Maced J Med Sci **electronic publication ahead of print,**
published on March 04, 2010 as doi:10.3889/MJMS.1857-5773.2010.0088

Macedonian Journal of Medical Sciences. 2010 Mar 15; 3(1):XXX-XXX.
doi:10.3889/MJMS.1857-5773.2010.0088
Macedonian Medical PhD

Macedonian Medical Doctor of Philosophy (PhD) Theses Defended in 2009

Faculty of Medicine, University "Ss Kiril and Metodij", Skopje, Republic of Macedonia

Citation: Macedonian Medical Doctor of Philosophy (PhD) Theses Defended in 2009. Maced J Med Sci. 2010;3(1):XXX-XXX. doi.10.3889/MJMS.1957-5773.2010.0088.

Key words: Docotor of Philosophy (PhD); Medical research; Republic of Macedonia.

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Received: 08-Jan-2010; Online first: 04-Mar-2010

Competing Interests: The authors have declared that no competing interests exist.

Abstract

We present English abstracts of PhD theses defended in 2009 at the Faculty of Medicine, University "Ss Kiril and Metodij", Skopje, Republic of Macedonia. English summaries are published as they are translated by authors and included in the final version of defended PhD. Macedonian Medical Doctor of Phylosophy (PhD) theses are deposited in the Central Medical Library and National and University Library "St. Kliment Ohridski" in Skopje.

At the Faculty of Medicine in Skopje 24 PhD theses there were defended in 2009, six of them wihout English abstract (25 %): two from the Institute for Forensic Medicine; two from the University Clinic of Orthopedic Surgery, one from the Institute of Epidemiology and Biostatistics with Medical Informatics; and one from the University Clinic of Ophtalmology. Eleven PhDs are without Key words (45.83%), and only two of them are with structured abstracts (8.3%).

Editorial Board does not take any responsibility either for the content, nor the quality of the abstracts.

We have to repeat that primary responsibility for the quality of the PhD theses belongs to the mentors, to the institutions they are representing, and to the Vice-Dean of science. All of them should be more actively involved in the preparation of Doctor of Phylosophy theses in order international standards to be achieved.

Gordana Jankoska. Phenotypic and molecular investigations of the virulence of the uropathogenic bacteria from species enetococci [PhD Thesis]. *Skopje, Republic of Macedonia: Institute for Microbiology and Parazitology, Faculty of Medicine, University "Ss Kiril and Metodij"; 2009.*

At least 10 species of enetrococci, *Enterococcus faecalis (E. faecalis)* as most frequent, are known as cause of human infections, often localized in the urinary tract. Phenotypic and molecular investigations of enetrococci show the role of virulence markers in pathogenesis of infections. Expressive resistance of enetrococci to antimicrobial agents is an actual problem for therapy of human infections. The aim of our study was to estimate if there are specific uro-pathogenic enetrococci by proving the presence of the most often examined factors of virulence in enetrococci obtained from different sources and depend of that results. At the same time we analyzed the possible relationship of some virulence factors with resistance to antimicrobial agents. Total of

137 strains of *Enetrococcus spp.* were examined: 172 strains from human specimens and 1 strain from probiotic Linex (lek,Skopje). Enetrococci from different sources were included in the investigation: causes of urinary tract infections (the main examined group), causes of extra urinary infections and conditionally pathogenetic enetrococci from the normal micro flora. Different enetrococcal species were incestigated: *E. faecalis* (131 strain), *E. faecium* (36 strains), *E. gallinarum* (3 strains) and *E. avium* (2 strains). Isolation of the strains was done on UTI and blood sheep agar (Oxoid). Differentiation of the species was done by Vitek automated system (GPI-card). The presence of virulence factors: enetrococcal surface protein, aggregation substance, gelatinase and hemolysin are proved by detection of gene for its expression using polymerase chain reaction (PCR). Production of gelatinase and hemolysin was detected phenotypical: gelatinase on tripticase soy agar supplemented with 1.5% skim milk and hemolysin on Columbia CAN agar. Antibiotic sensitivity to cefriaxone, ampicillin, nitrofurantoin, ciprofloxacin and vancomycin, was examined by agar diffusion method, according to CLSI standards. All 4 examined virulence factors were present in high percent in our strains of *E. faecalis*; the most frequent was gelatinase (64.12%), then enterococcal surface protein (61.07%). The difference in presence of factors depend of the species was significant; in *E. faecalis* were more often detected all factors then in *E.faecium*. There weren't detected any factors in the other examined species: *E.gallinarum* and *E. avium*. The investigations of enterococcal isolates from different sources is of special interest for finding differences between strains that cause infection and strains which are a part of normal micro flora. Urinary tract isolates were the richest with virulent factors, than isolates which are cause of infections out of the urinary tract. Enetrococci from normal micro flora were with low presence of factors and in the strain from probiotic Linex wasn't detected any of examined factors. Analysis the combination of the factors, which was done to detect uro-pathogenic enetrococci shows that the most common presence of gelatinase and enterococcal surface protein, in 50% of the urinary strains. In enetrococci isolated from other human specimens there wasn't proved any specific combination of factors. Literature data point out spread of enterococcal resistance to vancomycin, but in out study all strains were susceptible to vancomycin and nitrofurantoin also. Resistance to other antimicrobial agent was different, but significant higher in *E.faecium* than in *E. faecalis*. Relationship between virulence factors and resistance to some antibiotic couldn't prove with certain. Significant statistical difference was found in *E. faecalis* resistant to ampicilin depend of gelatinase presence.

Key words: *enetrococci; Enterococcus faecalis; virulence; enterococcal surface protein; aggregation substance; gelatinase; hemolysin.*

Defended: January 8, 2009.

Mentor: Prof. Dr. Milena Petrovska

Jelka Masin Spasovska. Early ischemia-reperfusion injury and immunologic damages on the graft and their predictor values on the maintenance, clinical picture and result of the renal dysfunction in the patients after kidney transplantation [PhD Thesis]. *Skopje, Republic of Macedonia: Univeristy Clinic of Nephrology, Faculty of Medicine, University "Ss Kiril and Metodij"; 2009.*

Chronic allograft nephropathy (CAN) has become the leading cause of late kidney transplant failure. Its histological hallmarks are tubular atrophy, interstitial fibrosis, microvascular changes ang glomerulosclerosis. CAN is driven by a number of immunological and non-immunological factors such as pre-existing donor patology, ischemia reperfusion injury (IRI), dalayed graft function (DGF) and/or acute tubular necrosis (ATN), acute (AR) and subclinical acute rejections (SAR), hypertension and calcineurin inhibitor toxicity.

Ischemic damage during kidney transplantation might be associated with an increased incidence of DGF and AR, at the same time favoring development of chronic allograft nephropaty. Although precise mechanisms of IRI have not been clarified, some chemical mediators, such as oxygen radicals and platelet activating factors accompained by endothelial dysfunction, have been shown to be an early key event proceding IRI.numerous studies have inestigated features of allograft injury in renal protocol biopsies obtained in stable kindey transplants. Evolution of protocol biopsies has revealed a considerably high prevalence of SAR and CAN in protocol biopsy with long-term allograft failure ant the clinical relevance of this procedure. In addition, surveillance (protocol) bipsies performed in stable kidney allografts might allow timely recognition of pathological conditions causing a deterioration of graft function at later time point. Protocol biopsy may uncover histological signs of acute rejection without associated graft dysfunction (SAR and/or borderline changes (BC)) as well as the early occurrance of CAN, and may help to establish an individually targeted immunosuppresive regimen, which may include

antirejection treatment of SAR, the use of novel less toxic immunosuppresive or even reduction or withdrawal of immunosuppresive drugs.

The aim of this study was: 1) to evaluate the levels of vasoactive endothelial factors following IRI: endothelin (ET_1), nitric oxide (NO) and free oxygen radicals (FOR), and to estiamte the post-IRI effects on allograft function and hystology at 1 and 6 months after transplantation (Tx), and one year graft outcome; 2) to identify and evaluate SAR and BC, as well as the hystology of CAN in protocol biopsies performed at 1 and 6 months after living related kidney Tx, and to determine whether treatment of SAR and BC at 1-month posttranspant has a benficial effect on the graft hystology and function at 6 months, and their possible imlpications on graft function at first year after Tx.

Forty consecutive living related kidney transplant recipents were included in our study. Endothelial factors (ET_1, NO and FOR) were analyzed at variuos time points: before Tx, after Tx, at day 1,at 1, 2, 3 weeks and at 1 and 6 months after Tx. The clinical and biochemical data related to the graft function (serum creatinine-sCr and calculated clearence-cCrcl) were recorded at the same points, as well as at 12 months after transplantation.

During the first postoperative month patients with DGF who suffered post-transplant ATN or expirienced a clinical episode of AR were treated with hemodyalisis or pulse corticosteroids, respectively, whenever an increase in serum creatinine >20% or a decrease in urine output for 2 consecutive days was observed.

Biopsies of the graft were performed by indication whitin the 1-month after Tx. However, protocol biopsies in the stable kidney transplants (no change in serum creatinine >20% for at least 2 weeks before the first biopsy) were performed at 1 and 6 months after Tx and were blindly reviwed for evidence of acute and chronic changes by the same pathologistusing descriptive morphologic criteria according to the Banff 97 scoring schema on a scale from 0 to 3. CAN score was calculated as a sum of scores for individual histological markers of chrinicity: intestitial fibrosis, tubular atrophy, vascular fibrosis intimal thickening, arterial hyalinosis and chronic glomerulopathy. The histological index (HI) was calculated as total sum of scores for acute and chronic changes.

