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PROMISING MEDICAL SCIENTISTS WILLING TO LOOK BEYOND

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## ORAL PRESENTATIONS

### *Anaesthesiology*

#### **Local versus systemic endomorphin 2 in a carrageenan model of inflammatory hyperalgesia**

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Inflammation effectively increases the activation of opioid receptors on peripheral terminals of sensory neurons. The resulting inflammatory hyperalgesia responds by decreasing in intensity to local treatment with opioid analgesics. Comparatively, much less is known about the efficiency of opioid peptides administered peripherally at the inflammation site. The study examined the antihyperalgesia elicited by endomorphin 2, a mu agonist, when administered peripherally in a model of acute inflammation with carrageenan. The effects of endomorphin 2 administered locally in three different doses were investigated with behavioral assays. The local paw injection of endomorphin at the site of inflammation induced an antihyperalgesic effect. For confirmation of the peripheral mechanism of analgesia, the peptide was systemically administered (i.p.) and at the dosage we used endomorphin 2 was not significantly antihyperalgesic. Endomorphin 2 was antihyperalgesic without significantly affecting edema. In conclusion, the antihyperalgesic effect of the endomorphin 2 is not secondary to a reduction of edema, since reduction of edema does not occur. These observations plead for an action at the level of opioid receptors on nerve terminals rather than on immune cells.

#### **A Comparison of five scoring systems of mortality risk among the ICU admitted patients in Tehran**

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*Introduction:* The aim of this study is to compare the performance of five applied general severity scoring systems and their ability to predict mortality rate for each of the intensive care unit patients (Simplified Acute Physiology Score II (SAPS II), Mortality Probability Model at admission (MPM II 0) at 24hours (MPMII 24) and 48hours after admission and also MPM overtime for patients with more than 48 hours of staying in ICU). These scoring systems have been developed in response to an increase emphasis on the evaluation and monitoring of health care services and also making cost-effecting decisions.

*Method and materials:* all of the scoring systems were applied to the same 121 patients through the retrospec-

tive data collecting. Predicted mortality rate for each of them were calculated separately. Standardized mortality ratio (SMR), correlation, logistic regression and Discrimination (ROC Curves) were evaluated.

*Results:* Predicted mortality by all systems was significantly correlated and they were not significantly different from actual mortality [SMR for SAPS II: 0.79; MPM II0: 1.09, MPM II24: 1.35, MPM II48: 1.05, MPM II overtime: 1.00]. Discrimination was best for MPM II24 (ROC AUC: 0.927) followed by SAPS II (0.903), MPM II0 (0.899), MPM overtime (0.861), MPM II48, (0.848).

*Conclusion:* All five general ICU mortality predictors have got accurate standardized mortality ratio. MPM II24 has the best discrimination which is followed by SAPS II. The local performance of MPM II24 in addition to its ease-to-use makes it an attractive model for mortality prediction in our study. ICU : Intensive Care Unit, MPM: Mortality Probability Model , SAPS: Simplified Acute Physiology Score

#### **The Influence of N-methyl-acetazolamide on acute hypoxic pulmonary vasoconstriction (HPV) in conscious dogs**

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*Aim:* The carbonic anhydrase (CA) inhibitor acetazolamide (Acz) can prevent acute hypoxic pulmonary vasoconstriction (HPV). Recent studies propose that the effect of Acz is independent of CA inhibition. Thus the question was raised whether n-methyl-acetazolamide (NMAcz) - a molecule in which the CA-inhibitory properties are inactivated - might inhibit HPV comparable to Acz. In a study of pulmonary smooth muscle cells (PASMC) NMAcz inhibited the hypoxia induced intracellular Ca<sup>2+</sup> response and thus the PASMC's contraction. But does NMAcz also inhibit HPV in conscious dogs?

*Methods and Material:* The study was approved by the animal protection committee of Berlin (LAGetSi - 0084/04). Ten Beagle dogs were kept under standardized conditions. Protocols: 1) Control, 2a) NMAcz low (250 mg bolus and 5 mg/kg/h continuously) 2b) NMAcz high (10 mg/kg bolus and 10 mg/kg continuously) 3) Acetazolamid (2mg/kg bolus, 2 mg/kg/h continuously). Three dogs underwent all protocols in randomized order. Another 4 dogs were studied in protocol 1), 2a), 4) and 3 dogs in 1), 2b) and 4). During all experiments, the dogs were conscious and breathed spontaneously via a ventilator circuit. During the first hour of normoxia the FiO<sub>2</sub> was 0,21; during the following two hours of hypoxia the FiO<sub>2</sub> was lowered to 0,1. Respiratory parameters, systemic and pulmonary haemodynamics were recorded. Arterial blood gas analysis were performed at the end of each hour.

The data are pre-sented as means  $\pm$  SEM;  $p < 0,05$  (GLM-ANOVA).

**Results:** The results were analysed in two groups taking all dogs treated with a low dose of NMAcz (2a) together and all dogs with a high dose of NMAcz (2b). The comparison was made between the normoxic and the hypoxic period. During hypoxia the arterial PaO<sub>2</sub> was 34,5 -36,2 mmHg (Controls) and 36-39 mmHg (NMAcz low, high, and Acz), PaCO<sub>2</sub> was 27-30 mmHg in all groups. Mean pulmonary artery pressure (MAP) increased about 6 mmHg in Controls, about 4 mmHg in NMAcz low and high and about 2 mmHg in Acz. The pulmonary vascular resistance increased by 117-180 dyn·s·cm<sup>-5</sup> in Controls, by 100-128 dyn·s·cm<sup>-5</sup> in NMAcz (low), by 68-91 dyn·s·cm<sup>-5</sup> in NMAcz (high), and by 17-27 dyn·s·cm<sup>-5</sup> in Acz.

**Conclusion:** NMAcz partially inhibits HPV in conscious dogs. The weak effect of NMAcz could not be significantly improved by increasing the amount of NMAcz given. This may lead to the conclusion that there is an additional factor preventing HPV under Acz-treatment. Probably, the inhibition of the endothelial CA contributes to the stronger effect. On the other hand the amine of the sulfonamid moiety (SO<sub>2</sub>NH<sub>2</sub>) in Acz, which is replaced by a methyl group in NMAcz, may also influence a different cellular receptor or channel than the CA.

### Anesthesia-induced neurodegeneration in different regions of the developing brain in rats

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**Background:** Neuronal death induced by anaesthetics in the developing brain was evident in previous pre-clinical studies (1,2,3,4). However, these studies were done in the absence of noxious stimuli, indicating that such side effects in neonates may not represent the clinical situation in the operating theatre. Also the neuronal cell types involved in anaesthetic-induced neuronal cell death remains elusive (5).

**Aims:** 1). To determine the impact of noxious stimuli on anaesthesia-induced neuronal cell death; 2). To investigate glutamatergic, GABAergic, cholinergic and dopaminergic neuronal cell apoptosis induced by anaesthetic exposure in specific brain regions in rats.

**Method:** Separate cohorts (n = 4/group) of 7-day-old SD rat pups were randomly assigned to six groups: (1) Naive; (2) Anaesthetics alone (70% nitrous oxide and 0.75% isoflurane exposure for 6 hrs); (3) Surgical incision alone (midline 1cm cut to the left hind paw); (4) Formalin injection alone (subcutaneous injection with 10  $\mu$ l 5% formalin into the left hind paw); (5) Anaesthetics + surgical incision (A+S); (6) Anaesthetics + formalin injection (A+F). The rats were euthanized after 6-hr treatments, perfused, and then the brains were removed, dehydrated and sectioned into 30 $\mu$ m thick slices. Brain slices containing the cingulate cortex were selected and subsequently subjected to immunofluorescence staining for caspase-3. In the anaesthetics-alone group, brain slices that contained the

basal forebrain were selected and subsequently subjected to double immunofluorescence staining for caspase-3 and choline acetyltransferase (ChAT); substantia nigra for caspase-3 and dopamine; CA1 subarea of hippocampus and cingulate cortex for caspase-3 and either vesicular glutamate transporter 1 or glutamic acid decarboxylase 67. The positive-staining cells were viewed under fluorescence microscope and photomicrographs were captured for data analysis.

**Results:** In the cingulate cortex, noxious stimuli caused a significant increase of anaesthesia-induced neuroapoptosis (No. of caspase-3 positive cells: naïve  $2 \pm 1$ ; A alone  $10 \pm 2$ ; A+S  $20 \pm 7$ ; A+F  $21 \pm 5$ ;  $p < 0.05$  vs. A alone). In the anaesthetics alone group, 54% and 14% of the apoptotic cells in the CA1 subarea of hippocampus were GABAergic and glutamatergic neurons respectively. In the cingulate cortex, 30% and 37% of apoptotic cells were GABAergic and glutamatergic neurons respectively. In the substantia nigra, 22% of apoptotic cells were dopaminergic neurons. The basal forebrain was spared from anaesthesia-induced neuroapoptosis.

**Conclusion:** Noxious stimuli appear to have a detrimental impact on anaesthesia-induced neurotoxicity observed in the cingulate cortex. Different types of neurons exhibit different vulnerabilities to anaesthesia-induced neurotoxicity in different brain regions.

**Key words:** Anaesthetics, Neurons, Apoptosis, Caspase-3, Nitrous oxide, Isoflurane, Pain, Inflammation

## Biochemistry

### Association of glycated hemoglobin with dyslipidemia and risk of atherosclerosis in type 1 diabetic patients

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Premature atherosclerosis is a risk factor for cardiovascular disease, particularly coronary artery disease and seems to be related to the changes in plasma lipid profile and to poor metabolic control.

**Aim:** The aim of this study was to determine whether the dyslipidemia is associated with glycated hemoglobin (HbA1c), then the relationship of dyslipidemia and glycated hemoglobin with atherosclerosis, then to study the gender difference in dyslipidemia.

**Materials and Methods:** Twenty five clinically diagnosed type 1 diabetic children and adolescents in the age group of 7-18 years and twenty five age and sex matched healthy children and adolescents constituted the study population. HbA1c was measured using HPLC. Result: HbA1c levels were compared with lipid levels by Pearson's coefficient which shows - HbA1c levels are negatively associated with total cholesterol which is not of significance. HbA1c is negatively associated with total triglyceride as well as VLDL in control but it is positively associated in the diabetic cases, which is strongly significant of the distortion caused by the disease in these levels, HbA1c is positively associated with HDL as well as LDL levels but

there was statistical significant difference in controls and cases. HbA1c is negatively associated with atherogenic index in both control as well as cases but the value is significantly different. The gender difference was calculated shows that females are more prone to changes in lipid profile with the change in HbA1c levels as compared to males.

**Conclusion:** It is concluded that type 1 diabetes mellitus patients are at increased risk of premature atherosclerosis due to associated dyslipidemia with increased levels of glycosylated hemoglobin. The major risk factor which can lead to atherosclerosis as revealed in our study is significant decrease in HDL levels which shows negative correlation with HbA1c levels that is with the increase in glycosylated hemoglobin associated with significant decrease in HDL levels which in turn increases the risk of atherosclerosis to many fold. Females are at increased risk of atherosclerosis than males because of higher prevalence of dyslipidemia among females as compared with males.

