most frequent complication after thyroid surgery with wide range of incidence from 1.2 to 40%. Permanent hypoparathyroidism is less common and occurs in 3 to 10% patients. Postoperative hypoparathyroidism is traditionally detected by serial measurement of serum calcium, requires multiple venepunctures and prolonged hospitalization. In recent years, iPTH assay has been under investigation for thyroid surgery as an early prediction of postoperative hypocalcaemia, guiding the surgeon for parathyroid autotransplantation, and calcium substitution therapy. Still there are many controversies about it, and there are no unique guidelines for PTH use.

HYPOTHESIS: iPTH is a better predictor of postoperative hypocalcaemia than serial measurement of ionized calcium (iCa).

AIMS: This study aims to determine the role of iPTH assay in comparison to ionized calcium as a predictor for hypocalcaemia and its impact on future management of patients with these complications. Further goals are to determine: predictive values of PTH assay in early postoperative diagnosis of hypocalcaemia; optimal number and time of sampling blood for PTH; sensitivity and specificity of the method as a screening test.

MATERIALS AND METHODS: A 100 patients going for a thyroid surgery will be followed up prospectively. 63 patients in main group scheduled for thyroidectomy, and 37 patients in control group scheduled for partial resection of thyroid e.g. lobectomy. Exclusion criteria: underage patients, previous thyroid or neck surgery, renal insufficiency, parathyroid disease. Blood samples for iPTH and iCa measurement will be taken 8 times according to the planned time table (during, and after surgery, as well as in outpatient follow up).

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: Early iPTH measurement may be of value in prediction of postoperative hypocalcaemia, and in guiding surgeon whether to perform parathyroid autotransplantation. iPTH can serve as useful tool for selecting patients requiring substitution therapy and safe discharge home.

Acknowledgements: I would like to thank my mentor, nurses and technicians from the Department of ENT and Nuclear medicine, as well as to OR personnel for their help and patience during collecting and processing samples for this investigation.

MeSH / Keywords: Parathyroid hormone, thyroid surgery, hypocalcaemia, complications, hypoparathyroidism

RELATIONSHIP OF ADIPONECTIN AND OMENTIN IN NECK ADIPOSE TISSUE AND SERUM WITH METABOLIC RISK

PhD candidate: Danijela Grizelj, MD

Mentor: Vlatka Pandžić Jakšić, MD, PhD, research associate

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INTRODUCTION: Increasing amounts of adipose tissue which we recognize clinically as obesity are associated with the development of insulin resistance, diabetes, dyslipoproteinemia, atherosclerosis and cardiovascular disease. Ectopic compartments of adipose tissue, which originate from its redistribution in obesity, are the source of predominantly proinflammatory adipocytokines. They shift the metabolic balance toward chronic inflammation and contribute to the development of metabolic disorders. Increased waist circumference has revealed the visceral adipose tissue as a large compartment of ectopic fat, but recent data have shown that the increased neck circumference is also associated with metabolic risk.

HYPOTHESIS: Expression of adiponectin and omentin in the neck adipose tissue is different in subcutaneous and paracarotid section and is associated with their serum levels and metabolic risk factors.

AIMS: We want to investigate the level of adiponectin and omentin expression in different sections of neck adipose tissue, to compare them to their serum levels and to examine their association with several metabolic risk factors.

MATERIALS AND METHODS: The study will include 40 participants. Samples of adipose tissue will be taken during routine neck surgery. Gene expression of omentin and adiponectin in the tissue will be analyzed by RQ-PCR method. Serum samples will be taken preoperatively for the analysis of adipocytokine levels by ELISA and for the analysis of standard laboratory parameters. Standard anthropometric measurements, body composition analysis by bioelectrical impedance and ultrasound measurement of intima-media thickness of common carotid artery will be also made.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: There is no known research of comparing adipocytokines' gene expression between different neck adipose tissue sections. We have assumed that at least part of the adipose tissue of the neck acts as an ectopic compartment. In this context, we want to investigate whether the expression of adiponectin and omentin differ in neck adipose tissue compartments and whether they may be related to serum adipocytokine levels and metabolic risk factors.

MESH / Keywords: adiponectin, omentin, neck, adipose tissue

INTERFERON GAMMA FROM LYMPHOCYTES OF CHILDREN UNDER FIVE YEARS WITH LATENT TUBERCULOSIS INFECTION

Part of thesis: Interferon gamma from Lymphocytes of Children under Five Years with Latent Tuberculosis Infection

PhD candidate: Ivan Pavić MD

Mentors: Professor Neda Aberle, MD, PhD; Professor Slavica Dodig, PhD

Affiliation: Clinical Hospital Center „Sestre milosrdnice“ Children's Hospital Zagreb

INTRUDUCTION: Children who have been in contact with active tuberculosis (TB), has a high risk of infection with Mycobacterium (M.) tuberculosis. In children aged up to 5 years, the risk of developing TB within 2 years of infection is 20-40%. In response to the infection host's immune system reacts with nonspecific and after with specific immune responses. Cellular immune response could be examined: in vivo (tuberculin skin test (TST) and ex vivo (interferon gamma (IFN- γ) released from T lymphocytes upon stimulation with M.tuberculosis-specific antigens).

HYPOTHESIS: The concentration of released IFN- γ depends on the age of children. Measuring the concentration of IFN- γ contributes to the diagnosis of LTBI in children < 5 years, which reduces unnecessary prophylaxis.

AIMS: The aim of the present study was to improve diagnosis of LTBI in BCG-vaccinated children < 5 years. The objectives were to assess: 1) the influence of infectivity of adult source case [proximity of contact, cavitory lesions, acid fast bacilli staining, culture status], 2) the impact of age on results of IFN- γ , and 3) the agreement between IFN- γ and TST results.

MATERIALS AND METHODS: Children 1 month to 5 years of age who will be examined because of history of exposure to a case of TB. Exclusion criteria include children \geq 5 years, immunocompromised children, inadequate blood sampling and diagnosis of TB.

IFN- γ , using a commercial QuantiFERON-TB Gold In Tube test (Cellestis Ltd., Carnegie, Australia) was measured according to the manufacturer's instructions. TST was performed with two tuberculin units of standardized purified protein derivative (PPD) solution (Tuberculin PPD RT 23, Statens Serum Institute, Copenhagen, Denmark).

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: The results of our research will contribute to better understanding the impact of exposure of children < 5 years in contact with TB patients. The results of TST and IFN- γ could help in the assessment of cellular immunity mediated by T lymphocytes in children with LTBI. Based on the results of the determination of IFN- γ after stimulating with M. tuberculosis-specific antigens got to see the specific reactivity of immunocompetent effector T-lymphocytes in children with LTBI.

Keywords: children, interferon gamma release assay, latent tuberculosis infection, tuberculin skin test

PROPRIOCEPTION IN OSTEOARTHRITIS

Part of a thesis: Effects Of A Proprioceptive Therapeutic Training Program On Pain And Functional Ability Compared With Standard Therapeutic Training Program In Patients With Knee Osteoarthritis: Single-Blind Controlled Study

PhD candidate: Lana Bobić Lucić MD

Mentor: Professor Simeon Grazio MD, PhD

Affiliation: Special Hospital for Medical Rehabilitation Lipik, Lipik

INTRODUCTION: Osteoarthritis (OA) is a chronic noninflammatory rheumatic disease and can have significant impact on functional ability or lead to severe disability of the patient. Clinical guidelines induce support for training programs under the supervision of a physiotherapist in order to reduce the pain and improve function and finally quality of life. Many of clinical trials are researching training programs that are unspecific in relation to the nature of the damage. The efficacy of proprioceptive training in prevention, rehabilitation and improvement of a number of motor characteristics is proven. Leading body in situations which could induce activation of proprioceptors, the optimal response in emergency situations that could cause injury is enabled, which is the basic meaning of proprioceptive training. Compared with a healthy population, people with knee OA have reduced proprioceptive function of the affected joint. This research aims to evaluate the effectiveness of proprioceptive training, in addition to standard training, and these effects will be compared with the effects of standard therapeutic training program.

MATERIALS AND METHODS: The study will include a total of 140 subjects both men and women, 50 to 80 years of age in whom OA have been diagnosed according to the criteria of The American College of Rheumatology. They will be randomly assigned to 1 of 2 groups that performed exercises for 12 days under the supervision: a standard-exercise group and a proprioceptive-exercise group. The outcome measures included a visual analog scale (VAS) for pain, the Western Ontario and McMaster Universities Osteoarthritis Indeks (WOMAC), The Short Form Health Survey (SF-36), manual muscle test, range of motion, muscle power and a few balance tests - Tinetti Performance Oriented Mobility Assessment (POMA), FICSIT-4 (Frailty and Injuries: Cooperative Studies of Intervention Techniques) and The Activities-specific Balance Confidence (ABC) Scale. Measurements will be recorded by a blinded researcher at baseline, 12 days, 1 and 3 months after initiating the intervention.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: The present study will help better understanding of proprioceptive training as a supplement to conventional practice and their effects on functional ability in persons with OA of the knee.

Acknowledgements: I would like to thank Mr.D.Kelemen, a director of the hospital, for financial support; my mentor Prof.S.Grazio, MD, for his guidance, encouragement and corrections and my colleague Ms.S.Slivar Rendulić, MD, for her very helpful comments and suggestions.

MeSH / Keywords: osteoarthritis, knee, proprioception, exercise

SERUM GHRELIN, LEPTIN AND PROINFLAMMATORY CYTOKINES LEVELS IN INFLAMMATORY BOWEL DISEASE

Part of a Thesis: Cross Sectional Study On Serum Ghrelin, Leptin And Proinflammatory Cytokines Levels In Inflammatory Bowel Disease

PhD candidate: Ana Kunović, MD

Mentor: Associate Professor Željko Krznarić, MD, PhD, FEBGH

Affiliation(s): Clinical Hospital Centre Zagreb

INTRODUCTION: Crohn's disease and ulcerative colitis are chronic, idiopathic and relapsing forms of inflammatory bowel disease (IBD). The pathogenesis of IBD likely involves genetic, environmental, and immunological factors. Treatment largely focuses on targeting local and systemic inflammation, including administration of steroids, aminosalicylates, immunomodulating agents, and biologic medications such as monoclonal antibodies to inflammatory cytokines. There is number of studies investigating blood chemistry markers in inflammatory diseases.

HYPOTHESIS: In IBD patients serum ghrelin, leptin and proinflammatory cytokines (IL-6, TNF α) levels are related to activity of the disease, nutritional status and different therapy modalities.

AIMS: Our aim is to investigate the relation between ghrelin, leptin and proinflammatory concentration and activity of the disease; to determine a possible role of these hormones and cytokines as a biomarkers for evaluation of inflammatory activity and efficiency of therapy. In addition, the aim of this study is to determine the correlation between serum ghrelin, leptin and proinflammatory cytokines levels and nutritional status of patients with inflammatory bowel disease receiving different medications.

MATERIALS AND METHODS: Ghrelin, leptin and proinflammatory cytokines levels will be measured in 150 to 250 patients and 50 healthy volunteers with inflammatory bowel disease using commercially available enzyme-linked immunosorbent assays. Ghrelin, leptin and proinflammatory levels will be correlated with disease activity, type, localization, treatment and nutritional status. Measurements will be repeated in three months and correlated with previous values and disease activity and nutritional status.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: To discover a role of ghrelin, leptin and proinflammatory cytokine levels as a potential biomarkers of the activity of the disease, nutritional status and drug effect on the course of the disease.

MeSH / Keywords: Inflammatory bowel disease, ghrelin, leptin, proinflammatory cytokines

QUALITY OF LIFE AND FUNCTIONAL PROSTHETICS REHABILITATION OUTCOME

Part of a Thesis: Evaluation of quality of life and functional prosthetics rehabilitation outcome in patients with unilateral lower limb amputation

PhD candidate: Tamara Vukić, M.D.

Mentor: Assistant Professor: Ida Kovač, MD, PhD

Affiliations: Institute For Rehabilitation And Orthopaedic Devices, University Hospital Centre - Zagreb

INTRODUCTION: In this research we will use two chosen questionnaires to evaluate the functional outcome of prosthetic rehabilitation and to analyze quality of life of patients with lower limb amputation

HYPOTHESIS: Patients who will have a better functional outcome after prosthetic rehabilitation at PPA questionnaire analysis will also have a better quality of life with prosthesis at SF-36 questionnaire analysis.

AIMS: The aim of this study was to analyze the functional outcome of prosthetic rehabilitation of patients with unilateral lower limb amputation and analysis of quality of life of these patients.

MATERIALS AND METHODS: We will analyze a group of 80 patients who spent their first prosthetic rehabilitation and prosthetic supply in the Institute For Rehabilitation and Orthopaedic Devices University Hospital Centre Zagreb. Conditions that patients must meet to be included in this study are: age of patients above 18 years, unilateral lower limb amputation, and the period which has elapsed since the first prosthetic supply must be at least one year. Participants will complete two questionnaires. The first questionnaire Prosthetic Profile of the Amputee (PPA) is a questionnaire specific to persons with amputation. This questionnaire will determine the functional outcome of prosthetic rehabilitation and the success of prosthetics supply. The second questionnaire the Short Form (36) Health Survey - (SF-36) is a generic questionnaire used to assess the quality of life of patients, and in this case to analyze the quality of life of patients with a prosthesis and their general life satisfaction. From these two questionnaires we will conclusively analyze the important predictors for the success of prosthetic rehabilitation.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH : This research will contribute to the analysis of functional outcome of prosthetic rehabilitation and to the analysis of the quality of life of patients with prosthesis. The results of two questionnaires will be analyzed and compared. From this analysis we will draw important predictors for successful prosthetic rehabilitation of patients with lower limb amputation.

To our knowledge this kind of research was not yet conducted or published.

Acknowledgements: I would like to thank my mentor Assistant Professor Ida Kovač and also great thanks to all colleagues who participated in this study.

MeSH / Keywords: prostheses, rehabilitation, quality of life, questionnaires

HEALTH-RELATED QUALITY OF LIFE IN INFLAMMATORY BOWEL DISEASE

Part of a Thesis: Quality Of Life In Children With Inflammatory Bowel Disease: Role Of Disease-Specific And Generic Questionnaires

PhD candidate: Slaven Abdović, MD

Mentor: Professor Sanja Kolaček, MD, PhD

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

INTRODUCTION: Health-related quality of life (HRQOL) assesses how disease and therapeutic management affect health, psychological status, social interaction and coping with symptoms in respect to the patient's own perceptions and expectations. Significant increase in the prevalence of inflammatory bowel disease (IBD), relapse-remitting course, debilitating symptoms, and frequent complications make this disease very important in respect of the patient's quality of life, particularly in children. Impact-III is the only disease-specific HRQOL instrument for use in children with IBD, and it has not been adapted for use in Croatia.

HYPOTHESIS: Impact-III and SHS are valid assessment tools for evaluation of HRQOL impairment in children with IBD. HRQOL questionnaires are valid in prognosis of future complications and treatment outcome.

AIMS: To linguistically and psychometrically validate Impact-III as a part of cross-cultural adaptation. Investigate appropriateness of different disease-specific and generic instruments for evaluation of HRQOL in children with IBD and to compare it to the control, healthy sample. Analyze correlation of proxy reporting in HRQOL to child's self-reporting. Assess which factors strongly influence HRQOL in children with IBD and investigate relevance of HRQOL instruments as treatment and disease-course outcome measures.

MATERIALS AND METHODS: One hundred children and adolescents aged 9-18 with IBD from three pediatric gastroenterology departments in Croatia will be recruited. HRQOL will be self-assessed using two disease-specific (Impact-III and SHS) and a generic questionnaire (PedsQL™). Parent will fulfill proxy version of the generic instrument. Demographics, data on disease type, extent, activity, as well as current and previous therapy, anthropometry and complications will be noted. Assessments will be repeated after 6 and 12 months.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: By using IBD-specific (IMPACT-III, SHS) and generic questionnaires (PedsQL™) we will assess quality of life in 100 pediatric IBD patients. In relation to the activity indices, disease extent and anthropometrical measures we will report applicability of quality of life scales in projection of future complications of disease course. Implementing generic questionnaires, quality of life will be compared to the control sample, while proxy reporting will assess appropriateness of parental evaluation of child's quality of life.

Acknowledgements: I would like to thank Professor Mladen Persic from University Hospital Center Rijeka and Dr. Irena Senecic-Cala from University Hospital Center Zagreb for participation in this research.

MeSH / Keywords: inflammatory bowel disease, Crohn's disease, ulcerative colitis, health-related quality of life, questionnaires, children and adolescents

GAMMA-GLUTAMYL TRANSFERASE IN DETECTION AND ESTIMATION OF SEVERITY OF NON-ALCOHOLIC STEATOHEPATITIS

Part of a Thesis: Value Of Elevated Gamma-Glutamyl Transferase In Detection And Estimation Of Severity Of Non-Alcoholic Steatohepatitis

PhD candidate: Neven Baršić, MD

Mentor: Professor Marko Duvnjak, MD, PhD

Affiliations: University of Zagreb School of Medicine, University Hospital Centre 'Sestre Milosrdnice'

INTRODUCTION: Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease, with potentially progressive form, non-alcoholic steatohepatitis (NASH), present in a significant proportion of patients. NAFLD is commonly unveiled by accidental finding of elevated aminotransferases, and liver biopsy is the only reliable method which can confirm the diagnosis and determine disease severity in a given patient. Diabetes is the major risk factor for presence of progressive disease forms. Published data on aminotransferase elevations in diabetics are inconclusive. Gamma-glutamyl transferase (GGT) is often excluded from studies of NAFLD, notwithstanding the evidence for good correlation with disease characteristics in some studies. Despite frequent occurrence in common clinical practice, there is no published data on prevalence of GGT elevation in NAFLD, or histological spectrum of disease and clinical characteristics in the subgroup of patients with isolated GGT elevation.

HYPOTHESES: GGT is a significantly more prevalent enzyme elevation found in diabetic population compared to elevated ALT or AST. When compared to ALT and AST, GGT shows better correlation with disease characteristics. Histological spectrum of disease in patients with isolated elevation of GGT is not less severe when compared to patients with elevated ALT/AST.

AIMS: The aim of the first part of the study is to define and compare prevalence of aminotransferase elevation in a large cohort of diabetics. In the second part, aim is to prospectively characterise the elevation of aminotransferases in NAFLD patients and define and compare correlation of histological spectrum and clinical parameters with enzyme levels, including evaluation of the subgroup of patients with isolated GGT elevation.

MATERIALS AND METHODS: The first part of the study used electronic registry of outpatient diabetes patients seen at our hospital. Prospective evaluation will include all patients (n ~ 90) evaluated for suspected NAFLD who underwent liver biopsy at our department. Detailed histologic evaluation and correlation calculation will include all important pathologic and clinical characteristics of the disease.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: The study would show that elevation of GGT is frequently the only indicator of steatohepatitis and that its value should not be disregarded in clinical practice and in epidemiological and other studies of NAFLD, which is currently often the case.

Acknowledgements: I would like to thank my mentor, as well as the staff of Department of gastroenterology and hepatology and Department of pathology at Sestre milosrdnice University hospital centre for their support and help in conduction of this study.

MeSH / Keywords: nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), diabetes.

CALPROTECTIN IN ASSESSMENT OF ACTIVITY OF ULCERATIVE COLITIS AND CROHN'S DISEASE

PhD candidate: Dora Grgić MD

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

Affiliation(s): University of Zagreb School of Medicine, University Hospital Centre-Zagreb

INTRODUCTION: Inflammatory bowel diseases (IBD)(Crohn's disease and ulcerative colitis) are chronic conditions characterized with recurrent episodes of inflammation in the gastrointestinal tract. The diagnostic grounds for IBD are endoscopic methods, but due to minimized invasiveness, new diagnostic markers have been implemented into clinical practice. Calprotectin is a calcium-binding protein, very stable and resistant to proteolytic degradation in stool, found in abundance in neutrophils. In inflammatory and infectious conditions calprotectin's concentration rises up to 100 fold. Recent studies have suggested calprotectin as a good marker in distinguishing inflammatory bowel diseases from similar irritable bowel syndrome. However, intensity of endoscopic lesions and fecal calprotectin haven't been compared yet.

HYPOTHESIS: Positive correlation exists between concentration of calprotectin in stool and Crohn's disease activity index in patients with Crohn's disease. Positive correlation exists between concentration of calprotectin in stool and Truelove Witts index in patients with ulcerative colitis.

AIMS: 1. Analyze correlation of fecal calprotectin concentration in patients with IBD with disease activity; 2. Determine concentration of fecal calprotectin in patients with ulcerative colitis; 3. Determine concentration of fecal calprotectin in patients with Crohn's disease ; 4. Determine values of fecal calprotectin and correlation with different stages of disease activity; 5. Analyze correlation of fecal calprotectin with laboratory parameters and indexes of activity of IBD

MATERIALS AND METHODS: 164 patients with diagnosis of IBD (82 patients with Crohn's disease, 82 patients with ulcerative colitis) either hospitalized in Department of Gastroenterology either as outpatients will be included in our study. After agreement and written consent to be part of a study 5 grams of stool and 5 ml of blood will be taken for analysis. Elisa method will be used for quantitative measurement of fecal calprotectin.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: This research will explore the relationship between fecal calprotectin in IBD with the degree of inflammatory activity and also evaluate diagnostic value of fecal calprotectin in active IBD.

MesH / Keywords: calprotectin, inflammatory bowel disease, faecal markers

INFLUENZA VACCINE EFFECTIVENESS ESTIMATE

Part of a Thesis: Influenza Vaccine Effectiveness Estimate in Prevention of Seasonal Influenza 2010-11

PhD candidate: Sanja Kurečić Filipović, MD, MSc

Mentor: Professor Ira Gjenero-Margan, MD, PhD

Affiliation: Croatian National Institute of Public Health

INTRODUCTION: Influenza vaccination has been a main strategy in prevention and control of seasonal and pandemic influenza over the last 60 years. Influenza vaccine effectiveness (IVE) is defined as likelihood of the intervention preventing influenza under the field conditions. Seasonal IVE estimates vary from season to season because of virus variation and the variation in the influenza epidemics characteristics. This observational study uses test-negative case-control method in order to estimate IVE in the season 2010-11 in preventing RT-PCR laboratory confirmed influenza.

HYPOTHESIS: 2010-11 seasonal influenza vaccine decreases the odds of RT-PCR laboratory confirmed influenza.

AIMS: To collect characteristics of individuals who had an influenza-like illness (ILI) in season 2010-11 and whose clinical samples were tested by RT-PCR method for influenza confirmation at WHO National Influenza Centre. To estimate overall IVE and IVE in specific groups (age groups, risk groups recommended for vaccination) in preventing RT-PCR confirmed influenza in the season 2010-11 using test negative case-control method. **MATERIALS AND METHODS:** Study population consists of individuals who consulted physicians due to influenza-like illness (ILI) in season 2010-11 and whose respiratory sample had been taken and sent to WHO National Influenza Centre at Croatian National Institute of Public Health in order to detect influenza virus. Out of all individuals whose samples have been tested by RT-PCR, 1000 individuals were randomly selected to be contacted by phone in order to collect data on vaccination status and other patient characteristics using a standardized questionnaire. The sample size is based on the assumption of the vaccine effectiveness of 60% (OR: 0.40) and vaccine coverage of 11% among controls. Cases are defined as ILI cases with a respiratory sample positive for influenza and controls are defined as ILI cases tested negative for influenza by RT-PCR. Test-negative case-control method will be used to estimate influenza vaccine effectiveness in preventing RT-PCR confirmed influenza. Vaccine effectiveness (VE) will be computed using the following equation:

$$VE = 1 - OR * 100.$$

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: The study results of this research conducted in a specific epidemiological setting will be used for making further recommendations of seasonal influenza vaccination.

Acknowledgements: I would like to thank all medical professionals nationwide for their cooperation in providing the data as well as all individuals involved in interviews for their great contribution in gaining additional knowledge about influenza. I would also like to thank my mentor for her support and advice and colleagues from the Infectious Disease Epidemiology Service.

MeSH / Keywords: Influenza, influenza vaccine, vaccine effectiveness, case-control study

ULTRASOUND SHEAR WAVE ELASTOGRAPHY OF PLACENTAL TISSUE

Part of a Thesis: Evaluation of the elastic properties of placental tissue in fetal growth restriction

PhD candidate: Maryna Kharchenko, MD

Mentor: Docent Berivoj Miskovic, MD, PhD

Affiliations: Department of Obstetrics and Gynecology, Medical School University of Zagreb, Sveti Duh Clinical Hospital, Zagreb. Health center Ozalj, Ozalj.

INTRODUCTION: The aim is to study elasticity of placental tissue using new, noninvasive ultrasound method, Shear Wave Elastography (SWE) in pregnancies with Intrauterine Fetal Growth Restriction (IUGR) and in normal pregnancies. Elasticity of tissue is a new diagnostic parameter that depends on the microscopic and macroscopic structure of the tissue and therefore may vary significantly in normal and pathological tissues. Until now, quantitative measurement of elastic properties of placental tissue has not been carried. It is known that in cases of IUGR there are common pathological changes to the placenta including stromal fibrosis, intervillous thrombosis, increased fibrinoid deposition, villitis, etc. We assume that elasticity of placenta measured by SWE method differs for normal pregnancies and pregnancies with IUGR.

HYPOTESIS: The elasticity of the placental tissue determined by ultrasound „Shear Wave” elastography differs in normal pregnancies and pregnancies with intrauterine fetal growth restriction.

AIMS: To investigate the placental tissue elasticity by ultrasound „Shear Wave” elastography in pregnancies with intrauterine fetal growth restriction and compare it with normal pregnancies.

MATERIAL AND METHODS: This is a cross sectional study. The study group consists of pregnant women with singleton pregnancies and verified fetal growth restriction. The control group consists of pregnant women with eutrophic fetuses of the same gestational age. Elasticity will be investigated in one central part of the placental tissue, at a distance of 2 cm from umbilical cord insertion. Initially, the placental tissue will be visualised using ultrasound B-mode, avoiding the large blood vessels with color doppler. The selected part of placenta will be investigated by SWE. We will qualitatively analyze elasticity of the placenta by color (blue - red), and quantitatively, through obtained values of the elastic module. Collected data will be described with ordinary methods of descriptive statistics. Results will be shown in tables and graphs. Software support JMP 9.0.2, SAS Institute Inc. and software support R will be used for analysis.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: The first data on the elastic properties of placental tissue in normal pregnancies and pregnancies with fetal growth restriction.

MeSH / Keywords: elastography, placenta, fetal growth restriction

INFLUENCE OF IMMUNOGLOBULIN G (IGG) GLYCOSYLATION ON THE EFFICACY OF RITUXIMAB THERAPY IN PATIENTS WITH LYMPHOMA

PhD candidate: Nevenka Cigrovski, MD

Mentor: Igor Aurer, MD, PhD

Affiliations: School of Medicine, University of Zagreb and University Hospital Centre, Zagreb

INTRODUCTION: Use of rituximab in treating patients with lymphoma is a form of immunotherapy. Rituximab is a chimeric monoclonal antibody whose mode of action is not completely clear, but antibody dependent cellular cytotoxicity (ADCC) is considered to be the most important. Studies have shown that glycosylation of monoclonal antibodies affects the binding affinity to effector-cell receptors, thus affecting its therapeutic efficacy. Monoclonal (therapeutic) and endogenous (patient's) antibodies compete for the same receptor, thus endogenous antibodies could inhibit the therapeutic effect of the monoclonal antibody. **HYPOTHESIS:** IgG glycosylation influences therapeutic effectiveness of rituximab in the treatment of patients with lymphoma. Patients whose IgG do not contain fucose respond less well to the combination of rituximab and chemotherapy.

AIM: To determine whether glycosylation of endogenous IgG influences the efficacy of rituximab in treating patients with lymphoma.

MATERIALS AND METHODS: The study will include over 100 patients with lymphoma treated over a three-year period with a combination of rituximab and chemotherapy. Blood samples from patients will be obtained twice: before and after therapy. Endogenous IgG will be isolated and its glycosylation pattern analysed. The results will be correlated with patients' characteristics at diagnosis, histopathological features of lymphoma, international prognostic index (IPI), response to therapy and survival.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: This research will show to what extent glycosylation of immunoglobulin G affects the outcome of the usual treatments of patients with B-cell non-Hodgkin lymphoma. This could have not only practical consequences, but could also help to clarify the influence of protein glycosylation on the regulation of immune reactivity.

Keywords: lymphoma, immunotherapy, ADCC, monoclonal antibody, rituximab, glycosylation

EXPRESSION AND PROGNOSTIC SIGNIFICANCE OF LMO2 PROTEIN, ANGIOGENESIS MARKER IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

Part of a Thesis: Angiogenesis In Non-Hodgkin Lymphoma

PhD candidate: Andjela Prolic, MD

Mentor: Associate Professor Antica Duletic-Nacinovic, MD, PhD

Affiliations: University of Rijeka School of Medicine, University Hospital Centre - Rijeka

INTRODUCTION: Angiogenesis has a major role in the pathogenesis of malignancies. Studies involving the role of angiogenesis have been most commonly performed in solid tumors. However, studies related to hematopoietic neoplasia and angiogenesis are relatively limited. LIM-only transcription protein 2 (LMO2) is a member of a transcription factor family of proteins characterized by their LIM domains. Its expression is required early in hematopoiesis, and angiogenesis. Early in development of the primitive vascular network formed by vasculogenesis does not undergo maturation into functional vascular structures in the absence of LMO2. **HYPOTHESIS:** Expression of LMO2 correlates with favorable patients' outcome. Expression of LMO2 correlates with decreased vascularisation of DLBCL. Angiogenesis in DLBCL is enhanced by vascular endothelial growth factor (VEGF) expression and connected with poor patient outcome.

AIMS: To assess the expression and prognostic significance of LMO2 protein and correlation with VEGF, micro vessel density (MVD) and patients' outcome.

MATERIALS AND METHODS: The present study investigate the expression of LMO2 and VEGF factors by immunohistochemical staining of routinely formalin-fixed, paraffin-embedded slices from 100 patients with diffuse large B-cell lymphoma (DLBCL). Follow up of the patients will be at least two years. Statistical assessment for patients' characteristics will be done by χ^2 test and nonparametric statistics (Mann-Whitney U test), analysis of variance (Cox regression analysis) and survival analysis (Kaplan-Meier and log-rank test).

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: Angiogenesis plays a differential role in diffuse large B cell lymphomas. Aggressive lymphomas express the potential molecular therapeutic targets VEGF and have higher MVD. LMO2 expression might have a role in prognostic advantage. Therapeutic strategies aimed at angiogenesis should take into account lymphoma heterogeneity and therapy adjustments could be provided.

MeSH / Keywords: LMO2, Lymphoma, Non-Hodgkin, VEGF

THE ROLE OF CAVEOLIN-1 AND TRANSFORMING GROWTH FACTOR BETA IN NONALCOHOLIC FATTY LIVER DISEASE

Part of a Thesis: Expression and Distribution of Caveolin-1 and Transforming growth factor beta in Hepatocytes of Patients with Nonalcoholic fatty liver disease

PhD candidate: Marija Gomerčić Palčić, MD

Mentor(s): Professor Marko Duvnjak, MD, PhD; Professor Božo Krušlin, MD, PhD

Affiliation(s): University of Zagreb School of Medicine, *Sestre milosrdnice University Hospital*

INTRODUCTION: Nonalcoholic fatty liver disease (NAFLD), a hepatic manifestation of metabolic syndrome is considered to be the most common liver disease in Western countries and also leading cause of cryptogenic cirrhosis. Insulin resistance, of all components of metabolic syndrome, plays the major part in fibrogenesis. Progression of NAFLD is characterized by enhanced accumulation of intrahepatic triglycerides, oxidative stress in hepatocytes and promoted fibrogenesis due to insulin resistance, causing abnormalities in expression and distribution of caveolin-1 and transforming growth factor beta (TGF- β). **HYPOTHESIS:** expression of caveolin-1 and TGF- β is increased and their distribution is altered in hepatocytes of patients with metabolic syndrome, both correlating with progression of the disease.

AIMS: This study will be conducted to investigate expression and distribution of caveolin-1 and transforming growth factor beta in hepatocytes according to each NAFLD stage, and their relations with noninvasive biochemical and imaging (abdominal ultrasound) tests. In addition, we will investigate their relations with each component of metabolic syndrome (arterial hypertension, central obesity, atherogenic dyslipidemia and insulin resistance).

MATERIALS AND METHODS: This is a prospective study that will involve sixty-five patients of both genders, aged 18 to 65 years, with NAFLD pathohistological findings and a control group without NAFLD. Blood tests and physical examination will be performed in order to determine whether metabolic syndrome is present in patients with NAFLD, as well as abdominal ultrasound to establish presence of NAFLD. Pathohistological analysis of liver tissues obtained by liver biopsy together with immunohistochemical staining using antibodies on caveolin-1 and TGF- β will be performed in both groups. Relations between expression and distribution of caveolin-1 and TGF- β with pathohistological findings, components of metabolic syndrome and imaging (abdominal ultrasound) tests will be evaluated.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: This would be a first human study investigating role of caveolin-1 in patients with NAFLD. Estimation of expression and distribution of caveolin-1 and TGF- β in hepatocytes in patients with different stages of NAFLD will help in understanding pathophysiology of the disease and natural history, as well to improve diagnostic and therapeutic possibilities.

MeSH / Keywords: nonalcoholic fatty liver disease (NAFLD), caveolin-1, transforming growth factor beta (TGF- β), insulin resistance, noninvasive tests of liver fibrosis

COMPARISON BETWEEN THE CRITERIA IN THE DIAGNOSIS OF GESTATIONAL DIABETES

Part of a Thesis: Comparison between World Health Organization criteria and HAPO study in the Diagnosis of Gestational diabetes

PhD candidate: Mato Pavić, MD

Mentor: Associate Professor Ivana Pavlić Renar, MD, PhD

Affiliation: University of Zagreb School of Medicine, University Hospital Centre - Zagreb, Department of Gynecology and Obstetrics

INTRODUCTION: Gestational diabetes mellitus (GDM) is a state of impaired glucose tolerance of different levels to be first diagnosed during pregnancy. High perinatal mortality and morbidity caused by GDM, confirmed the importance of diagnosing. The most important risk factors for GDM are obesity and age.

HYPOTHESIS: It is expected higher incidence of GDM according HAPO criteria in relation to WHO criteria. Treatment of GDM established by HAPO criteria should reduce the number of complications of pregnancy and improve perinatal outcomes.

AIMS: Find the difference in incidence of GDM according criteria for WHO and HAPO, to determine the degree of insulin resistance in pregnancies and newborns with GDM according to HAPO criteria, to analyze specific perinatal outcomes.