Patients with histology at 1-month biopsy of BC or SAR (AR type I or IIA) and an increase in serum creatinine between 10 and 20 %from baseline (serum creatinine 2 weeks prior the biopsy) were consenquently treated with pulse corticoid therapy. The patients with histology of BC or SAR followed by rise in serum creatinine <10% from baseline were not treated.

When compared according to the occurance of DGF and AR during the first postransplant week, Group 2 – with clinical manifestation of IRI (DGF and/or AR), was with significantly longer cold ischemia time (CIT), and expectedly, significant higher ET_1 and FOR levels after Tx and at day 1 post Tx, folowed by a significantly lower NO levels at the same time points. At 1-month biopsy a higher percentage of acute histological changes (SAR and BC) was found in G2 (with DGF and/or AR) when comapred to G1 (without DFG and AR); (83% vs. 75%). As expected this group (G2) had a significantly higher score of acute histologic lesions found at 1 and 6 month biopsy, as compared with G2. Importantly, the groups differed significantly in the mean HI score at 6 month biopsy (9.1 ± 4.9 (G2) vs. 7.2 ± 2.9 (G1), $p<0.05$). Thereby, a significantly higher proportion of patients with CAN progression were found in G2 (75% vs. 57%). However, there was no significant difference in the graft function, i.e. calculated creatinine clearance at 1 and 6 month after Tx, in both groups. Hence, the group with clinical manifestation of IRI, had a significantly higher serum creatinine and lower calculated creatinine clearance at 12 months after Tx.

Among all biopsies only 7.5% showed no histopathologic lesions. BC was found in 32.5% and 30% and SAR in 40% and 147.5% of patients, on 1 and 6 month biopsies, respectively. The mean HI, increased significantly at 6 month biopsy 5.3 ± 2.9 vs. 7.8 ± 3.6 ($p<0.001$). Similarly the mean CAN score of 2.1 ± 1.5 at 1 month, increased significantly to 4.6 ± 2.3 ($p<0.000$) at 6 month biopsy. When divided according to the treatment of BC and SAR, the group of treated BC/SAR found at 1 month had mean HI score of 7.0 ± 1.8, which remained almost at the same value 7.3 ± 2.4 at 6 month biopsy. The proportion of these changes in untreated BC/SAR group have been increased from 5.0 ± 2.0 to 8.4 ± 4.3 ($p<0.001$). However there was no significant difference in graft function, i.e. calculated creatinine clearance from 1 to 6 months, in both groups. In 27 patients (33.8%) no CAN was present in both biopsies, 27 (67.5%) showed progression of CAN and 13 (32.5%) were with stable CAN, at 6 month biopsy.

When compared according to the progression of CAN, the group with progression of CAN (pCAN) had mean HI score of 5.0 ± 3.0 at 1 month, which increased significantly to 9.5 ± 2.8 ($p<0.001$) at 6 month biopsy. The proportion of these changes in the stable CAN group (sCAN) was 5.5 ± 3.0 to 4.10 ± 3.2, respectively. The pCAN group had a significantly higher proportion of DGF (29.6% vs. 15.4%, $p<0.05$), and a higher percentage of acute histological changes (AR, SAR and BC) found at 1

and 6 month biopsy (81% vs. 62% and 92% vs. 54%, p<0.01, respectively).

Moreover, the groups differ significantly in the percentage of treated BC and SAR, 25% in pCAN group vs. 50% in sCAN group, as well as in the scores of acute histological lesions, 1.97 ± 0.95 vs. 0.40 ± 0.64 (p<0.01) at 6 month biopsy.The groups differed significantly in the mean CAN score at 6 month biopsy, 5.4 ± 1.8 (pCAN group) vs. 3.0 ± 2.3 (sCAN group), p<0.01. However, there was no significant difference in the graft function, i.e. calculated creatinine clearance from 1 to 6 and 12 months, in both groups.

In conclusion, post IRI in mediated by endothelial release of vasoactive factors such as endothelin, nitric oxide and free oxygen radicals, potentially key molecules in the link of IRI, DGF and AR. IRI, followed by endothelial dysfunction, initiates an inflammatory response that provokes an increased level of acute host immunological reactivity. This would explain the apparent synergy between DGF and episodes of acute rejection early after transplantation, and the development of CAN. In fact, the group with DGF and AR early after Tx shown higner percentage of acute histological deterioration on the 6-months biopsy, which in turn results in accelerating progress of CAN, and the late graft dysfunction. Namely, the determination of graft function at 12 month after Tx found in the group with clinically manifested IRO in our study, confirmed the association between DGF and poor short-and long-term graft survival. Based on our findings, we could suggest that 1-month protocol biopsies may be valuable in uncovering and determining the high incidence of BC, SAR and presence of early CAN in stable renal allografts, which infect on the impact od evolution on renal allograft function at the later time point. The presence of an untreated BC and SAR found at 1 month biopsy showed greater susceptibility for acute and chronic histological deterioration on the 6 month biopsy, accelerating the process of CAN. Therefore it can be recommended that a histological diagnosis of SAR and BC at 1 month should be treated for rejection. It remains to be demonstrated whether the treatment of BC and SAR provides a long term benefit of the graft outcome and survival. Additionally long term clinical, histological and treatment follow up are necessary to confirm our findings that untreated BR or SR have been at much worse prognosis for graft survival. Finally, protocol biopsies may be helpful to optimize the level of immunosuppression among patients with SA or BC, perhaps as a step toward a more individualized approach to patients follow up.

Key words: *kidney transplantation; chronic allograft nephropathy; ischemia-reperfusion injury; endothelin; nitric oxide; free oxygen radicals; protocol biopsy; delayed graft function; acute rejection; subclinical acute rejection; borderline changes.*

Defended: January 9, 2009.

Mentor: Prof. Dr. Ninoslav Ivanovski

Marijan Bosevski. Factors of prognosis and progression of diabetic polyvascular disease [PhD Thesis]. *Skopje, Republic of Macedonia: University Clinic of Cardiology, Faculty of Medicine, University "Ss Kiril and Metodij"; 2009.*

The study was aimed to define the factors of prognosis and progression of atherosclerosis in pts with diabetic polyvascular disease. Based on the presence of vascular disease three cohort groups were enrolled in the study: 1st 294 pts with type 2 diabetes (T2D) and polyvascular disease (DPD); 2nd 46 pts with T2D and coronary artery disease; and 3rd pts with T2D without vascular disease. Study population was followed up 31.35 ± 10.59 months for onset of cardiovascular events and progression of atherosclerosis. Cox logistic regression and linear regression models were built to define predicting factors.

Study population with T2D and vascular disease was on age 60.28 ± 27 years (231 men and 109 women) and mean diabetes duration of 8.58 ± 6.17 years (y). High prevalence of metabolic syndrome and its components: arterial hypertension, low HDL-cholesterol and hypertriglyceridemia were found. Cross sectional analysis at the beginning of the study found age, T2D duration, non HDL-cholesterol, waist and microvascular complications as predictors for development of DVD. Diabetes duration, neuropathy and glycemia were found to be factors of peripheral arterial disease (PAD); and age, non HDL-cholesterol and PAD for presence of carotid artery disease.

Cohort follow up in 1st and 2nd groups detected incidence of cardiovascular events of 54.7% estimated for 100 pts. The predictors for events were defined as followed: increased weight, LDL-cholesterol, PAD and carotid stenosis. For period of 31 months estimated rate of cardiovascular death was 8.7% and for death of all causes 9.5%. Age, arterial hypertension, obesity, stenosis of carotid internal artery and Gensini score were defined as predictors for cardiovascular death and ankle-brachial index (ABI) for all-cause of death. Dynamic of DPD and progression of peripheral atherosclerosis was detected in 41.3% (measured as decreased of ABI), in 86.6% measured

as progression of carotid IMT and in 17.8% measured as increased of carotid stenosis. Dynamic range of ABI value was determinate with initial value of ABI, microvascular complications and history of cerebrovascular disease. Change of carotid IMT over time of three years was influenced independently with values of diastolic blood pressure and body mass index (BMI). Values of fibrinogen and CRP (estimated in 62 pts) influenced independently on progression of ABI value. Increased CRP>3 mmol/L was defined as predictor for new angina. Microvascular complications, dyslipidemia and low value of ABI were defined as predictors for progression of coronary atherosclerosis, which was found with incidence of 67.5% for 3 years. Endothelial dysfunction (when flow mediated peripheral vasodilatation measured in 171 pts) was found as factor for progression of coronary atherosclerosis. Built models for peripheral and coronary revascularization showed absent peripheral pulses, carotid lumen diameters and carotid plaque' area as predicting factors.

In the population with T2D, and without vascular disease (age 53.3 ± 7.3 y, mean diabetes duration 7.5 years), progression of PAD and carotid artery disease were found with incidences of 62.5% for the period of 3 years. As predictors for progression of PAD were defined: HDL-cholesterol, urea, systolic blood pressure and diabetes duration. Progression of value of carotid IMT was determine with BMI, weight, diastolic blood pressure and endothelial dysfunction. In this group incidence of 0.1 event per pt for 3 y was detected. As predictors of events were found: HDL-cholesterol, its value < 1.03 mmol/L, CRP and smoking.