### The role of GSTM1 polymorphism in susceptibility to renal cell carcinoma

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**Aim:** Cytosolic glutathione transferases (GTSs) are superfamily of enzymes that protect normal cells by catalyzing conjugation reactions of electrophilic compounds, including cancerogens, with glutathione. Since differential glutathione transferase expression significantly influences anti-carcinogenic potential of tissues, GSTs are currently being investigated as biomarkers of risk for various cancers, including renal cell carcinoma (RCC). Among polymorphic GST classes, GSTM1, GSTT1 and GSTP1 polymorphisms have been investigated with regard to interindividual differences in susceptibility to RCC. The case control study was designed to test the association between genetic polymorphism of GSTM1 and susceptibility to RCC independently or in conjunction with cigarette smoking, high relative body weight and high blood pressure which are known risk factors for this carcinoma.

**Methods and materials:** Genomic DNA was isolated from blood of 56 controls and 65 patients with RCC, who completed a structured interview. Genotypes of GSTM1 were determined by multiplex PCR. Data obtained on GSTM1 polymorphism were statistically analyzed with respect to known RCC risk factors.

**Results:** GSTM1 null genotype (GSTM1\*0) was found in 48,2 % controls and 58,5% in cases. The results have shown that frequency of GSTM1\*0 is not increased in RCC. However, the frequencies of GSTM1\*0 genotype and smokers were higher in patients with RCC. Analysis of demographic data in the group of RCC patients have shown that older patients had higher risk for RCC and that the number of RCC patients decreased with increase in body mass index. Moreover, the frequencies of GSTM1\*0 genotype within the lowest tertile of BMI were higher in patients with RCC.

**Conclusion:** Further studies are necessary to clarify the role of GSTM1 polymorphism as risk factor for RCC,

independently or in conjunction with other known risk factors for this carcinoma.

### The eventual association of CBS 844ins68 mutation and recurrent spontaneous abortion in a group of Romanian patients

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Cystathionine b-synthase (CBS) is an enzyme involved in the transsulfuration pathway of the homocysteine metabolism, that catalyzes the irreversible reaction between homocysteine and serine to form cystathionine in the presence of pyridoxal 5-phosphate, an active form of vitamin B6. Mutations in the CBS gene can lead to an impaired function of the coded enzyme, which leads to the accumulation of homocysteine by insufficient transsulfuration, and homocystinuria. The 68-bp (844ins68) insertion in the coding region of exon 8 in the CBS gene apparently leads to a poor transcription of the affected allele. Hiperhomocysteinemia is presently considered to be risk factor for many pathologies. The aim of our study was to analyze the distribution of the CBS 844ins68 allele in a group of 131 female patients with recurrent spontaneous abortion and 164 healthy Romanian females as controls, in order to investigate the possible association of this insertion and recurrent spontaneous abortion. We used the polymerase chain reaction (PCR)-simplex technique to investigate the allele and genotype distribution of the CBS 844ins68 mutation in both patients and controls. The frequencies of the mutant allele (21 vs. 13, odds ratio [OR] = 1.723, 95% confidence interval [CI] = 0.8437-3.517, p = 0.1568), heterozygote genotype (21 vs. 11, odds ratio [OR] = 2.152, 95% confidence interval [CI] = 0.9929-4.664, p = 0.0594). The 844ins68 homozygous genotype was found in only one subject in the control group. Our results indicate that the CBS 844ins68 mutant allele is not a risk factor for recurrent spontaneous abortion. At the same time, results were very close to the statistically relevant border, suggesting that in order to draw further conclusions about the association of the CBS 844ins68 mutation and recurrent spontaneous abortion it is necessary to enlarge the study group, consider eventual gene to gene interactions and measurements of the plasma homocysteine levels should also be taken into account.

## Cytotoxic activity of O,O'-diisobutyl-(S,S)-ethylenediamine-N,N'-di-2-propanoat ligands and corresponding platinum(II) complexes in vitro

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**Aim:** Because of cisplatin's impressive impact on cancer chemotherapy, great efforts have been made to develop new derivatives with improved pharmacological properties, better antitumor activity and less side effects. The aim of this study was investigation of cytotoxic effects of two novel platinum(II) compounds, as well as their ligand precursors (branched-chain ester hydrochlorides). In order to understand possible mechanisms of cytotoxicity, production of reactive oxygen species and level of autophagy in cells after the treatment with complexes were measured.

**Materials and Methods:** Two novel ligand precursors, O,O'-diisopropyl- (1a) and O,O'-diisobutyl-(S,S)-ethylenediamine-N,N'-di-2-propanoat dihydrochloride monohydrate (1b) and the corresponding dichloroplatinum(II) complexes (2a and 2b) were used in this study. Experiments were conducted on primary rat astrocyte culture, C6 glioma cell line and L929 fibrosarcoma tumor cells. Time of exposure was 24h, 48h and 72 hours. Viability rate of treated cells was determined with acid phosphatase assay. Cells were analysed on flow cytometer - for production of reactive oxygen species using dihydrorodamine, and for quantifying autophagy using acridine orange.

**Results:** Both ligand precursors (1a and 1b) showed very low cytotoxicity against tested cell lines for all measured times (IC<sub>50</sub> [microM] values >200). Platinum(II) compound 2a also showed low activity against astrocytes and C6 cells for all measured times (IC<sub>50</sub> [microM] values >200), while its effect against L929 cells was significant-IC<sub>50</sub> [microM] values were 198, 182.8 and 136.9 for 24h, 48h and 72-hour treatment, respectively. Platinum(II) compound 2b showed low cytotoxicity against astrocytes, for all measured times (IC<sub>50</sub> [microM] values >200), while that activity was significant against C6 cells (IC<sub>50</sub> [microM] values were 94.2, 94.2, and 68.5) and L929 cells (IC<sub>50</sub> [microM] values were 61.4, 54.6, and 38.1), after 24h, 48h and 72-hour treatment, respectively. Complex 2a, concentration 200 microM, produced increase of detected fluorescence intensity (Geo Mean value 32.36) in L929 cells after 24-hour exposure, comparing to untreated cells (Geo Mean value 21.01), what speaks in favour of increased production of ROS. Complex 2b, concentration 50 microM, also increased detected fluorescence intensity (Geo Mean value 34.14) in L929 cells after 24-hour exposure, comparing to untreated cells (Geo Mean value 18.87). Complex 2a, concentration 200 microM, used against L929 cells for 24 hours, produced increase of orange/green fluorescence intensity ratio (FL3/FL1=1.28), comparing to untreated cells (FL3/FL1=1.00), what speaks in favour of increased level of autophagy. Complex 2b, concentration 50 microM, used against L929 cells for 24 hours also induced increase of orange/green fluorescence intensity ratio (FL3/FL1 = 1.49), comparing to untreated cells (FL3/FL1=1.00).

**Conclusion:** Both precursor ligands and both Pt(II) com-

plexes show no significant cytotoxic activity on primary rat astrocytes. Both precursor ligands and complex 2a also show no significant activity against C6 cells, while complex 2b shows distinctive cytotoxicity. Precursor ligands 1a and 1b show low activity against L929 cells, while complexes 2a and 2b show considerably higher effect. Both examined Pt(II) compounds induce production of ROS, as well as increase of autophagy level in L929 cells after 24-hour exposure.

## Imidazole metalloporphyrins as photosensitizers for photodynamic therapy: Role of molecular charge, central metal and hydroxyl radical production

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**Introduction:** In this study we report a novel structure-function relationship among photodynamic therapy (PDT) agents as effective photosensitizers (PS) against cancer cells in vitro. An anionic Zinc porphyrin and a homologous series of three cations Zinc(Zn), Palladium(Pd) and Indium(InCl) were used, as variations were done in overall charge but no dramatic change was done in the structure. However dramatic differences were observed in the effectiveness of the compounds as PS agents against mouse and human cancer cells.

**Material and Method:** The in vitro photodynamic therapy activity of four imidazole-substituted metalloporphyrins has been studied using human (HeLa) and mouse (CT26) cancer cell lines. Induction of apoptosis was demonstrated using a fluorescent caspase assay. The localization of porphyrin in lysosomes and mitochondria was assessed by fluorescence microscopy. The porphyrins were studied using fluorescent probes to see intracellular ROS production.

**Results:** A dramatic difference in photo toxicity was noted: Pd cationic > InCl cationic > Zn cationic > Zn anionic. HeLa cells were more susceptible than CT26 cells. The anionic Zn porphyrin localized in lysosomes while the cationic Zn porphyrin localized in lysosomes and mitochondria of the cells. Studies using fluorescent probes suggested that the cationic Pd porphyrin produced more hydroxyl radicals as the reactive oxygen species.

**Conclusion:** We have demonstrated that varying the central metal atom in a series of cationic porphyrins makes a substantial difference in the photo toxicity and the cationic Pd porphyrin has high potential as a photosensitizer because of its greater propensity to efficiently generate the highly toxic hydroxyl radical upon illumination, and that this particular ROS is more effective in killing cells than the more commonly studied ROS, singlet oxygen. Overall this study gave good insights into characteristics for improved molecular designs to develop PS agents.



## Requirements for substrate cleavage of the GXGD-protease active site motif of Alzheimer's disease-associated presenilin 1

ESC-ID 372

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**Background:** Alzheimer's disease (AD) is the most frequent form of dementia worldwide affecting about 10% of the population over 65 years. Besides neurofibrillar tangles consisting of tau, another invariant pathological hallmark of AD is the accumulation of extracellular neuritic plaques composed of the 40-42 amino acid amyloid-beta (A-beta) peptides. A-beta40 is the predominant species whereas the minor but slightly longer A-beta42 species is more prone to aggregation. Before aggregation into insoluble plaques, A-beta peptides form soluble synaptotoxic oligomers, which are believed to play a major role in AD pathogenesis. Most early onset forms of familial AD (FAD) shift the cleavage specificity toward an increased production of A-beta42. A-beta is generated by stepwise proteolysis of the beta-amyloid precursor protein (APP), a type I transmembrane protein. In the amyloidogenic pathway, APP is first cleaved by beta-secretase, which removes the bulk of the APP extracellular domain. The remaining C-terminal fragment is subsequently cleaved by gamma-secretase within the membrane resulting in the secretion of A-beta. Besides APP, the most important gamma-secretase substrate is Notch1, whose intracellular domain released by gamma-secretase cleavage has an important signalling function for cell differentiation at all stages of life. Gamma-Secretase is a protein complex consisting of four essential subunits, nicastrin, APH-1, PEN-2 and presenilin (PS) 1 or 2. PS represents the catalytic subunit and PS mutations account for most cases of FAD. PS belongs to a novel class of intramembrane cleaving aspartyl proteases, which are characterised by a highly conserved GXGD active site motif. Our mutagenesis studies on PS1 G382 and G384 of the motif have shown that both glycine residues are essential for catalytic activity, maximally tolerating the subtle glycine to alanine mutation to retain proteolytic activity. Furthermore, L383 in PS1, which corresponds to position X of the motif, was found to affect APP/Notch1 substrate selectivity when it was exchanged with a phenylalanine which naturally occurs at the X position in the most distant PS homologue, SPE-4.