MATERIALS AND METHODS: The study is divided in two parts. The retrospective study analyzes the history of 2000 pregnant women who made OGTT with 75 g glucose and who gave a birth, in the clinic, for period 2001 - 2010. WHO and HAPO criteria are used for the diagnosis of GDM. According to WHO criteria GDM is diagnosed if fasting plasma glucose values are ≥ 7.0 mmol / L or ≥ 7.8 mmol / L measured 2 hours after 75 g glucose load. According to HAPO criteria GDM is diagnosed if fasting plasma glucose values are ≥ 5.1 mmol / L and / or measured 1 hour ≥ 10 mmol / L and / or measured 2 hours 8.5 mmol / L after 75 g glucose load. The prospective study includes 200 pregnant women with GDM according to HAPO criteria and 40 with normal OGTT. The study group is divided in two subgroups: subgroup with fasting plasma glucose in venous plasma between 5.1 and 7.0 mmol/L; subgroup with fasting plasma glucose in venous plasma >7 mmol/L. The second group is control group (about 40 pregnant women) with normal OGTT. After birth, from mothers venous blood and cord blood, glucose, insulin, C-peptide, insulin resistance will be determined.

EXPECTED SCIENTIFIC CONTRIBUTION: The research could further elucidate the relationship between hyperglycemia in pregnancy and specific perinatal complications. We should find out new information related to degree of insulin resistance in those pregnant women and infants that were not previously diagnosed as GDM, and who appear as a risk group because of the new diagnostic HAPO criteria.

Keywords: hyperglycemia, gestational diabetes, glucose tolerance test, HAPO study

THE ROLE OF CYTOKINES IN PROSTATITIS SYNDROME PATIENTS

Part of a Thesis: The Research of Proinflammatory and Antiinflammatory Cytokines in Prostatitis Syndrome Patients

PhD candidate: Adela Kolumbić Lakoš, MD

Mentor: Professor Višnja Škerk, MD, PhD

Affiliation: University Hospital for Infectious Diseases „Dr. Fran Mihaljević, PLIVA CROATIA Ltd.

INTRODUCTION: The causes of infection and inflammation cannot always be proved in prostatitis syndrome (SP). In accordance with the NIH classification, the SP can be divided into acute and chronic bacterial prostatitis with proven agents of infections, „chronic pelvic pain syndrome”, i.e. type III, and type IV prostatitis (asymptomatic with proven inflammation). Type III is further divided into IIIA (inflammatory) and IIIB (non-inflammatory) depending on the number of leukocytes in VB3 and EPS. Leukocytes, which are considered markers of inflammation, are not a reliable diagnostic parameter. Inflammatory markers such as cytokines have been increasingly studied and the immunology of prostate develops into a new scientific field. The available literature highlights the role of the immune system in the pathogenesis of SP. The imbalance of pro- and anti-inflammatory cytokines may partly determine the symptoms and outcome of the inflammatory process. This research, which will measure pro-inflammatory cytokines IL-6, IL-8, IL-17 and anti-inflammatory cytokine TGF- β , aims to provide new insights on the role of cytokines in patients with SP.

HYPOTHESIS: The levels of pro-inflammatory cytokines (IL-6, IL-8, IL-17) and anti-inflammatory cytokine (TGF- β) are higher in patients with chronic bacterial prostatitis and inflammatory form of chronic pelvic pain syndrome compared with patients with non-inflammatory chronic pelvic pain syndrome.

AIMS: To investigate the levels of proinflammatory and anti-inflammatory cytokines in patients with chronic bacterial prostatitis, inflammatory and non-inflammatory form of chronic pelvic pain syndrome.

MATERIALS AND METHODS: Inclusion criteria: men older than 18 years with chronic prostatitis syndrome confirmed according to NIH criteria. Exclusion criteria: absence of anatomical and functional abnormalities as well as of abscess and prostate cancer. The methodology involves detailed history, clinical status, filling out the NIH-CPSI questionnaire, digital-rectal examination, urogenital tract ultrasound, the PSA test, „four glasses test”, biochemical and microbiological examination of urine and EPS. Cytokines will be determined in EPS and/or VB3 with ELISA.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: New insights on cytokine levels in patients with chronic bacterial prostatitis and inflammatory and non-inflammatory form of chronic pelvic pain syndrome, and its potential benefit in differentiating various types of SP in conjunction with other diagnostic methods.

Acknowledgements: This research is part of the project „Investigation of Etiology, Epidemiology, Diagnosis and Treatment of Patients with Prostatitis Syndrome”, Research Projects of the National Foundation for Science, Higher Education and Technological Development No4/30

MeSH / Keywords: prostatitis, cytokines

OSTEOTOMY LINE IN PLANNING OF CORRECTIVE OSTEOTOMIES OF MALUNITED DISTAL RADIUS FRACTURE

Part of a Thesis: Importance and Optimisation of Osteotomy line in Planning of Corrective Osteotomies of Malunited Distal Radius Fracture

PhD candidate: Jadranko Kovjanic, MD, MSc

Mentor: Professor Ranko Bilic, MD, PhD

Affiliation: General hospital „Dr. Ivo Pedisic“ Sisak

INTRODUCTION: Corrective osteotomy is the method of choice for treating distal radius fracture malunion in young and active patients. It has previously been demonstrated that there is a correlation between the quality of anatomical correction and overall wrist function. However, surgical correction can be difficult in relation to complex anatomy associated with this condition. Clinical outcomes largely depend on the preoperative planning seeking the quality of restoration of normal anatomy. None of present surgical planning methods determine osteotomy line in relation to deformity. Our 3D computer-assisted planning method can find optimal osteotomy line for restoration of normal distal radius anatomy.

HYPOTHESIS: Computer defined osteotomy line in planning of corrective osteotomy of distal radius malunion will result in a better anatomical outcome compared with conventional osteotomy line.

AIMS: The objective of this study is to virtually compare outcomes after corrective osteotomy for distal radius malunion between preoperative computer-assisted defined osteotomy line and most frequently used osteotomy line (2,5 cm from articular surface, parallel to the articular surface in sagittal plane).

MATERIALS AND METHODS: This study is designed as a prospective evaluation of new computer-assisted method for planning osteotomy line for corrective surgery of the distal radius. CT scans of the wrists of 40 patients (20 man and 20 woman) will be taken and virtual corrective osteotomies with two different levels of osteotomy line will be done to each patient: test operation with osteotomy line determined by computer and control operation with most frequently used osteotomy line. Virtual postoperative bone shape will be compared between methods, expressed as sum of squares of a surface deviate from normal radius.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: Osteotomy line determined by our new computer method achieve better distal radius anatomy that makes functional results of this challenging procedure more reliable.

MeSH / Keywords: distal radius corrective osteotomy, distal radius malunion, osteotomy line, distal radius fracture

THERAPEUTICAL POSSIBILITIES IN PATIENTS WITH BRAIN ABSCESS

Part of a Thesis: The estimation of therapeutical possibilities in patients with brain abscess with propensity score

PhD candidate: Ana Pangerčić, MD

Mentor(s): Professor Bruno Baršić, MD, PhD, Professor Pavle Miklič, MD, PhD

Affiliation(s): University Hospital Centre „Sestre milosrdnice“, University Hospital for Infectious Diseases „Dr.Fran Mihaljević“, University Hospital Centre Zagreb

INTRODUCTION: There are no controlled randomized clinical studies which would estimate the differences in survival between groups of patients with brain abscess who were differently treated. Data about survival of these patients are usually given by multivariate analysis which is prone to bias.

HYPOTHESIS: Patients with brain abscess who were treated with stereotactic aspiration have less neurological sequel than patients treated with open craniotomy.

AIMS: To determine the correlation between treatment possibilities and outcome of patients with brain abscess. Specific aims are: to determine the association between therapeutic possibilities and co-morbidities, GCS and brain abscess characteristics and to define the population of patients who need more invasive surgical approach.

MATERIALS AND METHODS: Patients with brain abscess diagnosis treated at the University Hospital for Infectious Diseases „Dr.Fran Mihaljević“ in Zagreb from 1st June 2000 until 1st June 2010 will be included in this study. Patients will be divided into 2 groups based on the type of neurosurgical intervention: invasive (craniotomy in combination with antimicrobial therapy) and less invasive group (brain abscess aspiration in combination with antimicrobial therapy). According to treatment outcome, the patients will be divided into 2 groups: favorable outcome (GOS 4 and 5) and unfavorable outcome (GOS 1-3).

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: Evaluation of therapeutical possibilities in the treatment of patients with brain abscess will help to estimate which method has better outcome in treating these patients. To diminish the bias of observational study, patients' groups will be adjusted for propensity score for open craniotomy and then compared using multivariate analysis.

Acknowledgements: I would like to thank to my mentor professor Bruno Baršić, for guiding me through the scientific pathways since my student days.

MeSH/Keywords: brain abscess, craniotomy, stereotactic aspiration, antimicrobial treatment, propensity score

SUBARACHNOID INFLAMMATORY REACTION AND OUTCOME OF COMMUNITY-ACQUIRED BACTERIAL MENINGITIS IN ADULT PATIENTS

Part of a Thesis: Subarachnoid Inflammatory Reaction And Outcome Of Community-Acquired Bacterial Meningitis In Adult Patients

PhD candidate: Vjerislav Peterković, MD

Mentor: Dragan Lepur, MD, PhD

Affiliations: Department of Intensive Care Medicine and Neuroinfectology, University Hospital for Infectious Diseases "Dr. Fran Mihaljevic", Department of Neurosurgery, University Hospital Center Zagreb

INTRODUCTION: Community-acquired bacterial meningitis (CABM) in adults is an acute meningitis whose pathogenesis isn't linked with hospital admission or surgical CNS procedures. The importance of the disease lies in its severity. The antimicrobial agents ensure CSF sterilization within 24-48 hours but that doesn't necessarily mean good clinical outcome.

HYPOTHESIS: The hypothesis of this study is that the intensity of subarachnoid inflammation is an important determinant in the outcome of patients with CABM and the suppression of inflammatory reaction results in more favorable outcome in the daily clinical practice.

AIMS: The main objective of this study is to assess the predictive value of subarachnoid inflammation indicators and the efficacy of dexamethasone in adult CABM in daily practice.

MATERIAL AND METHODS: The study consists of two parts: A. Retrospective prognostic observational study - More than three hundred patients with community-acquired bacterial meningitis aged 18 years and more, admitted to the University Hospital for Infectious Diseases „Dr. Fran Mihaljević“ will be included in this retrospective cohort study. The observational period will be between 1990 and 2010. The original written patient medical records will be used to collect the data. Adjusted relative risks (RR, dexamethasone vs. no dexamethasone [control]) of Glasgow Outcome Scale (GOS)=1 (death) and GOS=5 (full recovery) at discharge/end of specific treatment will be estimated considering demographics, co-morbidity, CABM pathogenesis and on-admission characteristics and cerebrospinal fluid (CSF) inflammation indicators, causative agent and antibiotic timing. B. Systematic review and meta-analysis of the observational studies dealing with the influence of dexamethasone on the outcome of CABM and the predictivity of subarachnoid inflammation markers on disease outcome.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: The results of this study should be helpful in further clarification of dexamethasone clinical benefit in CABM patients. The inflammatory concept and the use of dexamethasone are logically connected, thus the results of this study should also evaluate the scientific value of the anti-inflammatory concept.

MeSH/Keywords: community-acquired bacterial meningitis; adults; S. pneumoniae, dexamethasone, clinical outcome

THE ROLE OF ANTIMICROBIAL PEPTIDES IN RESPIRATORY SYNCYTIAL VIRUS INFECTION

Part of a Thesis: Antimicrobial Peptides in Bronchoalveolar Lavage Samples from Infants with Severe Respiratory Syncytial Virus Lower Respiratory Tract Infection

PhD candidate: Srđan Roglić, MD

Mentors: Assistant Professor Goran Tešović, MD, PhD; Krešo Bendelja, PhD

Affiliations: University Hospital for Infectious Diseases „Dr. Fran Mihaljević“, Institute of Immunology, Clinical Hospital Centre „Sisters Of Mercy“

INTRODUCTION: Respiratory syncytial virus (RSV) causes acute respiratory tract illness across all ages while lower respiratory tract infection is usually a consequence of primary infection in infants.

Antimicrobial innate defense along with transferred maternal immunoglobulins play a significant role in infants while immune system undergoes conditional maturation. Beta-defensins are small antimicrobial peptides produced by epithelial cells and mononuclear leukocytes with wide pathogen specificity, displaying neutralization/inactivation capability. Their ability to activate innate as well as specific immunity helps in viral clearance as well as in disease pathogenesis.

HYPOTHESIS: Recent studies have shown that innate defense, including alpha- and beta-defensins, plays an important role in pathogenesis of RSV lower respiratory tract infection. We believe that levels of defensins are high in RSV respiratory tract infection and that they contribute to inflammatory response and disease severity.

AIMS: Aim of the study is to determine the role of antimicrobial peptides and pro-inflammatory cytokines IL-1 β , TNF- α and IL-6 in the pathogenesis of RSV lower respiratory tract infection.

MATERIALS AND METHODS: During the study we will determine levels of beta-defensin 1-4, IL-1 β , TNF- α and IL-6 in bronchoalveolar lavage and sera samples from infants with RSV-caused lower respiratory tract infections and compare it with values measured in healthy children in order to determine a profile of secreted antimicrobials.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: Immunopathogenesis of RSV infection is still not well defined and there is no (universally accepted) effective treatment. This study should yield new information on changes of the immune system during RSV infection and contribute to the better understanding of pathogenesis. This could lead to new treatment possibilities.

MeSH / Keywords: respiratory syncytial virus (RSV), lower respiratory tract infection (LRTI), bronchoalveolar lavage (BAL), cytokines, defensins

CHEMOKINES IN CENTRAL NERVOUS SYSTEM DISEASES IN CHILDREN

Chemokines CXCL10, CXCL11 And CXCL13 In Aseptic Meningitis, Neuroborreliosis And Acute Disseminated Encephalomyelitis

PhD candidate: Lorna Stemberger, MD

Mentors: Goran Tešović, MD, PhD, Assistant Professor, Snježana Židovec Lepej, mag.mol.biol., PhD

Affiliations: University Hospital for Infectious Diseases „Dr.Fran Mihaljević“

INTRODUCTION: Chemokines are low molecular weight basic proinflammatory proteins implicated in a variety of diseases within the central nervous system. They play important role in host defense by attracting and activating leukocytes thus modifying process of microbial invasion and have been associated with acute and chronic inflammation as well as with immunologically mediated diseases.

HYPOTHESIS: Concentrations of chemokines CXCL10, CXCL11 and CXCL13 in cerebrospinal fluid and sera of patients with aseptic meningitis (AM), neuroborreliosis (NB) and acute disseminated encephalomyelitis (ADEM) differ between themselves and in comparison to group of patients without inflammatory process in CNS; there is a „cut off“ value of particular or combination of chemokines which could serve in rapid classification of these diagnoses before definitive diagnosis.

AIMS: The aim of this study is to help in revealing the importance of chemokines CXCL10, CXCL11 and CXCL13 in pathogenesis of AM, NB and ADEM in children. 1. To determine whether there is a concentration gradient of these chemokines between cerebrospinal fluid and sera. 2. To determine if these concentration gradients differ between themselves and in comparison to the control group. 3. To find a „cut off“ value of particular or combination of chemokines which could serve in rapid classification of these diagnoses before definitive diagnosis.

MATERIAL AND METHODS: About 70 patients, 20 with diagnosis of AM, 20 with ADEM, 15 with NB and 15 without inflammatory process in CNS will be included in study. In control group lumbar puncture was made as a routine protocol because of strong clinical suspicion of inflammatory CNS process, which is later excluded. Inclusion criteria were age younger than 18 years and definite clinical/laboratory/radiologic diagnosis of AM, NB and ADEM. To all of these patients lumbar puncture was made in first 24 hours after admission and 1ml of CSF and 2ml of blood was taken to determine chemokine concentration with quantitative enzyme immunoassay and bead-based flow cytometry.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: Data on role of these cytokines is very deficient and to the best of our knowledge there is no study which connects and compares them. Our study will enrich existing knowledge about these cytokines in inflammatory CNS diseases. If we find „cut-off value“ which could sort these diseases, clinical use will be also possible in future.

MeSH/Keywords: aseptic meningitis, neuroborreliosis, acute disseminated encephalomyelitis, chemokines CXCL10, CXCL11, CXCL13

DYNAMICS OF NATURALLY ACQUIRED IMMUNITY AGAINST *COXIELLA BURNETII* IN PROFESSIONALLY EXPOSED PERSONS

Part of a Thesis: Correlation of the incidence of Coxiella infection in people depending on the naturally acquired immunity or manifest disease in animals.

PhD candidate: Branka Sep-Ševerdija MD, MSc

Mentors: Assist. Prof. Goran Tešović, MD, PhD, Silvio Špičić, DVM, PhD

Affiliations: Institute of Public Health of Istria County - Pula; Croatian National Institute of Public Health; Microbiology Service Istituto Zooprofilattico Sperimentale delle Venezie, Sezione territoriale di Udine, Laboratorio di sierologia

INTRODUCTION: Q-fever is one of the most frequent anthroozoonoses in the world. Its causative agent is *Coxiella burnetii*, a highly infectious and very resistant bacterium. For man, the most important reservoirs of the disease are sheep, goats and cattle. The disease is endemic in the southern part of the Istrian peninsula. Numerous seroepidemiological studies show that professionally exposed persons have higher levels of seropositivity compared to control groups, but no longitudinal studies of immunity have been published so far.

HYPOTHESIS: The investigator's hypothesis is that there is a statistical difference in naturally acquired immunity between professionally exposed persons working in the southern part of Istria, compared to persons working in the northern part.

AIMS: 1. To investigate the dynamics of naturally acquired immunity to *Coxiella burnetii* in professionally exposed persons 2. To determine the interdependence of the incidence of *Coxiella* infection in people and the presence of infection in animals 3. To confirm that Q-fever is a seasonal and professional disease 4. To determine the statistically significant difference in the serological status between the subjects in the southern part of Istria and those in the northern part.

MATERIALS AND METHODS: The study will involve 120 persons professionally exposed to *Coxiella* infection in the area of the Istria County. They will be tested on the presence of antibodies to *Coxiella burnetii* at the beginning of the study and their serological status will be monitored for two years. The indirect immunofluorescence test (IFA Vircell Microbiologist *Coxiella burnetii* I and II IgG/IgM) will be used. Simultaneously, around 350 animals on farms (sheep and goats) will be subjected to serological examination in the first and second year of study using the Chekit Q-Fever enzyme-linked immunosorbent assay (ELISA).

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: We expect (1) to determine the incidence of asymptomatic infections with *Coxiella* in professionally exposed persons, (2) by monitoring of the dynamics of antibodies in immunologically experienced professionals to determine the frequency of re-infection (3) to determine the interdependence of the incidence of *Coxiella* infection in people and the presence of infection in animals.

MeSH/Key words: Q fever, professionally exposed persons, goats, sheep, Istria County, serological survey

SHEAR WAVE ELASTOGRAPHY (SWE) FOR EVALUATION OF LIVER FIBROSIS IN PATIENTS WITH CHRONIC VIRAL HEPATITIS

Part of a Thesis: Elastographic assessment of liver fibrosis in patients with chronic viral hepatitis

PhD candidate: Matija Crnogorac, MD

Mentor: Ivica Grgurević, MD, PhD

Affiliation(s): Dubrava University Hospital

INTRODUCTION: Sonoelastography is a new non-invasive method for assessment of liver fibrosis. So far diagnostic gold standard was liver biopsy which is however invasive procedure with potentially serious complications and also provides limited data on fibrosis of the entire liver.

HYPOTHESIS: Shear wave elastography (SWE) is a new real-time kvantitative ultrasound elastography method which allows reliable non-invasive assessment of liver fibrosis in patients with chronic viral hepatitis.

AIMS: Aim of this research is to assess diagnostic reliability of Shear wave elastography (SWE) for evaluation of liver fibrosis in patients with chronic viral hepatitis. Potential correlation between pathohistological stage of liver fibrosis and liver elasticity values obtained by this method will be tested and cut-off values of liver elasticity will be calculated that could distinguish healthy subjects from those with liver fibrosis, earlier from advanced stages of fibrosis as well as cirrhosis from other stages of fibrosis.

MATERIALS AND METHODS: Research shall include healthy individuals, patients with chronic viral hepatitis B or C without liver cirrhosis, patients with chronic viral hepatitis B or C and liver cirrhosis. Liver biopsy as well as Shear wave elastography will be performed on patients with chronic viral hepatitis B or C without liver cirrhosis, with pathohistological staging of liver fibrosis being done using Ishak score. Liver cirrhosis will be diagnosed either according to biopsy results or by clinical criteria including patients history of chronic liver disease, imaging methods, lab results compatible with cirrhosis, endoscopic signs of portal hypertension. Patients with liver disease caused by conditions and diseases other than viral hepatitis B or C, diagnosed with adequate lab tests and other diagnostic tools, shall not be included in the research. STATISTICA version 10.0 (www.statsoft.com) will be used for analysing acquired data.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: Results of this research should offer new and useful data on SWE value in assessing liver fibrosis in patients with chronic viral hepatitis.

Acknowledgements: special thanks to my mentor for all the patience he displays in working with me

MeSH/Keywords: elastography, liver fibrosis, liver biopsy, viral hepatitis

INTERATRIAL CONDUCTION AND LONE ATRIAL FIBRILLATION: IS THERE A LINK?

Part of a Thesis: Interatrial Conduction In Patients With Lone Atrial Fibrillation

PhD candidate: Margarita Brida, MD

Mentor: Professor Anton Šmalcelj, MD, PhD

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

INTRODUCTION: Atrial fibrillation is the most common arrhythmia in the clinical practice. Lone atrial fibrillation represents unique entity by lacking risk factors as well as known cardiovascular comorbidities. Interatrial block is one of the most common electrocardiographical abnormalities - still its roll is yet to be defined in the pathogenesis of atrial fibrillation, especially in lone atrial fibrillation.

HYPOTHESIS: Interatrial conduction delay is a cryptogenic factor in etiology of lone atrial fibrillation.

AIMS: To investigate distribution of interatrial conduction delay in patients with paroxysmal and persistent lone atrial fibrillation. To analyze P-wave length and morphology in relation to echocardiographically measured atrial electromechanical conduction time. To explore correlation of interatrial conduction block with development of persistent form of lone atrial fibrillation.

MATERIALS AND METHODS: Eighty adult outpatient or in-patient participants with diagnosed lone atrial fibrillation according to the established criteria will be included. Each participant will have age and gender case-matched healthy control. Signed informed consent will be obtained before each enrollment. Full 12-channel electrocardiography and echocardiography will be investigated during sinus rhythm or seven days after successful cardioversion for patients with paroxysmal or persistent form of atrial fibrillation, respectively. Complete interatrial block will be defined as wide (>120 ms) or biphasic (+,-) P-wave in the inferior leads (II, III, aVF). P-wave ≥ 110 ms will be defined as partial interatrial block. Dimension of heart chambers, valve and myocardial function will be determined by M-mode, 2D and Doppler echocardiography. Atrial electromechanical conduction time will be measured by tissue Doppler echocardiography. Parametric (independent t-test) or nonparametric (Mann Whitney U) statistical tests will be used. Analysis will be performed using computer program SPSS 17.02 version (Illinois, USA) with statistical significance set at 0.05. Research will be conducted according to the Ethical Declaration of Helsinki.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: This research will analyze distribution of interatrial conduction time delay (electrocardiographically) and electromechanical conduction time (echocardiographically) in patients with lone atrial fibrillation, which will contribute to the better understanding of etiology in patients without otherwise known risk factors or comorbidities for development of atrial fibrillation.

MeSH/Keywords: atrial fibrillation, interatrial conduction, interatrial block, atrial electromechanical conduction time, electrocardiography, echocardiography

MYOCARDIAL BRIDGING

Part of a Thesis: The role of MSCT coronary angiography in the diagnosis and future therapeutic guidelines of congenital coronary artery myocardial bridging

PhD candidate: Mladen Jukić, MD

Mentor: Professor Aleksander Ernst, MD, PhD

Affiliation: Polyclinic Sunce, Zagreb

INTRODUCTION: Myocardial bridging (MB) is an entity known for more than 200 years. However, we don't know the frequency of MB, whether MB is a hereditary condition or not. Consequently, its possible clinical significance is still not appropriately understood. There are no guidelines on how to diagnose MB, and whether it should be treated or not, and if yes in what fashion.

HYPOTHESIS: CCTA is a rather new, non-invasive diagnostic modality, which has been proven as a method of choice for diagnosis of congenital coronary artery anomalies, including MB. Invasive CA (ICA) has been proven insufficient in this respect. CCTA can allow morphological and clinical characterization of MB, and as such can have influence on further management of patients.

AIMS: The aim of this study is to determine the frequency of MB in patients with clinically suspected coronary artery disease. We will also investigate possible relationship of the clinical presentation of these patients with the characteristics of MB as depicted on CCTA, and possible impact on future therapeutic strategy.

MATERIALS AND METHODS: Study will include 100 pts with suspected but not known CAD and with indication for CT or invasive coronary artery angiography. Based on clinical presentation and CCTA findings patients are divided in two groups of 50 subjects. First group will consist of patients with clinically manifest angina, but with negative or unequivocal findings on stress- and/or perfusion-tests. These patients have no CAD on CCTA, but have MB at any location in coronary artery tree. Second group will consist of patients who are conditionally „healthy“ with precordial oppressions and polyvalent disturbances of idiopathic etiology. These patients have neither CAD nor MB on CCTA.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: As the frequency of MB is still not fully elucidated, and as there are no studies up to date that would analyze MB with its characteristics on CT, we believe that this research will enable an original scientific contribution that will try to elucidate the possible role of CT in diagnosis of MB, help in further clinical understanding of this condition, and possibly indicate future therapeutically guidelines.

Acknowledgements: I would like to thank my family and colleagues at the extraordinary support they have given me.

MeSH / Keywords: Coronary CT Angiography (CCTA), Myocardial Bridging (MB), Invasive Coronary Angiography (ICA)

COMPARISON OF TWO POSTOPERATIVE REHABILITATION PROTOCOLS FOLLOWING TOTAL KNEE ARTHROPLASTY

Part of a Thesis: Comparison of two postoperative rehabilitation protocols following total knee arthroplasty

Phd candidate: Ozren Kubat, MD

Mentor: Professor Domagoj Delimar, MD, PhD

Affiliation: Department of Orthopaedic surgery, Clinical Hospital Centre Zagreb, University of Zagreb Medical School

INTRODUCTION: Osteoarthritis (OA) of the knee is a chronic, progressive disorder of the joint. Today, conservative, pharmacological and surgical therapeutic modalities are used in OA treatment. An integral part of the surgical treatment of knee OA, Total knee arthroplasty (TKA), is a very effective way of treating end-stage knee OA, both primary and secondary. Rehabilitation always follows surgery, and is a vital part of the whole treatment. The main goals of rehabilitation are achieving at least 90° flexion in the operated knee, and adequate patient autonomy needed for everyday activities. Today, an abundance of rehabilitation protocols exist worldwide, as there is still no consent on the best modality and intensity of rehabilitation following knee OA. Use of so-called clinical pathways, and aggressive, „fast-track“ (FT) protocols is widespread. This study will compare a standard FT rehabilitation protocol to one designed in our Department.

HYPOTHESIS: Patients in the „delayed“ FT rehabilitation group will achieve faster improvement of knee range of motion and functional tests, when compared to patients in the standard, „early“ FT rehabilitation group.

AIMS: To determine any differences in knee flexion, postoperative pain, functional tests scores, KSS and WOMAC score results between patients subjected to two rehabilitation protocols.

MATERIALS AND METHODS: A hundred patients undergoing TKA in our Department will be stratified into two groups, group R subjected to the standard „early“ FT rehabilitation used at our Department, and group T subjected to the tested „delayed“ FT rehabilitation. The difference between the two protocols is a full 4 days of resting the knee, without any flexion permitted, in the tested „delayed“ FT rehabilitation. Daily measurements of visual analog scale for pain and degrees of knee flexion will be made. On the 8th postoperative day, a measurement of functional knee tests („Timed up and go“, 6 minute walk, stair climbing) will be made. KSS and WOMAC scores will be gathered in different, set, time points. All of the gathered data will be processed and presented using appropriate statistical methods.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: Evaluation of a new rehabilitation protocol designed at our Department.

Acknowledgements: I would like to sincerely thank my mentor and teacher, professor Domagoj Delimar and my colleague Goran Bićanić, MD, PhD for their invaluable help in making this thesis proposal.

Keywords: Osteoarthritis, Knee, Endoprosthesis, Rehabilitation

FEASIBILITY OF AN INTRACARDIAC ELECTROGRAMS BASED METHOD FOR OPTIMIZING CARDIAC RESYNCHRONIZATION THERAPY

Part of a Thesis: Cardiac Resynchronisation Therapy Optimizaton with Intracardiac Electrograms Based Method

PhD candidate: Borka Pezo Nikolić, MD

Mentor: Professor Jadranka Šeparović Hanževački, MD, PhD

Affiliation: University of Zagreb School of medicine, University Hospital Centre- Zagreb

INTRODUCTION: Cardiac resynchronization therapy (CRT) with biventricular pacing has become an established electrophysiologic solution for patients with medically refractory congestive heart failure (CHF) due to asynchronous cardiac contractions. Optimization of atrioventricular (AV) and interventricular (VV) delays have been shown to influence hemodynamics. The most common, proven and tested method for AV and VV optimization is echocardiography. A new intracardiac electrogram (IEGM) based method was developed to calculate optimal AV i VV delays, which can be preformed easily during routine device follow- ups. This study compares this IEGM based method with the current echocardiography (ECHO) method. The comparability of IEGM to the best-known ECHO procedure for determining optimal conduction delays will be verified.

HYPOTHESIS: Applying the IEGM method we can determine optimal AV i VV delays with great impact on cardiac function. Optimal VV delay reduces total electrical activation time in left ventricle, which will then synchronize the electrical activation and presumably the mechanical contraction pattern.

AIMS: 1. Determine optimal AV i VV delays using intracardiac electrograms; 2. Determine the efficacy of the IEGM method compared to conventional ECHO guided optimisation;

MATERIALS AND METHODS: This study will be based on prospectively collected data from 30 patients previously implanted with a CRT. All patient are going to both echocardiographic and IEGM-based delay optimization. First, a routine follow-up of the CRT device will be conducted. Then, the IEGM evaluation will be performed. Finally, all patients will be tested with the echo-guided optimization. Statistical analysis will be performed using the concordance correlation coefficient (CCC), to assess the agreement between the two measurements.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: Applying the IEGM method we are going to have better insight in impulse conduction, electrical and mechanical delay correlation and better understanding of electrostimulation effect on diseased myocardium. Using the IEGM method we are going to improve CRT optimization and follow up of the patients achieving greater number of CRT responders.

MeSH/ Keywords: Cardiac resynchronization, AV i VV delay optimization; Echocardiography, Intracardiac electrogram

EFFECT OF CYP2C19 POLYMORPHISM AND MEASURING PLATELET REACTIVITY TO CLOPIDOGREL IN DETERMINATING CLINICAL OUTCOME AND JUSTIFICATION OF INDIVIDUALIZED APPROACH TO ANTIPLATELET TREATMENT OF PATIENTS WITH ACUTE CORONARY SYNDROME

Part of a Thesis: Individualized Approach In Clopidogrel Treatment Based On Pharmacogenetic Analysis And Measuring Platelet Activity Improves Clinical Outcome In Acute Coronary Syndrome

PhD candidate: Jure Samardžić, MD

Mentor: Professor Davor Miličić, MD, PhD, F.E.S.C.

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

INTRODUCTION: Patients who undergo percutaneous coronary intervention (PCI) are treated with dual antiplatelet therapy to reduce the occurrence of major adverse cardiocerebrovascular events. Aspirin and clopidogrel are still the cornerstone of antiplatelet treatment for these patients. The administration of standard doses of antiplatelet drugs to patients with ACS achieves different degrees of platelet activity inhibition. Some CYP2C19 allele variations are associated with unoptimal platelet response to clopidogrel. Current guidelines recommend universal antiplatelet therapy regimen.

HYPOTHESIS: Individualized approach to clopidogrel treatment based on pharmacogenomic analysis (CYP2C19) and/or platelet function test (Multiplate®) in vitro can improve outcome of patients with ACS.

AIMS: To investigate whether combining pharmacogenetic analysis (CYP2C19) and platelet function test can indicate individualized clopidogrel dosing to improve clinical outcome in patients with ACS. To determine CYP2C19 polymorphism of patients with ACS in Clinical Hospital Centre Zagreb. To show whether clopidogrel dose adjustment in patients with diminished response will lead to better platelet activity control and clinical outcome.

MATERIALS AND METHODS: Multiplate® function analyzer is a point of care device used to measure platelet aggregation. It will be used to determine high on-platelet activity in consecutive patients with ACS (STEMI, NSTEMI, unstable angina) day after successful PCI. Hyporesponders to clopidogrel will be divided in study group which will receive additional clopidogrel loading dose clopidogrel (600 mg) and control group which will receive standard maintenance dose (75 mg/day). Multiplate® measuring will be performed the following day and based on the results, patients in study group will receive new loading dose (if still hyporesponsive) or 150 mg/day if normoresponsive. Measurements will be performed on day 7, 30, 60, 90 and every three months until the end of first year. Clopidogrel maintenance dose will be tailored on each measurement in study group (75-300 mg) while control group will have standard dose regimen all time (75 mg/day). CYP2C19 genotyping will be performed to all patients with abnormal thrombocyte activity as this enzyme participates greatly in clopidogrel biotransformation.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: This study will contribute to better treatment of ACS and point to the usefulness of individualized approach to antiplatelet treatment.

Acknowledgements: I would like to thank my mentor professor Davor Miličić.

MeSH / Keywords: acute coronary syndrome, myocardial infarction, antiplatelet drugs, clopidogrel, drug resistance, platelet function tests, genetic polymorphism, CYP2C19

RISK STRATIFICATION FOR SUDDEN CARDIAC DEATH AFTER MYOCARDIAL INFARCTION

Part of a Thesis: Comparison of different methods of risk stratification for sudden cardiac death in patients after myocardial infarction

PhD candidate: Vedran Velagić, MD

Mentors: Associate Professor Davor Puljević, MD, PhD, Professor Davor Miličić, MD, PhD

Affiliations: Department of Cardiovascular Medicine School of Medicine & University Hospital Centre Zagreb Zagreb, Croatia

INTRODUCTION: Sudden cardiac death (SCD) is defined as an unexpected cessation of cardiac activity that results with hemodynamic collapse. Usually, it is caused by malignant ventricular arrhythmias in the setting of coronary heart disease and its consequences. Despite the contemporary treatment modalities (early revascularization and modern drug therapy), a large portion of patients after myocardial infarction (MI) remain at risk for SCD. Assessment of the degree of left ventricular systolic dysfunction (by echocardiography) is commonly used method for stratification of risk for SCD. Given the known shortcomings of echo derived parameters (primarily low predictive values), this method is considered inadequate and there is a consensus that new methods are needed.

HYPOTHESIS: 1. Certain invasive and noninvasive tests can predict SCD risk in postinfarction period
2. By combining the different methods it is possible to get clinically relevant positive and negative predictive values for the risk assessment of SCD

AIMS: To determine and compare the usefulness of certain methods in assessing the risk of SCD after MI in the era of early invasive reperfusion therapy and modern drug treatment. Try to develop a scoring system for risk stratification that would be useful in the clinical practice.