Building the models for dynamic of diabetic polyvascular disease in type 2 diabetic patients has clinical utility in defining risk factors and target areas for stopping progression of atherosclerosis. Prognostic models in this population are multifactorial, as its risk reduction should be. Ankle-brachial index and carotid ultrasound have incremental values in prognosis of these patients.

Key words: Not available.

Defended: January 9, 2009.

Mentor: Prof. Dr. Ljubica Georgievska-Ismail

Aleksandra Gutevska. Comparative analyzes of qualifications of body injuries according to the articles from the Phelony Law of Republic of Macedonia and Phelony Laws of the other countries. [PhD Thesis]. ***Skopje, Republic of Macedonia: Institute for Forensic Medicine, Faculty of Medicine, University "Ss Kiril and Metodij"; 2009.***

No abstract available.

Key words: Not available.

Defended: January 12, 2009.

Mentor: Prof. Dr. Zdravko Chakar.

Verica Popovska. Determination of the time of death in early post mortal period [PhD Thesis]. ***Skopje, Republic of Macedonia: Institute for Forensic Medicine, Faculty of Medicine, University "Ss Kiril and Metodij"; 2009.***

No abstract available.

Key words: Not available.

Defended: January 12, 2009.

Mentor: Prof. Dr. Biljana Janevska.

Violeta Vasilevska. The evaluation of radiological stadium and histopathological correlation in management of muscle skeletal soft tissue tumors [PhD Thesis]. ***Skopje, Republic of Macedonia: Clinic for Surgical Diseases "St. Naum Ohridski" - University Clinic of Radiology, Faculty of Medicine, University "Ss Kiril and Metodij"; 2009.***

A prospective radiological evaluation was done at 100 patients with muscle skeletal soft tissue tumor lesions aiming to determine the important radiological parameters in the differentiation of benign versus malignant tumors, in order to assess the radiological stadium with the histopathologic correlation in the management of soft tissue musculoskeletal tumors, as well as the value of the radiological diagnostic methods. All the patients were treated in the Clinic for Surgical Diseases "St. Naum Ohridski", the University Clinic for Orthopaedic Surgery, the University Pediatric Clinic and the Institute of Oncology and Radiotherapy at the Medical Faculty, University "Ss Kiril and Metodij" in Skopje.

Radiological imaging procedures were done at the Radiological Department of the Clinic for Surgical Diseases "St. Naum Ohridski" in Skopje at the University Clinic of Radiology at the Faculty of Medicine in Skopje. The Institute of Pathology at the Faculty of Medicine in Skopje was also included by performing the histopathologic

examination of the biopsy and/or operative material.

According to the histopathological findings, the patients were grouped in 4 groups: benign tumors (53), primary malignant musculoskeletal soft tissue tumors (26), tumor-like lesions (17), and secondary malignant musculoskeletal tumors (4).

The following radiological diagnostic procedures were performed in all patients: radiography, ultrasound (US), computed tomography (CT), and magnetic resonance (MR). Whereas angiography was done in selected patients.

The following parameters were analyzed: dimensions, contours, presence of a capsule, compartmental localization, presence of necrosis, calcification/tumor matrix mineralization and haemorrhage, as well as affection of neurovascular structures, bones and joints. As for the structure of the lesion, different parameters were analyzed depending on used method.

Having done the investigations, the radiological diagnosis was made. US or CT guided biopsy was performed in all patients.

The biopsy and operative material was processed at the Institute of Pathology, Faculty of Medicine in Skopje. TNM classification was used in order to determine the stadium of disease.

The following *benign tumors*: superficial lipoma (13), intra and intermuscular lipoma (9), fibrolipoma (1), fat tissue hypertrophy (4), desmoplastic fibroma (1), hemangioma (4), hemangiopericytoma (1), lymphangioma (1), synovial Backer's cyst (3), ganglion cyst (5), benign angiomatous fibrous histiocytoma (1), shwanoma (2), neurofibroma, neurofibromatosis (4), calcifying epithelioma (1), extraskeletal osteochondroma (2) and one mixoma were evaluated.

Out of all *malignant tumors*: liposarcoma(6), leomiosarcoma(1), rabdomiosarcoma(1), fibrosarcoma(5), fibromixosarcoma(1), synovial sarcoma (2), malignant fibrous histiocytoma (2), one malignant tumor of a peripherial nerve, one extraskeletal osteosarcoma, one case of extraskeletal Ewing sarcoma and two sarcomas were evaluated.

Tumor-like lesions examined in this series showed: one case of fasciitis, one case of fibrosis, ossificated myostits (4), abscess (2), primary musculoskeletal hydatid disease (3), one haematoma, two a-v malformations, and one case each of post traumatic aneurism, fat necrosis and panniculitis.

A satisfactory radiological accuracy in the terms of the determination of the stadium of malignant musculoskeletal soft tissue tumors was reached, and it was 63.6%. Difficulties in the radiological diagnosis as well as determination of the stadium resulted from the fact that the benign tumors can have malignant characteristics and vice versa, one malignant tumor can have benign characteristics.

Using radiography some of the soft tissue tumors and some of their characteristics could notify. However it could not give the final conclusion for the tumor character. Therefore, further examinations are necessary.

US is accessible and east method to perform, it is economically payable, it can enable assessment of the tumor structure and homogeneity, and in a great number of cases to differentiate a benign form a malignant lesion. Ultrasound has a great contribution in the performing of preoperative US guided biopsy.

CT is a diagnostic method, which present the soft tissue musculoskeletal tumors in two plains, having the possibility for their reconstructions. It clearly shows their structure, size, and insularity of the tumor formation, but not always because of the weak contrast resolution. In that case, the application of contrast improved the presentation, and the localization and tumor character can be determined. Performing of the lesion biopsy can also be done with this method.

MR is of great diagnostic help in the determination of the lesion character, with high accuracy shows the tumor, compartmental localization, size, contours, bordering as well as affection of the neighbouring structures. The weak side of MR is the insufficient accessibility and high price.

After all used methods the radiological imaging diagnosis shows sensitivity of 92.3% and specificity of 87.8% in determining a primary malignant soft tissue tumor. Global accuracy of MR in determining of a primary malignant soft tissue tumor is 89%.

Radiological staging or the exact determination of radiological stadium of primary malignant musculoskeletal tumors was done in 63.6% of the cases.

Radiological diagnostic algorithm gives us a direction to evaluate the musculoskeletal lesions, in order not to make unnecessary examinations, as well as not to miss a malignant lesion. All this imposes the fact that each musculoskeletal lesion should be approached seriously, should be completely examined radiologicaly,

and on top of all should be preoperative US or CT guided biopsy.

Key words: Not available.

Defended: January 22, 2009.

Mentor: Prof. Dr. Antonio Gligorievski.

Zoran Bozinovski. The influence of tenotomities on repositioning of the dislocated hip in patients with spastic cerebral paralysis.[PhD Thesis]. *Skopje, Republic of Macedonia: University Clinic of Orthopedy, Faculty of Medicine, University "Ss Kiril and Metodij"; 2009.*

No abstract available.

Key words: Not available.

Defended: January 23, 2009.

Mentor: Prof. Dr. Jordan Kamnar

Lidija Petkovska. Effects of immunosuppressive therapy with calcineuronics inhibitors and corticosteroids on the insulin sensitivity and resistance in patients with transplanted kidney.[PhD Thesis]. *Skopje, Republic of Macedonia: University Clinic of Toxicology, Faculty of Medicine, University "Ss Kiril and Metodij"; 2009.*

Modern immunosuppressive therapy (IST) enables better control of immunologic phenomenon, longer graft ant patients survival, and better quality of life after renal transplantation. As immunosuppressive therapy is life long, many adverse effects such as post-transplant hyperlipidaemia, diabetes mellitus and hypertension are well known. These side-effects are conventional risk-factors for cardiovascular morbidity and mortality, and also for chronic allograft nephropathy.

Post- transplant glucose disorders and PTDM are one of the most serious complications of IST, because of its macrovascular and microvascular complications. PTDM is also connected with IR, which is independent risk-factor of atherogenesis.

The aim of this study was to determine the type and frequency of glucose disorders, the correlation of maintenance IST with glucose disorders and IR, to determine the relative importance of IR and insulin deficiency in pathogenesis of glucose disorder after transplantation, to find out independent risk-factors for IR, and finally to analyze the impact of glucose disorders and elevated IR to graft function after a certain period of time.

A group of 59 renal allograft pts with a good graft function and without previous glucose disorder was included in the study. All pts were under triple maintenance immunosuppressive therapy (CsA, MMF, MP) in appropriate doses. OGTT was performed in all pts with normal or impaired fasting glucose levels in order to classify glucose disorders and to divide pts in group with and without glucose disorders. The basal values of glucose (G_0) and insulin (I_0) were used to calculate indexes of IR and beta-cell function according to the homeostasis equations. For evaluation of the renal function, the Cockcroft-Gault formula was used. BMI, CsA levels, lipoproteins and other analyses within the framework of regular biochemical monitoring were made among all pts.

For data processing we correlated the doses/ levels of IST and other potential risk-factors for glucose disorders and IR, and analyzed independent risk-factors for IR. In the prospective part of the study we followed the effect of impaired glucose metabolism and IR on the graft function, clinical parameters and need for changes in the therapy.