**Methods:** Molecular biology methods were used in combination with protein biochemistry.

**Results:** In contrast to the highly restrictive G382 and G384 residues, our data indicate that amino acid changes at position X383 can modify substrate selectivity. Aliphatic residues at this position allowed efficient processing of all substrates comparable to PS1 wt. In contrast, aromatic substitutions showed a distinct processing behaviour. APP was still cleaved in an efficient manner, but gamma-secretase activity was strongly reduced for Notch1 and its homologues and completely abrogated for CD44.

**Conclusions:** Since within the GXGD-protease family mainly aliphatic amino acids (L, I, M) are conserved at the

X position, we propose that in combination with G382 and G384, an aliphatic residue at X383 provides the structural requirements to cleave a wide range of substrates. Position X383 could be part of a final substrate entry site, which has been proposed to be very close to but distinct from the active site. Changes to less flexible aromatic amino acids seem to alter the binding properties, preventing certain substrates from entering the active site possibly due to steric hindrance effects.

## Modulation of Fas-induced apoptotic markers in conditions of epidermal growth factor (EGF) action

ESC-ID 615

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**Introduction:** Epidermal growth factor (EGF) antagonizes damage-induced apoptosis by interaction with the plasma membrane receptors and downstream apoptotic signaling in the liver tissue.

**Aim:** The aim of the present study was to explore the involvement of EGF in membrane phospholipids phosphatidylserine external translocation, Bcl2 and Bax expression during Fas-antibody induced apoptosis.

**Material and methods:** Primary rat hepatocyte cultures were isolated and four experimental groups were allocated: I Control, II anti-Fas mAb treated; III EGF treated; IV simultaneously treated with anti-Fas mAb and EGF. Concentrations of Bcl2 and Bax proteins were examined using Flow-cytometer.

**Results:** In response to the Fas-ligand apoptogenic stimulus, the anti-apoptotic ratio was almost three times lower. Obtained results have shown that EGF interferes with the intrinsic pathway of apoptosis at the mitochondrial level, since Bcl2 and Bax were near control value when hepatocytes were treated simultaneously with anti-Fas mAb and EGF. Beside this, EGF did not allow the translocation of the membrane phospholipids phosphatidylserine and early apoptosis of cells was inhibited. EGF decreased late apoptosis as well.

**Conclusion:** This evidence provides a mechanism that may explain how EGF, through interaction with the extrinsic and intrinsic pathway interferes with apoptosis, thus modulating the hepatocyte cell life/death balance. It may have a favorable impact on regeneration and restoration of liver tissue during injury. Key words: epidermal growth factor (EGF), Fas-induced apoptosis, Bcl2, Bax

## In vivo characterization of vascular targeting strategies using F8-SIP antibody against the ED A of fibronectin

ESC-ID 876

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Antibody based targeting has become a promising approach for specially targeting solid tumors. The alternatively spliced extra domain A (ED-A) of fibronectin has

been shown to be promising neoangiogenic marker in this concept [1]. The aim of our study was to characterize microvascular binding and biodistribution characteristics of an ED-A small immunoprotein (F8-SIP) during tumor angiogenesis and to further evaluate this process during anti angiogenic treatment. Additionally, we analyzed the microcirculatory effects of ED-A mediated photo dynamic therapy (PDT). For these purposes SF126 glioma cells were implanted into dorsal skinfold chamber (DSC) preparations of nude mice (n=5 per group). Host vasculature of mice without tumor bearing DSCs were used as control group. Microvascular and interstitial accumulation of F8-SIP, microvascular blood flow rate and preferential binding sites were analyzed at t0 h, t2 h, t4 h and t24 h after intravenous application of fluorescently labelled F8-SIP using intravital microscopy on day 7 after tumor cell implantation. To characterize the binding properties during and after anti-angiogenic therapy treatment with Sunitinib (i.p.) was initiated on day 6 after tumor cell implantation and was applied daily for 6 days (40 mg/kg/day). Intravital microscopic analyses were performed on day 8 (acute phase) and on day 12 (end phase) after tumor cell implantation and compared to tumors without therapy (basic group). In PDT experiments red light irradiation in a total of 150j/cm<sup>2</sup> light dose was applied 4 hours after the administration of photosensitizer coupled F8-SIP and analyzed by intravital microscopy. F8-SIP binds specifically to tumor vessels reaching its maximum binding capacity 4 hours after injection (t0h: 33,71 ± 18,75 vs. t4h: 91,46 ± 6,19; p <0,05). In control vasculature no binding was observed. Extravasation of F8-SIP into tumor interstitium was observed reaching its maximum 4 hours after injection (t0h: 22,34 ± 12,25 vs. t4h: 57,38 ± 3,98; p <0,05). Microvascular binding was flow-dependent with significantly increased binding in high flow blood vessels (HF ≥60 nl/sec) compared to low flow blood vessels (LF ≤20 nl/sec) by 44%. F8-SIP binding occurred preferentially in angiogenic sprouts compared to remaining tumor vasculature. At the end of Sunitinib treatment, F8-SIP showed significantly increased binding as compared to the basic group. Extravasation of F8-SIP into tumor interstitium was significantly increased from the beginning in both therapy groups compared to the basic group. PDT resulted in edema formation, vascular stasis and shut down of microvessels. In conclusion, F8-SIP represents a useful tool to specifically target tumor microvessels. Our results provide the biodistribution basics for future clinical applications.

### Examination of the interaction between neural proteins Caskin1 and Abi2

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*Aim:* It has recently been confirmed that beside the regulation of enzyme activity specific protein-protein interactions also play a significant role in intracellular signalling pathways. Large cytosolic proteins which consist of several domains called scaffold proteins, facilitate the formation of multi-protein complexes. Our research team

has been involved in research investigating scaffold proteins including Caskin1. Caskin1 is expressed in neural synapses, especially in postsynaptic density. Considering the structure of the protein with the several domains like SH2, SH3, SAM and a long proline-rich region we hypothesized that Caskin1 may have an important role in a number of protein-protein interactions. It appears that to date only its interaction with Cask has been described and its exact function is still unexplored. In this study we tried to identify possible interacting partners of Caskin1 and thus shed light on the protein's role in intracellular signalling.

*Methods and Materials:* In order to identify the possible interacting partners we used yeast-two-hybrid system applying human embryonal brain cDNA library. Among the several possible partners we examined the interaction with Abi2 (Abl-interactor protein 2). Abi2 is expressed mainly in CNS and constitutes part of a protein complex regulating the actin cytoskeleton. Abi2 knockout mouse displayed severe malfunctions of the nervous system. Considering the fact that the yeast two-hybrid system often results in false positive interactions, we had to prove the existence of Caskin1-Abi2 relation using other, both in vitro and in vivo methods. As an in vitro method, we used GST-precipitation. We expressed both the complete Caskin1 and its domains as GST-fusion proteins. We incubated the proteins with Cos7 cell extract overexpressing GFP-Abi2 and rat brain cell extract. In the western blot we used anti-GFP antibody. We made further experiments in order to accurately localize the Abi2-binding spot on Caskin1. Next, we expressed the SH3 domain of Abi2, incubated it with rat brain extract, then developed the membrane with anti-Caskin antibody. As an in vivo method we used immunoprecipitation, where we used Cos7 cell extract overexpressing tagged Caskin and GFP-Abi2, then immunoprecipitated Caskin1 from the lysate and developed the membrane with anti-GFP antibody.

*Results:* The yeast-two hybrid screening helped us identify several possible interacting partners, among which we worked with Abi2. Using in vitro methods, we found that Abi2 can link to the proline rich region of Caskin1 with its SH3 domain. The interaction has also been confirmed in an in vivo system.

*Conclusion:* Our research team identified a new interaction between neural proteins Caskin1 and Abi2 which delineated a new path for further research. Caskin1 is the first neuronspecific interacting partner of Abi2. The investigation of this interaction might contribute to a better understanding of the phenomena shown by the Abi2 knockout mouse. Further research is in progress in order to clarify the exact role of the Caskin1/Abi2 complex in CNS.

### The physiochemical method to compare effectiveness of drugs applied to treat urolithiasis

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*Introduction:* The aim of our investigation was to develop the new physiochemical method to compare effectiveness of medicines that are applied in clinical practice to prevent the formation of kidney stones. The medicines on

study were subdivided into several categories:  $\alpha$ -amino acids (methionine), vitamins A, E, C (antioxicaps), medicines of plant origin (prolyte and cyston), allopurinol.

*Method of investigation:* We added the daily therapeutic doses of the medicines to the colloidal solutions prepared by ultrasonic degradation of kidney stones, and studied the dynamics of their coagulation initiated by electrolytes. The coagulating process was examined by photometric measuring of turbidity in solutions after the addition of definite amounts of a coagulator. To some extent the turbidity coefficient characterized the rate of calcium phosphates, urates and oxalates sedimentation (these salts are the main components of kidney stones). We represented the turbidity coefficient as a function of the amount of electrolyte-coagulator and, using this graph, calculated the critical coagulation concentrations of a coagulator (c.c.c) and the rate constants of the slow step of coagulation. Both parameters can be used as the criteria for colloidal stability of solutions that are simplified models of biological fluids (blood and urene). The greater c.c.c and smaller rate constants of coagulation the higher is colloidal stability of solutions, and lower is a thread of stones precipitation.

*Discussion of experimental data:* The experimental data revealed that: (1)all examined medicines increased c.c.c of coagulating agents thus they all raise the colloidal stability of calcium salts in the model solutions. For example, the c.c.c of colloidal solution which was not stabilized by a medicine is less than 0.33 mmol/L, while the c.c.c values for solutions that contained medicines were in the range 0.67-1.67 mmol/L. Allopurinol exhibited the maximum c.c.c value (1.67 mmol/L) thus being the most effective in preventing kidney stones precipitation, and methionine was proved to be less effective (0.67 mmol/L) but still the stability of its solutions is twice greater than that in a not stabilized systems. (2)the rate constants of coagulation took the following values (min<sup>-1</sup>): allopurinol and cyston – 40; prolyne and methionine – 30; antioxicaps – 28. The rate constant of a process that occurred without a medicine is much greater and is equal to 55 min<sup>-1</sup>. Thus we came to belief that anti urolithiasis medicines retard the rate of coagulation preventing the formation of kidney stones. (3)There is a linkage between c.c.c of an electrolyte-coagulator and rate constants of the coagulating process: the medicines that increase c.c.c simultaneously decrease the rate of stones coagulation from model solutions. Thus the anti urolithiasis medicines on study proved their effectiveness in prevention of stones in kidneys.

*Conclusions:* The new physiochemical method to compare effectiveness of drugs applied to treat urolithiasis was developed. The photometric determination of turbidity in colloidal solutions after their coagulation gave an opportunity to calculate the physical parameters that characterize the ability of medicines to prevent the kidney stones formation.

## Global analysis of circadian protein stability

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*Introduction:* The circadian clock adapts many physiological processes like hormone secretion, core body temperature and the sleep-wake cycle to the daily environmental rhythm. Moreover, circadian rhythms are essential to health and their disruption is associated with various diseases. On a molecular level, endogenous transcriptional/translational feedback loops drive the circadian rhythm. Especially protein stabilities of core-clock proteins, which are tightly regulated by post-translational mechanisms within a 24-hour day, have been found to be important for a fine-tuned, non-diseased circadian phenotype. In addition, previous results demonstrated that 20% of the hepatic proteome are subjected to circadian control, including pathways of the urea and sugar metabolism. To further elucidate the impact of circadian protein stabilities on health and disease, we aim to identify global circadian protein stabilities in an high-throughput analysis.