MATERIALS AND METHODS: The research is planned as a prospective observational study that will enroll at least 200 adult patients younger than 70 years with verified acute MI. All patients must be treated with primary percutaneous coronary intervention and optimal drug therapy. The study includes two hospital visits when the following tests will be preformed: echocardiography, 12-lead ECG, Holter ECG and signal averaged ECG with the derived parameters. First visit is during the index event (acute MI) and the second visit is planned two months later when microvolt T-Wave alternans and electrophysiology study will be performed. Follow up period will be two years during which telephone check-ups will be preformed. The main study endpoint is SCD.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH:

Better understanding of SCD and development of risk assessment tools which can be useful in determination of eligibility of MI survivors considering the cardioverter-defibrillator implantation which is currently the best mode of SCD prevention.

Keywords: sudden cardiac death, risk assessment, myocardial infarction

CORRELATION BETWEEN DIFFERENT TYPES OF CONSERVATIVELY TREATED FRACTURES OF THORACOLUMBAR JUNCTION OF SPINE WITH THE INTENSITY AND PATTERNS OF PAIN DISTRIBUTION, AND WITH THE PATIENT'S QUALITY OF LIFE

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INTRODUCTION: The greatest number of vertebral compression fractures affects the last two thoracic and first two lumbar vertebrae. Fractures observed in this study are considered stable, and conservative, non-surgical treatment is indicated. Typically, the immobilization with thoracolumbar orthosis is administered, which reduces further compression of injured vertebra and minimizes micro-movements between fracture fragments, thereby reducing the sensation of pain. However, abovementioned orthosis cannot correct the existing deformity, nor can restore vertebral body height.

HYPOTHESES: 1. Different subgroups of fractures (A 1.1., And 1.2. And 1.3.) are associated with different intensity of pain, as well as with different patterns of its distribution. This difference exists before and after the treatment with thoracolumbar orthosis. 2. Patients with different subgroups of fractures will have a different average score on the test for the quality of life.

AIMS: To analyze the patterns of pain distribution and pain intensity in relation to the type of vertebral compression fractures (subgroups A 1.1., A 1.2, and A 1.3., according to AO/ASIF-classification) and the resultant impact of different types of fractures on patient's quality of life.

MATERIALS AND METHODS: This study includes patients with A1.1, A1.2 and A1.3 type of fracture, according to AO / ASIF-classification. Intensity of pain will be assessed by visual analogue scale (VAS), and pattern of pain distribution will be determined according to the classification published by the Doo TH, etc. The results will be analyzed using chi-square test. Patients' quality of life will be assessed using WHOQOL-BREF questionnaire, and analysis of results will be carried out by ANOVA.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: Assessment of association between different subgroups of spinal compression fractures with different intensities and patterns of pain distribution, and, consequently, the quality of life of patients will give a contribution to identifying generators and mechanisms of pain in the studied fractures. It will also show whether the current treatment, i.e. immobilization with thoracolumbar orthosis, is equally effective in all subgroups of studied fractures, or there is a need to consider the other forms of treatment.

MeSH / Keywords: Spinal Fractures, Pain Measurement, Quality of Life.

PROGNOSTIC CHARACTERISTICS OF NEW SCORING SCALE SYSTEM FOR TRAUMA PATIENTS

Part of a Thesis: Prognostic Characteristics of New Scoring Scale System for Trauma Patients

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INTRODUCTION: Scoring scale systems (SSS in further text) applied in clinical medical practice are designed to objectively quantify clinical data. They are utilised in assessing mortality risks, clinical decisions and quality of management of patients. They enable a quantification of patient outcomes thus rendering treatment results comparable. An ideal scoring scale has the following characteristics: 1. adequate correlation with predictable consequences (mortality, length of hospitalization, expenses); 2. clinical data needed in the SSS are readily available; 3. simple application

There are seven SSSs widely used globally: anatomical (ISS , NISS, ICISS), physiological (TS, RTS, PTS) and combined (TRISS, ASCOT, PRI). In Croatia, on the other hand, apart from the Glasgow Coma Scale, SSSs are not widely implemented.

HYPOTHESIS: A new scoring scale - to be applied in assessing the risks and treatment outcomes for trauma patients- can be designed based on criteria already used in established SSSs and the said new SSS will have better prognostic characteristics and everyday applicability.

AIMS: To conduct a meta analysis of literature referring to prognostic quality of trauma SSSs widely applied for patients with multiple trauma. To design a new SSS based on a combination of characteristics previously used in older SSSs. To implement the new SSS in everyday practice and test it's prognostic value.

MATERIALS AND METHODS: Most commonly used, well established SSSs, Simplified Acute Physiology Score (SAPS); Glasgow Coma Scale(GCS); Injury Severity Score(ISS), will be applied parallel to estimate the following physiological, neurological and anatomical characteristics for patients with multiple trauma: 1. number of days in ICUs; 2. mechanical ventilation; 3. complications (local, systemic); 4. organ donation; 5. treatment expenses; 6. length of hospitalization. By using the three most significant SSSs parallel with each other, a new simplified and more precise scoring system will be devised. The total number of allocated points in the new SSS is achieved by adding one third of the points scored by applying each of the three previous SSSs.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: The research will yield a new SSS that will be better applicable in everyday practice than the previously existing ones.

Acknowledgements: The author would like to thank his family for understanding and support, his mentor for guidance and his hospital for financial assistance.

MeSH / Keywords: scoring scale systems, multiple trauma, treatment outcomes, risk assessment

ANATOMICAL LOCALIZATION OF UMBILICUS

Part of a Thesis: Determination of an Anatomical Localisation of Umbilicus Using Mathematical Model

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Mentor: Associate Professor Davor Mijatović, MD, PhD

Affiliations: University of Zagreb School of Medicine, Univesity Hospital Centre - Zagreb

INTRODUCTION: The umbilicus with its looks and position is essential to the aesthetic appearance of the abdomen. There is a large number of publications describing various neoumbilicoplasty or reinsertion techniques of umbilicus after abdominoplasty but very few about its exact localisation.

HYPOTHESIS: Determination of an exact localisation of umbilicus is possible with specific mathematical model brought out from relations between umbilicus and adequate anatomical fixed points around abdominal wall.

AIMS: 1. To build mathematical model for calculating exact localisation of umbilicus using relations between umbilicus and adequate anatomical fixed points around abdominal wall in haelthy and young population. 2. To define if there is a difference in anatomical localisation of umbilicus regarding to gender (1) and age (2, only for women). 3. To define if it is possible to use the same mathematical model for localisation of umbilicus in different subpopulations of this research. 4. To define correspondence between exact and anticipated localisation of umbilicus and flaw of anticipation.

MATERIALS AND METHODS: In this study 150 people will be included and divided in 3 groups: (1) Young women between ages 18 and 25 with BMI 19-25 kg/m², (2) Young men between ages 18 and 25 with BMI 19-25 kg/m², (3) Women between ages 35 and 45 with BMI>25 kg/m². (i) Anthropometric measures of height, weight, distances between umbilicus and adequate anatomical fixed points, skinfolds, waist and hips measurements. Tools: anthropometer, measure tape, weight scale, caliper. (ii) Building of a mathematical model(s). (iii) Statistical analysis.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: Building a mathematical model for calculating exact localisation of umbilicus is of great scientific value because there are very few publications about exact localisation of umbilicus by now. Exact localisation of umbilicus is an essential in it's reconstruction or reinsertion procedureds so there is also a great clinical and practical contribution of this research too.

Acknowledgements: I would like to thank to my Mentor Davor Mijatović and students Ivana Stipić and Oliver Šuman for all their help and support during this research and also to Johnson&Johnson Croatia for funding.

MeSH / Keywords: umbilicus, exact localisation, mathematical model, anthropometry.

QUALITY INDICATOR „USE OF BLOOD“

Part of a Thesis: Use of Blood As A Quality Indicator In Infrarenal Aortoiliacal Procedures

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Mentor: Jasna Mesarić, MD, PhD

Affiliations: University Hospital Merkur, University Hospital Dubrava, University Hospital Sv. Duh, University Hospital Centre Rijeka

INTRODUCTION: Surgical treatment of infrarenal aortoiliac occlusive disease and aneurysms is associated with blood loss. In our literature, there are no reports on the quality of transfusion and surgical practice, and on blood transfusion correlation with surgical treatment outcome. The published studies leave open questions as indication for perioperative blood transfusion (transfusion trigger), use of autologous transfusion, indications for cell-saver, and indicate the great variety in transfusion practice which may require reevaluation and standardization. The Performance Assessment Tools for Hospital (PATH) program of the World Health Organization Regional Office for Europe provide a quality indicator „Use of blood“ as a tool for quality measurement of transfusion practice in different fields of surgery. This indicator was modified for vascular surgery and aortoiliacal procedures.

HYPOTHESIS: Quality indicator „Use of blood“ as a tool for evaluating outcome of elective infrarenal aortoiliacal procedures, will define areas of surgical and transfusion practice that require improvement in preoperative patient set-up, ordering and use of blood pre-, intra- and postoperatively.

AIMS: To analyze current surgical and transfusion practice in elective aortoiliacal procedures; to define areas for improvement; to compare transfusion practice between hospitals involved; to justify allogenic transfusion; and to analyze correlation between transfusion and postoperative complications including local infection, pneumonia and sepsis.

MATERIALS AND METHODS: This prospective, cohort, multicentric study will include at least 100 patients undergoing elective aortoiliacal procedures at 4 hospitals. Patients with known coagulation abnormalities, with prothrombine time < 0,35, those with reoperation for the same initial diagnosis and patients submitted to more than one type of surgical procedure during the same hospital episode were excluded from this investigation. Demographic, clinical and data on surgical and transfusion practice will be collected by a modified list of the „Use of Blood“ indicator from the Performance Assessment Tools for Hospital (PATH) program of the World Health Organization Regional Office for Europe.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: This research will evaluate the role of quality indicator „Use of blood“ in quality assessment of transfusion and surgical practice with aim to improve it, and so contribute to standardization of transfusion practice in aortoiliacal procedures.

Keywords: infrarenal aortoiliac procedures, AAA, indicators, transfusion practice

UNIDIRECTIONAL BARBED SUTURE AS FLEXOR TENDON SUTURE (EX VIVO ANALYSIS)

Part of thesis: Biomechanical characteristics of unidirectional barbed suture as flexor tendon suture (ex vivo analysis on animal model)

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Mentor: Professor Zdenko Stanec, MD, PhD

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INTRODUCTION: The treatment of flexor tendon injury in the area of palm and fingers is demanding because of early mobilisation protocol implementation needed to enable tendon gliding, differential gliding, prevent tendon adhesions to soft tissue, and enable better healing. Today suture materials slide in both directions, so we use a number of configurations to make the repair stronger. Suture materials that slide in one direction and resist sliding in other are in clinical use. In theory they show advantages in joining together two ends of tendon (both of them having tendency to diverge).

HYPOTHESIS: Unidirectional barbed suture has biomechanical properties that are preferable for flexor tendorrhaphy comparing to other conventional suture material.

AIMS: To analyse biomechanical characteristics of unidirectional barbed suture as core suture for flexor tendorrhaphy. To find the most suitable configuration to utilise biomechanical advantages of unidirectional barbed suture for flexor tendorrhaphy.

MATERIALS AND METHODS: Forty-six fresh porcine flexor digitorum profundus tendons will be measured first to unify the tendons and then divided randomly into four groups (with the first group as control group comprised of intact tendon). Tendons will be transected and repaired on following way: four-strand knotless technique with 1 cm purchase, four-strand knotless technique with 2 cm purchase, four-strand double Kessler technique. The cross-sectional area before and after repair, ultimate tensile strength, and the way of repair failure will be recorded.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH:

To find the advantages and disadvantages of unidirectional barbed suture as core suture in flexor tendorrhaphy, the best way of its clinical application and thus expanding the knowledge in the field of flexor tendon repair surgery.

MeSH / Keywords: Flexor tendorrhaphy, core suture, unidirectional barbed suture

REMOTE ISCHEMIC PRECONDITIONING IN CARDIAC SURGERY

Part of a Thesis: Remote ischemic preconditioning as a method of myocardial protection

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Mentor: Professor Željko Sutlić, MD, PhD

Affiliations: Department of Cardiac Surgery, Dubrava University Hospital - Zagreb

INTRODUCTION: Remote ischemic preconditioning (RIPC) is a preconditioning of a non-cardiac organ or tissue that can increase myocardial tolerance to ischemic-reperfusion injury during the subsequent prolonged ischemia.

HYPOTHESIS: RIPC has beneficial effects on myocardium during the controlled cardiac arrest shown as reduced ischemic-reperfusion injury, an improvement of myocardial function and better clinical outcomes.

AIMS: Primary outcome is the total cardiac troponin-I (cTnI) released during 72h after surgery. Secondary end-points are: cTnI at 6h after surgery, peak value of cTnI, postoperative cardiac index (CI), postoperative left ventricular stroke work index (LVSWI), inotropic score, postoperative left ventricular ejection fraction (LVEF), incidence of low cardiac output syndrome (LCOS), peak value of C-reactive protein (CRP), duration of mechanical ventilation, intensive care unit stay, perioperative mortality and incidence of other postoperative complications.

MATERIAL AND METHODS: This randomized controlled trial will include 60 adult patients scheduled for elective surgical aortic valve replacement and randomized to a RIPC or a control group. Exclusion criteria will be concomitant coronary artery disease, emergency or redo surgery, age of >80y, diabetes mellitus with insulin therapy, severe renal or hepatic disease, severe peripheral vascular disease of upper extremities. RIPC protocol will include 3 cycles of 5 minutes of right arm ischemia achieved by 300 mmHg cuff inflation/deflation, followed by 5 minutes of reperfusion. Standard aortic valve replacement through median sternotomy will be performed in normothermia and using cold blood cardioplegia to achieve cardiac arrest. cTnI will be measured before surgery and at 1h, 6h, 24h, 48h and 72h after surgery. Hemodynamic parameters will be measured before surgery, immediately after and 24h after surgery. CRP will be sampled before surgery, immediately after and at 24h, 48h and 72h after surgery.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: This research will prove the efficacy of RIPC on human model that is more representative than previously used: no patients with coronary artery disease will be included and relevant hemodynamic and ultrasound parameters will be measured to precisely evaluate postoperative myocardial function.

MeSH / Keywords: remote ischemic preconditioning; cardiac surgery

EPIGENETIC CHANGES IN DEGENERATIVE CALCIFIC AORTIC VALVE STENOSIS

Part of a Thesis: Epigenetic Changes in Degenerative Calcific Aortic Valve Stenosis

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INTRODUCTION: Calcific aortic valve stenosis (CAVS) is the most common indication for surgical valve replacement. CAVS is not just an age related disease but an active biological process with some established similarities to the risk factors and the process of atherogenesis. Still little is known about the mechanisms of its etiology and progression. Thus there is an unmet scientific need to closely determine pathological mechanisms and identify new approaches for CAVS treatment.

Deregulation of epigenetic mechanisms in the aortic valves of elderly patients with CAVS causes altered gene expression promoting the progression of disease.

MATERIALS AND METHODS: Research will encompass elderly patients (n=25) undergoing valve replacement for severe calcific aortic stenosis and heart transplant recipients (n=5) without any visible clinical and morphological aortic valve lesions. Extraction and quantification of DNA and RNA molecules from tissue and peripheral blood samples (5ml), and subsequent determination of global DNA methylation pattern, mRNA, protein, and miRNA expression and histone code analysis will be performed using appropriate commercial kits and immunohistochemical procedure.

DISCUSSION: The role of epigenetic regulatory pathways represents a fundamentally new perspective on CAVS. This perspective is exciting given the potential for therapeutic intervention and reprogramming of cells at the epigenetic level. Determination of epigenetic factors relevant for the degenerative calcifying aortic stenosis will contribute to clarification of the pathophysiological mechanisms responsible for its etiology and progression and allow the discovery of new potential biomarkers for the pharmacological therapy. This would further enhance the possibilities of conservative treatment, enable the reduction of morbidity and mortality and reduce the medical and economic burden on the healthcare system associated with the disease.

MeSH / Keywords: CAVS, epigenetics, miRNA, histone code, gene expression, DNA methylation

IMPACT OF DEPRESSIVE SYMPTOMS ON THE OPERATING RISK IN PATIENTS UNDERGOING CORONARY ARTERY BYPASS GRAFTING (CABG)

Part of a Thesis: Impact of depressive symptoms on the operating risk in patients undergoing coronary artery bypass grafting (CABG)

PhD candidate: Stjepan Ivanković, MD.

Mentors: Assistant professor Vedran Ćorić, PhD., MD.; Professor Alma Mihaljević Peleš, PhD., MD.

Affiliation: The University *Hospital Centre Zagreb* (KBC *Zagreb*), Department of Cardiac Surgery, Zagreb, Croatia

INTRODUCTION: WHO predicts that by 2020. depression will become second health problem world-wide. In most countries the number of people who suffered from depression is 8-12%. Depression is present in 20% of patients with ischemic heart disease and in 35% of patients with congestive heart failure. In CABG patients the number is even higher 32% -38%. The impact of depressive symptoms on operative risk in patients who require CABG was studied in several studies, but has not yet been sufficiently explored. Some criticisms of the studies: tests were conducted on a small number of patients and included only one gender. In this study we investigate whether and to what extent the symptoms of depression, affect the operating risk in CABG patients.

HYPOTHESIS: CABG patients that have a higher prevalence of depressive symptoms have a higher operating risk.

AIMS: Assess the extent to which these depressive symptoms affect the operating risk in CABG patients

MATERIALS AND METHODS: The prospective study will include patients, hospitalized at the Department of Cardiac Surgery, KBC „Zagreb“. Consecutive sample of CABG patients will participate in the study. EuroSCORE II will be evaluated. Severity of depressive symptoms will be determined by standardized questionnaire: PRIME MD test and the BDI-II test. During the operation we will measure: time of extracorporeal circulation, length of myocardial ischemia. After the operation we will measure: the length of postoperative mechanical ventilation, the levels of inflammatory markers (CRP) and we will determine postoperative mortality (death within 30 days after surgery). As predictors will be taken EuroSCORE II, the weight of depressive symptoms, the characteristics of cardiac surgery and as dependent variables: duration of mechanical ventilation after surgery and CRP. The study will include up to 200 patients with estimated number of predictors in the regression analysis (to 10). **EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH:**

Taking depressive symptoms in the assessment of operative risk (EuroSCORE review)

Medical surveillance and optimal treatment of patients that have higher prevalence of depressive symptoms would result in a faster recovery. This would result with: shorter hospitalization, lower costs and would decrease mortality and postoperative complications, ensuring longer survival with better quality of life.

Keywords: Depressive symptoms, CABG patients, Operating risk

GLYCEMIA CONTROL AND ENDOTHELIAL DYSFUNCTION IN PATIENTS UNDERGOING CORONARY ARTERY BYPASS GRAFTING

Part of a Thesis: Influence of Glycemia Control by Continuous Insulin Infusion on Endothelin-1 Expression During Coronary Artery Bypass Grafting

PhD candidate: Daniel Unić, MD, MSc

Mentor: Professor Željko Sutlić, MD, PhD

Affiliations: University of Zagreb School of Medicine, Dubrava University Hospital - Zagreb

INTRODUCTION: Perioperative hyperglycaemia is associated with increased morbidity and mortality in patients undergoing coronary artery bypass grafting (CABG). Systemic inflammatory response and endothelial dysfunction are among the most important consequences of hyperglycemia responsible for adverse outcomes. Endothelin-1 (ET-1) represents both a mediator in systemic inflammatory response as well as a marker of endothelial dysfunction.

HYPOTHESIS: Continuous insulin infusion will decrease ET-1 expression in systemic and coronary circulation in diabetic patients undergoing CABG.

AIMS: To investigate the influence of continuous insulin infusion as a method of perioperative glycemia control on ET-1 and nitric oxide (NO) expression in systemic and coronary circulation in patients undergoing CABG. To investigate the influence of glycemia control methods on hemodynamic parameters and myocardial damage markers.

MATERIALS AND METHODS: Fifty diabetic patients scheduled to undergo CABG will be randomized in two groups according to perioperative glycemia control method: continuous insulin infusion (CII) or bolus (BOL). Endothelin-1 and NO levels will be determined from arterial and coronary circulation at 4 perioperative time points using ELISA method. Glucose levels will be determined every 2 hours postoperatively and insulin therapy (CII or BOL) administered to keep glucose levels between 5.5-8.3 mmol/L. Systemic and pulmonary pressure, potassium levels and cardiospecific enzymes will be determined at specific time points. CRP as an additional inflammatory marker will also be determined.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: Results of this study should further explain the role of glycemia in endothelial dysfunction in patients undergoing CABG and determine the optimal method of glycemia control which could decrease endothelial dysfunction.

MeSH / Keywords: Endothelin-1, aortocoronary bypass, endothelial dysfunction, perioperative hyperglycaemia

THE INFLUENCE OF HOMOLOGOUS BLOOD TRANSFUSION ON MEMORY AND ATTENTION IN PATIENTS UNDERGOING CARDIAC SURGERY

Part of thesis: Influence of homologous blood transfusion on memory and attention in patients undergoing cardiac surgery

PhD candidate: Jakov Vojković, MD

Mentor: Associate Professor Vedran Ćorić, MD, PhD

Affiliation: University of Zagreb School of Medicine, University Hospital Centre - Zagreb, Department of cardiac surgery

INTRODUCTION: One of the causes of cerebral injury in patients undergoing cardiac surgery is the transfusion of homologous blood. Previous studies have shown that homologous blood transfusion leads to increased ischemic complications in patients. In cardiac surgery, neurologic complications lead to prolonged hospital stays in the hospital and mobilization of significant material and human resources. My hypothesis is that homologous blood transfusions to be associated with a higher incidence of neurological damage. Testing will be consecutive, observational and prospective in all consenting patients who are scheduled to undergo elective surgical myocardial revascularization using cardiopulmonary bypass, with no known neurological disease or carotid artery disease. Pre- and postoperative test of manual dexterity, memory, attention and orientation will be preformed,

HYPOTHESIS: Transfusion of homologous blood will cause patients having lower scores in memory and attention test.

AIMS: The main aim of this research is to determine how the transfusion of homologous blood affects cognitive functions in cardiac surgery patients.

MATERIALS AND METHODS: In the preoperative phase, we will be collecting demographic data, and do laboratory tests to determine the level of hematocrit in the blood. He will also perform pre-operative cognitive tests of manual dexterity, memory, attention and orientation. We will use the Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA), Trail Making Test, Perdue pegboard test, Audio-Visual Learning Test (AVLT). During the operation hematocrit will be monitored and the lowest value will be used as a reference. It will also monitor the amount of homologous blood transfusions administered. In the period after surgery to monitor the neurological deficit of type I (focal) and type II (cognitive). Before mentioning cognitive tests will be run again after surgery.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: The meaning of the incidence of neurological deficit in the postoperative period, and the negative health implications for patients and burdens health care system have already been emphasized. So far there are no clear findings how transfusions of homologous blood affect brain blood flow and thus cognitive functions. Therefore, there is a need for evaluation of consequences of homologous blood transfusion and its impact on cognitive function of patients.

MeSH / Keywords: executive functions, blood transfusion, cardiac surgery

INTERNAL JUGULAR VEIN PATENCY FOLLOWING NECK DISSECTIONS

Part of a Thesis: Influence of Sternocleidomastoid Muscle on Internal Jugular Vein Patency Following Neck Dissection

PhD candidate: Damir Sauerborn, MD

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Affiliations: University of Zagreb School of Medicine, University Hospital Dubrava - Zagreb. General Hospital „Dr. Josip Benčević“ - Slavonski Brod

INTRODUCTION: Classic radical neck dissection is a procedure that removes all lymphatic tissue of the neck together with internal jugular vein (IJV), accessory nerve and sternocleidomastoid muscle (SCM) because of treatment and control of metastatic disease in the neck. Modern perception of the neck dissection includes preservation of all important neck structures with the purpose of minimizing morbidity and increasing quality of life. Preservation of IJV in unilateral dissection remains unclear since unilateral ligation of vein is not associated with severe morbidity. Preservation of vein prolongs surgical procedure while, in many studies, vein becomes thrombotic in postoperative period (0 to 30% of dissections). According the literature, there are many different factors that may cause IJV thrombosis. In our analysis of literature we didn't find any study that includes preservation or sacrifice of SCM and its relationship to IJV thrombosis. In this prospective study we compared preservation and sacrifice of SCM according to patency of the IJV after neck dissection.

HYPOTHESIS: Neck dissections with sacrifice of the SCM cause changes in IJV patency (decrease in blood flow and increase in flow velocity) in greater extent than neck dissections with preservation of the SCM.

AIMS: General aim is to determine influence of preservation and sacrifice of SCM on IJV patency following neck dissection. Specific aims are: to define changes in diameter, flow velocity, flow rates and appearance of thrombosis of the IJV according to neck dissections with preservation and sacrifice of the SCM, and also to determinate influence of postoperative radiotherapy on IJV patency.

MATERIALS AND METHODS: Research compares 2 groups of examinees, first with preservation and second with sacrifice of SCM during neck dissection. Number of examinees in both groups is 30. Doppler ultrasonography is used in assessment of IJV patency. Beside appearance of thrombosis, measurement will encompass vein diameter and flow velocity, while total volume flow will be calculated from obtained data. Measurements will be performed before surgery, 2 weeks, 3-4 months and 9-12 months after surgery.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: To quantitatively determinate changes in IJV patency after neck dissections with preservation and sacrifice of SCM.

MeSH / Keywords: Internal jugular vein patency, internal jugular vein thrombosis, sternocleidomastoid muscle, neck dissection

VIRULENCE AND CLONAL SPREAD OF FLUOROQUINOLONE-RESISTANT STRAINS OF UROPATHOGENIC *ESCHERICHIA COLI* IN OUTPATIENTS FROM ZAGREB REGION IN FIVE-YEAR STUDY PERIOD

Part of a Thesis: Fluoroquinolone-resistant Uropathogenic *Escherichia coli* Strains in Zagreb Region in Five-year Study Period

PhD candidate: Tatjana Marijan, MD, MSc

Mentor: Professor Jasmina Vraneš, MD, PhD

Affiliation: Dr. Andrija Štampar Institute of Public Health - Zagreb

INTRODUCTION: *Escherichia coli* (*E. coli*) is the most prevalent uropathogen, accounting for more than 70% of all urinary isolates. Fluoroquinolone-resistance of *E. coli* in Croatia is now exceeding 10%, with especially high resistance rates in older patients. Besides selection of resistant mutants under antibiotic pressure, clonal dissemination of fluoroquinolone-resistant (FR) strains considerably contributes to rise in fluoroquinolone-resistance. In recent years, intercontinental dissemination of FR uropathogenic *E. coli* clonal groups O25:H4-ST131 and O15:K52:H1 has been described.

HYPOTHESIS: Based on results from the pilot study conducted in 2007, the hypotheses of this research are: a) FR *E. coli* clonal groups O25:H4-ST131 and O15:K52:H1 are widespread in Zagreb outpatients with urinary tract infection (UTI) with different prevalence in two study-periods, b) FR *E. coli* strains carry lesser virulent potential than fluoroquinolone susceptible (FS) strains, c) FR strains are due to their presumed lower virulence mostly extended in older patients with complicated UTIs.

AIMS: This research aims to assess the outspread of O25:H4-ST131 and O15:K52:H1 clones in Zagreb outpatient population in two study-periods. Furthermore, the aim is to compare O serotype distribution and possession of virulence genes with their phenotypic expression in FR and FS *E. coli* strains.

MATERIALS AND METHODS: A total of 446 *E. coli* isolates recovered from urine samples of patients with significant bacteriuria will be included in the survey. Antibiotic susceptibility testing, O-serotyping, molecular and phenotypic virulence profile analysis will be conducted on 69 FR and 73 FS *E. coli* strains from the first study period (2006/2007) and 202 FR and 102 FS strains from the second study period (2011/2012). Serogroup O15 and O25 strains will be further assessed for their belonging to O25:H4-ST131 and O15:K52:H1 clonal groups as well as for their pulsed-field gel electrophoresis profile similarity. Statistical analysis will be performed using Fisher's exact test and χ^2 test with threshold for significance of < 0.01 .

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: First description of O25:H4-ST131 and O15:K52:H1 clonal groups in Croatia, analysis of putative link between fluoroquinolone-resistance and *E. coli* virulence with consequential better understanding of UTI pathogenesis in older patients.

Acknowledgements: I would like to express my gratitude to dr Ana Mlinarić-Džepina for collecting strains included in this research and to ing Marko Šenjug for technical assistance.

MeSH/Keywords: urinary tract infections, uropathogenic *Escherichia coli*, virulence, antimicrobial drug resistance, fluoroquinolones

THE CORRELATION BETWEEN SUBCLINICAL HYPOTHYROIDISM AND MORBIDITY AND MORTALITY OF PATIENTS IN END-STAGE RENAL DISEASE

Part of a Thesis: The Correlation between Subclinical Hypothyroidism, Constant Inflammation and Malnutrition with Morbidity and Mortality of Patients treated with Haemodialysis and Peritoneal Dialysis

PhD candidate: Nataša Črne, MD

Mentor: Assistant Professor Draško Pavlović, MD, PhD

Affiliations: University Hospital Centre „Sestre milosrdnice“, Zagreb, General Hospital „Dr Tomislav Bardek“, Koprivnica, General Hospital Bjelovar

INTRODUCTION: Incidence of chronic kidney disease (CKD) rises. Factors like constant inflammation, vascular calcification and malnutrition influence the incidence of morbidity and mortality of patients treated with dialysis. Low triiodothyronine (T3) syndrome is also one of the factors that influence the course of CKD. Patients with that syndrome have normal or higher level of thyreotropine-stimulating hormone and lower level of T3. In that way the latent hypothyroidism develops, which has a negative effect on the prognosis of CKD. **HYPOTHESIS:** Low levels of thyroid hormones, especially triiodothyronine, in comparison with constant inflammation and malnutrition, are significantly correlated with morbidity and mortality of patients treated with dialysis.

AIMS: The general aim is to research the correlation between levels of thyroid hormones, inflammation parameters and nutritional status of patients treated with haemodialysis and peritoneal dialysis with general and cardiovascular morbidity and mortality and their mutual correlation. The specific aim is to research which of these parameters mostly contributes to mortality and morbidity of these patients.

MATERIALS AND METHODS: The research will be carried out on patients treated with dialysis in three hospitals (about 230 patients) in one year time. Patients that will be acutely ill in the beginning of the research, will not be included. In the beginning of the research data concerning gender, age, the main cause of CKD, anamnesis, the duration of dialysis treatment, the sort of dialysis, the presence of AV fistula or central venous dialysis catheter and medical therapy will be collected. In the beginning of the research, 6 months and one year after, and in case of acute illness of a patient, the levels of thyroid hormones and parameters of inflammation and malnutrition will be determined from blood samples. In terms of statistical analysis, descriptive statistical methods, Kolmogorov-Smirnov test, Fisher test, Pearson correlation coefficient, multivariate, Kaplan-Meier and Cox regression analysis will be applied.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: The expected contribution is to explore the rankness of correlation between low T3 syndrome and mortality and morbidity of patients treated with dialysis and to contribute to the results of other studies in this area of medical science.

MeSH/Keywords: chronic kidney disease, low triiodothyronine syndrome, inflammation, malnutrition.

LOW LEVEL OF THE IRON AND FREQUENCY RLS IN THE PATIENTS ON THE HAEMODIALYSIS

Part of Thesis: Iron, Restless leg syndrome, Haemodialysis

PhD candidate: Sonja Hodžić MD, MS

Mentor: Professor Petar Kes, MD, PhD

Affiliation: Clinical hospital center Zagreb, Institution for the arterial hypertension, nephrology and dialysis
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INTRODUCTION. Iron has considerable influence on the frequency of the restless leg syndrome in the patients on the haemodialysis. Is it so and how really iron acts on the frequency of the RLS is the main goal of this examination.

MATERIALS AND METHODS: In this examination I shall study all important determining elements and their correlation in the frequency of the restless leg syndrome in our patients. It would be done by controlling laboratory measurements, for example CRP, KKS TRC, urea, creatinine, uric acid, ferritin, UIBC, TIBC, ferritin. Also, I have to see how it depends on the age of our patients, sex, therapy, dosage of the erythropoietin, how long they are on the haemodialysis. The information about eating habits, physical activities (Kornofsky score) I intend to get by the interviews. Of course, it would be restless leg syndrome rating scale done.

RESULTS: Results would show the statistical significance in the correlation of the low level of the iron and the frequency of the RLS in the patients on the haemodialysis. In the same time all results would be helpful in the better prevention of the restless leg syndrome.

DISCUSSION: In the discussion I shall show if we have statistically significant results or not. Also, by these results will see if we got our goals or not. Results we got in this way we could compare with the results in the professional and science articles.

Acknowledgments: I would like to thank my mentor PhD Petar Kes and Institution for the arterial hypertension, nephrology and dialysis.

MeSH/Keywords: Iron, Restless leg syndrome, Haemodialysis

INFLUENCE OF DRUSEN MORPHOLOGY ON DISRUPTION OF PHOTORECEPTOR LAYER IN AGE-RELATED MACULAR DEGENERATION

Part of a Thesis: Influence of Drusen Morphology on Disruption of Photoreceptor Layer in Age-related Macular Degeneration

PhD candidate: Krešimir Mandić, MD

Mentor: Nenad Vukojević, MD, docent

Affiliations: University of Zagreb School of Medicine, University Hospital Centre - Zagreb

INTRODUCTION: Senile macular degeneration or age-related macular degeneration (ARMD) is a major cause of significant vision loss and blindness in adults over 50 years of age in developed world. About 10% of the population between age of 65-75 and 19% of population older than 75 years of age have some form of age-related macular degeneration. There are two forms of the disease, non exudative („dry”) with prevalence of roughly 85-90 % with better visual prognosis and „exudative („wet”) forme with prevalence of 10-15 % and worse prognosis. In period of 5 years patients with intermediate „dry” ARMD have 18 percent chance of progressing into advanced „dry” or „wet” ARMD. The characteristic feature of „dry” ARMD are drusen made of clusters of extracellular material that accumulates between the choroid and retina. Drusen over time may progress and change its morphology, leading to functional disorders of photoreceptors making it a direct cause of the deterioration or loss of visual function. Although AREDS study conducted in 1990-s found that „intermediate, large, soft and confluent” drusen have greater risk of progressing to „wet” form, still it is has not been evaluated which of the morphologic characteristics of drusen in dry” form cause more progressive photoreceptor layer attenuation.

AIMS: Our objective is to determine which of the morphological characteristics found on optical coherence tomography (OCT) cause most significant attenuation of the photoreceptor layer.

MATERIALS AND METHODS: This prospective study will be conducted at Eye Clinic of the University Hospital Centre - Zagreb in period of 2 years. 30 patients with „dry” form of ARMD will be examined with OCT at the beginning of study and 2 years after. OCT is noninvasive, noncontact device that uses light rays to gain ultra high quality images of macula. Using OCT we shall determine overall drusen number, shape, reflectivity, height, width, homogeneity and photoreceptor layer thickness.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: By determining which of the drusen morphological characteristics found on OCT cause most significant attenuation of the photoreceptor layer over 2 years we hope to offer patient with „dry” ARMD more precise prognosis of their visual deterioration.