The results of all examined parameters showed significant statistical (ss) correlation between CsA levels, shorter period after transplantation, lower GFR, TL, LDLc and postprandial glicaemia. Also, CsA level positively correlates with the basal insulin level and IR index ($p<0.01$). So, we concluded that glucose disorders and elevated IR after transplantation are connected with higher therapeutic concentrations of CsA, but not with the daily MP doses. Multiple regression analyzes were used to determine independent risk-factors for IR and showed that G_0, I_0, GFR, HOMA-b, CsA level and shorter period after transplantation were ss associated with IR. The both groups showed high and almost equal prevalence of IR, but numeric value of IR index was ss higher in the group with glucose disorders. Both, impaired insulin secretion and IR are involved in the pathogenesis of post-transplant glucose disorders, but the defect in insulin action seems to be a prerequisite. IR presents significant factor for worsening of the renal function expressed as a GFR.

Among other side effects of IST, glucose metabolism disorders and IR are significant factors of morbidity and mortality after renal transplantation. Modern IST need to be adapted to the individual risk profile of the patient.

Key words: *renal transplantation; glucose disorders;*

insulin resistance; graft function.

Defended: June 6, 2009.

Mentor: Prof. Dr. Ninoslav Ivanovski.

Ljutvi Zuljbeari. Profil of dyslipidemia and apoproteins aberration in patients treated with repeated haemodialyses [PhD Thesis]. *Skopje, Republic of Macedonia: Nephrology Clinic, Faculty of Medicine, University "Ss Kiril and Metodij"; 2009.*

In the paper we analyzed 240 patients (105 women and 132 men) treated with chronically repeated haemodyalisis in the regional center for chronic HD-sis in Tetovo and Nephrology Clinic of the Medical Faculty in Skopje. The patients were treated with HD-sis in the last 6 months or more. In the analyzed patients group, cross section of the lipids profile is made in the three (3) successive months and the mean values are appointed. The serum is preleved at the morning (8^{th} o'clock am, after 12 hours starvation). In the analyzed patients group the serum concentration of total lipids (g/l), triglycerides, total cholesterol, HDL-ch and LDL-ch(mmol/l) is performed. The obtained results are compared with the findings occurred in the control group matched in sex, age, nationality and religion with the investigated patients. The analyzed patients were not treated with the hypolipemics in the last six months before to be included in the study. The patients group was distributed and analyzed keeping in view the basic diseases, sex, age, nationality, religion, the time of dialysis treatment and the index of body mass (BMIx).

Having in view the results of the study, may be concluded that the serum concentration of total lipids statistically not differs from the same encountered in the control group. The crucial disturbance of the lipids profile is hypertrigliceridemia (encountered in the 95% of investigated patients). The hypercholesterolemia is not a litlle presented (55%), while the blood concentrations of HDL-ch and LDL-ch are diametrically opposed (72% from the patients have diminished values for HDL-ch and 75% of them presents augmeneted serum concentrations for LDL-ch).

The variations data for serum TCh and HDL-ch in patients group aren't statistically different from the controls ($p<0.413$; $p<0.194$). The plasma concentration of Lp(a), LPL, tHcy and all analyzed apoproteins in our patient (N°=120) was statistically higher ($p<0.0001$) compared to controls. The autor concludes involvation of cited parameters in the genesis of accelerated uremic atherosclerosis. The accelerated atherosclerosis encountered in the group of chronically dialyzed patients may be equally significantly associated with the diminished values for HDL-ch (38.3% in the investigated group) and increased blood level for LDL-ch (40.0% of the dialyzed patients).

The BMI_x is moderately positively associated with the serum concentration for LDL-ch in the all patients group ($r=0.36$). The women presents significantly higher blood levels for TG, TCh, HDL-ch and LDL-ch in comparison with the males. The mentioned disturbances in the women's lipid profile may be explained with the endocrinologically obviously evident climax praecox. The diabetes mellitus at one significantly associates more higher TG-emia in comparison with the other basal renal diseases leading to terminal chronic renal failure. The Albanian nationality, Islamic religion determinates significantly higher blood concentrations for TG and LDL-ch-mia. The appropriate treatment of the 'uremic' lipids perturbation is highly advisable.

Key words: *chronically dialyzed patients; serum lipid profile; arteriosclerosis; apolipoproteins*

Defended: June 8, 2009.

Mentor: Prof. Dr. Kocho Chakalarovski

Selim Gjulshen. Evaluation of the influence of traditional and uremia/dialysis related risk factors upon cardiovascular mortality and morbidity in patients on maintenance hemodialysis [PhD Thesis]. *Skopje, Republic of Macedonia: University Clinic of Nephrology, Faculty of Medicine, University "Ss Kiril and Metodij"; 2009.*

Contrary to the continuous improvement in dialysis treatment and technology, as well as introduction of new pharmacotherapy in the last twenty years, mortality in patients on hemodialysis (HD) remains still extremily high worldwide, with a yearly rate of approximately 20%, where cardiovascular diseases (CVD) remain the most frequent cause of death and morbidity.

The pathogenesis of CVD in patients on HD is related to multiple different risk factors that, to a great deal, qualitatively and quantitatively differ from those in the general population. The high incidence of CVD in patients on HD could not only partially be a result of the increased prevalence of traditional risk factors, as the non-traditional, uremia and dialysis related risk factors are additionally associated with in. However, the high prevalence of CVD

at initiation of HD indicates that the predialysis stage of chronic kidney disease (CKD) is also a condition with high cardiac risk, thus it is clear that the mechanisms leading to cardiovascular events act rather early in the predialysis stage of CKD.

Identifying cardiovascular risk factors in HD patients is of great importance in terms of establishing prevention strategies for this group of patients at highest risk for CVD.

The aim of this study is to evaluate the impact of predyalisis nephrological care (PDNC), as well as traditional (hypertension, dyslipidemia, diabetes mellitus, body weight, smoking, sonography finding of left ventricular hypertrophy, predialysis and intradialysis cardiovascular events such as myocardial infarction, cerebrovascular insult and congestive heart failure) and non-traditional, i.e. uremia and dyalisis related cardiovascular risk factors(anemia, calcium and phosphate impairment, malnutrition/inflammation, ultrafiltration, duration of dyalisis, adequacy of HD) upon all-cause and cardiovascular mortality in patients on HD.

Two hundred and sixty one prevalent hemodialysis patients were investigated in the study, with time spent on dialysis more then 3 months. Laboratory assessment was performed each one and half month (for some parameters each 3 and each 6 months) in a five-year period, starting January 2002 up to December 2006, or until death of cardiovascular event or death of any cause. Regarding predialysis nephrological care defined as time interval in months starting at the moment of nephrology referral until day one of maintenance HD, patients were divided into two groups: nephrological care more than 6 months and nephrological care more than 12 months. The data where the outcome is death are analyzes using Kaplan-Meier survival curves, separately for all-cause and cardiovascular mortality. To asses the variables significantly influencing all-cause and cardiovascular mortality, a univariate Cox-proportional hazard model is used, and subsequently, a multivariate Cox-analysis, with age adjustment, separately for traditional and non-traditional risk factors. The predialysis nephrological care and predialysis cardiovascular morbidity are included in the multivariate Cox-analysis for both, traditional and non-traditional risk factors. In the end, the most significant variables in the multivariate Cox-analysis of traditional and non-traditional risk factors are included in the final Cox-analysis, where the outcome is all-cause or cardiovascular morbidity.

The results of the study showed as follows:

- Out of 261 patients, 117 died (44.8%); CVD were cause of death in 63.2%, whereas cardiac disease only in 44.4%

- Regarding PDNC as a predictor of cardiovascular mortality, it appears that longer then 12 months nephrological care is protective providing maintenance of the cardiovascular system in good condition in order to cope with the influx of numerous uremic and dialysis related risk factors

- Out of all traditional risk factors, predictors for all-cause mortality are cardiovascular events before initiation of maintenance HD, low predialysis diastolic blood pressure, high pulse pressure and left ventricular hypertrophy, whereas predictors for cardiovascular mortality are cardiovascular event before initiating maintenance HD and high pulse pressure.

- Out of all non- traditional risk factors, predictors for all-cause mortality are C-reactive protein (CRP), spKt/V and the duration of the hemodyalisis session, whereas predictors for cardiovascular mortality are age, PDNC, CRP and spKt/V.

- The appearance of CRP as a marker of inflammation and some dyalisis parameters as predictors for all-cause and cardiovascular mortality in the final Cox-model, suggest that the non- traditional risk factors, i.e. chronic inflammation in addition to hemodyalisis itself are the main cause for adverse outcome in hemodyalisis patients. Merely the high pulse pressure out of all traditional risk factors showed association with cardiovascular mortality in the final model.

The complexity of cardiovascular risk factors in patients on HD suggests that only combined analysis of traditional and uremia/dialysis related risk factors can identify patients with high risk for cardiovascular mortality, who differ substantially form those in the general population.

Kye words: Not available.

Defended: June 12, 2009.

Mentor: Prof. Dr. Olivera Stojceva-Taneva

Milena Golubovic-Arsovska. Evaluation of various techniques of laser photocoagulation in the treatment of diabetic maculopathy. [PhD Thesis]. ***Skopje, Republic of Macedonia: University Clinic of Ophtalmology, Faculty of Medicine, University "Ss Kiril and Metodij"; 2009.***

The subjects of this study are 120 patients with

diabetes mellitus type 2, which were treated at the Laser-photocoagulation Treatment Centre with the Ophtalomology Clinic between 2002 and 2007.