*Methods/ Results:* In order to analyse circadian protein stabilities we favoured a method based on a flow-cytometry read-out. To this end, we used a retroviral vector backbone including the fluorescent protein DsRed, followed by an IRES (internal ribosome entry site) coupled EGFP, which can be N-terminal fused to any protein of interest. As both proteins are simultaneously transcribed and translated, stability of the fusion protein can be monitored by the change of EGFP fluorescence compared to the long-lasting DsRed fluorescence. To analyse circadian protein stabilities, retrovirus pools of this vector including constructs covering a genome-wide protein library will be used to transduce human osteosarcoma cells (U-2OS). This cell line is well characterized to exhibit circadian transcriptional/translational properties. In regular 4-hour intervals cells will be harvested and protein stabilities will be analysed via the ratio of EGFP to DsRed fluorescence. First validation experiments have been performed showing differences in stabilities of two control proteins with known half-lives of 1- and 4-hours, respectively. Furthermore, as proof-of-concept we were able to show well characterized stability changes of the core-clock protein PER2 in different pharmacological and over-expression studies.

*Perspective:* Herein, we established a flow-cytometry based method in order to investigate global circadian protein “stabilomics”. Analyzing the circadian proteome will further broaden our knowledge of the impact of day-time dependent protein degradation in health and disease. Furthermore, revealing a circadian regulation of protein stabilities of various players within different metabolic pathways will deliver important information for further diagnostic and therapeutic applications.

## Inflammatory and apoptotic biomarkers in patients with ischemic heart disease

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*Introduction:* Atherosclerosis is the cause of ischemic heart disease in 90% of cases. It is known that inflammation plays a key role in atherogenesis. Apoptosis is present in the atherosclerotic plaque as well as in the myocardium. The main regulator of apoptosis is the Bcl-2 family of proteins. The aim of study: To determine the concentration and diagnostical value of hsCRP (marker of inflammation) and Bcl-2 (marker of apoptosis) in sera of patients with ischemic heart disease.

*Materials and methods:* The study included 4 groups of subjects (124 persons in total) according to their diagnosis: controls (C), stable (SAP), unstable angina pectoris (UAP) and acute myocardial infarction (AMI). The levels of Troponin I, hsCRP and Bcl-2 in the serum were evaluated.

*Results:* The level of serum Troponin I in AMI patients is significantly higher ( $p$  less than 0,001) than in C group, SAP and UAP patients. The level of hsCRP in serum of AMI patients is significantly higher ( $p$  less than 0,01) than of C group and SAP patients. Significantly higher values of Bcl-2 protein were found in SAP and UAP compared to those of control group ( $p$  less than 0,05). The sensitivity and specificity of the three evaluated biomarkers was analysed using the Receiver Operating Characteristic (ROC) curves. The biggest area under the ROC curve (AUC) in SAP and UAP patients was for Bcl-2 in comparison to the other two biomarkers. As far as AMI group is concerned, Troponin I showed the best result.

*Conclusion:* Inflammatory marker hsCRP increases gradually with the degree of ischemic disease. Bcl-2 has established itself as the best marker in the evaluation of the atherosclerotic plaque activity, and the marker with the biggest diagnostical value in SAP and UAP patients. **Keywords:** Bcl-2, hsC-reactive protein, Troponin I, ischemic heart disease

## A mammalian circadian interactome

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The mammalian circadian clock is an endogenous timing mechanism that regulates daily rhythms in physiology, metabolism and behavior. On the molecular level, clock cells contain an auto-regulatory genetic network constructed of transcription-translation negative and positive feedback loops based on protein-protein interactions and their resulting posttranslational modifications. Besides already described interactions within the circadian oscillator, we hypothesize that so far unknown interactions are crucial for the molecular regulation of circadian rhythms. Therefore, we have performed a systematic

matrix interaction mapping with 46 circadian candidates using high-throughput Yeast-Two-Hybrid (Y2H) robotic approaches. This led us to a reproducible binary interaction data set and allowed us to score the discovered interactions. The result of over 11,000 individual interaction experiments (6 independent repetitions) is a circadian protein-protein interaction network composed of 189 relevant interactions including a large number of interactions so far uncharacterized. As a proof of concept of our screen, 22.3% of all discovered interactions were previously described. Network topology already allowed us to identify 12 hubs (proteins with at least 10 interactors) whose relevance for the circadian system is currently being investigated. To identify false-positives of the Y2H system we are now validating the interactions using co-immunoprecipitation studies with epitope-tagged proteins in human cell culture. However, interaction maps in general have a static character and are not able to give us a temporal and spatial resolution of dynamic processes that underlie complex circadian phenotypes within cells. Overexpression studies and RNAi mediated knockdowns of our network components are underway to study the relevance of our candidates (e.g. hubs) for the molecular oscillator. In addition, the integration of (i) rhythmic RNA and protein expression profiles of our network components, (ii) co-localization studies from database mining plus (iii) predicted functional modules should create a basis for a better understanding of the circadian oscillator at the systems level.

## Isolation and Characterisation of Interferon-gamma induced Ubiquitin-conjugates in human Cells

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Ubiquitin (Ub)-mediated protein modification is a critical post-translational regulatory mechanism that occurs in all eukaryotic cells. Conjugation of Ub to substrate proteins is mediated by a cascade of enzymes that build up an isopeptide bond between the amino-groups of lysine side chains of a target protein and the C-terminal glycine of the Ub. Importantly, Ub itself contains 7 lysines and is often a substrate for ubiquitination, thereby leading to the formation of poly-Ub chains. Depending on the lysine residue, the covalent attachment of Ub to a substrate protein may lead to various outcomes. The widely known fate of ubiquitinated proteins is degradation by the 26S proteasome via lysine(K)48-linked Ub chains. However Ub labelling is not always fatal for the protein, with several non-proteolytic functions associated with the addition of a single Ub molecule (monoubiquitination) or specific cases of ubiquitination (K63-linked Ub chains). Up to date, our understanding about the regulation the Ub pathway is limited. However, recent studies in our group reported an increased accumulation of Ub conjugates in HeLa cells in response to IFN-gamma, which is an anti-inflammatory cytokine whose excessive release is associated with the pathogenesis of chronic inflammatory and autoimmune diseases. Interestingly, most of these IFN-gamma-induced Ub conjugates are oxidised and contain

K48-linked Ub chains, thereby suggesting that they are proteasomal targets.

*Aim:* It is assumed that alterations of the Ub pathway are at the heart of the aetiology of many malignancies, such as cancers, neurodegenerative disorders or genetic disorders. Therefore, a global understanding of ubiquitinated proteins in vivo is a key to unravelling the biological significance of ubiquitination. There are, however, a few effective screening methods for rapid analysis of ubiquitinated substrates. The focus of my project is to design a proteomic approach for the identification of Ub-conjugates in HeLa cells following IFN-gamma treatment. This requires the development of a potent isolation method for Ub-conjugates prior to their characterisation by mass spectrometry (MS).

*Materials and Methods:* The Ub-conjugates were enriched from IFN-gamma-treated cells through affinity purification (immunoprecipitation and FPLC) using Ub antibodies, specific ubiquitin-binding domains and systems with N-terminal tagged Ubs. The isolated Ub-modified substrates were next digested in-gel (1D-/2D-electrophoresis) or in solution with trypsin and analysed by LC-MS/MS measurements.

*Results:* A pool Ub-targeted proteins could be identified. Nevertheless, because of a GlyGly76-tag left following tryptic miss cleavage, the ubiquitination sites could not be determined yet. All candidates were compared with databases of known ubiquitinated target proteins and some selected proteins were further checked via immunohistochemistry to confirm the relevance of the MS data. An overview of the developed isolation technique and selected MS and biochemical results will be presented.

*Conclusion:* The isolation of Ub-conjugates in IFN-gamma-treated HeLa cells is feasible. Our method is a valid one for identifying potential Ub target proteins. However, due to their heterogenic composition and to their low concentration in cells, MS analysis does not lead to their direct identification. Future studies coupling this approach with additional other biochemical methods will increase our knowledge of the regulation of the Ub pathway.

## Cardiology

### Complete atrio-ventricular block following the isolated replacement of the aortic valve

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*Background:* Heart surgeries, especially the valve ones, seem to be in many cases followed by transient conduction disorders. Nonetheless, the need of pacemaker implantation on account of complete AV block is rather rare and varies between 2.5 and 5.7% for isolated aortic valve replacement (AVR). Although conduction disorders may be caused by mechanical trauma to the conduction system during surgical manipulation in the area of its anatomical structures, the perioperative risk predic-

tors of complete AV block are still not well investigated. *Aim:* The aim of the study was to identify the anatomical, clinical, and surgical factors that may be predictive for the incidence of complete AV block.

*Materials and methods:* The study included 598 patients (42.6% women, 57.4% men), who underwent isolated AVR in the centre between the years 2004-2008. Patients who required any additional procedures were excluded from the study. Those enrolled were divided into group A – patients with complete AV block (n=17) and group B – patients with sinus/nodal rhythm (n=581). Several parameters (age, gender, prosthesis suturing technique, presence of bicuspid valve, rhythm at the end of the procedure, cross clamping time, presence of calcifications, prosthesis size, and the need for reoperation) were analysed to investigate their influence on the need of permanent pacing because of complete AV block. Patients who postoperatively developed complete AVB were observed for 7- 10 days before permanent pacemaker implantation.

*Results:* Perioperative transient conduction disorders were present in 55 patients (9.2 %). Complete AV block, demanding permanent pacing occurred in 17 patients (2.8%). Permanent pacing turned out to be necessary in 11 patients (20%) with perioperative AV block. In the majority of cases the indication for AVR was aortic stenosis (67.7%), aortic regurgitation, combined disease and infectious endocarditis, which occurred in 8.5 %, 21.1%, and 2.8% respectively. The necessity for perioperative pacing because of conduction disorders (in group A- 64.7%, B- 7.6%, p <0.01) was the only parameter that was significantly more frequent in group A. Accordingly, it is thought to be predictive for complete AV block incidence. No other parameters examined reached statistical significance and therefore they are not to be treated as a predictive factor for complete AV block incidence.

*Conclusions:* Irreversible AV block requiring permanent pacemaker implantation is an uncommon condition following aortic valve replacement. This study seems to confirm what has been claimed in recent reports: finding any anatomical and surgical predictors for complete AV block is difficult, and whether they exist at all still remains controversial. The need for perioperative pacing was the only factor from all those examined in this study that could have an influence on complete AV block incidence.

### The anatomy and the clinical importance of the sinoatrial and atrioventricular nodal arteries

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*Background:* Arrhythmias caused by decreased blood supply of the SA and AV nodes are common complications of stent implantations or heart surgery. After stent implantation, coronary branches arising in the stented segment may embolize spontaneously or may be blocked by the wire mesh itself.