MeSH / Keywords: drusen, photoreceptors, age-related macular degeneration, optical coherence tomography

FIBROBLAST GROWTH FACTOR 23 AND ACUTE KIDNEY INJURY

Part of Thesis: Fibroblast growth factor 23 augmentation and postoperative acute kidney injury progression

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Mentor: Associate professor Nikolina Bašić-Jukić, MD, PhD

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INTRODUCTION: Fibroblast growth factor 23 (FGF23) is a novel peptide hormone with the regulatory role of phosphate metabolism. Present studies have shown that in patients with chronic kidney disease FGF23 is significantly elevated and responsible for the development of Chronic Kidney Disease - Mineral and Bone Disorders. FGF23 is a strong predictor of progressive development of chronic kidney disease and lethal outcome. Studies about the prognostic role of FGF23 in acute kidney injury (AKI) are insufficient and contradictory. Early identification of the new onset of reversible AKI imports early initiation of the treatment, which includes dialysis as well.

HYPOTHESIS: FGF23 augmentation in plasma is early prognostic indicator of postoperative AKI progression.

AIMS: Aim of this study is to investigate FGF23 trends in plasma regarding postoperative AKI progression, and evaluate the role of FGF23 as early predictor in AKI outcome in contrast to RIFLE/ AKIN criteria. Additional goals are comparing FGF23 values with the healthy control subjects and, moreover, researching trend relationship between FGF23 with serum and urinary values of phosphate, calcium and creatinine.

MATERIALS AND METHODS: Prospective, cohort study will be conducted. Two groups of adult subjects will be included in the study: AKI subjects admitted in the Intensive care unit who developed AKI after elective and emergency operations (N=120) and healthy control subjects (N=30) according to inclusion and non-inclusion research criteria. During first 5 postoperative days AKI progression will be followed as clinical and laboratory indicators and FGF 23 will be measured. AKI function will be reevaluated like recovery or failure on the 7th and 14th postoperative day. FGF23 will be measured by Enzyme-Linked Immunosorbent Assay (ELISA).

EXPECTED AND SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: For the first time insufficiently known FGF23 trends in AKI subjects will be followed and its role as early prognostic indicator in the AKI outcome. FGF 23 connection to serum and urinary phosphate, calcium and creatinine values will be searched. As well, for the first time FGF23 values in healthy control subjects will be determined.

Keywords: Acute kidney injury; Fibroblast growth factor 23, human; Intensive care, surgical

KIDNEY DAMAGE IN TWO DIFFERENT POPULATIONS OF ENDEMIC FOCUS

Part of a Thesis: Kidney Damage in Two Different Populations of Endemic Focus

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Mentor: Associate Professor Bojan Jelaković, MD, PhD

Affiliations: University of Zagreb School of Medicine, University Hospital Centre - Zagreb, Institute for Public Health, Brod-Posavina County, Slavonski Brod, Croatia

INTRODUCTION: Endemic nephropathy (EN) is a syndrome presenting as chronic tubulointerstitial nephropathy and/or upper urothelial cancers (UUC). EN affects the rural population of villages located along the Danube River tributaries. Today it is considered to be an environmental form of aristolochic acid nephropathy.

HYPOTHESIS: EN is diminishing from Croatian endemic focus due to a lack of exposure to environmental toxin because of changes in farming and milling practices.

AIMS: 1) To determine and compare kidney damage in immigrants from Bosnian non-endemic villages who have lived long enough in Croatian endemic villages to develop early signs of EN versus autochthonous peasants 2) To determine the prevalence of EN using 3 different EN criteria in order to compare the prevalence of EN in our focus with other affected countries. 3) To determine the number and age of EN patients currently undergoing dialyses and who have been operated on due to UUC in the last 5 years, and compare it with data from past period 4) To determine exposure to aristolochic acid nowadays and 20 years ago and to analyze the link between exposure to aristolochic acid and kidney damage.

MATERIALS AND METHODS: In this study 3,070 adults from 9 endemic and 3 non-endemic Croatian villages were enrolled on a door-to-door basis and were stratified to diseased, suspected of having EN, at risk for EN, non-affected („others”) and immigrants. An extended questionnaire and physical examination were performed; fasting blood and spot morning urine specimen were obtained to determine serum creatinine, hemoglobin, urinary α 1-microglobulin and albumin concentrations corrected to urinary creatinine. Glomerular filtration rate was estimated using MDRD (Modification on Diet in Renal Disease) formula.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: The most important contribution is to confirm that exposure to environmental toxin is no longer present, which will allow us to conclude that EN will disappear in the next decade.

Additionally, for the first time, using different criteria, it will be possible to compare the exact prevalence of EN in Croatia with other countries. This study will enable future planning of public health activities and confirm that EN is an environmental form of aristolochic acid nephropathy.

Acknowledgements: This research was supported by Grants 108-0000000-0329 and 098-0982464 from the Ministry of Science, Education and Sports of the Republic of Croatia and Fogarty International Collaboration Award (FIRCA) award number R03TW007042, award number P01ES004068 from the National Institute of Environmental Health Sciences.

MeSH / Keywords: endemic nephropathy, aristolochic acid nephropathy, epidemiology, immigrants

CEREBRAL HAEMODYNAMICS IN PATIENTS WITH POSTTRAUMATIC PERSISTENT VEGETATIVE STATE

Part of a Thesis: Cerebral Haemodynamics in Patients with Posttraumatic Persistent Vegetative State

PhD Candidate: Ivan Dubroja, MD, MSc

Mentor: Vida Demarin, MD, PhD, FAAN, FAHA, FESO

Affiliation: Special Hospital for Medical Rehabilitation Krapinske Toplice

INTRODUCTION: There is a functional disconnection between cortex and brainstem as a result of brain trauma in persistent vegetative state (PVS) patients. They are awake but unaware of self or environment. In healthy persons cerebral haemodynamics shows reactivity in time and space, i.e. it is time-dependent of activation/stimulation of particular neuron populations. There is an increase of cerebral haemodynamics during activation compared to basal conditions. Only few studies used transcranial doppler (TCD) to study PVS, mostly in hypoxic encephalopathy or stroke, but not in posttraumatic PVS.

HYPOTHESIS: Cerebral activation by auditory stimulation (listening to the music) increases haemodynamics in basilar artery (activation of cochlear nuclei) but not in middle cerebral artery (activation of primary auditory cortex) in posttraumatic PVS patients, due to cortico-mesencephalic dissociation, compared to healthy individuals. Cerebral activation by visual stimulation (eyes opening to daily light) increases haemodynamics in posterior cerebral artery (activation of visual cortex) in posttraumatic PVS patients as in healthy individuals.

AIMS: 1. To estimate cerebral haemodynamics in posttraumatic PVS patients in basal conditions and during cerebral activation and to compare it to healthy population. 2. To find if possible differences in cerebral haemodynamics have prognostic value for duration of coma and outcome in PVS patients.

MATERIALS AND METHODS: Fifteen patients with posttraumatic PVS in the patient group, and 50 healthy volunteers in the control group, age and sex matched. Cerebral blood flow will be monitored on cerebral arteries by transcranial doppler (TCD). For auditory stimulation classical music will be applied through the stereo headphones during 3 minutes. Visual stimulation will consist of eye opening and exposing to daily light during 3 minutes. Mean systolic velocities (MSVs), pulsatility index (PI) and resistance index (RI) in all arteries of circle of Willis will be registered by TCD at basal conditions (without stimulation). Then blood flow monitoring continues in areas of interest (MCA, BA, PCA) during stimulation and 3 minutes post stimulation. The data will be statistically compared within and among groups by Wilcoxon matched-pairs test.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: This research should gain new data on cerebral haemodynamics and underlying mechanisms of vascular autoregulation in posttraumatic PVS, and eventually give some answers regarding prognosis of coma in these patients.

Acknowledgements: To professor Vida Demarin for guiding through the study design, and asst. professor Dusko Cerovec, for institutional and technical support.

MeSH/Keywords: persistent vegetative state, posttraumatic, transcranial doppler, cerebral haemodynamics

VESTIBULAR EVOKED MYOGENIC POTENTIALS IN EVALUATION OF BRAINSTEM LESION IN PATIENTS WITH RELAPSING REMITTING MULTIPLE SCLEROSIS

Part of a Thesis: Vestibular evoked myogenic potentials in relapsing remitting multiple sclerosis

PhD candidate: Tereza Gabelic, MD

Mentor: Mario Habek, MD, PhD

Affiliation: University Hospital Centre Zagreb, Zagreb

INTRODUCTION: Vestibular evoked myogenic potentials (VEMP) are myogenic short latency responses evoked by sound or bone conducted impulse. Neuroanatomical basis of this test is based on stimulation of vestibular system by acoustic, electric or vibratory impulse and it's detection on stercleidomastoid and ocular muscles. VEMP is a useful diagnostic method in an evaluation of clinically silent lesions in patients with multiple sclerosis (MS), especially in lesions of lower pons and medulla oblongata.

Abnormal results of VEMP in patients with MS implicate lesion of the brainstem, despite normal MRI and/or neurological examination

HYPOTHESIS: Vestibular evoked myogenic potentials detect more brainstem lesions than neurological examination.

AIMS: Aim of this study was to evaluate latencies and corrected p13-n23 cVEMP and n10-p13 oVEMP amplitudes differences in VEMP between MS patients with and without brainstem lesions, detect proportion of patients with subclinical brainstem lesions and estimate differences in cervical VEMP (cVEMP) and ocular VEMP (oVEMP) in patients with multiple sclerosis.

MATERIALS AND METHODS: 100 patients with MS (50 patients with clinical sings of brainstem lesion and 50 without) and 50 healthy controls will be included. cVEMP and oVEMP in response to acoustic clicks of 1 ms duration at the intensity of 130 dB SPL and the stimulation frequency of 1 Hz will be studied. Signals are divided in segments of 120 ms duration (20 ms before the stimulus and 100 ms after the stimulus) and averaged.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: Vestibular evoked myogenic potentials are noninvasive and cheap method. Introduction of VEMP in evaluation of patients with multiple sclerosis might help in evaluation of brainstem lesions, patients follow up and evaluation of responce on specific MS therapy.

MeSH / Keywords: cervical vestibular evoked myogenic potentials, ocular vestibular evoked myogenic potentials, amplitudes, latencies, multiple sclerosis

LONG-TERM NEUROLOGICAL FOLLOW UP OF THE FIRST AND SECOND TWIN

PhD candidate: Maja Jurin, MD., Mr.sc.

Mentor: Professor Vida Demarin, MD,Phd

Affiliation: University Hospital Center Zagreb

INTRODUCTION: Twins and their parents experience increased, and sometimes unique, medical and psychological risks when compared to singletons. Twins have death rate four times higher than singletons. The main reason for that is preterm and very preterm birth in twins, resulting in low and very low birth weight children. Perinatal mortality and morbidity are also higher in monozygotic twins as compared to dizygotic. In addition to an increased risk to mortality, twins have higher rates of morbidity, specifically cerebral palsy and mental subnormality. Language and speech delays are more pronounced in twins, as are cognitive delays, motor development, behavioural problems and difficulties in parent-child interactions. Twins have also higher frequency of attention deficit hyperactivity disorder (ADHD).

HYPOTHESIS: Adult twins have frequent difficulties in speech and behaviour, higher frequency of ADHD and higher rates of epileptic and non-epileptic seizures resulting in poor school performance and lower academic degree.

AIMS: To analyse final psychomotor and emotional development, behaviour and scholastic success in first and second twin mutually and in comparison to singleton controls. Additional specific aims intend to analyse definitive language development, epilepsy frequency, presence of ADHD and non-epileptic episodic phenomena (sleepwalking-somnambulism, night terrors-pavor nocturnus, psychogenic seizures.), as well as the influence of neonatal complications on subsequent psychomotor development.

MATERIALS AND METHODS: Due to the previous power analysis and prevalence of neurologic disorders, 62 twin pairs and 62 controls born from single pregnancies were randomly chosen to take part in the research. All of them were born in the same Maternity hospital from 1980 until 1985. and were examinees in the first part of research 22 years ago. The data will be collected from existing medical records and by specially designed questionnaires.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH:

Estimation of ultimate psychomotor and cognitive development, behaviour and schooling achievements in adult twins. Long term neurological follow up will enable the study of neurological diseases in adult twins reciprocally and compared to control singletons as well as compared to the twins born in assisted reproduction.

MeSH/Key words: Twins, psychomotor and emotional development, speech and language development, behaviour, ADHD, epileptic and non-epileptic phenomena

INFLUENCE OF SNP RS3812718 ON LAMOTRIGINE EFFICACY IN PATIENTS WITH PARTIAL EPILEPSY

Part of a Thesis: Influence of SNP rs3812718 on lamotrigine efficacy in patients with partial epilepsy

PhD candidate: Ivana Marković, MD

Mentor: Silvio Bašić, MD, PhD

Affiliation: University hospital Dubrava, Department of Neurology

INTRODUCTION: Epilepsy is characterized by recurrent and unpredictable abnormal excessive or synchronous neuronal activity in the brain, manifested as epileptic seizures.

Despite the large number of antiepileptic drugs, one third of patients have bad response to antiepileptic treatment which is possible cause of interindividual genetic variations. Single nucleotide polymorphism rs3812718 of the SCN1A gene, specifically its genotype AA, was showed to be associated with bad response to the voltage gated sodium channel blockers phenytoin and carbamazepine. This reaserch is aimed to determine the association between genotype AA and response to the treatment with lamotrigine. These results will contribute to the use of pharmacogenetics in the field of treatment epilepsies with lamotrigine and antiepileptic drugs which act on voltage dependent sodium channels.

HYPOTHESIS: AA genotype of the rs3812718 polymorphism is associated with bad response to the therapy with lamotrigine and with shorter period without seizures in patients with partial epilepsy.

AIMS: In general, to determine the association of rs3812718 polymorphysm and response to the therapy with lamotrigine. More specific aims are to determine the association of AA genotype with bad response to the therapy with lamotrigine and shorter period without seizures; to determine influence of GA and GG genotypes of rs3812718 polymorphysm on the response to the therapy with lamotrigine.

PATIENTS AND METHODS: One hundred patients with partial epilepsy and on therapy with lamotrigine will be enrolled to the study. The seizure frequency before and after the introduction of lamotrigine will be analyzed and compared with results of genetic analysis. All patients will be asked to sign informed consent and all procedures will be explained before enrollment. All patients will be drawn 5 ml of venous blood which will be analyzed using TaqMan assay for rs3812718 genotyping (Applied Biosystems, Foster City, CA, U.S.A.).

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: Results of this study together with additional analyses will contribute to the further development and use of pharmacogenetics in the field of treatment of epilepsies with lamotrigine and possibly with other antiepileptic drugs which act on voltage dependent sodium channels.

Acknowledgements: I would like to thank Prof. Silvio Basic and Prof. Davor Sporis who have gained the access to the Register of patients with epilepsy in UH Dubrava.

MeSH / Keywords: epilepsy, pharmacoresistance, SCN1A, lamotrigine

PULSE WAVE VELOCITY IN PATIENTS WITH ENDEMIC (BALKAN) NEPHROPATHY UNDERGOING HEMODIALYSIS

Part of a Thesis: Arterial stiffness of large arteries in terminal stage of Endemic Nephropathy

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Mentor: Associate Professor Bojan Jelaković, MD, PhD

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INTRODUCTION: Arterial stiffness (AS) is a strong predictor of cardiovascular outcome, especially in hemodialysis (HD) patients. Hypertension (HT) was not considered to be part of early course of endemic nephropathy (EN), thus it could be proposed that cardiovascular morbidity has different clinical course. There are no data on differences in cardiovascular outcome between EN patients and patients with other chronic kidney disease.

HYPOTHESIS: Due to later development of HT, patients with EN undergoing HD have lower AS, peripheral and central blood pressure (BP) and consequently lower cardiovascular risk and longer life span.

AIMS: The aim of this investigation is to compare AS markers values (pulse wave velocity and augmentation index (PWV,Aix)), central and peripheral BP values between patients with EN undergoing HD vs patients with other end-stage-renal-diseases undergoing HD, and to determine whether AS and central BP are independent risk factors for development of subclinical target organ damage.

MATERIALS AND METHODS: In 80 EN and 80 non-EN adult patients being treated by means of HD at least three months, PWV and Aix along mean BP, pulse pressure and heart rate will be measured by Tensiomed Arteriograph. Brachial BP (the mean value of three measurements) will be measured using Omron device following ESH/ECS guidelines. Extended questionnaire and physical examination will be performed: fasting blood will be obtained to determine serum creatinine, glucose, electrolytes, lipids. The exclusion criteria are: amputation of at least one extremity, mentally ill patients or those with dementia, acute coronary incident in last 3 months, NYHA III and IV, atrial fibrillation, arrhythmias, ascites, acute disease or cancer.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH:

Assessment of cardiovascular risk in endemic nephropathy patients based not only on traditional risk factors, but also including markers of arterial stiffness. To our knowledge this is the first study on AS in EN, and also the first investigation in Croatia on AS conducted on patients undergoing HD.

Acknowledgements: This work was partly supported by grant of Ministry of Science Rep of Croatia 0108109

MeSH/Keywords: Endemic Nephropathy, Hemodialysis, Arterial stiffness, Arterial hypertension, Chronic renal disease

INFLUENCE OF UGT1A6 AND CYP2C9 POLYMORPHISMS ON VALPROATE ADVERSE EFFECTS IN EPILEPSY PATIENTS

Pharmacogenetics in epilepsy patients with valproate adverse reactions

PhD candidate: Zrinka Čolak, MD

Mentor(s): Silvio Basic, MD, PhD

Affiliation(s): University Hospital Dubrava, University Hospital Centar Zagreb

INTRODUCTION: Genetic polymorphisms of drug metabolising enzymes contribute to interindividual differences of clinical drug response, including antiepileptics. Valproate (VPA) is a widely used drug for generalized and partial epilepsy as well as for other neurologic and psychiatric conditions, such as bipolar and schizoaffective disorders or migraine. Its use is frequently limited by development of adverse reactions which sometimes can be fatal. Major VPA metabolic pathways comprise β -oxidation and glucuronidation. Cytochrome P450 (CYP) enzymes, mostly CYP2C9, mediate conversion of VPA to hepatotoxic metabolite 4-ene-VPA, and the influence of genetic variants on hepatotoxicity of VPA is still unknown. Since the glucuronidation is major metabolic pathway of VPA biotransformation, it is possible that uridine diphosphate glucuronosyltransferase isoenzyme 1A6 (UGT1A6) polymorphisms could influence the development of adverse effects.

HYPOTHESIS: Genetic polymorphisms of CYP2C9 and UGT1A6 enzymes influence the development of VPA adverse reactions.

AIMS: Aim of this study is to evaluate differences of CYP2C9 and UGT1A6 genetic polymorphisms in epilepsy patients that developed VPA adverse drug reactions and those who didn't.

MATERIALS AND METHODS: UGT1A6 and CYP2C9 genotypes will be determined in 100 epilepsy patients taking VPA (50 that developed adverse drug reactions and 50 that didn't). Possible association of wild-type or variant genotypes with adverse drug reactions will be studied, especially hepatotoxicity.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: Individual differences in genetic polymorphisms of CYP2C9 and UGT1A6 metabolizing enzymes could predict the possibility of VPA adverse reactions, leading to more rational treatment of epilepsy.

MeSH / Keywords: valproate, adverse drug reactions, epilepsy, CYP2C9, UGT1A6, pharmacogenetics

LASER IN SITU KERATOMILEUSIS (LASIK) WITH TWO DIFFERENT LASER PLATFORMS IN TREATING HIGH ASTIGMATISM

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Mentor: Professor Iva Dekaris, MD PhD

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INTRODUCTION: Laser in situ keratomileusis (LASIK) is the most common refractive procedure in the world. LASIK has been successfully used to correct myopia, hypermetropia and low to moderate astigmatism. There is less documentation of whether LASIK is acceptably effective, predictable and safe in correcting higher astigmatism, especially with regard to the effects of astigmatic corrections on higher-order aberrations (HOAs). Cyclotorsion compensation of the treated eye and eye-tracking systems of lasers are very important factors in getting good postoperative results.

HYPOTHESIS: Schwind Amaris 750S provides superior refractive results when treating high astigmatism compared to Wavelight Allegretto Eye-Q 400Hz. Aberation free program of Schwind induces less HOAs than Wavelight optimized treatment.

AIMS: To determine success of LASIK in treating patients with high astigmatism ($>2,5D$) and compare overall outcome of visual performances between two laser platforms. Investigate the influence of cyclotorsion on residual refractive error, the role of eye tracking in accurate position of laser shots and prevention of HOAs.

MATERIALS AND METHODS: 200 patients (400 eyes) will be enrolled in study. All patients will undergo LASIK performed by the same surgeon. All flaps will be created with Moria M2 mechanical microkeratome. Patients will be randomly assigned in two groups depending on laser platform performing tissue ablation. First group will be treated on Schwind Amaris 750S and second group on Wavelight Eye-Q Allegretto laser platform. During the six months follow up uncorrected and best corrected distance visual acuity, residual refractive error, contrast sensitivity, induction of high order aberrations, cyclotorsion effect, quality of eye tracker, depth of ablation, intraoperative and postoperative complications and patient's satisfaction will be measured and compared. Descriptive statistical methods will be used to describe respondents and test variables. Variations between groups will be compared with parametric (ANOVA with posthoc Scheffe test), non parametric (Kruskal-Wallis test), and t-test or Chi square test depending on data distribution. Statistically significant difference will be 5% ($p < 0,05$).

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH:

Better understanding of cyclotorsion effect on residual refractive error after LASIK, influence of 6D eye-tracker on method precision and effect of induced HOAs on overall quality of vision.

Keywords: LASIK, astigmatism, Schwind Amaris 750S, Wavelight Alegretto Eye-Q

POSTER TITLE: COMPARISON OF FEMTOSECOND LASERS AND MECHANICAL MICROKERATOMES FOR LASER IN SITU KERATOMILEUSIS

PhD candidate: Maja Bohac, MD

Mentor: professor Nikica Gabric, MD PhD

Affiliation: Eye Specialty Hospital „Svjetlost“, Zagreb, Croatia

INTRODUCTION: Laser in situ keratomileusis (LASIK) is mostly used refractive procedure today. The procedure implies photoablation of corneal stroma with excimer laser under the superficial corneal flap. Traditionally mechanical microkeratomers were used for flap creation. Recently femtosecond laser technology for flap creation rapidly evolved and became a worthy competitor to microkeratomers. Guiding idea of femtosecond laser technology is that lasers are more precise than mechanical microkeratomers and procedure is less surgeon and gear dependent and more software controlled. The idea implied that flaps of more reliable thickness and shape provide more accurate refractive and optical results. However, conclusion is still waiting for its complete scientific evidence since technology is relatively new and still not widely used.

HYPOTHESIS: There is no statistically significant difference in the number of intraoperative and postoperative complications, and final visual performances between femtosecond lasers and mechanical microkeratomers.

AIMS: To conduct a prospective research and compare complications and visual performances between mechanical microkeratomers and femtosecond lasers.

MATERIALS AND METHODS: 200 myopic patients (400 eyes) will be enrolled in study. The patients will be randomly assigned in four groups. All patients will undergo Laser in situ keratomileusis (LASIK). The only difference between groups will be the way of flap creation. First group will undergo flap creation with Zimmer Femto LDV, second group with Technolas 520F, third group with Moria M2 mechanical microkeratome, and fourth group with Moria SBK One Use Plus mechanical microkeratome. All ablations will be made with Schwind Amaris 750Hz excimer laser. Flap thickness, high contrast visual acuity, residual refractive error, contrast sensitivity, induction of high order aberrations, tear film abnormalities and patients satisfaction will be measured and compared during the six months follow up. Descriptive statistical methods will be used to describe respondents and test variables. Variations between groups will be compared with parametric (ANOVA with posthoc Scheffe test), non parametric (Kruskal-Wallis test), and t-test or Chi square test depending on data distribution. Statistically significant difference will be 5% ($p < 0,05$).

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH:

Proposed research should contribute to better understanding of new rapidly evolving femtosecond laser technologies and their advantages and disadvantages in refractive surgery.

Keywords: LASIK, mechanical microkeratome, femtosecond laser, corneal flap

CLASSIFICATION AND QUANTIFICATION OF THE RISK FACTORS AND TREATMENT STRATEGIES FOR OCULAR HYPERTENSION AND PRIMARY OPEN- ANGLE GLAUCOMA

Part of the thesis: Classification and quantification of the risk factors and treatment strategies for ocular hypertension and primary open-angle glaucoma: a systematic review of systematic reviews and meta-analysis of primary prognostic studies and randomized controlled intervention trials

PhD candidate: Qëndresë Daka MD

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

Affiliation(s): University of Zagreb School of Medicine, University Hospital Centre - Zagreb, University of Prishtina Faculty of Medicine, University Clinical Centre of Kosova

INTRODUCTION: POAG is the most common form of glaucoma, late detection with a consequent delayed treatment is a major risk factor for blindness. OHT is a major risk factor for development of POAG, but other factors may play a role in pathogenesis. Their individual or combined contributions to the risk of POAG and possible relationship with OHT have not been systematically evaluated thus far. There are five major classes of IOP-lowering drugs for the treatment of OHT and POAG and a number of laser-based and surgical procedures. A comprehensive quantification of the effects and classification of available treatment options based on their efficacy/safety has not been performed thus far. It is unclear if preventive treatments are cost effective in terms of long term avoidance of blindness.

HYPOTHESIS: There are other, apart from OHT, potentially modifiable risk factors with relevant individual contributions to the risk of POAG. IOP-lowering treatments delay the progression of OHT/POAG, but some are more effective than the others. IOP-lowering treatments are cost-effective for prevention of progression of OHT/POAG, but some have a better cost-effectiveness profile than the others.

AIMS: The primary objective is to systematically evaluate, classify and quantify the risk factors and treatment strategies for OHT and POAG. The secondary objective is to systematically evaluate cost-effectiveness of the IOP-lowering treatments.

MATERIALS AND METHODS: For the primary objectives, we will perform a systematic review of systematic reviews and meta-analysis of primary prognostic and randomized controlled interventional studies. For the secondary objective, we will perform a systematic narrative review of systematic reviews/Health Technology Assessments and semi-quantitative systematic review of primary studies dealing with cost-effectiveness of intraocular pressure-lowering treatment strategies.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: Identification and categorization of risk factors for POAG (in addition to IOP) should provide the basis for improved identification of subpopulations at the highest risk and, eventually, could enable identification of additional modifiable risk factors. Comprehensive evaluation of numerous currently available preventive treatments should help establish evidence-based criteria for their selection. This could be particularly useful for developing countries with restricted public health resources.

Acknowledgements: I would like to thank Professor Vladimir Trkulja, for agreeing to be my mentor on long distance and providing me with much needed advice regarding this research.

MeSH / Keywords: primary open angle glaucoma, ocular hypertension, risk factors, treatments

IMPACT OF DELAYED GRAFT FUNCTION ON LONG TERM KIDNEY TRANSPLANT OUTCOMES

Part of a Thesis: The impact of delayed graft function on long term graft survival and progression of chronic histological changes in kidney transplant recipients

PhD candidate: Bojana Maksimovic, MD

Mentor(s): Mladen Knotek, assistant professor

Affiliation(s): Renal Division, Department of Medicine, University of Zagreb Medical School and Clinical Hospital Merkur, Zagreb, Croatia

INTRODUCTION: Delayed graft function (DGF) is a manifestation of acute kidney injury. It has usually been defined as the need for dialysis within 7 days of the transplant. Incidence of DGF has increased over the time in deceased donors probably because of more often use of expanded criteria donors (ECD) and in some countries of donors after cardiac death (DCD). The incidence is approximately 30% in recipients of standard criteria deceased donor kidneys, 50 % for recipients of ECD and up to 70% recipients of DCD kidneys. The dominant cause of DGF is acute tubular necrosis. Risk factors for DGF are well defined, and they include cold ischemia time that exceeds 24 hours, calcineurin inhibitors use in the induction regimen and also factors related to the donor such as hypotension and dehydration. It is also known that there is higher incidence of acute rejection when DGF is present. The complex relationship between DGF and allograft survival and patient survival is still poorly understood.

HYPOTHESIS: Delayed graft function does not have negative impact on progression of chronic histological changes and long term renal function in kidney allograft.

AIMS: Primary end point is to establish correlation between DGF and progression of chronic allograft nephropathy (CAN) score. Secondary end point is impact of DGF on one year progression of components of CAN score and on long term renal function.

MATERIALS AND METHODS: Two groups of patients with transplanted kidney or combined kidney pancreas and kidney liver transplantation, transplanted between 2003-2012 will be included in the study. Group with established DGF compared to the group of patients without DGF. Glomerular filtration rate will be estimated by MDRD equation, at 6 months, one and 5 years after transplantation. Renal histology will be assessed at 0, 1, 6 and 12 months after transplantation. Impact of DGF on GFR and progression on chronic histological scores as defined by Banff classification will be analysed.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: This study will clarify the relationship between DGF and long term graft outcomes.

MeSH / Keywords: kidney transplant, delayed graft function, kidney biopsy

COMPARISON OF DIFFERENT PRESBYOPIA TREATMENTS: SURGICAL PROCEDURES ON LENS VERSUS LASER IN SITU KERATOMILEUSIS (LASIK) PROCEDURES ON CORNEA

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Mentor: Professor Iva Dekaris, MD PhD

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INTRODUCTION: Presbyopia is the most common refractive disorder of older age, related to a decrease of accommodative amplitude. The possibility of „curing” or reducing the effects of presbyopia is intensively investigated in modern ophthalmology. „Monovision” LASIK principle and implantation of accommodative or multifocal intraocular lenses (IOL) are current attempts for presbyopia treatment. Patient selection and patient education are critical elements in monovision success. Preoperative refractive error, patient age and occupation are factors that determinate a proper choice between the two surgical procedures.

AIMS: To evaluate the effectiveness of two surgical methods for presbyopia treatment and to establish the protocols of patients selection for one of the two surgeries.

MATERIALS AND METHODS: 200 patients (400 eyes) will be enrolled in study. Patients will be randomly assigned in two groups („RLE Group” and „Monovision Group”). Refractive lens exchange with multifocal diffractive intraocular lens implantation will be performed in first, „RLE Group”. Multifocal IOLs will be bilaterally implanted to 100 patients (N=200 eyes). Phacoemulsification of the clear lens will be performed by same experienced surgeons on same surgical machines. In second, „Monovision Group”, LASIK Monovision will be performed to 100 patients (N=200 eyes). LASIK will be performed in both eyes, dominant eye will be corrected for distance vision and non dominant eye for near vision. The targeted myopia will depend on the age, patient profession, expectations and life style. During the six months follow up near, intermediate and distance uncorrected visual acuity (logMAR), residual refractive error, spectacle dependency, intraoperative and postoperative complications rate, visual disturbances and patient’s satisfaction will be measured and compared. Descriptive statistical methods will be used to describe respondents and test variables. Variations between groups will be compared with parametric (ANOVA with posthoc Scheffe test), non parametric (Kruskal-Wallis test), and t-test or Chi square test depending on data distribution. Statistically significant difference will be 5% ($p < 0,05$).

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH:

Establishing an algorithm for proper patient selection for two available surgical options of the presbyopia treatment.

Keywords: refractive lens exchange, LASIK, presbyopia

COMPARATION OF AXIAL LENGTH MEASUREMENT OF SILICON OIL FILLED EYES BETWEEN OPTICAL COHERENCE BIOMETRY AND MAGNETIC RESONANCE IMAGING.

Part of a Thesis: The Most Accurate Biometry Of Silicon Oil Filled Eye

PhD candidate: Jasna Pavicic-Astalos MD

Mentor: Professor Zoran Vataavuk. MD, PhD

Affiliations: Ophthalmology Department, University Hospital „Sestre Milosrdnice“, Zagreb, Croatia;
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INTRODUCTION: Silicone oil endotamponade has been used in vitreoretinal surgery for complex cases of retinal detachment for almost 50 years. Cataract is the most common complication after intravitreal injection of the silicone oil. Silicone oil removal can then be combined with cataract extraction to avoid further surgery. This surgical procedure demands accurate axial length measurement and precise calculation of intraocular lens (IOL) power. Biometry of silicone oil-filled eyes is difficult to perform and measurements may not always be obtainable. In the recent years, optical coherent biometry has replaced the standard ultrasound biometry procedure as the most accurate method for IOL calculation, but with several disadvantages. The use of magnetic resonance for axial length measurement in silicone oil-filled eyes has many advantages in all patients, especially in patients with dense cataracts and poor fixation.

HYPOTHESIS: Magnetic resonance biometry is more objective than optical coherent biometry in axial length measurement in silicone oil filled eyes.

AIMS: The aim of the study is to compare the accuracy of optical coherent biometry and magnetic resonance biometry for the measurement of axial length in silicone oil-filled eyes

MATERIALS AND METHODS: This is a prospective randomised study of 70 patients. Biometry will be performed using MRI in 35 patients, and optical coherent biometry in 35 patients. The difference between predicted and final refraction will be measured and evaluated statistically.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: This study can establish new and accurate method for axial length measurement in silicone oil filled eyes.

MeSH / Keywords: Silicon oil, biometry, axial length.

PREDICTION AND PREVENTION OF CYSTOID MACULAR EDEMA AFTER PHACOEMULSIFICATION CATARACT SURGERY

Part of a Thesis: Possibilities of Prediction and Prevention of Cystoid Macular Edema after Phacoemulsification Cataract Surgery

PhD candidate: Ivana Romac, MD

Mentor: Professor Iva Dekaris, MD, PhD

Affiliation: University Eye Clinic „Svjetlost“, Zagreb

INTRODUCTION: Cystoid macular edema(CME) involves fluid accumulation in the outer plexiform layer secondary to abnormal perifoveal retinal capillary permeability. Although the exact cause of CME is not known, it may accompany a variety of diseases such as retinal vein occlusion, uveitis, or diabetes. It most commonly occurs after cataract surgery. About 2-10% of those who have cataract extractions will experience decreased vision due to CME, usually two to four months after surgery. In cases of complicated surgery (capsular rupture, vitreous incarceration), the incidence of CME is greater.

Various methods of investigation are utilized to detect CME. Fluorescein angiography is a dye test which shows „petalloid“ leakage in cases of CME. Optical coherence tomography(OCT) is a noninvasive imaging technique that obtains cross-sectional images of the retina. Treatment options of CME consist of nonsteroidal antiinflammatory drugs, corticosteroids, acetazolamide, triamcinolone, anti-VEGF and vitrectomy.

HYPOTHESIS: Preoperative application of nonsteroidal antiinflammatory drugs topically, triamcinolone subtenon or antiVEGF intravitreal decreases the incidence of CME after cataract surgery in patients with preoperative predisposing diseases like diabetes or inflammation of the eye.

AIMS: To determine appropriate treatment algorithm for nonsteroidal antiinflammatory drugs, triamcinolone and antiVEGF before cataract surgery in order to prevent postoperative CME and to define which patient has higher risk for postoperative CME and how can this risk be decreased.