The criteria to be satisfied in order to enter the research were the changes of the fundus in the macular area, on either or both eyes.

The goals of the scientific research were: to determine the incidence of the diabetic maculopathy, to determine the influence age and length of the diabetic condition have on the development of diabetic maculopathy, as well as the link between the entity with other micro- and macro- vascular changes, to determine the influence of metabolism, dislipidemia, and blood pressure have over the diabetic maculopathy, to determine the influence the laser-photocoagulation treatment over the recession of the macular oedema and the hard exudates, the influence the treatment has on the macular function, the vision acuity up close and at longer distances and the influence on the vision field and color vision.

Every patient had a personal record, including personalized polls and charts from the Laser-Photocoagulation Treatment Centre.

For the evaluation of the influence of the treatment techniques in the recession of the oedema and the hard exudates and regarding the macular function (vision clarity, central visual field and color vision) a case control study was conducted.

The treated eyes were grouped as follows:

· Group 1 – Eyes where a focal technique was used

· Group 2 – Eyes where grid technique was used

· Group 3 – Eyes where the modified grid technique was used

The analysis of the influence the length of the diabetes and the age did not show any statistic significance regarding the severity of the maculopathy.

Significant statistic data was recorded in the severity of the maculopathy with inadequate metabolic control of the diabetes, the dislipidemia (increased values of triglycerides, overall and LDL cholesterol) and poor blood pressure regulation.

Diabetic maculopathy usually occurs in patients with severe and very severe retinopathy and is correlated with the affection of the cardio-vascular system and kidney disease.

With respect to the resorption of the macular oedema and hard exudates after 12 month monitoring all three techniques have shown to be equally effective, although the modified gird technique showed to be most effective in the treatment of the macular changes, with respect to the anticipation of the level of oedema and hard exudates prior to the treatment.

Regarding the enhancements of the visual clarity, the modified grid technique produced far better results then the alternative methods, whereas the near vision improvements were greatest with the focal technique.

The analyses of the influence the laser technique had on the visual field showed that the modified gird technique is the most aggressive and a statistically significant loss in the central visual field up to 10 degrees was noted. With respect to the color vision measured pre-op, there is a significant difference regarding the color discrimination, which remains after the laser-photocoagulation. There was no significant statistical difference between laser techniques.

The disorders in the macular function hint to the need of a complex approach and laser treatment in due time when treating patients with diabetes mellitus.

Key words: Not available.

Defended: June 19, 2009.

Mentor: Prof. Dr. Elena Dzajkovska

Elizabeta Todorovska. Correlation between lipid status and parameters of fibrinolytic system. [PhD Thesis]. ***Skopje, Republic of Macedonia: Institute for Transfuzion Medicine, University "Ss Kiril and Metodij"; 2009.***

Reduced fibrinolytic capacity in lipid metabolism disorder causes impairment of intravascular thrombogenesis resulting in a thrombotic process. Thrombogenesis imbalance is done in the wall of vascular endothelial cells. That synthesize activators and inhibitors of the fibrinolytic system, they also participate in expression and degradation of different substances, among which circulatory lipids play an important role. Dyslipidemia and impaired fibrinolysis are risk factors for thrombotic processes that include coronary heart disease (CHD), cerebrovascular insult (CVI), diabetes mellitus (DM) and deep venous thrombosis (DVT).

Having this in mind, it was our aim to examine and evaluate concentrations of fibrinolytic parameters, such

as tissue plasminogen activator (t-PA), plasminogen activator inhibitor (PAI) and DD (D-dimers) on one hand, and on the other hand concentrations of lipid fractions, such as total cholesterol (TC), LDL cholesterol, lipoprotein (a) and HDL cholesterol. The principal aim of this study was to determine whether there were correlations between separate examined parameters of the fibrinolytic system and lipid fractions.

Differentiated nosologic subjects with CHD, CVI, DM and DVT were examined by applying ELISA for quantitative determination of the fibrinolytic parameters t-PA, PAI and DD and of the lipid fraction lipoprotein (a), whereas enzyme-spectrophotometric methods were used for quantitative determination of the lipid fractions.

The results obtained have shown decrease of t-PA concentration and increase of PAI-1 and DD concentration increase of TC, LDL cholesterol and Lp(a) concentrations and decrease of HDL cholesterol concentration in comparison with the control group.

Analysis of the results revealed several correlations between certain parameters on the fibrinolytic system and lipid fractions.

In order to precisely define diagnosis and prophylaxis of thrombotic processes, routine application of tests for determination of fibrinolytic system parameters and lipid profile is an imperative. Everyday use of these tests in prevention and treatment of thrombotic processes would significantly reduce the incidence rate of these diseases.

Key words: *thrombogenesis; fibrinolysis; lipids; lipoproteins; parameters; correlation.*

Defended: June 24, 2009.

Mentor: Prof. Dr. Stojanka Kostovska.

Antonio Georgiev. Association of coronary arterial disease with polymorphisms and mutations of the genes for lipoproteins lipaza and apolipoproteins [PhD Thesis]. *Skopje, Republic of Macedonia: Univeristy Clinic of Cardiology, Faculty of Medicine, University "Ss Kiril and Metodij"; 2009.*

Introduction: Coronary arterial disease (CAD) is the leading cause for the exeptionally high invalidity, mortality and morbidity in the world. In the etiology of CAD more factors are involved and they are result of the complex interaction between the genetic predisposition and environmental influences. Multigenetic nature of ateriosclerosis and CAD should also be forgotten. The frequency of separate mutations and polymorphism in the LPL gene varies significantly among different populations. LPL-PvuII, LPL-HindIII, LPL-MnII and ApoCIII-SstI polymorphisms increase the risk of CAD appearance.No study has shown the influence of the upper stated polymorphisms over the appearance of CAD in Macedonian population, as well as the mutual influence with the risk factors for the appearance of CAD.

Aim: Presentation of LPL-PvuII, LPL-HindIII, LPL-MnII and ApoCIII-SstI polymorphisms as independent risk factors, and as predictors of CAD in Macedonian population is the aim of this study.

Material and methods: In this original study 114 patients included as examined group (CAD group 87 men and 27 women), and they all had angiographically documented stenosis of coronary arteries over 70% of the artery lumen. The control group had 35 patients (21 men and 14 women) and they all had angiographically documented normal coronographic findings. The greatest part of the patients is at the age of 50 and 59, where the mean age of the CAD group is 59.4 and of the control group are 57.9. In our study amplification with polymeric chain reaction (PCR) is applied where LPL-PvuII, LPL-HindIII, LPL-MnII and ApoCIII-SstI polymorphisms are provoked by corresponding and successive restriction enzyme digestion in angiographic selected examined and control groups.

Results: Our analysis showed difference in the distribution of the stated genetic markers, namely there was higher presence in the examined group. The analysis of the risk factors influence on the two groups isolated the hyperlipidemia, the diabetes, and the use of antilipemics as statistically significant parameters. The research also showed that there was no significant dependence between the examined risk factors and examined genetic markers. Concerning the connection to the lipid status, LPL-PvuII showed significant association with the level of triglycerides and with the total lipids. LPL-HindIII polymorphisms had statistically significant influence on the increased values of tryglicerides and on LDL-cholesterol in the examined group, so it had direct influence over the appearance of CAD. The study also showed that ApoCIII-SstI had no important association with the level of lipid fractions. Statistically significant was the influence of LPL-MnII polymorphism over the appearance of triglycerides.

Conclusion: No one of the genetic markers was identified as an independent risk factor for the appearance of CAD in our study. The analysis on the patents

demonstrated existence of mutual influence of LPL-PvuII, LPL-HindIII, LPL-MnlI and ApoCIII-SstI polymorphisms to the risk factors and to the serum lipids, which means direct influence over the appearance of coronary arterial disease in Macedonian population.

Key words: *LPL-PvuII; LPL-HindIII; LPL-MnlI and ApoCIII-SstI polymorphisms; lipids; coronary arterial disease.*

Defended: June 26, 2009.

Mentor: Prof. Dr. Samuel Sadikario.

Svetlana Jovevska. Evaluation of the morphological development of the kidney in human fetuses [PhD Thesis]. *Skopje, Republic of Macedonia: Institute of Anatomy, Faculty of Medicine, University "Ss Kiril and Metodij"; 2009.*

Kidney is a parenchymal organ and it is always examined as morphological and functional entity. The development of the kidney is a complex process and has three phases: primary kidney (pronephros), middle or secondary kidney (mesonephros), and definitive kidney (metanephros). Kidney variations during fetal growth as well as individual variations of different segments of its structure are permanent and persist after birth.

The aim of this study was to present the changes in kidney size during gestation in fetuses from 3th to 10th lunar month and to evaluate the dynamics of their growth and development.

This study included a sample of 300 fetuses (154 males and 146 females). Fetuses were obtained from the Clinic of Gynecology and Obstetrics in Skopje. All fetuses are part of the collection of the Institute of Anatomy. The kidneys of all 300 fetuses were examined using common anatomical and histological methods. Macrodissection was used to extract both kidneys "en bloc" and they were carefully separated from surrounding tissue. Afterwards, length, width and thickness were measured by Vernier caliper gauge. Length is a distance between the most remote points of two poles and width plus thickness were measured at the level of the hilus. The renal volume was calculated from other kidney diameters using the ellipsoid formula:

$$\text{Volume} = \text{length} \times \text{width} \times \text{thickness} \times \pi/6.$$

All parameters were statistically analyzed and there are graphically shown. Numerical values of these measurements were compared with weight, height and gestational age of the fetuses.