*Aims:* Our aim was to localize and identify the SA and AV nodal arteries. It was also necessary to localize the other atrial arteries that do not take part in the blood supply of the nodes, because identifying them as nodal arteries

might lead to controversial statistical data. We have also examined the short- and long-term effect of stent implantation on the diameter of the coronary twigs branched off in the stented region.

**Methods:** During the post mortem CT angiography Lipiodol Ultrafluid solution (5%) or resin polymer (according to M. Kiss' method) was used as contrast material. After washing out the Lipiodol solution, the resin was injected into the coronary arteries and a repeated CT scan was performed and then corrosion casts were prepared. Some hearts were dissected after the resin injection, and the CT scan; the SN region was removed and processed for histology. We have localized the angiographically visualized, supposedly nodal arteries with careful dissection and later on the cast specimens, as well. The histological analysis has proved that in fact these arteries seen on the CT scan and on the cast preparations are really the SA node supplying vessels.

**Results:** Our results show, that 73% of the SNAs derives from the right coronary (according to Ortale's classification: R1: 50%, R2: 23%). Only a single branch was seen in 60% of the cases, which originates mostly (36%) in Ortale R1 localization. These areas are the typical sites of the stent implantations. The AV node is supplied by a single artery in 46%, and by two arteries in 39%. These arteries may stem off from the distal or proximal part of the right coronary artery, from the left circumflex artery and from the left anterior descending artery, as well. Stenoses were seen in 73% of the arteries leaving from the stented region. We have found that these stenoses correlated well with the measured instant restenosis. Where the stented region was at the origin of the nodal arteries, no SNA or AVNA was observed. Statistics were made about the origin and course of the SNA and AVNA analysing the corrosion specimens and the CT images.

**Conclusions:** With this new method we could localize the origin and course of the SNAs and AVNAs, which may prove to be clinically important. Our results indicate that stent implantation in the segments where the nodal arteries arise may reduce the perfusion of the SN and AVN and this may lead to serious arrhythmias.

### The role of Toll-like receptor 9 in coxsackievirus B3-induced myocarditis

ESC-ID 570

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**Aim:** The Toll-like receptor 9 is a member of the innate immune system and has been shown to influence myocardial function, but its role in myocarditis is hitherto unknown. We therefore investigated whether or not TLR9 plays a role in this disease in human endomyocardial biopsies (EMB) and in coxsackievirus B3 (CVB3)-induced myocarditis in mice.

**Methods:** Cardiac TLR9 expression in EMB from patients with acute CVB3-positive myocarditis and CVB3-negative inflammatory cardiomyopathy (DCMi) was detected by immunostaining. Left ventricular (LV) function, car-

diac immune cell infiltration, virus mRNA and cytokine activation were investigated in TLR9 deficient (KO) and wild-type (WT) mice after infection with CVB3.

**Results:** Human cardiac TLR9 expression was significantly increased in patients with AMC, but not in patients with DCMi. In the acute phase of CVB3-myocarditis, KOCVB3 mice displayed an improved LV function associated with reduced cardiac amounts of immune cells and selectively increased cardiac IL-6 mRNA levels when compared to WTCVB3 mice. In contrast, in the chronic phase, LV function and cytokine levels were not seen to differ among the groups.

**Conclusion:** Cardiac modulation in CVB3-induced myocarditis by TLR9 is, despite of its cardiac up-regulation in human and mice, only limited to a transient cardioprotection.

### Adhesive molecules as markers of atherosclerotic plaque stability

ESC-ID 631

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**Introduction:** Adhesive molecules play an important role in developing and progression of coronary atherosclerosis. Margination, adhesion and transendothelial migration of circulated white blood cells are very important processes in initiation and progression of atherosclerotic disease.

**Aim:** The aim of the paper was to analyze a soluble form of vascular cell adhesive molecules 1 and intracellular cell adhesive molecules 1 in patients with acute coronary syndrome, which was compared with patients with stable angina pectoris and patients without coronary disease.

**Materials and methods:** Blood samples were taken from 25 patients with acute myocardial infarct (AMI), 25 patients with unstable angina pectoris (UAP), 25 with stable angina pectoris (SAP) and from 15 patients without coronary disease. In all patients blood was taken for determination of ICAM-1 i VCAM-1.

**Results:** The level of VCAM-1 is significantly higher in patients with AMI ( $799.8 \pm 26.3$  ng/ml) compared with UAP ( $644.2 \pm 26.7$  ng/ml), SAP ( $526 \pm 32.5$  ng/ml) and control group ( $270 \pm 26.8$  ng/ml). In the patients with UAP, VCAM-1 is significantly higher compared with SAP and control group. The level of VCAM-1 is significantly higher in SAP group compared to control group. Serum concentration of ICAM-1 is similar in patients with AMI ( $424.1 \pm 15.2$  ng/ml), UAP ( $403 \pm 12.3$  ng/ml) and SAP ( $381.2 \pm 16.2$  ng/ml). Concentration of ICAM-1 is significantly higher in these groups compared with control group ( $244.3 \pm 11$ ).

**Conclusion:** Determination of VCAM-1 is useful for detection of coronary atherosclerotic plaque destabilization, while ICAM-1 is a useful indicator of atherosclerotic plaque presence in coronary vessels. Key words: adhesive molecules, coronary artery disease, ICAM-1, VCAM-1

## The investigation of left ventricular dysfunction using the systolic velocity of the mitral annular, measured with Tissular Doppler echocardiography

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Timisoara N-terminal pro-brain natriuretic peptide (NTproBNP) is recognized as a reliable marker of systolic and diastolic left ventricular (LV) function. The ratio between early diastolic transmitral velocity and early mitral annular diastolic velocity (E/Ea) reflects LV filling pressure in a variety of cardiac diseases. However this relationship was not validated in some categories of patients. We believe that combining the index of diastolic function (E/Ea) and a parameter that explores LV systolic performance (Sa, peak systolic velocity of mitral annulus) provides a close prediction of NTproBNP.

*Aim:* to assess the relationship between a new parameter, E/(Ea\_Sa), and NTproBNP level in patients with LV dysfunction.

*Methods:* We screened 145 consecutive patients with LV dysfunction in sinus rhythm referred for LV catheterism. Patients with inadequate echocardiographic image, paced rhythm, mitral stenosis, significant primary or organic mitral regurgitation, mitral prosthesis, severe mitral annular calcification, pericardial disease, acute coronary syndrome, coronary artery by-pass within 72 hours or renal failure were excluded. The remaining 113 patients (78 with heart failure with reduced ejection fraction –HFREF, 12 with HF with normal EF, 23 with isolated diastolic dysfunction) formed our study group. Echocardiography was performed simultaneously with NTproBNP measurement.

*Results:* Simple regression analysis demonstrated a significant linear correlation between E/(Ea\_Sa) and NTproBNP, superior to E/Ea correlation. Significant but weaker correlations were found between NTproBNP and pulmonary artery systolic pressure, Sa, LVEF, E wave, mitral E deceleration time and Ea. We couldn't demonstrate significant relationships between NTproBNP and left atrial (LA) diameter, LA surface or LA volume. Among analyzed parameters, E/(Ea\_Sa) was best correlated with NTproBNP levels in patients with Heart Failure with normal EF, and in those with isolated diastolic dysfunction.

*Conclusions:* E/(Ea\_Sa) had a good correlation with plasma NTproBNP level and can be a simple, reproducible and accurate echocardiographic index in patients with LV dysfunction in sinus rhythm.

## 64-multislice computed tomography, a noninvasive method for visualization of coronary artery bypass grafts

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*Introduction:* The progressive improvements in non-invasive cardiac imaging techniques have led to the question whether cardiac catheterization should remain the key clinical tool for assessing the anatomy and physiology of the heart and its associated vasculature.

*Aim:* To compare the accuracy of non-invasive 64-MSCT and invasive coronarography, in patients that previously had by-pass surgery, consequently to ischemic heart disease.

*Material and methods:* Twenty-seven patients who had undergone coronary artery bypass graft surgery (age 59 + 18 years) were included. All patients underwent MSCT and conventional coronary angiography for the evaluation of the status of their bypass grafts. Three-dimensional reconstructions of the heart and bypass grafts were compared with selective angiographic images of the bypass grafts. The bypass graft patency and the presence of stenosis as well as the proximal and distal anastomosis were evaluated by two experienced readers.

*Results:* A total of 55 coronary artery bypass grafts (23 internal mammary artery - 42%, 15 radial artery - 27% and 17 saphenous venous grafts -31%) were examined, all adding up to 142 segments. Five out of the 142 segments (4%), however, could not be properly explored by MSCT, either due to metallic clefts (3), severe coronary calcification (1) or stents(1), but invasive cardiac angiography did not prove the existence of relevant lesions in these segments either. After analyzing the entire group of patients, MSCT proved to have an 87% sensibility, 100% specificity, a positive predictive value of 100%, and a negative predictive value of 98% in identifying grafts with significant lesions or partial obstructions. MSCT detected occluded grafts with a sensitivity and specificity of 100%.

*Conclusion:* Three-dimensional coronary angiography obtained with 64 MSCT is a promising and useful diagnostic imaging technique for the evaluation of graft patency in patients who have undergone coronary artery bypass graft surgery. Dysfunctional bypass grafts can be detected with high diagnostic accuracy. This technology can be used as a noninvasive test for patients with suspected graft dysfunction.

### Ten- year survival of the patients with dilated cardiomyopathy treated with medicamentous therapy

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*Introduction:* Dilated cardiomyopathy is a progressive heart muscle disease, characterized by dilatation of the heart cavities and weakened systolic function of the left ventricle, in absence of the congenital coronary, valvular and pericardiac heart lesions. Etiology is unknown, and therefore the specific treatment doesn't exist, but symptomatic therapy for heart insufficiency, prevention of systematic embolisms and diverse arrhythmia is used along with the sparing the patients from greater efforts. The disease has a progressive course.

*Aim:* We wanted to determine a 10-year survival of the patients treated by medicamentous therapy, distribution and changes of medicamentous therapy.

*Methods:* Study had involved 126 sequential patients with idiopathic dilated cardiomyopathy, referred to Dedinje Cardiovascular Institute. We had been noting the gender and the age of the patients, heart-function condition (estimated at the beginning of the study by measuring ejection fraction, capillary lung pressure, heart rate, systole and diastole pressure and tolerance pressure estimated on the basis of NYHA classification) risk factors, cardiovascular therapy and death result. Patients had been monitored for 10 years since their inclusion in the study.

*Results:* This study shows that a 10-year survival of the patients with dilated cardiomyopathy is low (38,1%). The most applied therapy at the beginning of our study were diuretics (85,71%), ACE inhibitors (71,43%) and digoxin (68,25%). At the end of the study certain changes were noticed. ACE inhibitors became a part of polytherapy of all patients (100%,  $p=0,0004$ ), the appliance of  $\beta$ -blockers increased significantly (from 17,46 to 86,66%,  $p=0,001$ ), and digoxin and diuretics remained high applied.

*Conclusion:* This study shows long-term survival of patients with dilated cardiomyopathy is very small, less than a half survives 10 years. The most applied is polytherapy, most often diuretics, ACE inhibitors and digoxin, in later research the appliance of  $\beta$ -blockers also increased, and so did the presence of the medicamentous therapy in general. **KEY WORDS:** dilated cardiomyopathy, survival, therapy.