MATERIALS AND METHODS: 260 patients who underwent uneventful phacoemulsification cataract surgery are included in the study and divided in 3 groups: diabetic, nondiabetic and patients who had inflammation of the eye prior the surgery. Patients are divided in subgroups according to preoperative treatment (none, nonsteroidal antiinflammatory drugs topical, triamcinolone subtenon or antiVEGF intravitreal).

Patients are also divided in 2 groups whether the time of phacoemulsification was shorter or longer than 30 seconds. Visual acuity and macular thickness by OCT are measured on the day of surgery, after 7 days, 5 and 12 weeks after the surgery.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH:

To define the optimal algorithm for treatment and preoperative prevention of CME and to determine the factors that contribute in pathogenesis of CME in order to decrease the incidence of postoperative CME.

Keywords: CME, phacoemulsification , nonsteroidal antiinflammatory drugs, triamcinolone acetate, antiVEGF.

HIGH-MOBILITY GROUP A PROTEIN 2 (HMGA2) GENE EXPRESSION IN DIFFERENTIATED CARCINOMA AND BENIGN THYROID DISEASES

Part of a Thesis: HMGA2 expression in thyroid neoplasms

PhD candidate: Nina Dabelić, MD

Mentor: Professor Zvonko Kusić, MD, PhD

Affiliation: University Hospital Center „Sisters of Charity“, Zagreb

INTRODUCTION: High-mobility group A protein 2 (HMGA2) gene overexpression has oncogenic potential. It is proven to be linked with clinically aggressive disease in breast, lung, and some other cancers, but not with certainty in thyroid cancer.

HYPOTHESIS: 1.) HMGA2 gene overexpression is linked to clinically more advanced stages of differentiated thyroid carcinoma (DTC).

2.) HMGA2 gene expression levels differ between DTC, benign thyroid neoplasms (follicular adenomas), and healthy thyroid tissue.

AIMS: The aim of this study is to determine the level of HMGA2 gene expression by qRT-PCR in tissues of patients with different clinical stages of differentiated thyroid carcinoma, in benign thyroid diseases i.e. follicular adenoma, and in healthy thyroid tissues.

MATERIALS AND METHODS: In this retrospective study, paraffin embedded thyroid tissue from 200 patients who underwent surgery in our hospital in the last 10 years will be analyzed - 40 patients with DTC confined to thyroid, 40 patients with DTC and lymphogenic metastases, 20 patients with DTC and hematologic metastases, 50 patients with follicular adenoma, and 50 samples of healthy thyroid tissue. Total RNA will be isolated by commercially available kit. Purity and integrity of RNA will be checked by formaldehyde agarose electrophoresis. mRNA will be reversely transcribed to cDNA and the region of interest will be multiplied by polymerase chain reaction (PCR) in a process called real-time quantitative reverse transcription-PCR (qRT-PCR), which allows us relative quantification of HMGA2 mRNA in patients with different thyroid disorders and healthy thyroid tissue. The adequate statistical analyses will be used, according to data distribution.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: Revealing the higher HMGA2 gene expression in: a) malignant thyroid tumors compared to benign thyroid tumors, and b) malignant thyroid tumors of more advanced clinical stages compared to malignant tumors of less advanced clinical stages, will expand knowledge on thyroid tumor pathogenesis. This will possibly enable clinical use of HMGA2 gene expression analysis, both in diagnostics of thyroid cancer and in prognosis of clinical course of disease and selection of therapeutic procedures.

MeSH / Keywords: HMGA2; gene expression; reverse transcriptase polymerase chain reaction; thyroid carcinoma; thyroid neoplasm

IMMUNOHISTOCHEMICAL EXPRESSION OF ESTROGEN RECEPTORS BETA (ER β) IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

Part of a Thesis: Immunohistochemical expression of estrogen receptors beta (ER β) in head and neck squamous cell carcinoma

PhD candidate: Krešimir Gršić, MD

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

Affiliation(s): University hospital for tumors University hospital center „Sister of Mercy“, Zagreb

INTRODUCTION: Squamous cell carcinoma of the head and neck (HNSCC) is the fifth most common cancer in people worldwide. Emerging on the mucosa of the upper respiratory and digestive systems HNSCC is strongly influenced by the exposure to carcinogens in tobacco and alcohol, virus contacts (such as human papilloma virus), and genetic predisposition. Although modern principles of multimodal therapy were introduced in therapeutic procedures, survival of patients with HNSCC has not been significantly altered in the last 30 years. New researches are required to discover specific genetic and molecular changes responsible for the onset and development of HNSCC metastatic potential. Recent studies emphasize the important role of nuclear estrogen receptor beta (ER β) in HNSCC carcinogenesis.

HYPOTHESIS: Determination of estrogen receptor beta (ER β), which is supposed to have a protective role in the development of squamous cell carcinoma of head and neck, would provide extracting the group of patients with better prognosis.

AIMS: The survey aims to determine the immunohistochemical expression of estrogen receptor beta in squamous cell carcinomas of head and neck with respect to the site of the primary process (oral cavity, oropharynx, hypopharynx, larynx), disease stage, degree of tumor differentiation, and survival of patients.

MATERIALS AND METHODS: This retrospective study will be including patients with squamous cell carcinoma of head and neck who have been treated in the interval from 2000. to 2006. in our institution and have been monitored for at least 5 years. A total of about 200 patients with location of primary tumors in the oral cavity, oropharynx, hypopharynx and larynx will be enrolled in the research. Biopsy analysis from patients with known prognostic factors (TNM stage and tumor differentiation), and known course of the disease (local recurrence, regional recurrence, dissemination, survival) will reveal the immunohistochemical expression of estrogen receptor beta (ER β).

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: The study will define the importance of estrogen receptor beta (ER β) as possible predictors of locoregional aggressiveness. This would open the opportunity for potent drugs innovation, development and application. Moreover the existing multimodal procedures (surgery, chemotherapy and irradiation) will be revised in order to better efficiency.

Acknowledgements: I would like to thank Professor Božena Šarčević for his support and encouragement during this project.

MeSH / Keywords: squamous cell carcinoma of the head and neck, estrogen receptor beta, neoplastic cell transformation, survival

LIVER BLOOD FLOW ANALYSIS IN PATIENTS WITH COLORECTAL CANCER

Part of Thesis: Arterial and venous blood flow redistribution in patients with colorectal cancer

PhD candidate: Amir Ibukić MD

Mentor: Professor Leonardo Patrlj, MD, PhD

Affiliations: University Hospital Dubrava - Zagreb, University Hospital Center Sisters of Charity - Zagreb

INTRODUCTION: Colorectal cancer has an incidence of around 50 new cases per 100,000 population in Western countries and US. By the time of diagnosis 1/5 of patients already has liver methastases while in additional 25% of patients liver methastases occur within 2 years after diagnosis of colorectal cancer. Curability rates in stage I patients (Dukes A and B1) is 90 %, stage II (Dukes B2) is 75%, stage III (Dukes C and patients with lymphnode methastasis) is only 35 %. Recent studies revealed colorectal liver micromethastases in 30% of patients in whom standard preoperative diagnostic methods (CT, US, MRI) didn't show macroscopic signs of liver methastases. It is noticed that arterial and venous liver blood flow ratio is changed in patients with liver micromethastases. This ratio is defined as doppler perfusion index-DPI. Some studies has shown that liver arterial blood flow is higher than in healthy people.

HYPOTHESIS: The hypothesis of this research is that in patients with colorectal cancer there is sistematic disturbance in liver blood flow caused by circulating vasoactiv factor. If this divert in liver blood flow is caused by vasoactive tumor factor then it is expected that primary colorectal cancer consist clone cells that can produce the same vasoactive factor. This hypothesis could be determinated by measuring liver blood flow before and after surgical removal of colorectal cancer.

AIMS: To determine the nature of arterial - venous liver blood flow in patents with colorectal cancer and to determin if the surgical removal of primary tumor has an influence at this ratio.

MATERIALS AND METHODS: We will conduct our research on 3 gropus of exmaminees-patients with primary colorectal cancer, patients with liver methastases and healty examinees as a control group. Parameters of arterial and vein blood flow will be measured and statistically analised.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: The results of this study are expected to explain the nature of blood flow redistribution in presence of cancer cells, and to investigate possible usage of these results in early diganosis of colorectal cancer.

MeSH/Keywords: colorectal cancer, DPI, liver methastases

EXPRESSION OF PIWIL2 AND HMGA2 IN PAPILLARY THYROID CARCINOMAS WITH AND WITHOUT METASTASES

PhD candidate: Sandra Moslavac MD

Mentor(s): Professor Hrvoje Čupić, MD, PhD

Affiliation(s): University Hospital Centre 'Sestre milosrdnice', Polyclinic 'Sunce'

INTRODUCTION: Recent studies have demonstrated that Pivwil2 and HMGA2 are expressed in various types of human cancers with the lack of expression in the normal tissue. Unlike HMGA2 expression, Pivwil2 expression in thyroid has not been investigated. The aim of this study is immunohistochemical analysis of Pivwil2 and HMGA2 expression in papillary thyroid carcinomas (PTC) with and without regional lymph node (RLN) metastases, follicular adenomas, hyperplastic nodules and normal thyroid tissue.

HYPOTHESIS: The level of expression of Pivwil2 and HMGA2 is significantly higher in papillary carcinomas with metastases by comparison to papillary thyroid carcinomas without metastases.

AIMS: To analyse Pivwil2 and HMGA2 expression in PTC with and without RLN metastases and in follicular adenomas, hyperplastic nodules and normal thyroid tissue. To compare Pivwil2 and HMGA2 expression in PTC with RLN metastases with the expression in PTC without RLN metastases and in PTC with and without RLN metastases with the expression in follicular adenomas and hyperplastic nodules. To compare Pivwil2 and HMGA2 expression with histological grade and clinical parameters including age, gender and tumor size.

MATERIALS AND METHODS: Immunohistochemical expression of before mentioned markers in analysed groups will be compared and correlated with the histological grade and clinical parameters. The archival paraffin blocks of at least 30 patients with PTC and RLN metastases, 30 patients with PTC without RLN metastases, 30 patients with follicular adenomas and 30 patients with hyperplastic nodules will be used in the study.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: The subgroup of patients with PTC and higher risk of more aggressive biological behaviour could be identified based on the expression of Pivwil2 and HMGA2. Hypothetical results would allow less extensive therapy approach in patients with prospect of advantageous clinical course as well as adequate therapy in patients with more aggressive biological behaviour regarding the expression of analysed tumor markers.

Acknowledgements: financial support, contributors

MeSH/Keywords: papillary thyroid carcinoma, Pivwil2, HMGA2

CLINICAL SIGNIFICANCE OF METHYLTRANSFERASE EZH2 AND DEMETHYLASE JMJD3 EXPRESSION IN HYPOPHARYNGEAL SQUAMOUS CELL CARCINOMAS

Part of a Thesis: Clinical significance of methyltransferase EZH2 and demethylase JMJD3 expression in hypopharyngeal squamous cell carcinoma

PhD candidate: Alan Pegan, MD

Mentors: Associate Professor Koraljka Gall Trošelj, MD, PhD; Professor Mirko Ivkić, MD, PhD

Affiliations: „Ruder Bošković“ Institute, Zagreb; University of Zagreb School of Medicine, University Hospital Center „Sisters of Mercy“, Zagreb

INTRODUCTION: Histone modifications play an important role in the regulation of gene activity. One of them, methylation, represents a very important epigenetic mark. Two enzymes with antagonistic functions, EZH2 (histone methylase) and JMJD3 (histone demethylase), are responsible for methylation status on histone H3 lysine (K) 27. Trimethylated H3K27 is considered to be a very strong suppressive transcriptional mark, shown to be present in promoters of many tumor suppressor genes, in cancer cells. Hypopharyngeal squamous cell carcinomas (HSCC), which constitute 10% of all head and neck carcinomas, will be a model for investigating the ratio between EZH2 and JMJD3 expression, as prognostic parameters.

HYPOTHESIS: There is a change in the expression ratio between EZH2 and JMJD3. This imbalance (EZH2 increased, JMJD3 decreased, when compared with non-tumorous tissues) results in a worse prognosis for patients with a higher imbalance ratio.

AIMS: To investigate the gene expression ratio between EZH2 and JMJD3 on several levels: 1) genomic DNA: loss of heterozygosity (LOH) of JMJD3, 2) mRNA: a) the ratio of EZH2/JMJD3 in cancer and normal hypopharyngeal tissues, b) if only the cancer tissue is available, comparing the change of EZH2/JMJD3 ratio to housekeeping gene(s) expression; 3) immunohistochemistry analyses; 4) clinical significance - relate results to TNM status, differentiation stage, disease free and five years survival rate.

MATERIALS AND METHODS: sixty eight HSCC samples will be analyzed (37 HSCC samples without- and 31 HSCC with corresponding non-tumorous hypopharyngeal tissue) in addition to seven patient's lymph nodes. Genomic DNA will be extracted, and LOH analyses for p53 and JMJD3 will be performed using PCR, RFLP, PAGE and silver nitrate staining protocol, in addition to DNA sequencing. The mRNA will be reversely transcribed into cDNA, and two housekeeping genes (GAPDH and PAPA) will be amplified. Then, co-amplification of EZH2 and JMJD3, gel electrophoresis and densitometry will be performed. At the protein level, expression status of EZH2 and JMJD3 will be examined using immunohistochemistry.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: This research will help in further understanding the role of EZH2 and JMJD3 in the pathogenesis and clinical behavior of HSCC.

Acknowledgements: This work is supported by research grant No. 098-0982464-2511 (Epigenetic and immunomodulatory changes in head and neck carcinomas)

MeSH / Keywords: hypopharyngeal cancer, EZH2, JMJD3, p53, DNA, mRNA, protein, H3K27m3, clinical significance

CLINICAL SIGNIFICANCE OF BORIS AND MYC EXPRESSION IN HYPOPHARYNGEAL SQUAMOUS CELL CARCINOMAS

Part of a Thesis: Clinical Significance of BORIS and MYC Expression in Hypopharyngeal Squamous Cell Carcinoma

PhD candidate: Ivan Rašić, MD

Mentors: Renata Novak Kujundžić DVM., PhD, Professor Mirko Ivkić MD, PhD

Affiliations: Ruder Bošković Institute, Zagreb; University of Zagreb School of Medicine, University Hospital Center „Sisters of Mercy“, Zagreb

INTRODUCTION: Hypopharyngeal carcinomas amount to approximately 10% of all upper digestive respiratory tract cancers. Histopathologically, 90% of these cancers are squamous cell carcinoma. Risk factors include male gender, alcohol, tobacco smoke, human papilloma virus infections, nutritional and metabolic imbalances. Some of these risk factors cause global DNA hypomethylation, known to stimulate the expression of numerous genes including those relevant to carcinogenesis. During carcinogenesis, the activity of many genes is changed due to alterations in complex epigenomic network. Protein CTCF and its paralog BORIS are central in organizing chromatin into domains with different transcriptional activity. CTCF negatively regulates MYC, an important regulator of cell proliferation and differentiation, whereas different BORIS isoforms can either promote or suppress its transcription, leading to different clinical outcome.

HYPOTHESIS: Several transcript variants of BORIS are expressed in hypopharyngeal squamous cell carcinoma, with different role in regulation of proto-oncogene MYC expression, and therefore a different impact on disease outcome.

AIMS: The aims of this study are to demonstrate the influence of different BORIS transcript variants on MYC mRNA and protein expression in hypopharyngeal squamous cell carcinoma related to the classical prognostic factors (TNM, gradus) as well as recurrence-free and overall survival.

MATERIALS AND METHODS: In the proposed research, the expression of BORIS and MYC transcripts will be analyzed in 68 hypopharyngeal squamous cell carcinoma samples and available 31 adjacent surrounding tissue samples. Total RNA will be extracted, reverse transcribed into cDNA, and respective transcripts will be amplified by polymerase chain reaction (PCR). Products will be visualized on agarose gel and photographed for further quantification by EZquant software. Select amplicons will be reamplified and sequenced to document the identity of PCR products. Expression and localization of BORIS and MYC proteins will be analyzed by immunohistochemistry. Statistical analysis will be performed using analytical system R.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: The results will extend the knowledge on the role of BORIS in MYC expression to the hypopharyngeal squamous cell carcinoma model. The prognostic value of BORIS transcript variants and protein expression, in this type of cancer, will be determined statistically based on patients five-year follow up.

Acknowledgements: This work is funded by grant No. 098-0982464-2511 (Epigenetic and immunomodulatory changes in head and neck carcinomas) from the Ministry of Science, Education and Sport, Republic of Croatia.

Keywords: hypopharynx, squamous cell carcinoma, BORIS, MYC, transcript variant, protein, clinical significance

IMMUNOHISTOCHEMICAL EXPRESSION OF MATRIX METALLOPROTEINASES 1 (MMP-1) AND CYCLOOXYGENASE-2 (COX-2) IN CUTANEOUS SQUAMOUS AND BASAL CELL CARCINOMA AND ACTINIC KERATOSIS (AK)

PhD candidate: SANDA SMUĐ OREHOVEC, MD

Mentors: Professor Božena Šarčević, MD, PhD¹, Associate Professor Davor Mijatović, MD, PhD²

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INTRODUCTION: The most common non-melanoma skin tumors are actinic keratosis (AK), squamous cell carcinoma, and basal cell carcinoma. Cyclooxygenase-2 (COX-2) is critical for tumor formation, angiogenesis and metastasis. Matrix metalloproteinases (MMPs) are the members of zinc family (Zn) - and calcium-dependent endopeptidases that degrade the extracellular matrix.

HYPOTHESIS: Increased immunohistochemical expression of MMP-1 and COX-2 especially in AK could distinguish those patients who are at higher risk for developing squamous cell skin carcinoma, and could distinguish those patients with basal cell carcinoma who are at higher risk for recurrence due its histopatological subtypes.

AIMS: GENERAL OBJECTIVE: To determine the difference in immunohistochemical expression of MMP1 and COX2 in squamous cell carcinoma, and basalioma and AK
SPECIFIC OBJECTIVES: 1. Identify the difference in immunohistochemical expression of MMP1 and COX2 in squamous cell carcinoma of the skin depending on its degree of differentiation; 2. Identify the difference in immunohistochemical expression of COX2 and MMP1 in basalioma within its 3 subtypes: solid, adenoid, morfeiform; 3. Identify the difference in immunohistochemical expression of COX2 and MMP1 in AK between atrophic and hypertrophic subtype

MATERIALS AND METHODS: A retrospective analysis of 190 specimens which were excisional skin biopsies made in 190 patients for primary skin tumor (squamous cell carcinoma, basal cell carcinoma and AK). The resulting products will be revised with purpose to confirm the histopatological diagnosis and to determine the subtypes of tumors. From each specimen we shall make the two additional cuts for immunohistochemical processing of the COX-2 and MMP-1 using the mouse monoclonal antibodies.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: The proposed study could provide scientific contribution in determining the value of routine histopathological determination of MMP1 and COX-2 in AK which would lead to the exclusion of patients with a higher risk of squamous cells carcinoma. It would also be set aside their determination in patients with skin basalioma that have a higher risk for recurrence or in patients with squamous cell carcinoma who are biologically more aggressive tumors. All this could lead to changes in the current mode of treatment of patients with these skin tumors.

Acknowledgements: I would like to show my greatest appreciation to Prof. Božena Šarčević and Assist.prof. Davor Mijatović. I can't say thank you enough for your tremendous support and help.

MeSH / Keywords: MMP1, COX2, basal cell carcinoma, squamous cell carcinoma, actinic keratosis

CORRELATION BETWEEN PARABEN ESTERS AND BREAST CANCER

Part of a Thesis: Paraben Esters: Correlation of Concentrations in Human Breast Tissue with Tumour location, Histological type and ER status of Tumour

Candidate: Ana Šoštarić Zadro, MD

Mentor: Vesna Lesko Kelović, MD, PhD

Affiliation: General Hospital Karlovac

INTRODUCTION: The alkyl esters of p-hydroxybenzoic acid (parabens) continue to be used widely as antimicrobial preservatives in consumer products to which the human population is exposed, including not only pharmaceuticals and foods but also cosmetics.

Studies from 1998 onwards had begun to show parabens as possessing oestrogenic properties, and oestrogen is known to play a central role in the development, growth and progression of breast cancer.

It was suggested that low-level dermal absorption from personal care products applied to the breast region over the long term might have contributed .

In the current study will be collected 50 samples of primary breast cancer and measurement at four serial locations across the breast from axilla to sternum will be performed to enable assessment of the distribution of parabens at different locations within the breast and in relation to the site of the tumour.

HYPOTHESIS: Parabens can increase risk of breast cancer.

AIMS: We will measure concentrations of parabens esters and confirm its role in development, growth and progression of breast cancer.

MATERIALS AND METHODS: In prospective research, ethical approval for the study will be obtained from the Research Ethics Committee. After patient consent, 50 samples of human breast tissue will be collected at the General Hospital Karlovac, stored as fresh frozen aliquots of tissue at -80°C and transported on dry ice. Following mastectomy for primary breast cancer, samples of breast tissue were collected from each of four serial locations linearly across the breast from axilla to sternum.

Parabens will be extracted by a method analogous to that used to extract oestradiol from human breast tissue. The concentrations of five esters of p-hydroxybenzoic acid (parabens) will be measured using HPLC-MS/MS. The issue of conjugation is important to considerations of biological availability but in this study we will measure only total paraben (free + conjugated).

EXPECTED SCIENTIFIC CONTRIBUTION: The low-level dermal absorption of paraben, applied to the breast region over the long term may contribute development of breast cancer.

MeSH/Keywords: paraben, breast, oestrogen, breast cancer

METABOLIC SYNDROME, CHARACTERISTICS OF BREAST CANCER AND 25-HYDROXYVITAMIN D STATUS AT DIAGNOSIS

Part of a Thesis: Relation Of Metabolic Syndrome Factors And Characteristics Of Newly Diagnosed Breast Cancer To 25-Hydroxyvitamin D Status

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INTRODUCTION: Few studies performed in women with newly diagnosed breast cancer showed poorer prognostic characteristics in women with 25-hydroxyvitamin D deficiency and in those with metabolic syndrome (MetS), investigating them separately.

HYPOTHESIS: Poorer prognostic characteristics of newly diagnosed breast cancer (BCa) have women with both 25(OH)D deficiency and metabolic syndrome compared to women with only MetS.

AIMS: To compare BCa characteristics in women with both 25(OH)D deficiency and MetS vs women having only MetS.

MATERIALS AND METHODS: Cross-sectional study. Two groups of female patients with newly diagnosed BCa will be formed - one from those with 25(OH)D deficiency (< 50 nmol/l) and one from patients with ≥ 50 nmol/l 25(OH)D (i.e. group with 'normal' concentration). All clinical findings related to MetS and BCa characteristics will be compared between these two groups of patients.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: Prevalence for 25(OH)D deficiency and MetS in women with newly diagnosed BCa; possible comparison with general population in Croatia for overweight, central overweight, hypertension and diabetes. Data of characteristics of newly diagnosed BCa in women with both 25(OH)D deficiency and MetS compared with women with only MetS; association with 25(OH)D deficiency. Data source for future studies.

MeSH / Keywords: breast cancer, vitamin D deficiency, metabolic syndrome

RISK FACTOR'S INFLUENCING POLYETHYLENE WEAR AND POOR SURVIVAL OF INTRAPLANT HI/KS TOTAL HIP PROSTHESIS

Part of a Thesis: Intraplant HI/KS Total Hip Prosthesis Survival in Vivo Model of Early Polyethylene Wear in Relation to Patient and Biomechanical Properties

PhD candidate: Mislav Cimic, MD, MS

Mentor(s): Professor Domagoj Delimar, MD, PhD

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INTRODUCTION: Poor survival rate of total hip prosthesis Intraplant HI/KS used in single institution is observed. Some reports indicate that polyethylene sterilization in an oxygen environment leads to early PE failure. Polyethylene sterilization in the presence of oxygen has been used for Intraplant HI/KS, and therefore listed model of prosthesis presents a good in vivo model of early polyethylene wear. However, prosthesis survival varies considerably between individuals, suggesting that other factors to a greater or lesser extent have an influence on the polyethylene wear and prosthesis survival.

HYPOTHESIS: Survival of the prosthesis is not solely the result of prosthesis type, but there are other patient, surgery and implant related risk factors which alone or in combination affect the wear of polyethylene and prosthesis survival.

AIMS: The aim of the study is to detect patient, surgery and implant related risk factors that alone or in combination influence polyethylene wear and survival in an in vivo model of an early polyethylene wear.

MATERIALS AND METHODS: Data from 1400 total hip prosthesis Intraplant HI/KS with follow up >10 years will be analyzed. Data on age, sex, body weight, and activity level and surgery indication will be collected. Data on surgeon and surgical approach will be collected. New methods of measurement and detailed analysis of existing early and late postoperative X-ray images will be done. Using appropriate statistics patient and surgery related risk factors of polyethylene wear and poor survival are going to be detected.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: Using in vivo model of accelerated polyethylene wear patient, implant and surgery related risk factors that influence total hip survival are going to be detected. We expect that the application of the results of this research may enable the improvement of survival of total hip prostheses in the future.

Acknowledgements: I thank my mentor, and all colleagues for their great help in conducting this research

MeSH / Keywords: total hip, polyethylene wear, survival

COMPARISON OF BIOMECHANICAL PROPERTIES OF THE PLANTARIS AND SEMITENDINOSUS TENDONS FOR ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION

Part of a Thesis: Comparison of biomechanical properties of the plantaris and semitendinosus tendons for anterior cruciate ligament reconstruction

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INTRODUCTION: Anterior cruciate ligament rupture is the most common ligament injury of the knee causing subjective symptoms and chronic knee instability and in the longterm damage to the other structures of the knee joint like menisci or cartilage. In such cases there is a need to reconstruct the ligament. Reconstructive techniques utilizing hamstrings, patellar tendon or quadriceps tendon graft are generally used. All three grafts share common disadvantage: they are harvested from structures that have important role in biomechanics of the knee, thus additionally impairing stability of the knee.

HYPOTHESIS: The hypothesis of this research is that the plantaris tendon is suitable as a graft for the anterior cruciate ligament reconstruction, and that the plantaris tendon graft has comparable biomechanic properties with semitendinosus tendon graft used in anterior cruciate ligament reconstruction.

AIMS: The aim of this study is to compare biomechanical properties of the new plantaris tendon graft with biomechanical properties of the semitendinosus tendon graft commonly used in anterior cruciate ligament reconstruction.

MATERIALS AND METHODS: Semitendinosus and plantaris tendons will be procured from 30 cadavers in common fashion. Grafts will be made from each tendon and graft ends will be sutured with Krakow suture. Grafts will be placed in tensile testing machine and strenght and tension parameters will be measured. Biomechanical properties of the plantaris tendon graft will be compared with biomechanical properties of the semitendinosus tendon graft, which is currently gold standard in anterior cruciate ligament reconstruction. Depending on type of distribution data will be analysed using parametric or nonparametric statistical tests.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: create new graft for anterior cruciate ligament reconstruction without further compromising biomechanical relations of the injured knee and expand graft options for knee ligament reconstruction.

Acknowledgements: Authors would like to thank following institutions and their staff: Department of Orthopaedic Surgery University Hospital Centre Zagreb, Department of Forensic Medicine School of Medicine University of Zagreb, Department of Pathology and Cytology University Hospital Centre Zagreb, Faculty of Mechanical Engineering and Naval Architecture University of Zagreb

MeSH / Keywords: anterior cruciate ligament reconstruction, plantaris tendon graft

OPERATIVE VERSUS NON-OPERATIVE MANAGEMENT FOLLOWING ROCKWOOD GRADE III AND IV ACROMIOCLAVICULAR SEPARATION: RANDOMISED CONTROLLED TRIAL

Part of a thesis: Operative management following Rockwood grade III and IV AC separation results in better Constant score, faster ligament healing and lesser duration of a sick leave than non-operative treatment

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INTRODUCTION:Rockwood's classification of acromioclavicular dislocation is based on the degree and direction of clavicular displacement. Grades I and II are benign and are regarded as best managed conservatively. There is a general consensus that type V and VI lesions should be treated operatively. However, there remains controversy over optimal management strategy for grade III and IV injuries. There is a lack of well-designed studies in the literature to justify the optimum mode of treatment of grade III and grade IV acromioclavicular dislocations.

HYPOTHESIS: operative management following Rockwood grade III and IV AC separation results in better Constant score , faster ligament healing and lesser duration of a sick leave than non-operative treatment

AIMS: to prove that surgical treatment brings better results than conservative treatment

MATERIALS AND METHODS: The prospective study will be done. Patients with acromioclavicular separation of the grade III and IV by Rockwood as diagnosed in the initial MR examination, will be included in this study. They will be randomized in an operative and a non-operative group. The Constant score of both groups will be checked after 3 and 6 weeks and control MR examination will be performed after 6 weeks.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: The goal of this study is to answer the question which treatment is better for that injury

MeSH / Keywords:acromioclavicular separation,Rockwood classification, operative treatment of AC separation

INDIVIDUALIZED ANTIAGGREGATION THERAPY IN PATIENTS WITH MYOCARDIAL INFARCTION

Part of a Thesis: Evaluation of individualized antiaggregation therapy on clinical outcome in patients with acute myocardial infarction

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Menthor: Professor Davor Miličić, MD, PhD

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INTRODUCTION: Contemporary therapy of acute myocardial infarction is funded on urgent opening of the occluded coronary artery with placement of intracoronary stent paralleled with antiaggregation therapy with clopidogrel and acetylsalicylic acid. Response to antiaggregation therapy measured by aggregational laboratory tests shows interindividual variability. Insufficient response to therapy is connected with inferior clinical outcome. Hence, the term „resistance“ to antiaggregation therapy has been introduced. Between 5.5% and 45% of patients are resistant to acetylsalicylic acid and between 4% and 34% show insufficient response to clopidogrel. Several well-designed studies have shown that modification of the standard antiaggregation therapy can modify clinical outcome with higher doses of clopidogrel having been associated with better clinical outcome. Impact of modified dosing of acetylsalicylic acid has not been clearly established.

HYPOTHESIS: Patients with acute myocardial infarction resistant to antiaggregation therapy treated with higher doses of clopidogrel and acetylsalicylic acid will have significantly better clinical outcome than patients treated with standard doses.

AIMS: General aim of the study is clinical follow-up of the patients with acute myocardial infarction treated, according to platelet aggregation study results, with standard or higher doses of clopidogrel or acetylsalicylic acid. Aggregation studies in defined subsequent intervals and modification of the antiaggregation therapy are specific aims of the study.

MATERIALS AND METHODS: Eighty patients with acute myocardial infarction and non-satisfactory aggregometry results following standard dual antiaggregation therapy with loading dose of clopidogrel and acetylsalicylic acid will be enrolled. Fourty patients will repeat loading doses of clopidogrel or acetylsalicylic acid and continue to take double daily dose of clopidogrel or acetylsalicylic acid. Remaining fourty patients will take standard doses of clopidogrel and acetylsalicylic acid. Aggregometry is going to be performed with Multiplate bedside aggregometer (Verum Diagnostica GmbH). Follow-up will take one year with obligatory periodic aggregometry testing. Clinical outcome will comprise cumulative incidence of repeated myocardial infarction, need for revascularization of the target lesion, ischaemic stroke and cardiovascular death.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: This is the first prospective, randomized, interventional long-term clinical follow-up study based on aggregometry in patients with acute myocardial infarction which may result in important facts for everyday practice.

Acknowledgements: I would like to thank JGL d.o.o. for their financial support.

MeSH / Keywords: Myocardial Infarction; Clopidogrel; Acetylsalicylic acid

CONTRIBUTION OF COMBINING CLINICAL TESTS TO INCREASED ACCURACY OF KNEE MENISCAL TEAR DIAGNOSTICS

Part of a Thesis: Combination of three clinical tests increase accuracy of knee meniscal tear diagnostics

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INTRODUCTION: So far there are dozens clinical tests described for making diagnosis of meniscus tear which help examiner to distinguish this type of injury from other injuries in the knee. The most famous are the McMurray test, joint line tenderness, Steinmann I test, Ege test, and Thessaly test. Recent studies indicate that the combination of two clinical tests for assessing meniscal tear gets a higher sensitivity and specificity of any clinical test separately. **HYPOTHESIS:** The hypothesis of this research is that using a combination of three clinical tests used for the evaluation of meniscal tear (Thessaly test, joint line tenderness, McMurray test), combination being positive if two of three test are positive, during the examination achieves higher accuracy, specificity, sensitivity, positive predictive value and negative predictive value than other combinations of tests (Ege test, Steinmann I test, atrophy of thigh muscles) and in relation to each of these six tests individually.

AIMS: The aim of this research is to compare combination of three clinical test used for the evaluation of meniscal tear (Thessaly test, joint line tenderness, McMurray test) with other combination of clinical test used for the evaluation of meniscal tear (Ege test, Steinmann I test, atrophy of thigh muscles) and with each of these clinical tests individually.

MATERIALS AND METHODS: Research will be conducted on a group of 80 consecutive patients aged 16-55 years who were diagnosed with meniscal tear and who are therefore subscribed for surgical treatment. The final evaluation of whether there is a meniscal tear will be done during the arthroscopy.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: The expected scientific contribution of the proposed research is to determine combination of clinical test used for the evaluation of meniscal tear which would increase the accuracy of clinical diagnosis and that would be used in everyday use in orthopaedic and traumatology practice. In this way, the need for making MRI for all meniscal injury would be reduced, but would only remain reserved for doubtful cases.

Acknowledgements: I would like to thank Department of Orthopaedic surgery University Hospital Centre Zagreb for allowing me to conduct my research, my mentor for guiding me through it and my family for support.

MeSH / Keywords: meniscus, meniscal tear, clinical tests, diagnosis of mensical tears

VALIDITY OF PREOPERATIVE AUTOLOGOUS BLOOD DONATIONS FOR TOTAL HIP ARTHROPLASTY

Part of a Thesis: Validity of preoperative autologous blood donations for total hip arthroplasty

PhD candidate: Zrinka Orešković, MD, MSc

Mentors: Assistant professor Branko Tripković, MD, PhD, Associate Professor Domagoj Delimar, MD, PhD

Affiliation: University Hospital Centre - Zagreb

INTRODUCTION: Total hip arthroplasty operation is often accompanied by heavy perioperative and postoperative bleeding. To avoid using of large amounts of allogenic blood products, the methods of preoperative autologous blood donation are developed. The method consists of taking blood or blood components of patient-donor and the preparation of blood components intended for transfusion to same patient. The aim is to prevent transmission of viral diseases and avoiding a number of other possible complications that may occur as part of allogenic blood transfusion. In addition to benefits there are many complications associated with autologous blood donation.

HYPOTHESIS: Patients who preoperatively donate autologous blood do not profit of that because they need additional doses of allogenic blood. The reason is iatrogenic anaemia developed after blood donation.

AIMS: There is a need to develop algorithms individually for each patient depending on the expected blood loss and its condition (diagnosis, laboratory parameters). The aim of this study is to investigate the validity of use preoperative autologous blood donation in patients which undergoes total hip arthroplasty.