The analysis of the mean values of both the left and the right kidney of the examined series showed that there were significant statistical differences in relation to the lunar month. The length of both kidneys statistically significantly increased from one to the next lunar month.

The differences between the mean values of the length of the left kidney successively between the gestational weeks has shown that between 9 and 10 as well as between 10 and 11 gestational week, they were not significant. From 12 to 13 gestational weeks starts the intensive growth of the length of the left kidney with statistically significant differences between the previous and the next gestational week. The growth of the kidney decreased between 32 and 37 week, followed by a significant growth in length in 38 and 39 gestational week.

The results of the examination of the right kidney are the same as for the left kidney.

There were statistically significant and important differences between kidney dimensions (maximum longitudinal length, witdth, anteroposterior diameter and volume) in relation to gestational age.

Testing of the correlations between the analyzed parameters showed direct positive correlation between renal dimensions (maximum longitudinal length, width, anteroposterior diameter and volume) in relation to weight, height and gestational age of the fetuses. In this study we did not detect significant differences in the dimensions of the fetal kidneys between sexes.

The analysis of the distribution of the congenital malformations showed that there was no significant connection of these malformations in relation to sex. In both sexes, they were equally distributed. Of the total of 63 congenital malformations in male fetuses and 56 malformations in female fetuses, the trifurcation of the pielon was most frequently registered.

Such a difficult embryologic development can produce many renal and ureteral anomalies. In this study we found out that ectopic kidney and horseshoe kidney were more common than other anomalies.

Only one ectopia was registered on the left kidney in both female and male fetuses, and 2 ectopias in male and 3 in female fetuses were registered on the right kidney.

The kidney is a well vascularized organ. Congenital malformations in the renal artery were registered in 12 (7.8%) of a total of 154 male fetuses and in 14 (9.6%) a total of 146 in total of female fetuses. This finding has shown that there was no statistically significant dependence

in the appearance of congenital malformations according to sex.

Although there were more cases of congenital malformation of the kidney pelvis in female fetuses, still it was not of statistical importance.

The congenital malformations of ureters were more frequent in male fetuses, but without statistical significance.

Examinations have shown that kidney is an organ that undergoes complicated changes during embryonic development. Therefore, knowledge of these measurements may contribute to earlier diagnosis of a variety of abnormalities.

Key words: *fetus; kidney; anatomy; fetal development; malformation.*

Defended: June 29, 2009.

Mentor: Prof. Dr. Dobrila Tosovska-Lazarova

Irina Pavlovska. Epidemiological method used in examinations of the roll of some risk factors in pulmonary and laryngeal carcinoma [PhD Thesis]. *Skopje, Republic of Macedonia: Institute of Epidemiology and Biostatistics with Medical Informatics, Faculty of Medicine, University "Ss Kiril and Metodij"; 2009.*

No abstract available

Key words: Not available.

Defended: July 3, 2009.

Mentor: Prof. Dr. Nikola Orovchanec.

Dimce Zafirov. Nephroprotective effects of epoetin alpha and amifostine in renal diseases caused by cisplatin in rats.[PhD Thesis]. *Skopje, Republic of Macedonia: Institute of Preclinical and Clinical Pharmacology with Toxicology, Faculty of Medicine, University "Ss Kiril and Metodij"; 2009.*

Cisplatin, a heavy metal complex, is one of the most active drugs used in treatment of many human malignancies. However, high-dose therapy with cisplatin is limited by its cumulative nephrotoxicity.

The main objectives of this study were to determine the nephroprotective effects of recombinant human erythropoietin (Epoetin alpha) and amifostine applied as monotherapy or concomitantly in nephrotoxicity induced by long-term administration of cisplatin (2 mg/kg/b.w./ week) during eight weeks in Wistar rats, as well as to evaluate the mechanisms of cisplatin nephrotoxicity and nephroprotective effects of epoetin alpha and amifostine. A total of 125 Wistar rats were randomly assigned in five groups, each including 25 rats. Group 1 (CP) received only cisplatin (2 mg/kg/b.w./week), group 2 (CP+EPO) received cisplatin and epoetin alpha, group 3 (CP+Ami) received cisplatin and amifostine, group 4 (CP+EPO+Ami) received cisplatin and epoetin alpha in combination with amifostine, whereas the last group 5 was a placebo-control group of animals. Test for evaluation of the renal function and renal damages, along with hematologic examinations were performed prior to the begging of the study, during and at the end of the study. Six animals from each group before initiation of the investigation, after 4 weeks and at the end of the investigation were sacrificed, both kidneys were removed and used for pathohistological and specific immunohistochemical analyses, which included assessment of cell proliferation (Ki-67), degree of apoptosis and expression of Bcl-2 specific anti-apoptotic protein.

The obtained results have shown that long-term cisplatin administration resulted in cumulative and dose-dependent nephrotoxicity, which was manifested with functional and morphological renal impairments associated with distinct inhibition of cell proliferation and apoptosis stimulation. The monotherapy with erythropoietin, as well as amifostine, although incompletely, greatly reduced and alleviated this nephrotoxicity. On the other hand, combined amifostine/ erythropoietin therapy has proved to be superior over the monotherapy with any of these two drugs. In completely prevented the nephrotoxicity and increased the tolerability of the animals to the maximum total cumulative dose of cisplatin, which was 16 mg/kg/b.w./ week in this study. At the same time, our results showed that there was a difference regarding the mechanism of nephroprotective effects of erythropoietin and, amifostine. Nephroprotective effects of erythropoietin were most probably due to its direct anti-apoptotic effect and its stimulating effect on cell proliferation. Both groups of rats that were given erythropoietin demonstrated significantly reduced apoptosis and increased tubular and interstitial cell proliferation when compared with the CP-group. On the contrary, nephroprotective effects of amifostine were mainly due to its direct cytoprotective effect.

Our results have undoubtedly proved the synergistic effects of erythropoietin (a stimulator of cell proliferation, with evident anti-apoptotic effects) and

amifostine (a direct cytoprotective agent) in prevention of nephrotoxicity induced by long-term administration of cisplatin.

Key words: *erythropoietin; apoptosis; cisplatin; amifostine; nephrotoxicity; rats.*

Defended: November 12, 2009.

Mentor: Prof. Dr. Stojmir Petrov.

Venjamin A. Majstorov. Prognostic importance of myocardial perfusion tomoscintigraphy and some serological biomarkers in coronary disease [PhD Thesis]. *Skopje, Republic of Macedonia: Institute of Pathophisiology and Nuclear Medicine, Faculty of Medicine, University "Ss Kiril and Metodij"; 2009.*

Coronary artery disease (CAD) represents a worldwide healthcare problem with high morbidity and mortality and tends to increase further in developing countries, including Republic of Macedonia. Having in mind the high costs for treatment of patients with CAD on one side, and limited financial resources of the healthcare systems on the other side, there is a need for precise and on time risk stratification of the numerous affected individuals, what could finally optimize the therapeutical approach and management of patients with different stadium of CAD.

The possibility to foresee the risk the future cardiac events in patients with CAD and to define the prognostic importance of perfusional and functional parameters obtained from myocardial perfusion scintigraphy (MPS), as well as that of some serological biomarkers (high-sensitivity C-reactive protein / hs-CRP, interleukin-6 / IL-6 and N-terminal pro-B natriuretic peptide / NT-proBNP), was the main objective to conduct these research.

In this prospective study 193 patients were involved, suspective of having CAD or with previously confirmed CAD, who were referred from Clinic of Cardiology for myocardial perfusion imaging between December 2005 and July 2006 at the Institute of Pathophysiology and Nuclear Medicine, Medical School in Skopje. Additionally, for NT-proBNT estimation, another 118 patients with the same characteristics were included, in the period from September 2006 to April 2007.

The patients were followed up for mean 25.5 ± 3.1 months for the subsequent occurrence of hard (myocardial infarction and cardiac death) and soft cardiac events (revascularization and occurrence of heart failure).

Our results have shown a significant association between MPS variables, especially perfusional variables that reflect the presence of myocardial ischemia, and future cardiac events. During the follow-up hard events were recorded in 2% of patients with normal stress/rest myocardial perfusion versus 4.7% in patients with abnormal stress myocardial perfusion. Soft events occurred cumulatively in 2% of patients with normal perfusion and significantly more, in 27.9% of patients with abnormal stress perfusion ($p<0.001$).

The risk for all cardiac events was estimated by univariate Cox regression analysis, which has shown 6.16 times ($p<0.01$) greater risk for patients with abnormal stress myocardial perfusion and 4.03 times ($p<0.001$) greater risk for patients with abnormal summed difference score (ischemic score) compared to patients with normal stress/rest MPS and normal SDS respectively. The wall motion scores and LVEF% after stress were also significantly associated with increased risk for all cardiac events.

In multivariate Cox regression model independent predictors for all cardiac events were ischemic score ($p<0.001$), hs-CRP ($p=0.001$) and abnormal stress myocardial perfusion ($p<0.05$). The only strong independent predictor for soft cardiac events was the ischemic score ($p<0.001$).