### Correlation of hemodynamic parameters and subjective evaluation of condition in patients with acute heart failure

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*Introduction:* Heart failure presents a clinical condition which is featured with heart inability to content metabolic demands of the organism. The consequences of this condition are changes in pulmonary circulation, followed by symptoms of dyspnea and tiredness.

*Objective:* To evaluate correlation between pulmonary capillary wedge pressure and pulmonary artery pressures, with subjective evaluation of condition in patients hospitalized due to acute heart failure.

*Methods:* The study enrolled 11 consecutive patients hospitalized due to acute heart failure in whom Swan-Ganz catheter was inserted. Hemodynamic parameters have been measured (pulmonary capillary wedge pressure, pulmonary artery pressure- systolic, diastolic and mean, and also cardiac index and right atrium pressure). These parameters were compared with subjective physician's and patient's evaluation of condition, using VAS-dyspnea test, euroqol-VAS and Likert scale. For evaluating of having correlation between hemodynamic parameters and subjective methods valuation of dyspnea was used statistical method of linear correlation.

*Results:* There was significant correlation between pulmonary circulation and right atrium pressures, with subjective methods (VAS-dyspnea and euroqol-VAS), (the values of correlation are between 0,344 and 0,693, respectively,  $p < 0,05$ ). On the other hand, no correlation was found between changes in pulmonary capillary wedge pressure and subjective methods (Likert scale and physician's evaluation).

*Conclusion:* Subjective evaluation of condition in patients with acute heart failure correlates partly with objective hemodynamic parameters, measured by Swan-Ganz catheter. The physician's and patients evaluation of changes in patient condition doesn't correlate with changes of hemodynamic parameters.

*Key words:* Heart failure, hemodynamic parameters, dyspnea

### The predictors of left ventricle remodeling and erythrocytes functional profile in heart failure patients

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*Introduction:* Pathogenesis of chronic heart failure (HF) progression is multicomponent, irreversible and associated with poor prognosis. Endothelial dysfunction and microcirculatory disturbances are of great value in HF progression. Erythrocytes and endothelial cells are the active participants of microcirculatory processes, thus investigation of red blood cells and endothelial function in scope of HF progression and left ventricle (LV) remodeling is of great scientific and practical importance.

*Aim:* To research the predictors of LV remodeling and parameters of functional state of erythrocytes in HF patients (pts) with different level of endothelium-dependent vasodilatation (EDVD).

*Material and Methods:* 42 male patients (pts) of mean age  $55 \pm 11,7$  years with arterial hypertension, ischemic heart disease and NYHA II-IV HF were enrolled. Some indices of haemostatic parameters, lipid spectrum, lipid and protein oxidation, functional state of erythrocytes (relative erythrocyte viscosity index (RVI) and erythrocyte deformability index (EDI) have been assessed. LV remodeling parameters (LV myocardial mass index (LVMMI) and LV relative wall thickness (RWT) were assessed by echocardiography. Level of EDVD was assessed in 29 pts by ultra-



sound techniques with cut-off point 10% of diameter increasing (preserved EDVD (PEDVD) with  $\geq 10\%$  and reduced EDVD (REDVD) with  $< 10\%$ ). 72% of pts had preserved EDVD and 18% had reduced EDVD.

**Results:** RVI was significant predictor (SP) for IMMLV in PEDVD group ( $r = 0,62$ ,  $p < 0,05$ ), and EDI was SP for RWT in REDVD group ( $r = 0,97$  ( $p < 0,05$ )). Left common carotid artery intima-media thickness (IMT) was SP for EDI in group with REDVD ( $r = -0,81$ ,  $p < 0,05$ ). Level of diene conjugates (DC) was SP for RVI in REDVD group ( $r = -0,83$ ,  $p < 0,05$ ). LV ejection fraction (EF) ( $r = 0,60$ ,  $p < 0,05$ ) and XIIa-dependent fibrinolytic activity (XII-FA) ( $r = 0,66$ ,  $p < 0,05$ ) were SP for RVI in PEDVD group. RVI and EDI were SP for LVMMI in pts with preserved EDVD in multilayer perceptron artificial neural network model with 3 hidden layers (SD ratio 0,17).

**Conclusion:** Erythrocyte functional profile parameters are SP for remodeling indices in both PEDVD and REDVD groups. IMT, EF, level of DC and XII-FA are SP for erythrocyte functional profile parameters in both PEDVD and REDVD groups.

### Evaluation of total plasma homocysteine level and its genotype as a risk factor for coronary artery disease in patients undergo angiographic procedures

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**Background and objective:** In coronary artery disease, despite the established significance of the classical risk factors, it has recently identified that elevated plasma homocysteine levels are associated with a greater risk of CAD. Molecular defects (C677T Polymorphism) in the MTHFR gene might be associated with hyperhomocysteinemia. Positive correlation between elevated total homocysteine level and its genotype as a risk factor for CAD is the aim of this study.

**Materials and Methods:** The diagnosis of CAD in all studied patients was confirmed by coronary angiography. The plasma homocysteine level was measured by ELIZA and genotype was analyzed by PCR. A formatted questionnaire was completed for each patient. In 45 subjects of case group with CAD the frequency of homozygote (cc,tt) genotypes in three subgroups of SVD, 2VD and 3VD were 38.2%, 35.3% and 26.5% respectively whereas the heterozygote genotype (ct) in these three subgroups were 27.3%, 27.3% and 45.5% respectively. When genotype frequency was compared among patients with different numbers of stenotic coronary arteries the frequency of homozygote genotypes was not significantly higher in patients with 3VD (26.5%) than in patients with single- or double- vessel disease (38.2% and 35.3% respectively,  $P = 0.49$ ). In comparison of homozygote and heterozygote genotype in every effected subgroup. It was obviously clear that the majority of patients are belonged to homozygote category (SVD:382 versus 27.5%, 2VD:35.3 versus 27.3%, 26.5% versus 45.5%). The frequency of C allele in 15 patients of subgroup from at least one stenotic vessel to triple stenotic vessel suffered from CAD was

38.6%, 35.7% and 25.7% respectively. The frequency of T allele in these three mentioned groups was 33.3%, 33.3% and 13.2% respectively ( $P = 0.82$ ).

**Conclusion:** According to this study, elevated homocysteine levels was believed to be one of the modifiable risk factors of CAD, although the correlation between elevated homocysteine level and the CAD's severity in terms of the number of stenotic vessels hasn't been shown significantly. The homozygote genotypes (cc,tt) of MTHFR in particular were not also associated with the severity of disease. Key words: Homocysteine, CAD, Genotype, risk factor?

### Differential DC activation in a murine model of enterovirus myocarditis

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Coxsackievirus B3 (CVB3) infection is a frequent course of acute myocarditis. One potential late sequela of acute myocarditis is dilated cardiomyopathy, which is linked to virus persistence and chronic inflammation of the heart. A well-established mouse models mimics the human disease progression: C57BL/6 mice eliminate CVB3 following acute myocarditis, whereas A.BY/SnJ mice are susceptible to chronic disease. In an attempt to analyze susceptibility factors of chronic disease, dendritic cells (DC) were recently shown to exhibit differential chemokines expression kinetics. Here, we investigated the maturation capacity of DC, which eventually prime T cells, in resistant C57BL/6 mice and permissive A.BY/SnJ mice early upon CVB3-infection. C57BL/6 mice and A.BY/SnJ mice were infected with  $1 \times 10^5$  PFU CVB3 Nancy strain and mice were sacrificed 36 and 72h post infection (p.i.). The expression of co-stimulatory molecules, migration markers and MHC complexes was analyzed by FACS analysis revealing a significant up-regulation of CD80, CD86, CD40 and CCR7 in C57BL/6 mice 36h p.i. In contrast, up-regulation of these markers was diminished and delayed in DCs isolated from A.BY/SnJ mice. Further, TaqMan analysis of total splenocytes revealed enhanced mRNA expression of IL-6, IL-10, IL-12p40, TNF- $\alpha$  and IFN- $\gamma$  36h p.i. particular in C57BL/6, whereas cytokine expression was only slightly increased in A.BY/SnJ. Likewise, mRNA expression of TNF- $\alpha$ , IL-6 and IL-12p40 in isolated CD11+DCs was found to be significantly enhanced in C57BL/6 mice at 36 p.i. In addition, the type I IFN response was more pronounced in C57BL/6 mice, which was consecutively associated with enhanced OASL-1 mRNA expression in the resistant host. These data substantiate above mentioned findings of impaired maturation of DCs in mice being susceptible to chronic disease. In conclusion, in C57BL/6 and A.BY/SnJ mice substantial differences were observed in the maturation and cytokine expression kinetic of splenic DC upon CVB3-infection, thus being suggestive for impaired DC function in A.BY/SnJ mice in CVB3-myocarditis.

### Cardiohaemodynamic features in children with myocardiodystrophias

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The term "myocardiodystrophy" means metabolism disturbances in a myocardium in biochemical level, partially or completely reversible at elimination of the reason which have caused them. Long existing and progressing dystrophias of a myocardium conducts to depression contraction function and heart failure development. Till now there is a small experience of studying of disturbances of the central and peripheric haemodynamic in children with myocardiodystrophy. The purpose of the present research was studying of features endocardial hemodynamic in children with myocardiodystrophias.

*Patients and methods:* Under our observation were 38 boys and 15 girls at the age from 6 till 17 years which were hospitalized in the 2-nd Minsk city clinical children hospital with the diagnosis of myocardiodystrophias. Examination of children was spent under standard plans, including an electrocardiography and an echocardiography.

*Results:* The analysis of results echocardiography has shown, that at boys and at girls the regurgitation on valves the right departments of heart prevailed (in boys: on the three-cuspidate valve - in 93,8 %, on pulmonary - in 65,6 % of children; in girls: on the three-cuspidate and pulmonary valve equally often in 92,9 % of children, The regurgitation on valves of the left departments of heart was less often observed (in boys: on mitral valve - in 37,5 %, on the aortal valve - in 21,9 % of children, in girls: on mitral in 21,4 % and on the aortal valve in 7,1 % of children). Three-cuspidate regurgitation of the minimal degree was observed in 21,9 % of boys and in 50% of girls, I-st degree in 59,4 % of boys and in 42,9 % of girls, II-nd degree in 12,5 % of boys, regurgitation of II-nd degree in girls it was not observed. The regurgitation of the minimal degree on a pulmonary artery was observed in 34,3 % of boys and in 71,4 % of girls, I-st degree in 66,7 % of boys and in 21,5% of girls. In boys rate of a regurgitation on the three-cuspidate valve was  $2,22 \pm 0,3$  m/s with radius  $1,8 \pm 0,46$  sm, gradient of systolic pressure was  $14,6 \pm 4,9$  mm.Hg., in girls rate of a regurgitation on the three-cuspidate valve was  $1,73 \pm 0,29$ m/s., with radius  $1,47 \pm 0,42$ sm., gradient of pressure was  $11,1 \pm 4,9$ mm.Hg., in boys on pulmonary rate of regurgitation was  $1,39 \pm 0,29$ m/s, with radius  $1,58 \pm 0,45$ sm, gradient of diastolic pressure was  $8,3 \pm 3,58$  mm.Hg., in girls on pulmonary  $1,35 \pm 0,3$ m/s, with radius  $1,06 \pm 0,37$ sm, gradient of diastolic pressure was  $7,5 \pm 3,4$ mm.Hg.