MATERIALS AND METHODS: In this research will be included 75 patients undergo total hip arthroplasty. They will be a randomly divided into three groups. The first group will be patients who have not donated blood before surgery, in second patients who donated blood 72 hours preoperatively and third patients who donated blood 14 days preoperatively. Postoperative recovery will be assessed on the basis of estimates perioperative and postoperative bleeding (drainage, hematoma, local bleeding), and considering the need for intraoperative and postoperative transfusion (autologous / homologous). Also by comparing the value of postoperative values of blood parameters (E, Hb, Hct, Tr, Fe, TIBC, UIBC, reticulocyte count, PT, APTT) and postoperative values noninvasively measured parameters (BP, pulse, SpO₂). We will also compare duration of hospitalization between the groups of patients. **EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH:** The research will contribute to setting objective parameters to select patients who need to apply the method of blood donations. Based on these results preoperative donation will be reduced, which would avoid potential complications, accelerate patient recovery and optimize the operating costs of treatment.

MeSH/Keywords: transfusion, preoperative autologous blood donation, total hip arthroplasty, bleeding

COMPARISON OF HEALTH RELATED QUALITY OF LIFE WITH EXERCISE TESTING IN CHILDREN AND ADOLESCENTS WITH HEART DISEASE

Part of a Thesis: Evaluation Of Quality Of Life In Children With Heart Disease

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INTRODUCTION: Over the last few decades, great advances in pediatric cardiac interventions and intensive care have led to lower mortality rate for children with the most complex congenital heart disease (CHD). On the other hand, survivors suffer from morbidity resulting from their circulatory abnormalities and the medical therapies they have received. For all these reasons, new questions about the quality of life of these patients arise among patients, parents and doctors. Quality of life (QOL) analysis requires the use of appropriate validated questionnaires. Concerning that perception of QOL, as more subjective variable, can differ among patients, parents and medical providers, it seems reasonable to compare reported data with some more objective evaluation, which is exercise testing (ergometry).

HYPOTHESIS: 1. Quality of life and functional status of children a) with simple CHD are similar to those of healthy children and b) with complex CHD differ from those of healthy children, although CHD severity or complexity does not necessarily correlated regularly with the QOL. 2. There is positive correlation between QOL estimated with questionnaires and

ergometry, especially in prepubertal population. 3. Perception of QOL between patients, parents and doctors / pediatricians don't necessarily agree.

AIMS: 1. For children with heart disease, treated in Croatia, conduct proceedings of cross-cultural adaptation of Health-Related Quality of Life (HRQOL) questionnaire, with the aim of getting linguistic and psychometric valid instrument for use on Croatian linguistic area.

2. Using a validated questionnaire, evaluate HRQOL of children with heart disease, and then compare the questionnaires data with the results of exercise test.

PATIENTS AND METHODS: Study population - Children with congenital and aquired heart disease age 8-18 years and one of their parents. Anticipated number of children questioned: 150-200. Study design - observational cross-sectional clinical study. Methods - Using a validated questionnaire, evaluate HRQOL of children with heart disease treated in Croatia, and then compare the questionnaires data with the results of exercise test.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: This study will assess QOL perceptions in the subgroups of pediatric cardiac patients from the perspective of the patient and parent, comparing to results of ergometry.

MeSH / Keywords: Congenital heart disease, Acquired heart disease, Pediatrics, Quality of life, Health-related quality of life, Questionnaires

THE SIGNIFICANCE OF ALARMINs HMGB1 AND S100A12 AND THEIR RECEPTOR RAGE IN JUVENILE IDIOPATHIC ARTHRITIS

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INTRODUCTION: For years it was thought that the disorder of adaptive immunity is critical in the pathogenesis of juvenile idiopathic arthritis (JIA). However, the results of recent studies emphasize the importance of innate immunity and especially the role of alarmins, a heterogeneous group of proteins that are released into the intercellular space during tissue injury or inflammation. In that sense alarmins serve as endogenous „dangers signals“ and play a role in the pathogenesis of various inflammatory diseases, such as SLE and RA. On the other hand, their role in the JIA still remains unclear.

HYPOTHESIS: High levels of HMGB1 and S100A12 and low levels of sRAGE in patients with JIA indicate an active disease.

AIMS: The aim is to determine gene expression and the level of HMGB1, S100A12 and RAGE in the serum of children with JIA, depending on the type of JIA and compared with findings in the control group. We aim to try to examine the relationship between serum levels of these alarmins in patients with JIA compared to the routine laboratory parameters used to evaluate the activities JIA and to determine the role alarmins as potential biomarkers for monitoring activity of the disease.

MATERIALS AND METHODS: Peripheral blood will be obtained from 120 children with JIA at the time of diagnosis, before any therapy was introduced. Children admitted for non-inflammatory conditions will be used as controls. The gene expression of HMGB-1, S100A12 and their receptor RAGE will be assessed by quantitative RT-PCR while the protein levels will be determined by ELISA. STATISTICA 8.1 will be used for all the computations.

THE EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: The work will enable the introduction of levels and gene expression of alarmins HMGB1 and S100A12 and their receptor RAGE in patients with certain forms of JIA. It can contribute to better understanding of immunopathogenesis of the disease, and thus a better therapeutic approach. Furthermore, the determination alarmins levels in patients with JIA can be a useful guideline for determining disease activity.

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Keywords: juvenile idiopathic arthritis; HMGB proteins; S100A12 protein, RAGE.

ANALYSIS OF THE OUTCOME OF TREATMENT IN CHILDREN WITH HYPOPLASTIC LEFT HEART SYNDROME

Part of a Thesis: High Percentage of Patients with Hypoplastic Left Heart Syndrome (HLHS) Experiencing Adulthood – New Public Health Problem

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INTRODUCTION: Treatment of HLHS is carried out over the past 30 years. Outcome in developed countries (DC) are based on past 20-odd years, but until now, no comprehensive analysis of these data. In Croatia, the patients with HLHS defined by national and hospital population study waste to 2,3 % of all congenital cardiac defects (CHD). Improving the treatment of patients who have the most complex CHD, indicating an increased number of adult patients with cardiac problems for which still there are no optimal strategies for treatment. Estimate of the expected influx of such patients in Croatia in the next 10-y period is prerequisite for definition of new health care strategy.

HYPOTHESIS: in the first decade of 21st century outcome of children with HLHS in Croatia does not significantly lag behind the DC where it has witnessed a steady trend of improved survival and high percentage of patients experiencing adulthood. That becomes the new public health problem.

AIMS: Main is to assess outcome of patients with HLHS. Specific are: to estimate survival by stage of disease/treatment and total, in DC from 1990.-2000. and 2000.-present, and in Croatia (established cohort 1999.-2011), to estimate incidence of HLHS in DC and in Croatia and make projections on number of children with HLHS who will experience the adulthood in the next ten-year period.

MATERIALS AND METHODS: Systematic review and synthesis of information on survival of children with HLHS. Analysis of cohort of children with HLHS born in Croatia between 3/11/1999 and 01/09/2011. A systematic review of epidemiological studies on the incidence of HLHS in DC and evaluation of data for Croatia. Identifying significant predictors and survival data will allow the projection of number of children with HLHS who will experience adulthood.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: would be to generate „best evidence” assessment of the survival of children with HLHS in DC and the key predictors of outcome as comparison with outcomes in Croatia. On the basis of that, projections about the grown up congenital heart (GUCH) patients are first prerequisite for timely planning development strategies for their future care.

Keywords: hypoplastic left heart syndrome (HLHS), outcome predictors, survival, GUCH-patients

VISUAL EVOKED POTENTIALS - FOLLOWING AND PROGNOSTIC METHOD FOR NEURODEVELOPMENTAL OUTCOME OF CHILDREN WITH CONGENITAL CYTOMEGALOVIRUS INFECTION

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INTRODUCTION: Congenital cytomegalovirus (CMV) infection is the most common serious viral infection. Clinical consequences of CMV infection (as a result of placentitis and secondary chronic perfusion insufficiency) are IUGR and a wide spectrum of neurodevelopmental disorders. It is well known that congenital CMV infection can cause auditory dysfunction and choreoretinitis; a recent study shows that maturation of periventricular white matter is also affected.

Visual evoked potentials are a good prognostic factor of neurodevelopmental outcome, with high predictive values for visual function as well as neuromotor outcome. Visual evoked response undergoes changes during the first year of life, as consequence of brain maturation (synaptogenesis and myelinisation).

HYPOTHESIS: As is well known, most children with congenital CMV infection have delayed neuromotor development. From our clinical experience and results of a pilot study, they also have delayed maturation of visual pathways. It seems that is a good correlation of maturation of visual evoked response and neurological development in children with congenital CMV infection. Therefore, visual evoked potentials could be an effective diagnostic tool for the assessment of the clinical severity of neurodevelopmental dysfunction in children with congenital CMV infection and also a following and prognostic method for their neurodevelopmental outcome.

AIMS: To describe specific characteristics of visual evoked response in children with congenital CMV infection and to try proving that visual evoked response could be effective following and prognostic method for neurodevelopmental outcome in children with congenital CMV infection.

MATERIALS AND METHODS: Multidisciplinary prospective follow-up study of children with congenital CMV infection. Initial clinical examination will be performed in University Hospital for Infectious Diseases. Follow-up will be performed in Children's Hospital Zagreb with repeated neurological examinations, brain US and neurophysiologic tests (VEP, EEG), together with TCCD, ophthalmologic, ORL examinations, psychological and early communication evaluation.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: Research on the study will contribute to understanding the functional electrophysiological maturation of the visual pathway in children with congenital CMV infection. It will also prove if visual evoked potentials (painless, non-invasive and repetitive method) could be performed as useful assessment and prognostic method for neurodevelopmental outcome in children with congenital CMV infection.

MeSH / Keywords: Visual evoked potentials, Neurodevelopmental outcome, Congenital cytomegalovirus infection

LACTOBACILLUS REUTERI IN THERAPY OF FUNCTIONAL ABDOMINAL PAIN AND CONSTIPATION IN CHILDREN - RANDOMIZED, DOUBLE-BLIND, PLACEBO CONTROLLED STUDY

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Mentor: Professor Sanja Kolaček, MD, PhD

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INTRODUCTION: Functional abdominal pain (FAP) and chronic constipation are frequent problems in childhood. Beneficial therapeutic effect of probiotics has been reported in children with FAP and chronic constipation but the evidence for efficacy is limited.

HYPOTHESIS: Lactobacillus reuteri DSM 17938 is effective in treatment of FAP and chronic constipation in children.

AIMS: The aim is to evaluate the effect of L. reuteri on number and severity of episodes pain in children with FAP and on frequency and consistency of stools and episodes of pain in children with chronic constipation.

MATERIAL, PATIENTS, METHODOLOGY AND PLAN OF STUDY: Approximately 300 children with FAP (age 4-18 years) and chronic constipation (age 2-18 years) will be asked to join the study. Symptoms will be evaluated with the Rome III criteria for functional gastrointestinal disorders (FGIDs), visual-analogue scale (VAS) for pain and Bristol stool scale. Children with FAP will be randomly assigned into one of two groups. Group A will receive L. reuteri at a dose 10^8 CFU per dose for 3 months. Group B will receive placebo. Children with chronic constipation will be assigned into one of two groups. Group C will receive L. reuteri at a dose 10^8 CFU per dose plus lactulose in a dose 1-3 ml/kg, according to the stool frequency and consistency, for 3 months. Group D will receive placebo plus lactulose.

Primary endpoints will be frequency and severity of abdominal pain during treatment in children with FAP, and frequency of bowel movements, stool consistence and need for lactulose in children with constipation during treatment. The trial will be prospective, randomized, double blind, placebo controlled study. Ethics: study has been approved by Hospital Ethics Committee. A written informed consent will be obtained from at least one parent and a child, if the child is above 9 years.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED STUDY: The study is expected to define a potential role of the probiotic strain Lactobacillus reuteri DSM 17938 in treatment of FAP and chronic constipation in children.

Acknowledgements: I would like to thank Biogaia AB, Sweden, for providing probiotics and placebo.

MeSH / Keywords: Lactobacillus reuteri, abdominal pain, constipation, child

PROINFLAMMATORY CYTOKINES IN JUVENILE IDIOPATHIC ARTHRITIS

Part of a Thesis: Proinflammatory Cytokines In Juvenile Idiopathic Arthritis

PhD candidate: **Agneza Marija Kapović, MD**

Mentor: Alenka Gagro, MD, PhD

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INTRODUCTION: Juvenile idiopathic arthritis (JIA) is a heterogenous group of diseases characterised by arthritis of unknown origin with onset before age of 16 years. It is the most common childhood chronic rheumatic disease and causes significant disability. Chronic synovial inflammation is main pathological process in JIA, and is a result of activation of innate and adaptive immunity. These processes are mediated by secretion of numerous cytokines. JIA is an exclusion diagnosis so imaging techniques such as ultrasound and magnetic resonance provide useful information to supplement clinical and laboratory examination.

HYPOTHESIS: Investigation of serum and synovial fluid levels of proinflammatory cytokines, use of power doppler ultrasound (PD US) and contrast-enhanced magnetic resonance imaging (MRI) are improving algorithm for monitoring of disease activity and in the assessment of response to treatment in children with JIA.

AIMS: Investigation of levels of proinflammatory cytokines- interleukin (IL)-1, IL-17A, IL-18, IL-22, tumour necrosis factor (TNF)- α and vascular endothelial growth factor (VEGF) in serum and synovial fluid in children with JIA before therapy and in serum, 3 and 6 months after initiation of therapy. PD US will be performed at the same time as taking the samples for measuring cytokines, and contrast-enhanced MRI before, and 6 months after initiation of therapy. We will investigate PD US and MRI findings and correlate them with clinical investigation and levels of proinflammatory cytokines and markers of inflammation.

MATERIALS AND METHODS: Sera from 30 children affected with JIA and 10 age matched controls will be tested with a commercial ELISA for proinflammatory cytokines. Children with joint effusion will be tested for cytokines in synovial fluid. Furthermore, clinical examination, routine laboratory investigation and radiologic assessment (PD US, MRI) before initiation of therapy, as well as 3 and 6 months after initiation of therapy will be determined in all patients.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: This research will provide further information about the role of proinflammatory cytokines in children with JIA. We also plan to investigate which of the investigated parameters (cytokines, PD US, MRI) is most reliable in improving algorithm for disease activity monitoring and response to therapy in JIA.

Keywords: Juvenile idiopathic arthritis, proinflammatory cytokines, disease activity

THE FREQUENCY AND IMPACT OF SELF-INITIATIVE ELIMINATION DIET ON NUTRITIONAL STATUS AND CLINICAL COURSE OF DISEASE IN CHILDREN WITH CHRONIC INFLAMMATORY BOWEL DISEASES

Part of a Thesis: The frequency and impact of self-initiative elimination diet on nutritional status and clinical course of disease in children with chronic inflammatory bowel diseases

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INTRODUCTION:Inflammatory bowel disease, Crohn's disease and ulcerative colitis are chronic diseases that are characterized by relapsing-remitting course. Their exact etiology and pathogenesis have not been fully elucidated. Previous studies have shown that they are result of the interaction of abnormal immune response and environmental factors in genetically predisposed individuals. Mostly studied environmental factor is a diet, which is not only associated with the pathogenesis, but also with the influence in the course of the disease. It has been shown that patients often voluntarily avoid various kinds of food in order to prevent relapse. However, there are no relevant studies which prove the role of nutrition in the pathogenesis or the course of the disease. The consequences of diet limitations in children with inflammatory bowel diseases have not been determined.

HYPOTHESIS: Self-initiative elimination diet in patients with inflammatory bowel diseases reduce the quality of nutrition and have effect on the nutritional status of children and clinical course of disease.

AIMS: To assess the frequency of self-initiative elimination diets in children with chronic inflammatory bowel diseases and its impact on quality of nutrition, nutritional status and clinical course of disease.

MATERIALS AND METHODS: In this study it will be include 90 children with newly diagnosed inflammatory bowel disease and children with already diagnosed in which we will determine the anthropometric measures, biochemical measures, activity of disease, analyze the results of bioelectrical impedance and evaluate dietary habits using a food diary and validated dietary questionnaire. Their results will be compared with the healthy control of 90 children that will match by age and sex.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: This research will provide further information about frequency of self-initiative diet and their effect on nutritional status and clinical course of disease, as also determine the exact and detailed nutritional status of children with inflammatory bowel diseases, which we will compare with the healthy control group.

Keywords: Inflammatory bowel disease, nutrition, self-initiative diet

EFFECT OF BILIARY OBSTRUCTION ON GHRELIN, CHOLECYSTOKININ, INFLAMMATORY MARKERS AND NUTRITIONAL STATUS

Part of a Thesis: Effect of Biliary Obstruction on Ghrelin, Cholecystokinin, Inflammatory Markers and Nutritional Status

PhD candidate: Tajana Pavić, MD

Mentor: Professor Marko Duvnjak, MD, PhD

Affiliation: University of Zagreb School of Medicine, Clinical Hospital Center „Sestre milosrdnice“ Zagreb, Croatia

INTRODUCTION: More than 50% of patients with biliary obstruction have signs of malnutrition, but the mechanisms involved are not well understood. Concentration of cholecystokinin, an anorexigenic hormone, is increased in patients with obstructive jaundice. Ghrelin is the only known peripherally produced peptide with orexigenic, immunomodulating and anticatabolic properties. There is evidence for the functional antagonism of cholecystokinin and ghrelin, but the exact interplay concerning the secretion of both peptides remains unclear. There is no data regarding the role of obstructive jaundice on ghrelin.

HYPOTHESIS: Biliary obstruction causes alterations in circulating levels of ghrelin, cholecystokinin, inflammatory markers and nutritional status of patients with obstructive jaundice and those parameters are positively affected by endoscopic biliary drainage.

AIM: The aim of this study is to determine the effect of obstructive jaundice on the hormones controlling appetite and nutritive status of patients before and after endoscopic biliary drainage; to investigate the influence of duration, gravity and ethiology of biliary obstruction, H.pylori infection and general patient's characteristics on the biochemical parameters; to analyze the interplay between hormonal and biochemical parameters on nutritive status and appetite.

MATERIALS AND METHODS: This is a prospective study on patients with obstructive jaundice who are scheduled to endoscopic retrograde cholangiopancreatography (ERCP) in the Department of Internal medicine in Clinical Hospital Center „Sestre milosrdnice“ Zagreb. The study will include 55 patients with obstructive jaundice of benign and malignant ethiology and control group of 40 healthy age- and sex-matched volunteers. Initially patients will be assessed for appetite, nutritive status, level of ghrelin, cholecystokinin, TNF- α and IL-6, along with standard preparation for ERCP. 48 hours and 28 days after successful biliary drainage assessment of nutritive status, appetite, biochemical and hormonal parameters will be repeated. Data will be analyzed using statistical package MedCalc for Windows, version 11.3.1

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: We expect new evidence on factors influencing ghrelin secretion that can yield novel therapeutic targets in the treatment of malnutrition and wasting induced by various sub acute and chronic disorders.

MeSH / Keywords: biliary obstruction, ghrelin, cholecystokinin, IL-6, TNF- α , nutritional status, appetite

GROWTH AND DEVELOPMENT OF THE FOOT IN PRESCHOOL CHILDREN

Part of a Thesis: Anthropometric measurement of growing feet in preschool children

PhD candidate: Ozren Vrdoljak, MD

Mentors: Associate Professor Mirjana Kujundžić Tiljak, MD, PhD, Assistant Professor Mislav Jelić, MD, PhD

Affiliation(s): Children's Hospital Zagreb, Klaićeva 16, 10 000 Zagreb

INTRODUCTION: The anatomical specificity of children's feet change during their growth and development, and the foot therefore adapts to its function. When children start walking their feet begin to change shape, and they obtain their final shape when the feet stop growing. The outside shape of the foot is determined by anthropometric measurements: instep length, width, and height, heel width and toe length. Foot length is the basic unit of measurement for monitoring the development and growth of the foot. The existing standards of foot length and shape are not suitable for our population.

HYPOTHESIS: There is no such thing as a generalized, standardized human foot. This study will give us anthropometric measurements of preschool children's feet in Croatia, which will enable normal development and prevention of acquired foot deformities.

AIMS: Standards of growth and foot shape as well as the development of reference tables.

MATERIALS AND METHODS: The study will include preschool children between 2 (1.50-2.49) and 7 (6.50-7.49) years of age in Croatia who do not have any congenital or acquired deformities of the foot. The sample will consist of 150 female and 150 male children, totaling to 300 feet in each age group. Some of the data was collected as a part of the project „Anthropometric Measurement of Growing Feet“ (code 0072007/2001 of Croatia's Ministry of Science and Technology), a pilot study on a random sample of preschool children between 1.5 and 7.5 years of age. The rest of the data will be a supplement to the sample for each age group. The methodology was developed during the project „Anthropometric Measurement of Growing Feet“. The measurement technique will be standardized. Children's feet will be measured without shoes on a specially constructed device using a sliding caliper with a 15 cm range, a tape measure and a pedometer to determine the valgus of the heel.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: Anthropometric foot measurements of preschool children will allow us to obtain standards for the dynamics of foot growth and development, foot shape and foot deformities.

Keywords: growth and development, anthropometry, reference values

DEMORALIZATION IN SCHIZOPHRENIA

Part of a Thesis: Comparison of depression and demoralization syndromes as predictors of suicidality in chronic schizophrenia

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Mentor: Professor Vlado Jukić, MD, PhD

Affiliation: University Psychiatric Hospital Vrapče, Zagreb, Croatia

INTRODUCTION: Suicide is the main cause of death for people suffering from schizophrenia. Previous research has pointed to symptoms of depression as being the most powerful predictors of suicidal behavior. Since depression is defined as a syndrome, the question of suicidal potential of different depression symptom clusters, in different phases of schizophrenia, remains open. In recent years there is a noticeable trend of separating demoralization syndrome with cognitive determinant as an independent diagnostic category with already suggested criteria and rating scales.

HYPOTHESIS: 1. Depression and demoralization symptoms are significant predictors of suicidality in patients with schizophrenia. 2. Schizophrenic patients with longer duration of illness will have higher prevalence of demoralization than depression symptoms.

AIMS: General: To assess influence of depressive and demoralization syndrome symptoms on suicidality in schizophrenic patients. Specific: 1. To assess influence of depression and demoralization symptoms clusters as predictors of suicidality in relation to sociodemographic and clinical factors in schizophrenic patients. 2. To identify clinically relevant and applicable value of demoralization syndrome obtained by questionnaire that indicates increased risk of suicidality.

MATERIALS AND METHODS: Research will be conducted as prospective study among 146 remitted outpatients with diagnosis of schizophrenia treated in University Psychiatric Hospital Vrapče, Zagreb. Symptom severity and remission criteria, level of depression, level of demoralization and suicidality will be assessed by questionnaires Positive and Negative Syndrome Scale (PANSS), Calgary Depression Scale for Schizophrenia (CDSS), Demoralization Scale (Kissane et al.; J Palliat Care. 2004) and InterSePT Scale for Suicidal Thinking (ISST), respectively.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: Research will differentiate possible predictive values of depression and demoralization symptoms for suicidal behavior in schizophrenic patients. Possible distinction between depression and demoralization syndrome will provide better identification and prevention of suicidality in schizophrenic patients, especially when it is not connected with somatic symptoms of depression but with cognitive determinant of demoralization.

MeSH / Keywords: depression, demoralization, schizophrenia, suicidality

THE SIGNIFICANCE OF PERSONALITY TRAITS, ATTACHMENT STYLES AND THE QUALITY OF LIFE IN SELECTING SUBSTITUTION THERAPY FOR HEROIN ADDICTS

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Mentor: Docent Dolores Britvić, MD, PhD, psychiatrist

Affiliation: Public Health Institute, Department of Mental Health and Addiction Prevention and Out-patient treatment

INTRODUCTION: The treatment of heroin addicts in Croatia is based on the methadone substitution therapy or buprenorphine/naloxone therapy, by which the heroin abstinence is established and maintained.

HYPOTHESIS: 1. The drug users on therapy of buprenorphine / naloxon have more positive personality traits and more secure attachment style in relation to drug users on methadone maintains treatment; 2. The drug users on therapy of buprenorphine / naloxon more often have permanent partner relationships compare to the addicts on methadone maintains treatment; 3. More positive personality traits, more secure attachment style, partner relationships and parenting styles improved better quality of life of heroin addicts.

AIMS: General aim is to determine the importance of personality traits, attachment styles and socio-demographic characteristics of heroin addicts in the selection of substitution therapy, and to examine the contribution of these factors on quality of life. Specific aims are first to identify the differences in personality traits, attachment styles and demographic characteristics according to the type of substitution treatment of heroin addicts. Second to examine the contribution of personality traits, attachment styles and socio-demographic characteristics (age, marital status, parenthood, cohabitation) on quality of life of heroin addicts.

MATERIALS AND METHODS: The study will include 2 groups of 150 heroin addicts each. One group - heroin addicts on methadone maintenance therapy and another group treated with buprenorphine maintenance / naloxon. Both groups of patients are on stable maintenance therapy substitution and abstinent from heroin past 12 months, based on medical documentation. The patients will be implemented the standardized questionnaires as measuring instruments as follow: 1. Pompidou questionnaire (Hartnoll, R. (1994), 2. Personality Inventory BFI (John Donahne, Kent, 1991; by Benet-Martinez and John, 1998), 3. Experiences in Close Relationship Inventory - IIBV (Brennan et all.,1998), 4. Quality of Life Questionnaire - WHOQOL-BREF (Introduction, administration, scoring and generic version of The Assessment Field Trial Version 1996).

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: The expected scientific contribution is the introduction to studying on the relations between personality traits, the attachment styles and the quality of life by choosing substitution therapy.

MeSH/Keywords: personality traits, attachment styles, quality of life, methadone, buprenorphine/naloxone

THE RELATIONSHIP OF PEER PRESSURE, BODY SHAME AND BODY DISSATISFACTION WITH ADOLESCENT EATING BEHAVIOURS

Part of a Thesis: The Relationship of Peer Pressure, Body Shame and Body Dissatisfaction with Adolescent Eating Behaviours

PhD candidate: Jelena Mustapić, psychologist

Mentor: Assistant Professor Darko Marčinko, MD, PhD, psychiatrist and psychotherapist

Affiliations: Technical School Rudjer Bošković, Zagreb; School of Applied Arts and Design, Zagreb; Fifth Gymnasium, Zagreb; Head Office of Prison System Directorate, Department of Diagnostics, Ministry of Justice of the RoC

INTRODUCTION: Eating disorders are associated with serious biological, psychological, and sociological morbidity and significant mortality. Rapid physical growth and development in adolescence constitute the unique background for development of disordered eating behaviours and/or eating disorders at this stage of life. There is an increased value placed on peer acceptance and approval, and a heightened attention to external influences and social messages about cultural norms. The experience of body shame and body dissatisfaction can lead to poor health habits and low self-esteem. These negative feelings may affect health behaviors associated with poor eating habits, dieting, low self-esteem and eating disorders.

HYPOTHESIS: Disordered eating behaviours are positively associated with peer pressure, body shame, body dissatisfaction and body mass index.

AIMS: The purpose of this study was to determine the prevalence of disordered eating behaviours in non-clinical population of high school students and examine the relationships with peer pressure, body shame and body dissatisfaction and body mass index.

MATERIALS AND METHODS: A sample of 400 adolescent aged 15-17 years will complete self-reported measures of the mentioned concepts. The assessment tools include: Body Shape Questionnaire (BSQ), the Body Shame Scale (BS-OBCS), Eating Attitudes Test (EAT-26) Peer Pressure Questionnaire and Body Mass Index (BMI) which is calculated using person's weight in kilograms divided by the square of the height in metres (kg/m²).

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: The limitations and importance of these results will be discussed, and directions for future research and treatment of eating disorders will be indicated.

Acknowledgements: I would like to express my gratitude to all those who gave me the possibility to complete this thesis, especially to my supervisor, PhD Darko Marčinko, MD

MeSH / Keywords: Body Shame, Body Dissatisfaction, Peer Pressure, Eating Behaviours, Adolescents

DEPERSONALIZATION IN SCHIZOPHRENIA PATIENTS

Part of a Thesis: Relationship between depersonalization and positive and negative symptoms, depression, and cognition in schizophrenia patients

PhD candidate: Aleksandar Savić MD

Mentor: Professor Vlado Jukić, MD, PhD

Affiliation: University Psychiatric Hospital Vrapče, Zagreb

INTRODUCTION: Depersonalization refers to a complex phenomenon involving alteration in the perception of the self, of the body, or of the external world. It is placed on a spectrum ranging from depersonalization as a reaction to stress, depersonalization in other psychiatric disorders (depression, schizophrenia, anxiety disorders), to significant and persistent depersonalization that cannot be attributed to any other disorder. Recent research identified depersonalization as a clinical marker of disease severity in depression and anxiety disorders. Relatively scarce articles on depersonalization in schizophrenia confirm high prevalence of depersonalization in schizophrenia patients, and point to its possible relationship with positive symptoms, depression, alexithymia, and in general cognitive failures.

HYPOTHESIS: Presence of clinically significant depersonalization phenomenon in schizophrenia patients is a clinical marker of disease severity and outcome, making patients more likely to have longer disease episodes, poorer remission with more depression symptoms between episodes, and consequently poorer social functioning.

AIMS: Aim of this research is to evaluate depersonalization as a clinical marker of severity of disease in schizophrenia patients, and specifically the relationship of the phenomenon with positive and negative symptoms, depression, and cognition. Secondary aims include establishing the cut-off value on Cambridge Depersonalization Scale that points to poorer prognosis, and evaluating influence of drug-induced akathisia on depersonalization.

MATERIALS AND METHODS: 150 patients diagnosed with schizophrenia according to DSM-IV-TR and ICD-10 criteria will be interviewed. The Cambridge Depersonalization Scale will be used to assess depersonalization. In addition, to assess other symptoms patients will be given Positive and Negative Syndrome Scale (PANSS), Calgary Depression Scale (CDS), Barnes Akathisia Scale (BARS). Neurocognition and specific personality traits will be assessed through psychological testing (Wisconsin Card Sorting Test, Stroop test, letter/number sequencing subtest and digit span-backward from WAIS III UK-R, Benton Facial Recognition Test, Revised NEO Personality Inventory). Patients will be reevaluated six months and one year after the initial contact.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: Contribution of the proposed research would be identification of a phenotype that might be used as a clinical severity and outcome marker for schizophrenia patients, as well as clarifying the relationship between depersonalization and other symptoms in schizophrenia.

MeSH / Keywords: schizophrenia, depersonalization, cognition, outcome assessment

THE ROLE OF QUANTIFERON TEST IN DETECTION OF TUBERCULOSIS IN IMMUNOCOMPROMISED PATIENTS'

Part of a Thesis: The role of QuantiFERON test in detection of latent and active tuberculosis in patients' with rheumatoid arthritis and inflammatory bowel disease during treatment with TNF- α inhibitors

PhD candidate: Denis Baričević, MD

Mentor: Assistant professor Sanja Popović Grle, DMD, PhD

Affiliations: University Hospital Zagreb, Department for Respiratory diseases

INTRODUCTION: The introduction of new tumor necrosis factor- α (TNF- α) inhibitors in the treatment of severe non inflammatory diseases has increased the frequency of severe complications. Among them tuberculosis is one of the most common complications. Therefore it is of utmost importance to check every patient for latent tuberculosis in order to identify new patients' who would benefit from chemoprophylaxis's before starting biological therapy.

HYPOTHESIS: Patients' with positive QuantiFERON test can benefit from chemoprophylaxis to prevent disease manifestation.

AIMS: To identify the role of QuantiFERON test in the diagnosis of latent and active tuberculosis in immunocompromised patients' before starting biological therapy. To determine the frequency of latent infection in immunocompromised patients' with positive QuantiFERON but with negative clinical findings, radiological investigations and laboratory tests. To determine the frequency of disease manifestation in immunocompromised patients', candidates for biological therapy with QuantiFERON test with positive clinical findings, radiological investigations and laboratory tests. To determine the phenotype of QuantiFERON test in immunocompromised patients', candidates for biological therapy using longitudinal results of QuantiFERON tests before and after starting biological therapy. To determine whether isoniazid prophylaxis prevents disease manifestation in immunocompromised patients', candidates for biological therapy.

MATERIALS AND METHODS: In all patients' clinical examination will be performed and medical history will assess the following issues: co-morbidities, exposure history, family history of tuberculosis, occupational and environmental exposures, current and concomitant treatment, social status while BCG vaccination status will be assessed by examination for the presence of BCG scar. From each participant blood samples will be drawn for QTG test and in all patients' sputum test for BK 2X, ppd skin test, chest and heart radiography will be performed.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: Results of this study should be of great importance in developing guidelines for the use of the QuantiFERON test in diagnosis of latent and active tuberculosis in patients with rheumatoid arthritis and inflammatory bowel disease.

MeSH / Keywords: QuantiFERON test, latent and active tuberculosis, immunocompromised patients, rheumatoid arthritis, inflammatory bowel disease

RELATIONSHIP OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND ELEVATED CARDIOVASCULAR RISK

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Mentor: Professor Neven Tudorić, MD, PhD

Affiliation: Division of pulmonology, Department of internal medicine, University hospital Dubrava, Zagreb, Croatia

INTRODUCTION: Recent studies suggest a possible link between lung and systemic inflammation in chronic obstructive pulmonary disease (COPD) and the progression of atherosclerotic processes, which increases cardiovascular morbidity and mortality. No study has comprehensively investigated the relationship between lung function and arterial stiffness as an independent predictor of increased cardiovascular risk in patients with COPD and healthy smokers, and as a control group of healthy nonsmokers.

HYPOTHESIS: Patients with moderate to severe COPD are at increased cardiovascular risk compared to a control group of healthy smokers and healthy nonsmokers.

OBJECTIVES: The aim of this study is to determine whether there is an increased cardiovascular risk in patients with COPD compared to control group, to research the association between the variables of lung function (FEV1, FVC, respiratory failure) with elevated cardiovascular risk variables and to examine the relationship between the degree of difficulty (the world-accepted classification of GOLD II and III) and COPD phenotype with elevated levels of cardiovascular risk.

MATERIALS, SUBJECTS, METHODOLOGY AND PLAN: This will be a cross-sectional study with a total of 180 subjects, in three groups (COPD, non-COPD-smokers, non-COPD-non-smokers). Beside anthropometric measurements and traditional cardiovascular risk factors assessment, elements of COPD (phenotype of COPD, BODEx index, spirometry, bronchodilatation test, and hemoglobin oxygen saturation) will be examined with measurement of pulse wave velocity and aortic augmentation index using TensioMed arteriography. Subjects will be evaluated with SCORE and Framingham 10-year risk for cardiovascular disease.

EXPECTED SCIENTIFIC CONTRIBUTIONS: In our study, we will compare of the elements of arterial stiffness in patients with COPD and control groups, additionally evaluate cardiovascular risk using the variable arterial stiffness. We will determine to what extent the severity of COPD is associated with increased cardiovascular risk and test whether smoking is associated with changes in arterial stiffness. The results of this study should increase awareness of clinicians about the importance of preventing and reducing the increased cardiovascular risk in patients with COPD, which would ultimately lead to a reduction in morbidity and mortality.