Interleukin-6 has not shown significant association with future cardiac events in our study. NT-proBNP levels were significantly higher in patients with CAD, even though they had preserved LV function.

Comparative analyses of the clinical information, information obtained during the stress protocol with serological biomarkers and MPS parameters, has demonstrated that in the anticipation of various types of cardiovascular risk, independent predictors of greatest significance were the perfusional variables from MPS. Most important predictor was the amount of ischemia represented through the summed difference score.

Key words: *coronary artery disease; single-photon emission-computed tomography; prognosis; C-reactive protein; Interleukin-6; brain natriuretic peptide.*

Defended: November 17, 2009.

Mentor: Prof. Dr. Olivija Vaskova

Svetlana Koceva. Molecular basis of spinal muscular

atrophy, Duchenne/Becker muscular dystrophy and Friedreich's ataxia in Republic of Macedonia. [PhD Thesis]. ***Skopje, Republic of Macedonia: Resarch Center for Genetic Engineering and Biotechnology, Macedonian Academy of Science and Arts; 2009.***

Spinal muscular atrophy (SMA), Duchenne/ Becker muscular dystrophy and Friedreich's ataxia are inherited neuromuscular disorders in childhood with progressive clinical course and letal outcome. The discovery of the genes and the mutations, which are responsible for these disorders, enabled the diagnosis at the genetic level and prenatal diagnosis as a possibility for the prevention and control of these disorders.

The aim of this study is molecular characterization of spinal muscular atrophy, Duchenne/Becker muscular dystrophy and Friedreich's ataxia in Macedonian patients.

We have studied the molecular basis of 171 unrelated Macedonian patients of whom 38 patients with SMA (25 patients had SMA type I, eight had SMA type II and five patients had SMA type III), 96 patients with Duchenne/Becker muscular dystrophy and 50 patients with Friedreich's ataxia. The methods used for the molecular characterization included: Polymerase Chain Reaction (PCR), Restriction Fragment Length Polymorphisms (RFLP), Multiplex Ligation-dependent Probe Amplification (MLPA), analysis of Short Tandem Repeat (STR), Single Strand Conformational Polymorphism (SSCP), agarose/polyacrilamide gel electrophoresis and fluorescent capillary electrophoresis.

Homozygous deletions for at last part of the telomere copy of the SMN gene were found in 96% patients with SMA type I and in 87.5% patients with SMA type II. Of these patients, 87.5% were homozygous for deletions of axons 7 and 8, and 12.5% were homozygous for a deletions of axons 7 only.

Homozygous deletions of exon 5 of the NAIP gene were found in 44.0% of patients with SMA type I,11.1% patients with SMA type II and in 6.0% of the parents. Our data support the thesis that the telomeric SMN gene (SMN 1) may play a major role in determining the clinical severity of spinal muscular atrophy.

Molecular analysis of Duchenne/Becker muscular dystrophy showed deletions in 71.1% and duplications in 6.25% of DMD/BMD patients. The number of deleted exons was variable. Analysis of the breakpoint distribution pattern in the dystrophin gene showed that intron 44 was involved most frequently (37.8%) as a starting brealpoint. In the Macedonian patients, intron 53 (24.5%) in the second most frequently observed has a spot at the 3' end of the gene, those seem to be characteristic for the Macedonian patients. At the 5' end, the breakpoint is in intron two. As it was proposed by previously national studies, our findings also suggest that certain intronic sequences, characteristic for a population, probably determine the development of a preferential breakpoint profile in this disease.

Among the patients with clinical diagnosis of Friedreich's ataxia before 25 years, the GAA triplet repeat in the FRDA gene was expanded in 93.3% of patients.

The results of this study will be of the great help in clinical prognosis of the SMA, DMD/BMD and FRDA. Fifteen prenatal diagnoses in families with SMA and 15 prenatal diagnoses in families with DMD were performed as a practical achievement of the results of this doctoral thesis.

Key words: *Spinal muscular atrophy; Duchenne/Becker muscular dystrophy; Friedreich's ataxia.*

Defended: November 18, 2009.

Mentor: Academician Georgi Efremov

Irina Panovska Stavridis. Immunophenotypization of the cells from peripheral blood and bone marrow in diagnostic and in clinical stratification in patients with acute leukemia. [PhD Thesis]. ***Skopje, Republic of Macedonia: University Clinic of Haematology, Faculty of Medicine, University "Ss Kiril and Metodij"; 2009.***

The natural history of the acute leukemias and the response to therapy varies according to the type of blast involved in the leukemic process. Although, in many instances the lineage assessment of the different types of blast cells may be recognized by simple morphological and cytochemical stains, it is necessary to employ immunological analyses with monoclonal antibodies and cytogenetic or molecular biological techniques to identify their particular differentiation features. Correct diagnosis of the diverse subtypes of acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) plays a central role for individual clinical risk stratification and therapeutic decisions. The latest World Health Organization (WHO) classification of acute leukemias incorporates and interrelates to morphology, immunologic, cytogenes, and molecular genetics markers and pays major attention on the importance of genetic events in the classification and therapy of the AMLs. Its prognostic relevance is most

clearly demonstrated in the AMLs characterized by: t(15,17), t(8,21) and inv16, which generally have a favorable prognosis when treated with appropriate therapeutic agents. According to WHOs classification of ALL, a crucial role regarding the diagnosis, prognosis and clinical stratification of patients plays the immunophenotyping of the malignant cells. It is anticipated that advancements in diagnostic technology will reveal additional molecular markers in acute leukemia that will result in a more precise classification of this heterogeneous complex of disorders.

In order to establish and standardize a diagnostic algorithm and improve the diagnosis and management of acute leukemia in the Republic of Macedonia, we conduct a prospective study at the University Clinic of Hematology –Skopje. A total of 76 adult (>15 years) patients with acute leukemia, who were consecutively admitted at the Clinic, were enrolled in this study. The aim of our study is to establish the correct lineage assignment of the blast cells, to evaluate the incidence of the favorable genetic markers PML/RARα, AML1-ETO, CBFβ-MYH11 among the AML cases to test B-ALL cases for the presence of the BCR-ABL fusion gene. Then, the obtained results were correlated with the patient's age and performance status and a consecutive effective treatment strategy was selected for each single acute leukemia patient. For further improvement of the individual risk stratification of acute leukemia patient in diagnosis and evaluating the role of activating JAK2V617F mutations in the pathogenesis and clinical course of acute leukemia, we screened our patients for the mutations.

The multi-parameter flow cytometry (MPF) was introduced for the first time in the Republic of Macedonia and was performed at the beginning of this study at the Institute of Immunobiology and Human Genetics, Faculty of Medicine-Skopje and continued at the University Clinic of Hematology. The diagnosis of acute leukemia was made by standard morphological examinations and cytochemical analyses of bone marrow smears according to the criteria established by the French-American-British (FAB) Study Group and confirmed by immunophenotyping of bone marrow aspirates and/or peripheral blood samples following the criteria of the European Group for the Immunological Classification of Leukemia's (EGIL) and the British Committee for Standards in Hematology (BSCH).

Our results showed that the morphology and cytochemistry established lineage in 68 of patients, but not in 9 cases that were presented as acute leukemia, of which 4 were assigned as myeloid, 4 as lymphoid and in one patient a non-hematopoietic malignancy was indicated. Furthermore, immunophenotyping changes the lineage assignment based on the morphology and cytochemistry in three cases from lymphoid to myeloid. The results from out study showed that routine immunophenotyping improved the diagnosis in 12 cases (15.5%).

The exact lineage assignment of the blasts cells guides to implementation of specific molecular analyses in some subtypes of acute leukemia and their further definition, which is essential for more appropriate single patient therapeutic decisions.

The molecular analyses were performed, at the Center for Bimolecular sciences, Immunology and Pharmacogenetics, Faculty of Pharmacy-Skopje, according to standard procedures. Using RQ-PCR assay we detected the presence in of the fusion transcript PML/RARá in 5 patients. Ten patients were classified in the prognostic favorable AML genetic group Core Binding Factor (CBF)-AML according to the presence of the abnormal fusion transcripts AML1-ETO, CBFβ-MYH11. Further, we stratified three B-ALL patients into the very risk ALL group by the presence of the BCR-ABL oncogene. The allele-specific (AS)-PCR assay has revealed only one AML patient positive for the JAK2V617F mutation.

Our data supports and justifies implementations of MPF in the diagnostic evaluation of acute leukemia. The applied multimodal diagnostic approach consisted of minimal number of cytomorphological, cytochemical, immunological and molecular analyses enables an improved and more precise diagnosis and clinical stratification in 38.2% acute leukemia patients from our study.

Key words: Not available.

Defended: November 18, 2009.

Mentor: Prof. Dr. Lidija Cevrevska

Sanja Mancevska. Investigation of the effect of anxiety on attention and learning using EXG-paradigm. [PhD Thesis]. ***Skopje, Republic of Macedonia: Institute for Medical and Experimental Physiology with Antropology, Faculty of Medicine, University "Ss Kiril and Metodij"; 2009.***

In this thesis the prevalence of high trait anxiety and high state anxiety and the prevalence of mild and moderate depression, in a sample of 842 students from four faculties in the Republic of Macedonia, during early stages of their education, were estimated. An evaluation of cognitive functioning in 30 subjects with high level of trait

anxiety and 30 subjects with low levels of trait anxiety, using electrophysiological method EXG-paradigm with two levels of cognitive effort and a psychological memory test P-R, was made.