*Conclusion:* 1. At children with myocardial dystrophias disturbances cardiohaemodynamic from the right departments of heart are prevailed. 2. Disturbances cardiohaemodynamic in boys were more expressed, than in girls.

### Histomorphological examination of right and left atrium in patients with chronic atrial fibrillation

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*Aims:* The aim of the present study was to quantify the histomorphological changes of both (right / left) atrial chambers in patients with chronic atrial fibrillation. For this we performed a correlative quantitative morphometrical examination of the cardiomyocytes size, degree of interstitial changes and endocardial thickening in the right and left atrium in those patients.

*Methods:* Right and left atrial samples were taken from 16 patients (9 female, 7 male; average age 63 years, range 33 - 76 years) with clinical and electrophysiological secured chronic atrial fibrillation during elective open heart operations (valve surgery, bypass operation and radiofrequency ablation). Fifteen right and 15 left atrial samples from patients without atrial fibrillation formed the control group. After the excision the specimens were prepared for histological examination. Myocyte diameter was morphometrically evaluated on hematoxylin eosin-stained sections using a digital photcamera and the computer aided software Easy Measure. To evaluate the degree of fibrosis and scar tissue we used the KS 400 Imaging System. Endocardial thickening was measured at 30 different spots of the endocard in both chambers. The short diameter of the cardiomyocytes was measured at the nuclear level in at least 250 cells per patient and atrium side. Fibrosis and scars were evaluated in a myocard area of 4 mm<sup>2</sup>. Results were analysed using the t-test.

*Results:* The myocytes in the left atrium (myocyte diameter on average  $18,2\mu\text{m}$ ) showed strikingly more cell hypertrophy than the muscle cells of the right atrium (myocyte diameter on average  $16,2\mu\text{m}$ ). Conspicuous is the low standard deviation in both groups. The differences in the myocyte size are statistically significant. In comparison with a control group, the cardiomyocyte size is smaller in the control group. The amount of fibrosis and scar tissue are also larger in the left than in the right atrium. The control shows this tendency as well. Fibrose and scar tissue are lower in the control. Endocard thickening is higher in the left than in the right atrium. Here we find more endocardial thickening in the control group.

*Conclusion:* The histopathological findings for left and right atrial myocardium are obviously a consequence of the different atrial pressures and reflected the underlying valvular diseases. Atrial fibrillation seems to increase the alterations.

## Investigation of antihypertensive effects of natural polyphenol extracts of *Oenothera paradoxa*

ESC-ID 237  
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Arterial hypertension is one of most serious social and therapeutic problems. In spite of the large amount of antihypertensive drugs its treatment is not always successful. Necessity of finding new ways of treatment is very urgent. As oxidative stress plays a role in pathogenesis of arterial hypertension, the use of natural antioxidants represents a promising new approach. Polyphenols present in plants (e.g. fruits of *Oenothera paradoxa*) have significant antioxidative properties. Although their anti-hypertensive properties are unknown.

**Aims:** 1. To determine the influence of *O. paradoxa* extract on arterial hypertension development induced by angiotensin II. 2. To determine usefulness of animal model of angiotensin II – induced hypertension II as a model for testing efficiency of new antihypertensive drugs.

**Materials and Methods:** Studies were performed on C57BL/6J mice. Animals from experimental group (n=5) were orally administered *O. paradoxa* oil free extracts at 200mg/ kg. Control group (n=8) (without extract) received water. Hypertension in both groups was induced after 1 month of pre-treatment by infusion of angiotensin II (490 ng/min/kg body mass) using miniosmotic pump (ALZET, USA) for 14 days. Blood pressure in both groups was measured every 2 days using BP –2000 device (Visitech Systems, USA).

**Results:** No statistically significant difference was observed in average systolic blood pressure between both groups (with and without extract) in basic pressure level-before angiotensin II infusion ( $100.9 \pm 13.3$  vs  $98.3 \pm 13.2$  mmHg;  $p > 0.05$ ). In control group administration of Ang II caused increase of mice' systolic blood pressure from  $101.0 \pm 13$  to  $173.2 \pm 29$  mmHg. In experimental group this increase was slightly diminished but the difference did not reach statistical significance ( $167.2 \pm 31$  mmHg).

**Conclusion:** 1. Animal model of arterial hypertension induced by angiotensin II infusion is a valuable, cheap and easy method for preliminary testing of new antihypertensive drugs. 2. Extract from *O. paradoxa* caused a minor decrease in hypertensive response to angiotensin II, but didn't reach statistical significance. 3. Further research on mechanisms of *O. paradoxa* extract influence on cardiovascular system is warranted.

## Metabolic Therapy in Left Ventricular Hypertrophy Regression in Hypertensive Patients

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Hypertension is the issue of the day of contemporary medicine. The presence of left ventricular hypertrophy (LVH) is an important independent risk factor for total mortality and for cardiovascular morbidity and mortality. The ability to diagnose LVH has been improved by echocardiography. Nowadays scientists study effectiveness of combined therapy from the point of antihypertensive effect and heart chambers remodeling ability.

**Aim:** To study the influence of metabolic drugs on LVH regression in patients with the 2nd stage of hypertension.

**Materials and methods:** 50 hypertensive patients aged from 50 to 65 years (middle age -  $58.7 \pm 0.7$ ) with different disease duration (average duration -  $11.92 \pm 0.87$ ) were examined. All patients were divided in control and 3 investigated groups which were commensurable by age, sex and disease duration. Standard therapy which included beta-blocker and ACE inhibitor combination was prescribed to the patients of control group (n = 21). 1st investigated group of patients (n = 9) took basic therapy and a course of Thiotriazolol. Patients of 2nd investigated group (n=8) took basic therapy and a course of Trimetazidine. Patients of 3rd investigated group (n=12) took basic therapy and a course of Thiotriazolol with the subsequent reception of Trimetazidine. Patients underwent routine transthoracic echocardiography.

**Results:** Research of echocardiographic parameters of heart structure in control group have shown significant changes of ESD (end-systolic diameter), ESV (end-systolic volume), LA (size of left atrium) and IVSRT (interventricular septum relative thickness) 6 months later after the treatment started. ESD at second investigation was  $3.61 \pm 0.54$  cm vs.  $3.68 \pm 0.31$  cm. Analysis of ESV at second investigation shown the similar tendency to reduction:  $56.9 \pm 19.4$  vs.  $58.3 \pm 12.1$ . IVSRT also decreased from  $0.53 \pm 0.08$  to  $0.47 \pm 0.04$ . In this group of patients reduction of the size of LA from  $4.0 \pm 0.32$  to  $3.72 \pm 0.48$  was observed. In patients of 1st investigated group we observed the similar character of changes. ESD significantly decreased from  $3.68 \pm 0.31$  cm to  $3.60 \pm 0.54$  at second investigation. ESV decreased from  $58.3 \pm 12.1$  to  $55.9 \pm 19.6$ . At carrying out of the analysis of the LA sizes the similar tendency to its reduction was observed. Changes of echocardiographic parameters in patients of the second and third investigated groups at the first and second investigations gave the bases for a conclusion about more expressed changes of parameters of heart geometry and LVH regression. The absolute (posterior LV wall thickness, interventricular septum thickness) and relative (posterior LV relative wall thickness, interventricular septum relative thickness, LV relative wall thickness) sizes of LV walls were significantly decreased. **Conclusions:** The tendency to reduction of LV walls thickness was shown in all groups, especially in 2 and 3 investigated, that confirms the efficiency of Trimetazidine and combination of Trimetazidine+Thiotriazolol in hypertensive patients treatment.

## The Influence Of Pre-hospital Delay On Results Of Primary Percutaneous Intervention In STEMI

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*Introduction:* Pre-hospital diagnosis and quick transportation to reference centre may play an important role for long term outcomes of STEMI treatment.

*Aim:* The aim of the study was to assess the influence of pre-hospital and intra-hospital delay on recent results of primary PCI.

*Methods:* This was a prospective, single center study of 292 patients (age  $61,0 \pm 10,9$ , male 73,6%) with STEMI hospitalized between 2006 and 2007, treated with primary PCI. The study population was divided on two groups: the group A (n = 152) included patients with pain-to-door time 4 hours. The collected data included: basic demographics and risk factors, pre- and intra-hospital delay, Killip class on admission to hospital, as well as TIMI flow before and after the procedure. The primary endpoint was in-hospital mortality, while secondary endpoints were: maximum CK, CK-MB value, delta ST, left ventricular ejection fraction (LVEF) at discharge.

*Results:* There were no significant differences, except age (group A –  $58,9 \pm 9,96$ ; vs.  $63,1 \pm 11,54$  for group B); pted by primary angioplasty with the pain-to-door time rmore we analyzed risk factors influencing the pre- and intra-hospital mortality, which should be taken under consideration in procedural strategy. Moreover this study is the part of needs assessment and evaluation blueprint for public health project, which objective is to reduce the pre-hospital delay in STEMI patients by conducting social campaign among Silesia region inhabitants.

## Gata4 regulates the erythropoietin receptor expression in cardiomyocytes

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*Aim:* Various experimental studies indicate significant cardioprotective effects of erythropoietin (Epo) by binding to the Epo receptor (EpoR) and by activating various molecular mechanisms, including activation of Gata4, a transcription factor that induces anti-apoptotic genes. However, specific molecular mechanisms of EpoR regulation in cardiomyocytes are unknown. But since Gata4 is a positive regulator of EpoR gene in hematopoietic cells, we raised the question, whether Gata4 is involved in cardiac EpoR gene regulation.

*Methods and results:* Using reporter gene assays, we identified in murine HL-1 cardiomyocytes a 774 bp positive regulatory domain in the 5'-flanking region of the EpoR gene. Fragments that include an E-box, GC-rich elements, and binding sites for members of the GATA and Sp tran-

scription factor families contribute to EpoR promoter activity. Electrophoretic mobility shift assays (EMSA) indicated specific binding of Gata4 and Sp1 (a known interaction partner of Gata4) to the minimal EpoR promoter. Transient overexpression of Gata4 in HL-1 cells resulted in a 3-fold induction of EpoR mRNA expression, while Sp1 and/or Gata6 overexpression did not significantly affect EpoR expression. Depletion of Gata4 protein levels by treating mice with high-dose doxorubicin, a chemotherapeutic agent that causes cardiomyopathy, led to the downregulation of EpoR expression. A following upregulation of EpoR expression 5 days past doxorubicin-injection was correlated with recovery of Gata4. Furthermore, transgenic mice with an inducible shRNA against Gata4 confirmed the suppression of EpoR expression if Gata4 levels were reduced.

*Conclusion:* Our data indicate that Gata4 activates EpoR mRNA expression in cardiomyocytes. This is a novel mechanism that differs from EpoR regulation in neurons and hematopoietic cells. Moreover, it provides new insights into the role of Gata4 in cardioprotection by Epo.