Keywords: COPD, atherosclerosis, arterial stiffness, cardiovascular risk

ACUTE EXACERBATIONS OF COPD (CRONIC OPSTRUCTIVE PULMONARY DISEASES) - RELATIONSHIP OF INFECTIVE ETIOLOGY AND PULMONARY FUNCTION

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Mentor: Associate Professor J.Škrlin Šubić, MD, PhD

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INTRODUCTION: European COPD Coalition and UK deputy calls for more awareness of 'fifth biggest killer'. Start of antimicrobial treatment of acute lower respiratory tract infections acute exacerbations of COPD is usually empirical. Most of the guidelines in patients with no co-morbidity or risk factors recommend per os macrolide or doxyciclyn, and in patients with risk factors with combination of β -lactam and macrolide or quinolone. COPD is the fourth leading cause of adult death. COPD slowly damages airways. COPD mainly caused by smoking. Long-term exposure to other lung irritants, such as air pollution, chemical fumes, or dust, also may contribute to COPD.

HYPOTHESIS: The hypothetical is assumed that the isolates differ depending on the degree (0-IV) of COPD according to the GOLD (Global Initiative for Chronic Obstructive Lung Disease).

AIMS: Significantly, it would be determined at the Croatian to correlate bacterial, atypical bacteria and viruses in relation to lung function in AE of COPD according to GOLD. This could determine the appropriate antimicrobial therapy and guidance in the selection of antibiotics.

MATERIALS AND METHODS: We examined 110 patients with AE COPD and to treat bacteria, atypical bacteria and most frequently viruses. Results of sensitivity to antimicrobial agents was followed during years 2007, 2008 and 2009 for isolates of: *S. pneumoniae*, *H. influenzae*, *K. Pneumoniae* and *P. aeruginosa*. Sensitivity was tested with diffusion method according to CLSI standards.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: The scientific contribution of this paper consists in acquiring new knowledge about the etiology and pathogenesis of acute exacerbation of COPD in Croatia. This research will contribute to the actual state of acute exacerbation of COPD with the movement of viral or bacterial infections and their antibiotic sensitivity, also show how external factors, unknown etiology (smoke, allergens, etc.), the trigger for the start of acute exacerbations.

MeSH/Keywords: acute exacerbation; bacterial; viruses; chronic obstructive pulmonary disease; infective agents; pulmonary function;

MSCT CORONARY ANGIOGRAPHY IN PATIENTS WITH SUSPECTED CORONARY ARTERY DISEASE

Part of a Thesis: Evaluation Of The Clinical Use Of MSCT Coronary Angiography In Patients With Suspected Coronary Artery Disease

PhD candidate: Hrvojka Bošnjak, MD

Mentor: Assistant Professor Hrvoje Pintarić, MD, PhD

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

INTRODUCTION: MSCT coronary angiography is a new and still evolving noninvasive diagnostic procedure. Due to improvements in technology in the past decade and advanced spatial and temporal resolution of the CT devices, MSCT coronary angiography is a reliable diagnostic method for pathomorphological analysis of the coronary arteries. However, practice guidelines and firm indications for its application in the clinical practice are not yet available, due to lack of the large multicentre clinical studies with the adequate follow up period.

HYPOTHESIS: MSCT coronary angiography as a method of choice for detection of coronary artery disease is clinically relevant in patients with intermediate cardiovascular risk (PROCAM 10-20%).

AIMS: (1) To evaluate the present clinical use of the MSCT coronary angiography in symptomatic patients with suspected coronary artery disease, in correlation to their risk stratification group (PROCAM - low, intermediate, high risk); (2) To define the cut-off value of the PROCAM risk score as a predictor of the severity of stenosis detected by MSCT coronary angiography (<50% / ≥50%); (3) To define the prevalence of high Calcium score (>400) in each risk stratification group

MATERIALS AND METHODS: Patients with suspected coronary artery disease, who are referred for MSCT coronary angiography from January 2009 to March 2011, will be included in our retrospective study. Patients with prior myocardial infarction, CABG or PCI will be excluded. Patients' characteristics including age, sex, systolic and diastolic blood pressure, cholesterol, HDL, LDL, triglycerides, family history of CAD, diabetes and smoking will be recorded. According to the PROCAM risk score, patients will be divided into three risk stratification groups (low, intermediate, high risk). Calcium score, radiation dose and morphology of coronary arteries will be evaluated.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: The results of our study should define the selected group of patients with suspected, but not previously documented coronary artery disease, that may actually benefit from the MSCT coronary angiography. Defining the PROCAM cut-off value should improve the present clinical practice and reduce clinical dilemmas in indications for this diagnostic procedure. The results should contribute to the level of evidence for the clinical use of MSCT coronary angiography.

Keywords: MSCT coronary angiography, PROCAM, risk, CAD, radiation dose

RADIOLOGIC FRACTURE PATTERNS IN CRANIOFACIAL TRAUMA

Part of a Thesis: Role of defined computed tomography protocol in evaluation of the associated facial and skull base fractures

PhD candidate: Jasminka Igrac, MD

Mentor: Gordana Ivanac, MD, PhD

Affiliations: Clinical Institute for Diagnostic and Interventional Radiology, Clinical Hospital Dubrava; Department for Radiology and Ultrasound, County hospital Čakovec

INTRODUCTION: Craniofacial fractures are complex injuries of the head associated with the incidence of skull base fractures ranging from 4% to 71%. Because they are not initially diagnosed, part of the skull base fractures remains unnoticed with development of late complications.

HYPOTHESIS: In patients with blunt trauma of the head there are fracture patterns of associated injuries of the facial and skull base bones.

AIMS: To determine the value of the defined radiological CT protocols in the diagnosis of skull base fractures associated with fractures of facial bones; to establish the occurrence of radiological diagnosed skull base fractures associated with facial fractures in all examined groups; to describe the imaging characteristics of facial fractures expanding to the base of the skull in acutely traumatized patients; to classify certain types of fractures in the radiologic fracture patterns; to determine the relationship between radiologic findings of associated fractures of the skull base and facial bones, contusion markers, radiologically detectable, posttraumatic, pathologic changes of the neurocranium and viscerocranium and clinical outcome; to complement the existing global knowledge on epidemiological data of associated skull base and facial fractures.

MATERIALS AND METHODS: Study group includes adult patients of both sexes, with blunt head trauma, in which multislice computed tomography examination of the head was performed. **EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH:** Fracture pattern is the mode of blunt force translation through the bones of the skull base and facial bones which induces fracture. Reevaluation of radiologic findings in purpose of defining fracture patterns, correlation with radiologically detected associated injuries and clinical criterion will help determine the value of the defined radiological CT protocols in the diagnosis of skull base fractures associated with fractures of facial bones

MeSH/ Keywords: blunt head injury, facial bone fracture, skull base fracture, computed tomography, multiplanar reconstructions

CT PERFUSION IN HEAD AND NECK CANCER

Part of a Thesis: How Perfusion CT Parameters of Head and Neck Squamous Cell Cancers Correlate with Response to Chemo-radiotherapy

PhD candidate: Hrvoje Vavro, MD

Mentor: Professor Marko Radoš, MD, PhD

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INTRODUCTION: Squamous cell carcinoma (SCC) of head and neck makes up to 95% of malignant head and neck tumors, and is among most frequent malignant tumors. It is strongly associated with smoking and excessive alcohol consumption. The most frequent location is in the oropharynx, floor of mouth, anterior tongue and mandibular gingiva. The complexity of regional anatomy and non-specific post-treatment changes make initial staging and assessment of the treatment response difficult - there's often need for functional imaging, such as MR and CT perfusion and PET-CT. It has been shown before that perfusion CT (PCT) can discern benign from malignant neck lesions, and several studies show that there are perfusion differences within malignant lesions, as well. Baseline tumor perfusion values appear to have predictive value for chemotherapy and radiation therapy response.

HYPOTHESIS: Baseline tumor perfusion parameters are predictors of malignant head and neck tumor chemo- and radiation therapy response. Higher baseline blood flow (BF), blood volume (BV) and surface permeability product (PS) and lower mean transit time (MTT) indicate a better responding tumor.

MATERIALS AND METHODS: Thirty patients with pre-treatment contrast-enhanced CT (CECT) exams and biopsy-proven oropharyngeal SCC will be scanned ahead of therapy using perfusion-CT protocol on a 16-slice computed-tomography scanner. Fourty milliliters of non-ionic iodinated contrast media will be used per patient intravenously (injection rate 6 ml/s). Follow-up exam will take place within 4 weeks of therapy completion. PCT parameters, tumor volume, RECIST criteria for tumor response and midterm progression-free survival (PFS) will be described and analyzed by Kaplan-Meier curves, log rank tests and Cox proportional hazard regression models.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: If CT perfusion proves to be predictive for tumor (chemo)radiation response, it would become an integral part of treatment planning in patients with head and neck cancer. Reliable distinguishing residual malignancy from post-treatment changes by CT perfusion would potentially obviate the need for PET-CT exams.

Acknowledgements: I would like to thank Department of Radiology and Radiological Science, Medical University of South Carolina, Charleston, SC, USA, especially professor Zoran Rumboldt, M.D, Ph.D, for providing data and training on this research.

MeSH / Keywords: CT perfusion, head and neck cancer, SCC, BF, BV, MTT, PS

THE ROLE OF ALCOHOL IN VIOLENT DEATHS

Part of a Thesis: The Role of Alcohol in Violent Deaths of Persons Autopsied in the Department of Forensic Medicine and Criminology, Medical School University of Zagreb, from Year 2001 to 2010

PhD candidate: Petar Škavić, MD

Mentor: Professor Milovan Kubat, MD, PhD

Affiliation: Department of Forensic Medicine and Criminology, School of Medicine, University of Zagreb

INTRODUCTION: Health problems associated with the use and misuse of alcohol include increased mortality of natural causes and violent deaths. Violent death due to alcohol abuse can result from unintended events, accidents, or they may be caused by events with intent of ending ones life.-

HYPOTHESIS: Alcohol intoxication is a significant factor in all forms of violent deaths, in suicide and homicide, as well as in traffic accidents, fires, falls and asphyxias. Fatalities of sober persons in similar events are much rarer, as well as positive blood alcohol in people who die of natural causes.

AIMS: To prove that the frequency and alcohol intoxication level in persons who die of unnatural causes is greater than in those who die of natural causes.

MATERIALS AND METHODS: Materials are autopsy protocols and toxicological findings for persons died of unnatural deaths autopsied at the Department of Forensic Medicine and Criminology, Medical School in Zagreb from year 2001 to 2010. The observed data will comprise of demographic data, blood alcohol levels and subtype of violent death (1. suicide 2. homicide, 3. traffic accidents 4. nontraffic accidents). All persons will also be grouped in one of seven subgroups according to their blood alcohol levels at the time of death. The projected number of violent deaths in the time period is around 4000. We will have a matching number of persons who died of natural causes and their according alcohol levels. Statistical analysis will be done mostly by using χ^2 test and for some variables we will use frequency distribution and Student's t-test or Mann Whitney U-test where applicable. The occurrence with a p value of $p \leq 0.005$ will be considered statistically significant.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: This will be the first complete overview of effects alcohol has on unnatural deaths in this area. It will offer a possibility to compare effects of alcohol on various subtypes of violent deaths to each other as well as to alcohol levels in persons who die of natural causes. It will give objective, quantitative and statistically processed data that will indicate whether alcohol does and if it does in what measure it affects violent deaths in our population.

MeSH / Keywords: blood alcohol concentration (BAC), violent death

ACHILLES TENDONS ELASTOGRAPHY AFTER RUPTURE AND SURGICAL RECONSTRUCTION

Part of a Thesis: The Value of Ultrasound Elastography Assesses the Risk Achilles Tendon Rupture and Tendon Healing After Injury

PhD candidate: Domagoj Lemac, MD

Mentor: Associate Professor Gordana Ivanac, MD, PhD

Affiliation: University Hospital Dubrava-Zagreb

INTRODUCTION: Rupture of the Achilles tendon is a serious injury that usually occurs in previously healthy middle aged men in sports. Some are known risk factors as gender, age, increased body weight, heritage, taking certain medications (corticosteroids, levofloxacin) that correlate with tendon injury. Excluding gender no risk factor could be clearly linked to injury. It is known that the elasticity of tendons decreases with age and tendon with decreased elasticity are more prone to rupture.

HYPOTHESIS: Patients with ruptured Achilles tendons have altered the structure of the tendon tissue, which leads to a decrease in elasticity and thus to an increased risk of rupture.

AIMS: To compare the findings of ultrasound elastography in patients with ruptured tendon with the control group and try to estimate the increased risk of injury.

MATERIALS AND METHODS: The study included 35 patients with complete Achilles tendon rupture who were surgically treated in the Clinical Hospital Dubrava, Zagreb in the period from 2006-2011. All patients were male, mean age 37.3 (31-47) years. For the control group we selected 35 healthy male volunteers, mean age 36.3 (29-44) years. Achilles tendon ultrasound elastography is made to all patients and subjects in the control group on Supersonic ultrasound device, Aixplorer^R model. Both Achilles tendons were examined in both groups of patients (total 140 tendons). In repaired tendons elastography was performed in a different period after surgery, average 27.1 (3-57) months. We will compare the tendons after reconstruction with the findings in the control group, and particularly tendons on the contralateral side of patients with tendon injuries to the control group. All patients will fill out a questionnaire on subjective treatment outcome and functional results of treatment will be assessed by ankle-hindfoot scale (AOFAS).

EXPECTED CONTRIBUTION OF THE PROPOSED SCIENTIFIC RESEARCH: Ultrasound elastography is a noninvasive and simple method for testing of tissue elasticity. Our study will compare the elastographic findings of healthy tendon in patients with contralateral rupture with the control group and thus examine the value of the method in assessment of Achilles tendon rupture risk. Furthermore, we will try to determine the value of the elastographic findings to assess the healing of the tendon in different period after surgical reconstruction, and evaluate the correlation of findings with the functional and subjective results of treatment.

MeSH / Keywords: Achilles tendon, ultrasound, elastography, tendon injury

FAMILY VIOLENCE: PSYCHOSOCIAL TREATMENT OF FAMILY VIOLENCE OFFENDERS

Part of a Thesis: Effect of Psychosocial treatment of Family Violence Offenders to stop violence behavior

PhD candidate: Tatjana Katkic Stanic; social worker

Mentor(s): Mirjana Grubisic, MD, PhD.; Gordana Flander Buljan, Psychologist, PhD

Affiliation(s): Treatment Centers for a family violence offenders; Ministry of social policy and youth

INTRODUCTION: When the awareness of society on the problem of family violence increases, legislative regulations change and make it possible for state institutions to treat families with violence occurrence. Laws foresee the possibility for protection measures for the offender such as participation in psychological treatment for the elimination of violent behavior. Psychosocial treatment includes group procedures that are carried out by specially trained professionals in the aim of stopping and preventing violence on the family, and the procedures include achieving insight and accepting the responsibilities for one's violent behavior, studying social skills and changing convictions that lead to violent relationships.

HYPOTHESIS: Psychosocial treatment of family violence offenders is increasing security for family members exposed to violence and change their behavior and convictions.

AIMS: The aim is to investigate the effect of psychosocial treatment; to investigate the effect of learning the positive, equal, and non-violent forms of relationships; to investigate individual disturbance and pathology, stress result, substance abuse, and non-functional partner relationships; to collect evaluation tools; to redesign, recommend and propose criteria of psychosocial treatment, through which social and medical aspects of violence can be evaluated.

MATERIALS AND METHODS: At the beginning; in the middle and the end, and six month after psychosocial treatment by using methods of structured interview and questionnaire with violence offenders and wives I will assess the effects of treatment on stopping violent behavior during and after treatment which should have an effect on increasing security of entire family, and establish equal partner relationship. Also I will compare family violence offenders who were included in psychosocial treatment with those who were not included.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: The scientific contribution of this research is the assessment of the psychosocial treatment of family violence offenders is increasing security for the family members; prevention of future violent behavior, prevention of disturbances in behavior that occur with children exposed to family violence, as well as serious emotional and psychological disturbances and establish equal and functional partner relationship.

Acknowledgements: I wish to thank the representatives of family violence offenders and families, as well as all the experts in Treatment Centers

MeSH / Keywords: family violence, behavior, psychosocial treatment, security

COMPARISON BETWEEN REAL-TIME PCR TEST (*SEPTIFAST*[®]) AND BLOOD-CULTURES IN PATIENTS WITH FEBRILE NEUTROPENIA

Part of a Thesis: Comparison between Molecular and Standard Diagnosis of Bacteremia and Fungemia in Patients with Febrile Neutropenia and Malignant Disease

PhD candidate: Davorin Herceg, MD

Mentor: Professor Vanda Plečko, MD, PhD

Affiliation: University Hospital Centre - Zagreb

INTRODUCTION: Febrile neutropenia (FN) is an oncological emergency defined as temperature 38.3°C and more and as an absolute neutrophil count less than $0.5 \times 10^9/l$. Initial evaluation of FN should focus on determining potential sites of infection and causative microorganisms, and on assessing the patient's risk of developing infection-related complication. The most accurate prediction rule to assess risk of FN is from the Multinational Association of Supportive Care in Cancer (MASCC). Conventional microbiological diagnostics of bacteremia - blood cultures at initial evaluation of FN are positive in only 30%, therefore the empiric broad spectrum antibiotic therapy is standard of care promptly at first sign of infection. In order to improve the outcome of disease, better microbiological characterisation of FN is needed (more sensitive, specific and more rapid test). Novel real-time PCR-based molecular biological assays are emerging for rapid detection of pathogens causing bloodstream infections, but no test could substitute conventional cultures so far.

HYPOTHESIS: The first hypothesis in this study is that molecular assay of blood stream infection in patients with FN has better predictive value than blood culture, and second is that MASCC score ≥ 21 is connected with favourable final outcome of FN.

AIMS: First aim is to improve the identification of FN causative microorganisms and second is to verify the usefulness of MASCC score to determine the high risk group for complications of FN.

MATERIALS AND METHODS: The patients hospitalised due to FN will be randomised in the study. MASCC risk and other relevant clinical data at initial clinical evaluation, before initial empiric antibiotic therapy, will be assessed. Real-time PCR will be molecular test of choice. Based on the 16S rRNA gene sequences, primers for Gram-positive and Gram-negative bacteria and fungal pathogens will be used.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: Scientific efforts could not demonstrate contribution of molecular tests in detection of microbiological pathogens so far. Scientific contribution of the study could be new insights about the use of molecular techniques in diagnostics of blood stream infections in patients with FN, and the impact of MASCC score on the microbiological diagnosis of FN.

Acknowledgements: I wish to thanks to my mentor Professor Vanda Plečko for her engagement, advice and review of my PhD thesis.

Keywords: febrile neutropenia, MASCC score, bacteremia

PREVALENCE OF HPV INFECTION AND HIGH-RISK HPV GENOTYPING IN KOSOVAR FEMALE POPULATION

Part of a Thesis: Prevalence of HPV infection and High-risk HPV genotyping in Kosovar female population

PhD candidate: Pranvera Zejnullahu-Raçi MD

Mentor(s): Prof.dr. Adriana Vince MD, PhD

Affiliation(s): University Clinical Center of Kosova, and Department of Cell Immunology and Molecular Diagnostics at University Hospital for Infectious Diseases „Dr. Fran Mihaljevic“ Zagreb

INTRODUCTION: Statistical analyses released from the World Health Organization (WHO) suggest that cervical cancer is the second most common cancer in women worldwide. The most important risk factor for the development of cervical cancer is the presence of human papillomavirus, HPV type, and persistence of HPV infection. Recently, it has been demonstrated that over 99% of cervical cancers have detectable HPV DNA sequences, with the majority of cancers associated with specific HPV types, such as HPV 16 and 18. In Kosovo, till now, there are no data about the prevalence of HPV infection, neither which high-risk HPV types are related with cervical cancer.

HYPOTHESIS: HPV 16 and 18 are more prevalent high-risk HPV subtypes in Kosovar female population

AIMS: to analyze the prevalence of HPV infection and the prevalence and distribution of HPV high-risk subtypes among Kosovar female population.

MATERIALS AND METHODS: Our study is designed as observational cross-sectional study. The study will include in total 360 women from 5 regions of Kosovo to represent the real distribution and prevalence of HPV infection. Prior to enrolment in the study, all participants will fill out a questionnaire. The women included in our study the conventional PAP test smear will be performed, and analysis of PAP smear will be done in the Laboratory of Cytology, Clinic of Obstetrics and Gynecology in the University Clinical Center of Kosova. The samples collected for HPV detection and Genotyping will be collected, preserved and then will be transported in the Department of Cell Immunology and Molecular Diagnostics at University Hospital for Infectious Diseases „Dr. Fran Mihaljevic“ in Zagreb for further processing. HPV detection will be performed by Hybrid Capture 2 HPV test (Qiagen, USA, FDA approved) and samples that are HPV DNA positive will be further individually genotyped by using INNO-LiPA Extra Genotyping test (Innogenetics, Belgium).

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: As in Kosovo there has not been studied the prevalence of HPV, this study will help to reveal the real situation with HPV infection related with different socio-epidemiological factors. Important clinical contribution may be the fact that our results can help in developing a real overview on primary and secondary care for cervical cancer.

PENTADECAPEPTIDE BPC 157 AND ANAPHYLACTOID REACTION IN RATS AND MICE AFTER INTRAVENOUS DEXTRAN AND EGG WHITE ADMINISTRATION

Part of a Thesis: Pentadecapeptide BPC 157 antagonizes anaphylactoid reaction

PhD candidate: Božidar Duplančić, MD

Mentor: Associate Professor Predrag Sikirić, MD, PhD

Affiliations: University of Zagreb School of Medicine, University Hospital Centre - Split

INTRODUCTION: Anaphylactoid reactions have been recognized as infrequent but life-threatening responses to intravenous dextran therapy. Severe dextran-induced anaphylactoid reactions are characterized by bronchospasm, severe hypotension, cardiorespiratory arrest, and/or death. Likewise, they share the same characteristic presentation with egg white application in rats and mice. We will focus on stable gastric pentadecapeptide BPC 157 commonly shown to have marked anti-inflammatory effect in various inflammatory models while its effect on dextran and/or egg white-induced anaphylactoid reaction when given intravenously in rats and mice haven't been tested.

HYPOTHESIS: Pentadecapeptide BPC 157 prevents develop of dextran and/or egg white induced anaphylactoid reaction as well as treats developed one.

AIMS: It is expected that pentadecapeptide BPC 157 reduces dextran and/or egg white induced edema of the face, upper and lower lip, snout, paws and scrotum (presented with extreme cyanosis), poor respiration and the number of fatalities after dextran and/or egg white application.

MATERIALS AND METHODS: Male Wistar Albino rats or NMRI-mice (10 animals per each group) randomly assigned will be used in all experiments. Anesthetized animals will receive intravenously 6%, 10%, 20%, 40%, 60%, 80%, 90% dextran solution and/or egg white (1 ml/rat or 0.15 ml/mouse) into their tails. Medication (5 ml/kg) will be given intraperitoneally (BPC 157 10 μ g, 1 μ g, 10 ng, 10 pg/kg, chloropyramine 20mg/kg, cimetidine 10 mg/kg, alone or in combination), while controls will receive an equivolume of saline, immediately after challenge or, alternatively at 5 min after or 24 or 48 h before challenge. The effect will be assessed at 5, 10, 20 and 30 min after dextran and/or egg white challenge as follows: score 0 normal presentation; score 1 small suborbital edema; score 2 prominent edema involving face, upper and lower lip, snout; score 3 = score 2 + paws and scrotum; score 4 = score 3 + apparent cyanosis; score 5 = score 3 + extreme cyanosis and poor respiration. Also, the number of fatalities will be calculated.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: BPC 157 could have beneficial effect in prevention and treatment of other types of hypersensitivity reactions.

Mesh / Keywords: gastric pentadecapeptide BPC 157, anaphylactoid reaction, dextran, egg white, rats, mice

ROLE OF MICROBIOLOGICAL SEMEN ANALYSIS IN CHRONIC PROSTATITIS SYNDROME DIAGNOSIS

Part of a thesis: Rational diagnosis of the chronic prostatitis syndrome

PhD candidate: Dražen Kovačić, MD

Mentor: Professor Višnja Škerk, MD, PhD

Affiliations: University Hospital for Infectious Diseases (UHID) - Zagreb; Medical Affairs, PLIVA Croatia

INTRODUCTION: Prostatitis syndrome (PS) is heterogenous and complex disorder so knowledge about its etiology, diagnosis and therapy is constantly reviewed. Today's standard for research is a classification system approved by a NIH-NIDDK workshop committee in 1999 which divide PS into four categories: Category I Acute Bacterial Prostatitis (ABP); Category II Chronic Bacterial Prostatitis (CBP); Category III Chronic Prostatitis/Chronic Pelvic Pain Syndrome (CP/CPPS); Category IV Asymptomatic Inflammatory Prostatitis (AIP). To differentiate CBP from an abacterial form of PS i.e. CP/CPPS, traditional Meares and Stamey method of four-glass test are used for segmented analysis of urine and expressed prostatic secretions (EPS) specimens. This method is as standard recommended by European association of urology (EAU). Some authors offered method of two-glass test (synonym is pre-massage and post-massage two-glass test) as simplified procedure. As it is often suggested that these segmented tests do not display sufficient sensitivity, some authors have proposed the usefulness of adding semen analysis to a standard four-glass test (five-glass test) for the detection of prostatic pathogens. However, there are few published studies about semen analysis in PS, and these with controversial results.

HYPOTHESIS: Microbiological analysis of semen improves diagnosis of chronic prostatitis syndrome when it is used to complement standard four-glass test.

AIMS: To find frequency and type of traditional microorganisms identified in EPS and urine voided after prostatic massage (VB3) by method of four-glass test plus in semen, and then determine if adding microbiological analysis of semen to a standard four-glass test improve sensitivity of chronic prostatitis syndrome diagnosis.

MATERIALS AND METHODS: We plan to prospectively exam over 100 men 18 years of age and older with signs and symptoms of chronic prostatitis syndrome. All patients will be routinely subjected to the four-glass test, followed by semen culture and analysis. Two diagnostic scenarios will be used to compare relative sensitivity of four-glass test with a five-glass test where each patient will be control to himself.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: Significant contribution to the knowledge about rational diagnosis of chronic prostatitis syndrome, since research in this area is scarce with controversial results.

Acknowledgements: we are indebted to all patients included in this study. We thank Croatian Science Foundation for funding the project „Research of etiology, epidemiology, diagnosis and treatment of patients with prostatitis syndrome“ which this research is part of, and to Zdenko Sonicki MD, PhD, Senior Lecturer, for support in statistical methodology.

MeSH / Keywords: prostatitis syndrome, microbiological diagnosis, four-glass test, two-glass test, five-glass test, semen analysis.

INFLUENCE OF VALPROATE ON RAT POSTIMPLANTATION EMBRYO IN AN EX VIVO DEVELOPMENTAL MODEL (PHD THESIS APPLICATION)

Part of Thesis: Influence of valproate on rat postimplantation embryo in an ex vivo developmental model

PhD candidate: Milvija Plazibat, MD, MSc

Mentor: ¹Professor Floriana Bulić-Jakuš, MD, PhD

Affiliation: ¹University of Zagreb, School of Medicine, Department of Medical Biology, ²General Hospital Zabok, Department of Pediatrics

INTRODUCTION: Valproate causes teratogenic side-effects in humans. That activity has lately been assigned to Histone Deacetylase inhibition (HDAC inhibitor- an epigenetic drug class). Valproate exerted strain-specific differences in the mouse (Downing et al. 2010). In *Xenopus* and Zebra fish gastrulation, it significantly diminished growth of embryos, caused pericardial edema and ocular malformations more pronounced in Zebra fish (Gurvich i sur., 2005). In rat WEC (whole embryo culture) valproate caused apoptosis and neural tube defects associated to oxidative stress (Tung i Winn, 2011). In the ex vivo model established at the Department of Medical Biology, School of Medicine, Zagreb, dealing with the embryo-proper development, it is possible to follow up embryonic development for a longer period of time (14 days) than in WEC (2 days) (Šreb and Crnek 1980). Moreover, embryo-proper and ectoplacental cone can be investigated separately. Embryonic shield forms a teratoma-like structure in which main derivatives of three germ layers differentiate. In serum-supplemented medium, optimal growth is obtained, while in chemically defined conditions targeted differentiation and embryotoxicity is more easily demonstrated (Bulić-Jakuš et al. 1999; 2001). Depending on culture condition, differences in remaining developmental potential were discovered after transplantation in vivo (Bulić-Jakuš et al. 2006).

HYPOTHESIS: In the ex vivo model of rat postimplantation development, valproate will negatively affect development.

AIMS: The influence of valproate upon 9,5-days-old rat embryo-proper and ectoplacental cone ex vivo will be investigated. By the ectopic in vivo transplantation, the remaining developmental potential will be investigated.

MATERIAL AND METHODS: Under the binocular microscope, 9,5-day-old Fisher rat embryos will be microsurgically isolated. Embryonic shields and ectoplacental cones will be cultivated in Eagle's MEM and rat serum (1:1) at the air-liquid interface during two weeks with or without valproate (0,60; 1,2mMol/l). Some explants will be transplanted under the kidney capsule. Histological analysis, immunohistochemical assessment of PCNA expression, cleaved caspase-3 and acetylated histone-3, stereological quantification and statistical analysis will be performed.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH:

Original results about valproate impact upon the embryo and ectoplacental cone (contributing to placental development) of importance for better understanding of teratogenic effect of valproate are expected.

Acknowledgements: This thesis is supported by MZOŠ project No 108-1080399-0335 (project leader F. Bulić-Jakuš)

MeSH/Keywords: valproate, embryonic shield, ectoplacental cone, rat, ex vivo, transplant

INFLUENCE OF PENTADECAPEPTIDE BPC 157 AND ESTROGEN ON UROGENITAL ATROPHY IN OVARIECTOMIZED RATS

Part of a Thesis: Influence of pentadecapeptide BPC 157 and estrogen on urogenital organs in ovariectomized rats

PhD candidate: Karlo Tomičić, MD

Mentor: Associate Professor Hrvoje Vrčić, MD, PhD

Affiliations: University of Zagreb School of Medicine, University Hospital Centre - Zagreb

INTRODUCTION: Ovariectomy in rats causes a deficiency of estrogen which leads to changes like urogenital atrophy, osteopenia, changes in lipid and carbohydrate metabolism. Previous research of the application of BPC 157 in animal models of other diseases found that BPC 157 has a positive effect on genital atrophy but those findings were not systematically investigated.

HYPOTHESIS: Pentadecapeptide BPC 157 has a favorable effect on the urogenital organs of ovariectomized rats.

AIMS: To verify the effect of BPC 157 on vagina, vulva and urinary bladder in ovariectomized rats and to compare the effect with estrogen.

MATERIALS AND METHODS: Female albino Wistar rats will be randomized into 10 groups of 10 animals after ovariectomy. Abdominal cavity of anesthetized rat will be opened by medial longitudinal incision 3 cm wide and both ovaries will be ligated and removed. The control group will receive 1 ml 0.9% NaCl solution intraperitoneally (IP), while the treated groups of animals will receive BPC 157 IP at a dose of 10 mg/kg and 10 ng/kg; and estradiol at a dose of 0.5 mg/kg; and a combination of both drugs. Alternatively, the animals will receive BPC 157 in drinking water at dose of 10 mg/kg and 10ng/kg; and the combination with estrogen. Administration doses were chosen according to previous studies. The animals will be treated daily for 8 weeks and then sacrificed. Uterus, urinary bladder, distal part of the vagina and vulva are going to be removed. Evaluation will include animal weight at time of ovariectomy and sacrifice, distance between the clitoris and anus, uterine weight, gap between the two uterine horns and bladder weight. The organs will be fixed in formalin and then blend in paraffin wax so they could be cut, dyed and histologically analyzed. Nonparametric tests of Kruskal-Wallis and Mann-Whitney U test will be used for the statistical analysis. Fisher's exact test will be used for comparison of histological changes. A statistically significant value will be considered when $p < 0.05$.
EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: Establish a favorable effect of BPC 157 on urogenital organs after ovariectomy.

Acknowledgements: I would like to thank Professors Vrčić and Sikirić for their help and guidance that made this study possible.

MeSH / Keywords: BPC 157, estradiol, ovariectomized rat, urogenital atrophy

MOLECULAR CYTOGENETIC APPROACHES TO NEURODEVELOPMENTAL DISORDERS

Part of a Thesis: Molecular cytogenetic approaches to neurodevelopmental disorders

PhD candidate: Martina Rinčić, M. Sc.

Mentor: Associate Professor Lukrecija Brečević, PhD

Affiliations: University of Zagreb School of Medicine, Croatian Institute for Brain Research; Friedrich-Schiller-University Jena, University Hospital Jena, Institute for Human Genetics

INTRODUCTION: Intellectual disability (ID) and Autism spectrum disorders (ASD) are common neurodevelopmental conditions. Genetic factors, in particular chromosomal abnormalities are important etiological cause of ID. Recent studies suggest increasing involvement of subtle aberrations in ASD. Although extensively studied, in many instances the etiology of ID and ASD remains unexplained. It is thought that in most undiagnosed cases the genetic factors play a crucial role as well.

HYPOTHESIS: It is assumed that the latest molecular cytogenetic techniques will increase the detection rate of subtle chromosomal aberrations involved in ID and ASD, and identified new causes of neurodevelopmental disorders.

AIM: The objective of the study is to uncover and molecularly characterize subtle rearrangements associated with ID and ASD by applying a battery of novel molecular cytogenetic techniques.

MATERIALS AND METHODS: About 400 subjects with ID and ASD of unknown etiology will be studied. The research is divided into six phases. First phase encompasses a blood sampling, cell culture preparation and isolation of genomic DNA. In the second phase genome-specific screening will be carried out using specific Multiplex Ligation-dependent probe amplification (MLPA) technique. Specific MLPA probe sets are applied upon medical documentation and medical evaluation. In the third phase a high resolution karyotyping (≥ 550 bands/genome) will be performed in all 400 subjects. This is followed by verification/confirmation of all obtained positive results by Fluorescence In Situ Hybridization (FISH) techniques and/or DNA sequencing. In the fifth phase array Comparative Genomic Hybridization (aCGH) on 50-100 subjects with normal MLPA screening and normal hr-karyotype will be performed using Agilent oligonucleotide array chips 4x180K. Last phase represents processing and analysis of collected data.

THE EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: The proposed research could potentially discover new chromosomal regions/genes associated with ID and ASD. Identification of specific genetic loci would allow direct molecular characterization of the affected region and a better genotype-phenotype correlation.

Acknowledgements: I would like to thank Institute for Human Genetics, Jena for financial and technical support, also I want to express my gratitude to PD Dr. Thomas Liehr for all support and help during my research work.