For the purpose of clinical and psychological evaluation of the students, standardized psychological and psychiatric instruments (Taylor Manifest Anxiety Scale, Beck Anxiety Inventory and Beck Depression Inventory) in a form of self rating questionnaires and a psychiatric interview was used. The substance usage and facing stressors was evaluated with an in-standardized questionnaire designed for the purpose of the study.

The examination of the attention and learning in subjects with high levels of trait anxiety, in this study, is based on the concept of adaptation of the subjects to the changes of the environment and their interaction. For that purpose EXG paradigm has been used. It is an electrophysiological method based on the classical CNV paradigm, in which the dynamics of the process of expectancy expressed in the CNV potential is investigated. EXG paradigm places the subject in a varying environment expressed in the presence and absence of the S2 tone of the paradigm. During the experiment, in correlation of his/her cognitive capacity, the subjects adapt to the changing environment and in fact learn how to optimize his/her expectations and adjust to the requirements of the paradigm. Electrophysiological parameters of the associative learning such as duration of acquisition and extinction of the conditioned response, mean amplitude of the late contingent negative variation during the blocks of acquisition and extinction of the conditioned response and the speed of the motor response during the EXG paradigm are learning curves are also analyzed.

Psychological parameters of learning during the pattern-recognition test included: total number of mistakes, number of trials and time necessary for the P-R test to be completed, as well as the learning efficiency index.

There were high prevalence's of high levels of trait anxiety and moderate depression among students, on which facing stressors and substance use had negative impact.

The subjects with high levels of trait anxiety had impaired cognitive performance during more complex cognitive task within the EXG paradigm and the P-R test, compared to the subjects with low levels of trait anxiety.

Our data strongly suggest that a student counseling service which will provide mental health assistance and guidance in improving academic performance of students, should be established as an important part of the higher education facilities in Macedonia.

Key words: *anxiety; depression; attention; learning; expectancy; CNV potential; eletroexpectogram.*

Defended: November 19, 2009.

Mentor: Prof. Dr. Liljana Bozhinovska

Dejan Trajkov. Molecular defining of cytokine gene polymorphisms in Republic of Macedonia and their association with various duseases. [PhD Thesis]. *Skopje, Republic of Macedonia: Institute of Immunobiology and Human Genetics, Faculty of Medicine, University "Ss Kiril and Metodij"; 2009.*

Numerous SNPs is cytokine genes have been identified, and some of them are associated with qualitative or quantitative changes in protein production and susceptibility to various diseases, including autoimmune, infectious, allergic or cardiovascular diseases. Distribution of cytokine gene polymorphisms may vary significantly among different ethnic groups, and eventually contribute to observed differences in disease frequencies.

The aim of this study is to genotype 22 cytokine polymorphisms in the Macedonian population and to examine their possible role in host susceptibility to or protection against pulmonary tuberculosis, dilated cardiomiopathy, rheumatoid arthritis, bronchial asthma and chronic obstructive pulmonary disease.

The sample of the population comprised of 310 healthy unrelated individuals, 75 patients with pulmonary tuberculosis, 52 patients with dilated cardiomiopathy, 85 patients with rheumatoid arthritis, 74 patients with BA and 62 patients with chronic obstructive pulmonary disease. All of the patients and healthy individuals included in this study after signing a written consent. Blood samples were collected, DNA was isolated from peripheral blood leukocytes by the phenol-chloroform extraction method, and DNA samples were stored in the Macedonian Human DNA Bank (hDNAMKD). Cytokine genotyping was performed by polymerase chain reaction with sequence-specific priming (PCR-SSP) using the Cytokine Genotyping Kit (Heidelberg kit) (Dynal Biotech, Invitrogen, Brown Deer, WI; USA). Those SNPs that did not fit HWP were evaluated to determine whether there was an excess of homozygotes or heterozygotes. Comparisons of different genotypes for two groups were tested by the X^2 test, and crude odds ratio was calculated with 95% confidence

interval (CI).

Test of neutrality (Fnd) showed negative value, but was significantly different from 0 for *TGF-beta1 cdn10* and *IFN-gamma utr5644* (p of F=0.001 and 0.012 respectively). Several SNPs (*IL-1 alpha -889, IL-1 beta +3962, IL-2 +166, IL-4 -590, IL-4 -33, and IL-10 -592*) were not in HWP (p<0.005). Test of neutrality for cytokine haplotypes showed significantly difference from 0 only for IL-2 haplotypes (p=0.020). Susceptible association with TBC showed four cytokine alleles, five cytokine genotypes, three haplotypes and five diplotypes were found. Negative (protective) association with TBC and four cytokine alleles, four cytokine genotypes, two haplotypes and olnly one diplotype were found. Results showed two alleles, six genotypes, two haplotypes and six combinations of haplotypes were positively associated with dilated cardioiopathy, while negative association was identified for two alleles, three genotypes, one haplotype and two combinations of haplotypes. For RA results showed susceptible association for 4 cytokine alleles, 6 cytokine genotypes, one haplotypes and 4 diplotype, while protective association were found for 4 cytokine alleles, 3 cytokine genotypes, 3 haplotypes and only one diplotype. Susceptible cytokine polymorphisms for BA for six alleles, four haplotypes, and six combinations of haplotypes were found. Protective cytokine polymorphisms for BA for six cytokine alleles, seven cytokine genotypes, four cytokine haplotypes and six diplotypes was found. Positive (susceptible) association between patients with COPD and seven cytokine alleles, twelve cytokine genotypes, five haplotypes and six diplotypes were found. Negative (protective) association between patients with COPD and seven cytokine alleles, nine cytokine genotypes, two haplotypes and four diplotype were found.

These results suggests that some cytokine polymorphisms are significantly associated and affects host susceptibility/resistance to TBC, dilated cardiomiopathy, rheumatoid arthritis, bronchial asthma and chronic obstructive pulmonary disease in Macedonians. The results of cytokine polymorphisms in Macedonian population can be used for anthropological comparisons, as well as for association studies with different diseases.

Key words: *Cytokine gene polymorphisms; SSP genotyping; Macedonian population; TBC; dilated cardiomiopathy; rheumatoid arthritis; bronchial asthma; chronic obstructive pulmonary disease.*

Defended: November 23, 2009.

Mentor: Prof. Dr. Mirko Spiroski.

Elizabeta Zhogovska. The benefit of reconstructive methods in the outcome of the treatment of disraphic anomalies of the vertebral column. [PhD Thesis]. *Skopje, Republic of Macedonia: Clinical Centar, Clinic for Plastic and Reconstructive Surgery, Faculty of Medicine, University "Ss Kiril and Metodij"; 2009.*

The author present a retro-prospective study of reconstructive skin cover in the huge skin defect remained after myelomeningocela repair.

Myelomeningocoellae is a defect of the neural tube closure done from the 4th to 8th week of gestation. Caused by yet unknown cause, the closure of neural plate in the caudal or cranial region failed. Thus the neural plate remains uncovered by dural envelope, mesenhimal tissue and skin and a myelomeningocoellae occurred. In many cases the skin defect is very large and poses a serious reconstructive problem.

Material and method: The author presents two series of patients with toracolumbal, lumbal or lumbosacral huge meningocella over 5 cm, a series of 30 patients with direct skin closure after neural plate closure and dural envelope reconstruction, and a series of 59 patients where transposed, rotated or sliding flap is used for skin closure. Although the second series seems to be more heavily, both series are enough balanced for statistical analyze.

Results: The statistic analyze shows statistically well balanced two series according to the birth weight, sex distribution, the size of the defect, neurological symptoms, time and duration of surgery, although in second series there are more newborns with a smaller birth weight under 2500g. However postoperative local complications such as local skin infections, local skin disjunction, skin necrosis, cerebrospinal fluid fistula, are significantly increased in series with direct skin closure. Although the rate of meningitis is statistically insignificant (p=0.08) there is more cases with meningitis in the same series. The rate of mortality is higher in the series with direct skin closure than in the series with transposed, rotated or sliding flap used for skin closure.

Conclusion: The reconstructive skin cover techniques provide better surgical outcome, with lower rate of complications then direct skin closure for covering the skin defect in case of huge myelomeningocoela.

Key words: *skin defect; huge myelomeningocoela; skin flap; skin reconstruction; complications.*

Defended: November 25, 2009.

Mentor: Prof. Dr. Gorge Dzokic.

Karolina Blazevska Buzarovska. The evaluation of the effects of trabeculotectomy with and without use of 5-Fu in patients with glaucoma and the meaning of vascular factors in them. [PhD Thesis]. ***Skopje, Republic of Macedonia: University Clinic of Ophthalmology, Faculty of Medicine, University "Ss Kiril and Metodij"; 2009.***

No abstract available

Key words: Not available.

Defended: November 25, 2009.

Mentor: Prof. Dr. Elena Dzhajkovska.

Milan Samardziski. The evaluation of the value of the surgical methods for saving the extremities in patients with osteosarcoms and chondrosarcoms [PhD Thesis]. ***Skopje, Republic of Macedonia: University Clinic of Orthopedy, Faculty of Medicine, University "Ss Kiril and Metodij"; 2009.***

No abstract available.

Key words: Not available.

Defended: December 3, 2009.

Mentor: Prof. Dr. Gjorgji Zafirovski.