MeSH/Keywords: Intellectual disability, Autism spectrum disorder, chromosomal aberration, Karyotyping, Multiplex Ligation-dependent probe amplification, DNA Sequence analysis, Fluorescence In Situ Hybridization, array Comparative Genomic Hybridization

ANTI-GLUTAMATERGIC EFFECT OF CEFTRIAXONE-THE INFLUENCE ON BEHAVIOUR OF LABORATORY ANIMALS

Part of a Thesis: Anti-glutamatergic Effect of Ceftriaxone – The Influence on Behavior of Laboratory animals

PhD candidate: Marina Zmajevic Schonwald MD, MSc, neurologist

Mentor: Ante Tvrdeic, MD, PhD, Professor of Pharmacology

Affiliation(s): Department of Pharmacology, Medical School, University of Zagreb, Clinical medical center „Sisters of Mercy” Zagreb

INTRODUCTION: The classical effect of beta-lactam antibiotics on central nervous system was the epileptogenic effect. However, recent researches showed that several beta-lactams, especially the ceftriaxone (CFTX), increase the expression of GLT1 glutamate transporter, which inactivates synaptic glutamate. Consequently, CFTX blocks glutamatergic neurotransmission (ie. acts as antiglutamate drug).

HYPOTHESIS: CFTX affects spatial memory and anxiety in DBA/2J and C57BL/6J mice and modulates the audiogenic convulsions in DBA/2J mice and thermal pain sensation in WZ-5HT rats. Temporal pattern of behavior changes coincides with the change of GLT1 expression in appropriate brain region. Behavioral effects of CFTX are specific and do not depend on CFTX effect on locomotor activity.

AIMS: To shed the light on behavioral consequences of antiglutamate actions of beta lactams, effects of CFTX on behavioral paradigms for memory, anxiety, epilepsy and sensomotor activity in rodents, together with the brain expression pattern of GLT1 transporter, will be studied in details.

MATERIALS AND METHODS: Parallel design trial will be conducted using mice (DBA/2 and C57BL/6-Ly5) and rats (WZ-5HT). CFTX treated group will be receiving single daily intraperitoneal (ip.) injection of CFTX during 6 subsequent days (antiglutamate treatment protocol). During the same period, control group of animals will be injected with daily ip. injection of saline (0,9% NaCl in volume of 10ml/kg). Half hour after the last injection of CTFX or saline, locomotor activity (Open field test, Beam walking test), anxiety (Dark-light exploration test, Elevated plus maze), memory (Barnes labyrinth, Novel object recognition test), sensitivity to audiogenic convulsions or thermal pain will be tested. Animal behavior will be video recorded and analyzed by using Any-maze tracking software. Later, animals will be sacrificed to make the Western blot analysis and measure the expression of GLT1 transporter in appropriate brain region. For statistical analyses we will use GraphPad software and appropriate statistical tests, with level of significance set at $p < 0,05$.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: The proposed research should resolve the open dilemmas about the anti-glutamatergic neurotropic effects of CFTX, as one of the beta-lactam antibiotics. Thereby we can also estimate the possible usefulness and safety of CFTX given in new neurological indications.

MeSH / Keywords: Beta lactams, ceftriaxone, GLT1 transporter, memory, anxiety, convulsions, rodents

BIOREACTOR-BASED ENGINEERING OF CARTILAGE

Part of a Thesis: Structural analysis of autologous osteochondral graft engineered in bioreactor in an ovine animal model

PhD candidate: Andreja Vukasovic, MD

Mentor: Assistant Professor Alan Ivkovic, MD, PhD

Affiliation(s): Department of Histology and Embryology, School of Medicine University of Zagreb, Croatia; Department of Surgery, Orthopedics and Ophthalmology, Faculty of Veterinary Medicine, University of Zagreb, Croatia; School of Cellular and Molecular Medicine, University of Bristol; Holostem Terapie Avanzate S.r.l. Centro di Medicina Rigenerativa „Stefano Ferrari”, Modena, Italy

INTRODUCTION: Currently, cartilage repair remains major challenge both for basic scientists and clinicians due to its limited healing capacity. Untreated defects progress to osteoarthritis. Although autologous chondrocyte implantation (ACI) has become golden standard for treatment of localized chondral and osteochondral defects, there are still many drawbacks associated with this method. During this treatment, it is necessary to perform two surgical procedures (one to obtain small piece of cartilage tissue from the joint, second to implant expanded chondrocytes into the defect). Basic procedures for generating engineered tissues have been based around conventional manual bench-top culture techniques which are labor-intensive and possess inherent risks of contamination, high operator variability and limited scale-up potential. Nasal chondrocytes are easier to obtain, they have better expansion capacity in culture and are responsive to physical forces resembling joint loading. Bioreactor-based manufacturing process allows full automation of bioprocesses and production of functional cartilage grafts. Autologous three-dimensional osteochondral grafts engineered in perfusion bioreactor will be studied *in vivo*.

HYPOTHESIS: It is possible to engineer viable autologous cartilage tissue grafts from nasal chondrocyte and scaffolds in perfusion bioreactor which will have as good healing capacity as autologous grafts made of articular chondrocytes and scaffolds.

AIMS: To assess nasal chondrocyte-based grafts produced in perfusion bioreactor for the treatment of articular cartilage defects and compare regenerative capacity between nasal and articular chondrocytes.

MATERIALS AND METHODS: Full-thickness cartilage defects will be created on both condyles of the posterior leg of 16 sheep. At the same time nasal and articular cartilage will be harvested for bioreactor-based production of the grafts. After six weeks sheep will be divided in 4 groups (4 for articular cartilage grafts, 4 for nasal cartilage grafts, 4 for cell free grafts, 4 empty defects) thus allowing for 8 defects in each group. 2 animals of each group will be sacrificed after 6 months and the others after 12 months postoperatively. The explants will be analyzed histologically (HE, safranin-O, ICRS-Assessment Scale), immunohistochemically (collagen I, II, aggrecan) and biochemically (glycosaminoglycans).

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: Nasal chondrocytes could become first choice cell source for treating cartilage defects in joints.

Keywords: cartilage grafts, perfusion bioreactor, tissue engineering

PRIORITIES FOR NATIONAL MEDICINES POLICY IN THE EUROPEAN UNION

Part of a Thesis: National Medicines Policy in the European Union

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Mentor: Professor Stjepan Orešković, PhD

Affiliations: University of Zagreb School of Medicine, Krka-Farma d.o.o.

INTRODUCTION: The primary role of EU pharmaceutical legislation is to safeguard public health while encouraging development of industry through creation of single market for pharmaceuticals. On the other hand, division of competences within EU ensures that member states have responsibility for organization of their health care systems. Result is shared competences space between national and EU level where evidence on effectiveness and transferability of most policy interventions is very limited. In this setting Croatia needs to formulate set of new, coherent policies in order to achieve an adequate balance between cost-containment, innovation, patients' access to medicines and health outcomes. Moreover, these policies need to be well aligned with EU objectives while bearing in mind their feasibility in light of recent economic crisis and negative demographic trends. This research will apply systematic, consensus based approach through nationwide expert panel in order to define and prioritize policy components that will meet challenges of the next decade.

HYPOTHESIS: Croatian long-term policy priorities are well aligned by content and rank with main EU objectives.

AIMS: To define content of long-term policy components and rank them. To measure consensus regarding feasibility of implementing these components as policy measures.

MATERIALS AND METHODS: Research process will include literature review and primary research using two-round Delphi survey with a panel comprising of key experts in pharmaceutical sector. Components will be organized in topics and voted on ten-point Likert scale regarding two main measures, their importance and feasibility.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: This research will for the first time, through nationwide expert panel define, rank and analyse long-term priorities for medicines policy in new EU setting, in order to accomplish main objectives regarding cost-containment, innovation, patients' access to medicines and better health outcomes. In addition, it opens space for further research regarding benchmarking of best practices between countries in order to facilitate accession process in this policy area.

MeSH / Keywords: Croatia, European Union, pharmaceutical policy, priorities

PREVALENCE OF NEW DIABETES AND IMPAIRED FASTING GLUCOSE IN KOSOVO. SCREENING FOR DIABETES IN DEVELOPING COUNTRIES MAY NEED VERY PRAGMATIC APPROACH

PhD candidate: Linda Carkaxhiu-Huseyin, MD, Sci

Mentor: Associate Professor Marija Vrca Botica, MD, PhD

Affiliations: Five local general practices in the Kosovo, Center for diabetes-Gjilan, Kosovo

INTRODUCTION: Epidemiological studies in England, America and Australia suggest that 30-50% of the overall number of diabetes is undiagnosed, therefore more screening is recommended for Type 2 Diabetes in high-risk groups. Although lacking evidence from random research if screening is cost effective or not, it is indisputable that early diagnosis and proper treatment can reduce the risk of complications. This study is designed to systematically screen for type 2 diabetes in primary care, to determine the prevalence of new diabetes and impaired fasting glucose and to investigate the feasibility and performance of a very pragmatic system for identifying patients with these two conditions.

HYPOTHESIS: The prevalence of new diabetes, and impaired fasting glucose (IFG) in Kosovo, are higher than in region, because of social and economic reasons.

MATERIALS AND METHODS: The study is a opportunistic screening programme for type 2 diabetes in primary care. All patients at risk for undiagnosed type 2 diabetes, aged 45 and above , who will require health services for any reason previously assigned to any of these five general practices, the fasting capillary blood glucose is to be measured and we will repeat the test for each patient also in the next day. In the same day we will also undertake some anthropometric measurements.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: Active case detection and diagnosis of Type 2 diabetes should be considered for the following reasons: (1)Type 2 diabetes is a serious and costly health problem, (2) The natural history of Type 2 diabetes includes an asymptomatic phase which is not benign and during which it can be diagnosed and (3) early treatment of Type 2 diabetes reduces morbidity from long term complications. This is an epidemiological research by means of which the data on the number of undetected diabetes in Kosovo is provided and indirectly the data of diagnosed Diabetes is acquired, these data have been lacking until now in Kosovo. We hope that this research will raise awareness of the decision-making instances so that in a near future we can undertake a survey at a much larger scale, at the national level.

Acknowledgements: The author wishes to thank The Medical Group for the contribution of instruments and general practitioners from five local general practices in the Kosovo who participated in this study.

MeSH / Keywords: primary care, opportunistic screening, general practices, Kosovo

HEALTH RELATED BEHAVIOURS AND HEALTH PROFILE IN STUDENTS WITH DISABILITIES

Part of a Thesis: Health Related Behaviours and Health Profile in Students with Disabilities on the University of Zagreb

PhD candidate: Marina Dimov Di Giusti, MD, MSc

Mentors: Full Professor Vesna Jureša, MD, PhD and Full Professor Rea Fulgosi-Masnjak, Psychologist, PhD

Affiliation: Institute of Public Health „Dr. Andrija Štampar” - Zagreb

INTRODUCTION: At the University of Zagreb, there are more than 65.000 students, of which, according to the University of Zagreb Office for Students with Disabilities records, about 330 students (5‰) are with disabilities. According to the data available from literature, few research is done on relationship between health related behaviours and health profile of students with disabilities.

HYPOTHESIS: There is a correlation between health related behaviours and health profile in students with disabilities.

AIMS: To explore if there is a relationship between health related behaviours and health profile of students with disabilities.

MATERIALS AND METHODS: Study population consists of female and male students with disabilities who are studying on the University of Zagreb. Expected number of participants is 200 students. In collaboration with the University of Zagreb Office for Students with Disabilities and Organisations of Students with Disabilities, students with disabilities at the University of Zagreb who voluntarily agree to participate, will be enrolled in this study. To check the hypothesis, control group of students without disabilities, who will be paired by the age, sex and faculty to the students with disabilities, will also be enrolled. Data will be collected using the Personal Health Prevention Questionnaire and SF-36 Questionnaire officially translated into Croatian language. Individual interrogation will be organized and, if necessary, adapted to the specific kind of student's disability. Subjects will be informed about the purpose of the study and will be provided with Patient Information Sheet and Informed Consent Form to be signed, if they decide to participate. Study will be performed in only one time point.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH:

The study results in this research can contribute to new scientific knowledge and it is expected to be published in scientific papers and publications. In addition, based on the obtained results, it will be possible to create guidelines for support services specifically focused on different issues related to the persons with disabilities.

Acknowledgements: I would like to thank my two co-mentors for their great support and valuable consulting

MeSH / Keywords: Adolescent, young adult, student, quality of life, SF-36, persons with disabilities

PERINATAL HEALTH CARE ORGANIZATION IN CROATIA AND PERINATAL RISK ASSOCIATED WITH LOW AND EXCESSIVE BIRTH WEIGHTS

Part of a Thesis: Perinatal health care organization in Croatia and perinatal risk associated with low and excessive birth weights

PhD candidate: Tatjana Glivetić, MD, M.S.

Mentor: Assistant Professor Urelija Rodin, MD, PhD

Affiliations: Croatian National Institute of Public Health and General Hospital Zabok

INTRODUCTION: Maternal and perinatal health outcomes are important measures of the overall population health and health care services. Perinatal mortality rate in developed EU countries is very low, and further decrease is not expected with current medical knowledge and technology. Decreasing of perinatal mortality was significantly contributed to the specific organization of perinatal health care system - regionalization. In order to improve perinatal outcomes in newborns of low and/or excessive birth weight, and for a timely transport of the most vulnerable pregnancies to a tertiary center, it is necessary to have indicators of fetal growth according to national fetal growth-curves and regional distribution of birth weights. Croatia does not have its own national fetal growth curves, and the creation of such anthropometric standards in our own population of newborns would contribute to the quality of perinatal care.

HYPOTHESIS: On health outcomes of newborns with low and/or excessive birth weight affects the organizational level of neonatal facilities.

AIMS: The aim of this study is the evaluation of perinatal health care organization according to the PERISTAT methodology using birth databases from Croatian medical institutions. From this data national fetal growth-curves will be made allowing to define the proportion of high risk neonates (those under the 10th and above the 90th percentile).

MATERIALS AND METHODS: Data from birth and perinatal mortality databases of the Croatian National Institute of Public Health in the period from 2009.- 2010. (About 85,000 births and 650 perinatal deaths) will be analyzed according to Peristat methodology and fetal growth curves will be developed. Maternity ward levels will be determined using recommendations from the Croatian Society for Perinatal Medicine. Analyses of the perinatal care indicators and the comparison of maternity wards levels will be conducted with descriptive-statistical methods.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH:

Evaluation of perinatal care in Croatia based on health-statistical indicators of birth would be helpful for targeted improvement of the regional organization of perinatal care that would achieve a decrease in perinatal mortality and morbidity and its consequences in high-risk newborns.

MeSH / Keywords: Newborns, Health indicators; Regional differences; Birth weight, Birth length, Growth reference

STRESS, BURNOUT AND DEPRESSIVE DISORDER AMONG GENERAL PRACTITIONERS IN ZAGREB COUNTY

Part of a Thesis: Stress, Burnout and Depressive Disorder among General Practitioners in Zagreb County

PHD candidate: Tanja Grdić, MD

Mentors: Assistant Professor Branko Kolarić, PhD., Bojana Knežević, PhD

Affiliations: Community Health Centre of Zagreb County, Public Health Institute of Zagreb County

INTRODUCTION: Stress, burnout and depressive disorder among doctors working in hospitals and other facilities have been the subject of research in the last several years in different countries. Occupational stress has been identified as a significant professional challenge which disturbs physical and mental health and performance at work, leading to the burnout syndrome and, consequently, depressive disorder.

HYPOTHESIS: The null-hypothesis of the research is: „Stressors at work in primary health care have an impact on the occurrence of burnout and depressive disorders among doctors.“**AIMS:** For the purpose of research, it is necessary to study the stressful conditions identified by respondents, the intensity of stress identified by respondents, the frequency of the burnout syndrome among doctors, the frequency and degree of depressive disorder among doctors, is there a difference between specific medical specialties, is there a difference between the doctors who have private practices and those who don't, and is there a link between the identification of stress and the occurrence of burnout and depressive disorder among general practitioners.

MATERIALS AND METHODS: The research will cover all general practitioners in Zagreb County. It will be carried out in the period from 1 January 2012 to 1 January 2013. By type, this is a cross-sectional study. Participation in the research will be voluntary and anonymous, and respondents will be informed in writing and orally. Respondents will receive questionnaires measuring the levels of stress, burnout and depressive disorder. The questionnaires used are the Questionnaire on Stressors at Workplace (Milošević et al., 2009), the Maslach Burnout Inventory and the Inventory for Measuring Depression (Beck). The surveys will be analysed by descriptive statistical methods, using a Chi-squared test, a T-test, and a linear regression analysis.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: The original scientific contribution of this research is the impact of occupational stress on the mental health of general practitioners in Zagreb County with a proposal of preventive measures.

KEY WORDS: stress, burnout, depressive disorder, general practitioners, Zagreb County

INTERNET ADDICTION IN ADOLESCENTS

Part of a Thesis: Internet addiction in adolescents

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INTRODUCTION: Addiction is defined as a way of behaving which is connected in insistence in some activity, which causes damages in health, social, psychical functioning and life quality in a certain time period and in determined conditions. Internet addiction is a growing global generational biopsychological, health and bio-ethical problem, currently classified as obsessive-compulsive disorder. Science is lacking data on this phenomenon, especially those related to various nationalities within Europe.

HYPOTHESIS: Hypothesis 1: Addiction and the type of Internet addiction in adolescents depends on the economic development of the country and the degree of „internetization“. Hypothesis 2: The degree of dependance on the Internet (unproblematic use of the Internet, problematic Internet use, pathological addiction) is associated with quality of life, health status, psychosocial functioning and ethical use of Internet content.

AIMS: examine the frequency of Internet addiction in participating adolescents embed obtained results into the definition using health status, quality of life, bioethical determinants and the dimensions of personality according to the theory of „the five factor model“

MATERIALS AND METHODS: Subjects are students of primary and high schools from Croatia and some countries of European Union. Internet addiction was assessed by IAT (Internet Addition Test); The scale for evaluating the ethical norms on the Internet (SEI), the quality of life has been examined by the scales of quality of life by Kolesarić - Krizmanić; five-factors model and the questionnaire of personality. Data were analyzed using tables of frequency, arithmetical middle, standard deviation, hi-Quadrate test, multiple analyses of variance and the Leven's test of equality of variance.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH:

The analyses of these data would show the difference in health status, quality of life and bio-ethical dimension in different levels of internet addiction, as well as compare internet use in Croatian and European adolescents.

Acknowledgements: I would like to express my gratitude to my mentor, Prof.dr.sc. Stipe Orešković, whose expertise, understanding, and patience, added considerably to my graduate experience. A very special thanks goes out to Rector Johanna Erkkilä, Prof. dr.sc. Wiesław Walkiewicz , prof. Wolfgang Pink, Mayor of Vaterstetten Robert Niedergesäß, Mrs. Slavici Tavara

MeSH / Keywords: Internet addiction, Internet, biopsychological aspects, Internet ethic, usage of Internet

HLA CLASS II RISK ALLELES AND HAPLOTYPES AND THEIR FREQUENCY IN PEMPHIGUS VULGARIS PATIENTS

Part of a Thesis: HLA Class II Alleles and Haplotypes and Their Frequency in Pemphigus Vulgaris Patients

PhD candidate: Ines Lakoš Jukić, MD, MSc

Mentor: Prof. Branka Marinović, MD, PhD

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INTRODUCTION: Pemphigus vulgaris is a rare, organ-specific, most severe autoimmune bullous disease, clinically characterized by blister formation in the skin and mucous membranes, histologically with acantholysis and immunopathologically with in vivo-bound and circulating antibodies against keratinocyte cell surface components; desmoglein 1 and desmoglein 3. The etiopathogenesis of pemphigus vulgaris remains poorly understood with complex interaction of genetic and environmental factors which are responsible for development and exacerbation of pemphigus vulgaris. Various studies from different countries and populations have shown that different HLA antigens or alleles confer susceptibility to pemphigus vulgaris in different ethnic populations.

HYPOTHESIS: There is a HLA class II risk allele and haplotype in our population of patients with pemphigus vulgaris which is different (or identical) from risk allele and haplotype in other populations.

AIMS: To identify susceptible HLA class II alleles and haplotypes in our pemphigus vulgaris patients; to compare frequency of identified HLA allele and haplotype with frequency of the same alleles and haplotype associations in a control group of healthy unrelated blood donors; to compare results with studies in other populations.

MATERIAL AND METHODS: The study sampled all the patients who were treated for pemphigus vulgaris at our Department between January 1st 2007 and December 31 2010. The diagnosis was based on clinical, histological, immunopathological and confirmed by ELISA testing for autoantibodies to desmoglein 1 and desmoglein 3 (MESACUP, Medical and Biological Laboratories, Nagoya, Japan). HLA typing was performed using high resolution polymerase chain reaction with sequence specific primer (PCR-SSP) (Olerup-SSP, Švedska).

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: To the best of our knowledge, this is the first study investigating susceptible HLA class II alleles and haplotype among patients with pemphigus vulgaris in Croatia. The results of our study will be compared with results from other populations. Since there is a fairly strong genetic background to pemphigus vulgaris in ethnic group of Mediterranean and Middle East origin, with higher annual incidence compared to Western Europe, it will be interesting to correlate our results of HLA typing with studies from different ethnic population.

Key words: pemphigus vulgaris, HLA class II risk alleles, haplotype

ADOLESCENT SMOKING AND MEDIA

Part of a Thesis: Smoking Habits of Adolescents In Relation To Media Messages About Smoking

PhD candidate: Dijana Mayer, MD, M.Sc.

Mentor: Professor Josipa Kern, MD, PhD

Affiliation: Hrvatski zavod za javno zdravstvo

INTRODUCTION: Tobacco use is the leading cause of morbidity and mortality globally, including also Croatia, where 1/3 of adult population smoking. According to the latest conducted GYTS, among the Croatian students aged 13-15, 2/3 of them tried to smoke at least once, while 28% currently use tobacco products. Same time, almost 3/4 of them saw pro or anti-tobacco media messages, and about 67% of all students live in homes where others smoke in their presence. Current researches of mass media messages and environment influence to the smoking habits of adolescents, determined specific interconnection, but also emphasized the necessity of further surveys on this issue. The objective of this research is, by analysing the GYTS 2011. data, determine interconnection on one side media, and on the other environment with smoking habits of adolescents in Croatia.

HYPOTHESIS: Media messages about smoking are related to smoking habits of adolescents (current smoking and life prevalence), while category and intensity of relation depends on exposure to smoking in environment, age and gender of adolescents.

AIMS: Overall objective of the research is to identify relationship between media messages about smoking and smoking habits of adolescents, depending on smoking exposure in the environment. Specific objectives are to research, according to age and gender smoking habits and exposure of adolescents to smoking in environment (parents, home, friends, teachers), media messages that promotes and those against smoking, and analyze differences in smoking habits of adolescents in relation to media messages that promotes and those against smoking depending on smoking habits of environment.

MATERIALS AND METHODS: Research will be based on data obtained from „Global Youth Tobacco Survey” conducted in Croatia 2011., on 3551 students from 50 schools (7th and 8th grade primary, and 1st grade secondary school), aged 13-15, selected by 2-phase clustering method, conducted by Questionnaire with 83 questions related to smoking habits, environmental and media exposure of adolescents. Data will be processed by Descriptive statistic and Logistic regression analysis.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: The research will contribute to comprehensive understanding of environment and media as a risk, or preventive factor for adolescent smoking habits.

MeSH / Keywords: smoking, students, environment, media, GYTS

THE QUALITY OF LIFE ASSESMENT OF PARENTS WITH MENTALLY RETARDED CHILDREN

Part of a Thesis: The Quality of Life Assesment of Parents with Mentally Retarded Children in the City of Zagreb and Zagreb District

PhD candidate: Teodora Not, MSc, special education teacher

Mentor: Assistant professor Hrvoje Tiljak, MD, PhD

Affiliations: Centre for rehabilitation Zagreb

INTRODUCTION: Past researches about quality of life of parents with mentally retarded children (MR) i.e. intellectual disabilities (ID), speak of increased presence of depressive disorders, poorly evaluated psychic health and vitality. Growing up is never simple or uniform and is in a constant interaction with parents and the environment. Every incapability or limitation in a child's development is an additional factor of stress, and parenting is very demanding all by itself.

HYPOTHESIS: (1) There is a substantial difference between specific dimensions in quality of life of parents with MR children and in the quality of life of parents with healthy children. (2) Quality of life of parents with MR preschool children is better than the quality of life of parents with MR children in elementary school. (3) Quality of life of mothers with MR children is significantly lower than the quality of life of the fathers.

AIMS: General: to asses the quality of life of parents with MR children and the parents with healthy children. Specific: to examine the connection of quality of life of parents with MR children with: degree of MR, parental roles (mother, father), age of the child (preschool, elementary school), professional qualification

MATERIALS AND METHODS: The pattern of examinees will be made up of parents with children aged 1-15 with moderate and severe MR on rehabilitation in City of Zagreb and Zagreb district. Observed descriptive variables of the parents will be their gender, age, professional qualification and place of residence, while age and level of impairment will be the children's observed variables. A control group of parents with healthy children will be chosen by principle of matching pairs. For assesment of the quality of life we will be using the following instruments: SF-36 (the short form health survey questionnaire SF-36), Social Support, and DSQ-40 (Defence Style Questionnaire - 40).

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: The planned research points out the peculiarity of particular influences on the quality of life of parents with MR children with the assesment of strenght of individual influences. Further more, the research will show the interdependence of health within the family and the metodology of research of that area.

Keywords: quality of life, parents, children with mental retardation

APPLYING THE INTERNATIONAL CLASSIFICATION OF FUNCTIONING, DISABILITY AND HEALTH (ICF) IN THE PROCESS OF DISABILITY ASSESSMENT

Part of a Thesis: Possibilities In Applying The International Classification Of Functioning, Disability And Health (ICF) In The Process Of Disability Assessment In Republic Of Croatia

PhD candidate: Jasmina Ostojić, MSc

Mentor: Tomislav Benjak, MD, PhD.

Affiliations: Croatian Institute for Public Health, Ministry of Social Policy and Youth

INTRODUCTION: According to the Register of persons with disabilities, prevalence of disability in Croatia is 12%, being in line with estimated world's average. For disability assessment World Health Organization recommends the application of International Classification of Functioning, Disability and Health (ICF). In order to ensure equal opportunities for all persons with disability, Unified list of impairments as well as Unified list of functional abilities were created, each establishing common criteria for disability assessment according to international and national documents. According to the Government's conclusion, piloting of both lists has been approved.

HYPOTHESIS: ICF cannot be applied in the process of disability assessment without using adequate evaluation instruments, professional education and multidisciplinary approach.

AIMS: The aim is to investigate the possibility for the application of ICF in the process of disability assessment in Croatia; to investigate the applicability of the Unified list of functional abilities; to collect evaluation tools for assessing functional abilities, by which a new basis for the unified application of ICF can be created; to design a recommendation for common criteria of disability assessment, through which social and medical aspects of disability can be evaluated.

MATERIALS AND METHODS: Unified list of impairments was designed according to current provisions covering defined rates of disabilities and International Classification of Diseases, while the Unified list of functional abilities is based on ICF. In preparation of both lists a team of various experts together with representatives of persons with disabilities were included. After the test application of the unified lists, expert teams for specific disabilities will continue to work during 2012. The goal is to produce a final recommendation for the unified method for disability assessment.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: The scientific contribution of this research is the assessment of the applicability of ICF classification with regards to disability evaluation as a part of the disability assessment system. This research will offer an insight into the possibility of designing common criteria for disability assessment, which will in turn facilitate the implementation of international and national conventions aimed at providing equal opportunities for this vulnerable population.

Acknowledgements: I wish to thank the representatives of persons with disabilities, as well as all the experts that have contributed in the design of the two mentioned lists.

MeSH / Keywords: ICF, persons with disabilities, common criteria for disability determination

BIRDS AS CARRIERS OF CRYPTOCOCCUS NEOFORMANS

Part of a Thesis: Ecology of *Cryptococcus neoformans* species in Croatia and Kosovo.

PhD Candidate: Donjeta Pilana MD,

Mentor: Professor Emilija Mlinaric Missoni, MD, PhD

Affiliation: Croatian National Institut for Public Health

INTRODUCTION: *Cryptococcus neoformans* is opportunistic basidiomyceteous yeast that causes life-threatening infections primarily in immunocompromised hosts. The sources of this organism are bird's excreta and decaying wood.

HYPOTHESIS: The frequency of isolation and antifungal susceptibility profiles of *C. neoformans* will be different depending on the kind of sample and the location of collected samples. Serotypes, mating types, and genotypes of *C. neoformans* isolated from different kind of samples (bird's excreta and hollows of the trees) and from the different geographic locations will be the same, but their prevalence will be different in Croatia and Kosovo.

GENERAL AIM: To isolate *C. neoformans* and other pathogenic species of *Cryptococcus* genus (*C. gattii*) in hollows of the trees and bird's excreta in public places from different geographic locations in Croatia and Kosovo. To conduct the in vitro antifungal susceptibility testing of *Cryptococcus* spp. isolates from both kind of samples collected from the different geographic locations in Croatia and Kosovo. To determine the molecular characteristics of environmental *C. neoformans* isolates from different geographic locations in Croatia and Kosovo.

SPECIFIC AIM: To investigate the frequency of isolation of *Cryptococcus* spp. based on the kind of sample: bird's excreta vs. hollows of the trees; the frequency of isolation of *Cryptococcus* spp. based on geographic locations in Croatia and Kosovo; to compare antifungal susceptibility profiles of environmental and clinical isolates of *C. neoformans* from Croatia; serotypes, mating types, and genotypes of environmental and clinical isolates of *C. neoformans* from Croatia.

MATERIALS AND METHODS: During 2011-2012 will be collected around 600 samples of desiccated bird's excreta and swabs from the hollows of the trees from different geographic locations in Croatia and Kosovo. Samples will be collected and processed as described by Cafarchia et al., (2006) and Randhawa et al., (2005). Identification and antifungal susceptibility testing of isolates will be done with commercially available tests. Molecular analysis of isolates will be performed by PCR fingerprinting and AFLP method (Hagen et al., 2010).

SCIENTIFIC CONTRIBUTION: Also, for the first time, will be known the antifungal susceptibility profiles, serotypes, mating types and genotypes of *Cryptococcus* species isolated from environmental sources in different geographic locations of Croatia and Kosovo.

ACKNOWLEDGEMENTS: Project „Uzročnici gljivicnih u imunokompromitiranih bolesnika“ with HZJZ, approved by HZZO/ RH 2010/2012

Key words: *Cryptococcus neoformans*, environmental isolates, antifungal susceptibility, genotyping.

THE QUALITY OF OUTPATIENT UTILIZATION OF PSYCHOPHARMACEUTICALS IN ZAGREB 2001-2010

Part of a Thesis: The Quality Of Outpatient Utilization Of Psychopharmaceuticals In Zagreb 2001-2010

PhD candidate: Marina Polić Vižintin MD, MSc

Mentor: Assistant Professor Danijela Štimac, MD, PhD

Affiliation: Institute of Public Health Dr Andrija Štampar- Zagreb

INTRODUCTION: The rational utilization of psychopharmaceuticals has been inadequately studied. According to Intercontinental Marketing Service data, the leading groups of drugs utilized worldwide are cardiovascular drugs, followed by central nervous system drugs with a continuous annual rise. A similar pattern has been observed in Zagreb. The burden of mental disorders has been seriously underestimated by traditional epidemiological methods accounting only mortality, but not disability rate. Mental disorders accounted for about 9% of the total number of hospitalizations in Zagreb, and the share of these disorders as death causes in the total mortality rose for 183% from 1971 to 2008. A comprehensive insight into drug utilization as an economic and primarily a public health issue can only be acquired in the context of overall health state of the respective population.

HYPOTHESIS: During the study period the quality of prescribing psychopharmaceuticals becomes better.

AIMS: 1. To determine the trend in outpatient utilization of psychopharmaceuticals. 2. To assess the quality of prescribing by use of the Drug Utilization 90% (DU90%) method. 3. To assess the rational drug prescribing by use of the Eurostat Ratio indicators. 4. To determine the trends in hospitalization for mental disorders. 5. Implementation of the method for evaluation of the psychopharmaceutical prescribing quality

MATERIALS AND METHODS: Data on the outpatient utilization of psychopharmaceuticals were received from all pharmacies in Zagreb during 2001-2010. Using the WHO Anatomical-Therapeutic-Chemical classification system (ATC)/ Defined Daily Doses (DDD) methodology, the number of DDDs is calculated from data on the number and size of drug packages. Using the ATC/DDD methodology, the data obtained can be compared with other settings and between different time periods. The Drug Utilization 90% method is used as a criterion of prescribing quality. The cost/DDD within and beyond DU90% segment is calculated. The rate of hospitalization for mental disorders is analyzed by use of the hospitalization base data (according to age and sex). The rationality of prescribing is evaluated by use of the Eurostat Ratio indicators.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH: Implementation of the method for evaluation of the psychopharmaceutical prescribing quality and rational use, at the primary healthcare level.

MeSH/Key words: ATC/DDD methodology, psychopharmaceuticals, utilization, Drug-Utilization 90% method, Zagreb

QUALITY ASSESSMENT IN GENERAL PRACTICE - SELF EVALUATION OF GENERAL PRACTITIONERS

Part of a Thesis: Development of quality indicators in general practice

PhD candidate: **Slavica Sović, MD**

Mentor: Professor Hrvoje Tiljak, MD, PhD

Affiliations: University of Zagreb School of Medicine Andrija Štampar School of Public Health

INTRODUCTION: A quality system includes all activities performed to assess and improve quality of care. Developments of protocols and guidelines, assessment procedures, continual medical education, quality management are parts of the quality system. In view of demands for high quality care, assessment of the professional performance, practice management and organisation are seen as a crucial step for quality of care improvement. It can enable a practice to target its efforts and resources optimally according to the need of their patients. It is especially visible in General Practice (GP). However, GP is responsible for areas of great uncertainty and complexity, deals with many issues that are not easily measured. Use of quality indicator provides possibility to measure specific element of practice performance. Quality indicators should be adapted to health care organisation and socio-demographic characteristics of GPs. In Croatia, quality of care in GP has become matter of interest of professionals in last two decades. Former efforts in quality assessment were focused on description of structure and process of care, instruments development and adaptation of international instruments to local settings. It has been recognised that professionals should have the lead in the selection and use of indicators.

HYPOTHESIS: The selection of quality indicators in self evaluation process is influenced by working conditions, health demand of practice population and socio-demographic characteristics of GPs.

AIMS: To get insight in process of quality indicators selection within self-evaluation scheme of GP-a in Croatia. To assess relation between practice and doctors characteristics to quality indicators they chose within self-evaluation process.

MATERIALS AND METHODS: The study includes reports of 647 medical doctors, participants of Post-graduate study in Family medicine at the School of Medicine, University of Zagreb. As a part of the course, „Quality improvement” students submit a written description of their practice, addressing various quality aspects they find important. Analysing text quality indicators will be identified. Influences of some sociodemographic factors and work conditions on the selection of quality indicators will be analysed by appropriate descriptive and other statistical methods.

EXPECTED SCIENTIFIC CONTRIBUTION OF THE PROPOSED RESEARCH:

The study will propose quality indicators and criteria which can be used in systematic quality assessment and quality improvement in General Practice in Croatia.

Acknowledgements: I would like to thank my mentor Professor Hrvoje Tiljak for patient guidance and advices he has provided me throughout preparing my doctoral thesis.

MeSH/Keywords: primary health care, general practice, quality indicator, quality assessment, quality improvement

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