## Life style evaluation and prevalence of cardiovascular disease risk factors in Medical Students

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*Aim:* The aim of this study was to determine the prevalence of risk factors of cardiovascular diseases (CVD) in medical students, their knowledge and awareness regarding its risk factors and relevant preventive practices they adopt in their life style.

*Methods and Materials:* This cross sectional study was conducted on students of a medical college in Pakistan. A well-structured pre-tested questionnaire was filled for each participant evaluating the participants for the prevalence of different CVD risk factors (like increased BMI, blood pressure, smoking, family history of CVD and decreased physical activity). Blood pressure was noted following standard protocol and height/weight for calculating Body mass index (BMI) were taken using standard instruments. All the collected data was entered in SPSS 16.0 and results were then analyzed.

*Results:* Data was collected from 132 students out of which 57 (43.2%) were male. The mean age of participants was  $20.85 (\pm 1.21)$  years. The mean BMI was  $20.47 (\pm 3.02)$  kg/m<sup>2</sup>. Overall 37 (28%) students were underweight and 23 (17.4%) were overweight. A total of 75 students (56.8%) had a family history of CVD, 46.5% of which had a father or mother suffering from CVD. Elevated blood pressure was found in 27.3% of students, blood pressures in the pre-hypertensive range (taken as systolic BP between 120 and 139 mmHg or diastolic pressure between 80 and 89 mmHg) were seen in 11.4% and 15.9% had blood pressures in the hypertensive range (defined as systolic pressure  $\geq 140$  mmHg or diastolic BP  $\geq 90$  mmHg). Cigarette smoking was reported by 9.4% of students, 33%

were regular smokers while 66% reported that they smoked occasionally. Alcohol consumption was very low being reported by only 1.8% of students. Physical activity level was calculated using standardized analysis techniques as per WHO recommendations which showed that 29.5% students were highly active, 32.7% were moderately active while 37.9% had low physical activity level. According to metabolic equivalent (MET) the median physical activity of students was 1920 MET-minutes per week. Analysis of the respondents' answers revealed that 115 (87.1%) students had changed their diet on account of the beneficial or harmful effect of different food substances in development of cardiovascular disorders. A total of 52 (39.4%) students reported having reduced or completely stopped consumption of some food product due to its role in developing CVD and 31 (23.5%) students reported having increased or started additional intake of a food product due to its beneficial role in preventing CVD. **Conclusion:** There is high prevalence of CVD risk factors like family history and hypertension in our medical students which indicate much higher risk of CVD risk factors in other students and groups of the society as medical students have comparatively better knowledge about the prevention of these diseases. Awareness campaigns should be launched to educate the students about the risk factors and preventive measures for CVD. Medical students should in turn educate others so that CVD burden can be reduced from the society as it is the leading cause of death worldwide.

## Dentistry

### Application of Adipocyte Stem cells in regeneration of non space making bone defects around titanium Implant

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**Introduction:** Bone defects are important problems in dental implantations. Vertical augmentation is one of the best treatments in vertical defects that use different tools like autogenous grafts, allografts, and grafting materials. In this study we surveyed the application of adipocyte stem cell in reconstruction of vertical defects around the implantation site.

**Material and Methods:** We used 9 Wistar rats. After isolation of Adipose Tissue Stem Cells (ADSCs) and expansion of them, twice passaged cells were seeded on blade type implants in culture plates. Osteogenic induction of grafted cells started after attachment of cells and continued until 4 days then these scaffolds were implanted in femor. After 8 weeks histological evaluations were done by light and electron microscope. **Result:** We evaluated the osteogenesis with SEM: Presence of calcium which shows osteoblastic activity in upper site of titanium implantation in bone was evaluated & compared by the opposite site of the implant which was stem cell free. The results showed significant difference in mean of calcium density of control and treatment surfaces.

**Conclusion:** The treatment surface showed higher density. So adipocyte stem cell application can be a benefit way for regeneration of non spacing making bone defects.

**Key Words:** Adipocyte Stem cells. vertical bone defects, titanium Implant

### The comparison between two different phases of gutta percha after vertically compaction: (a bacterial leakage study)

ESC-ID **365**  
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**Introduction:** micro organisms play an important role in causing pulpo-pre apical lesions. There fore, obtaining three dimensional seal, from coronal to apical of the canal is mandatory. Beta phase gutta percha cone with sealer uses for obturating and sealing root canals with clinicians conventionally. Recently, alpha phase gutta percha cone has presented on market.

**Aim:** The aim of this study is to compare apical sealing ability of alpha and beta phase of gutta percha using a bacterial leakage model.

**Method and material:** fifty single rooted human premolars after taking buccolingual and mesiodistal radiographs were selected. The root canals were prepared with M two rotary %4 taper instruments to apical size 35. The roots were randomly divided into two groups containing 20 teeth each. the canals were obtured respectively by alpha phase gutta percha and AH26 sealer(G1) and beta phase gutta percha and AH26 sealer (G2) with warm vertical compaction technique. 10 prepared canals served as positive and 2 premolars with intact crowns as negative controls. Specimens were allowed to set for 72 hours at 37°C and %100 humidity. Then all the teeth were sterilized with ethylene oxide gas. After that bacterial suspension of *E. faecalis* in 0/5 Mac farland concentration was made. All teeth were mounted on a 2 chamber apparatus which containing SF broth and then exposed to bacterial suspension of *E. faecalis* every 3 days. The number of days required for the entire contamination of the root canals was recorded, observing the turbidity of the SF broth for a period of 30 days. The data were recorded.

**Results:** negative controls revealed no microbial leakage and positive controls displayed gross microbial leakage. There were no significant differences between the G1 and G2 groups (fisher exact test,  $p > 0/05$ ). The quality of the apical seal in the two groups tested was similar.

**Conclusion:** with regard to better thermal transmission and adaptability of alpha phase cone, our study revealed no significant differences between alpha and beta phases of gutta percha in warm vertically compaction. **Key words:** microbial leakage, gutta percha, apical seal

### **Analysing of enamel surface morphology after application of fluorid containing and fluoride-free bleaching agents**

ESC-ID **526**  
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*Introduction:* Tooth sensitivity after bleaching treatment may represent a big problem in clinical usage, which may be solved by fluoride application after the treatment. To avoid eventual tooth sensitivity from the beginning of the treatment until fluoride application, fluoride can be added to the bleaching agent.

*Aim:* To evaluate the effects of fluoridated bleaching agents and post-bleaching fluoridation treatment on the enamel surface morphology, and to find eventual differences in the whitening efficiency after the treatment.

*Methods and material:* Sixteen freshly extracted human anterior teeth were cut into halves and then divided into the following four groups: Group I: untreated control specimens (buffer saline solution) Group II: specimens treated with fluoride-free 10% carbamide peroxide (CP). Group III: specimens treated with 10% CP with additional topical fluoridation by using 2% neutral sodium fluoride gel for 30 min. Group IV: specimens treated with 10% CP containing 2% sodium fluoride gel. Groups II to IV were treated 8 hours per day for 14 days, then immersed in buffer saline solution for 2 weeks. Enamel morphology changes were observed under SEM. Changes in enamel colour were evaluated on Days 7 and 14, and compared with the key colour.

*Results:* After 2 weeks, an erosion pattern was noted on the specimens in Groups II and III. Specimens from group IV showed a milder demineralized pattern on enamel surface. All the bleached enamel specimens revealed increased whiteness.

*Conclusion:* The fluoridated bleaching agents produced less changes in surface morphology. The addition of fluoride did not impede the whitening effect.

### **Evaluation of Dentosept® mouthrinse influence on periodontal status and activity of egxoglycosidases in crevicular fluid in patients with chronic periodontitis**

ESC-ID **674**  
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 Coauthor **Malgorzata Pietruska, Stefan Sobaniec, Anna Skurska, Ewa Dolinska, Malgorzata Knas, Jan Pietruski, Marzanna Cechowska-Pasko, Slawomir Szajda**  
 Country **Poland**  
 Institution **Medical University of Bialystok**

*Introduction:* Dentosept® mouthrinse containing herbal materials is well known for many years and often used in patients with periodontal diseases.

*Aim:* The aim of this study was to evaluate Dentosept® mouthrinse influence on periodontal clinical status and activity of N-acetylo- $\beta$ -heksozoaminidaze (HEX) and  $\beta$ -glucuronidase ( $\beta$ G) in crevicular fluid in patients with chronic periodontitis (CP).

*Material and methods:* In the study 25 generally healthy individuals with CP was included. Depending on used treatment patients were randomly divided into two groups. In group I scaling with root planning was performed and additionally patients were using Dentosept® mouthrinse during two weeks. In group II professional teeth cleaning was performed without additional pharmacotherapy. Clinical and biochemical examination was done on the baseline and two weeks later.

*Results:* In group I API, BOP and PPD parameters decreased while CAL and GR remained on similar levels. In group II scaling caused significant improvement of API, PPD and CAL but BOP and GR didn't really change. Independently of used treatment, activity of HEX decreased, but differences wasn't statistically significant. Activity of  $\beta$ -glucuronidase after Dentosept® treatment slightly decreased. In group where only scaling was performed decrease of  $\beta$ -glucuronidase activity significantly decreased.

*Conclusions:* Use of Dentosept® after scaling doesn't have any influence on additional improvement of periodontal parameters and reduction of enzymatic activity.

### **Oral health status among mentally retarded children in Tanzania**

ESC-ID **508**  
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 Country **Tanzania**  
 Institution **Muhimbili University of Health and Allied Sciences**

*Aim:* The aim of this study was to determine the oral health status of mentally retarded children in Dar es Salaam.

*Design:* A descriptive cross sectional study.

*Subjects and methods:* The study was conducted in the three districts of Dar es Salaam. Clinical examination was done with the subject being seated on an office chair utilizing natural day light aided by a mouth mirror. Plaque was scored as present when one or more surfaces presented with plaque upon being scrubbed with an edge of the mouth mirror or a piece of gauze. Bleeding was recorded as present when a subject exhibited bleeding in one or more sites when touched lightly by the mouth mirror. Visual inspection was done for calculus and dental caries. Calculus was scored to be present when one or more tooth surfaces exhibited calculus. Dental caries was scored according to the WHO criteria.

*Results:* A total of 116 mentally retarded children aged 7 to 18 years (64.7% boys and 35.3% girls) were recruited. In the deciduous dentition, the mean dmft for the whole group was 0.57 while for those aged 7-10 years it was 1.6. In the permanent dentition the mean DMFT was 0.92. Plaque was observed among 94.8% of the study subjects, gingival bleeding in 21.6% and calculus in 44.8%. Few children had paralysis, 12.9% paralysis of upper limb(s), 4.3% paralysis of lower limb(s) and one child 0.9% paralysis of one side of the face. Only about 34% of MRC examined had attended to the dentist at least once in their life time.

*Conclusion:* The caries status of this group of mentally retarded children is low but the prevalence of periodontal conditions particularly dental plaque and calculus is extremely high.

## Application of adipocyte stem cells in Guided Bone Regeneration in bone defects around implant

ESC-ID **843**  
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 Institution **Shahed Dental School**

*Introduction:* The most important problem in edentulous patients or patients with not favorable jaw bone quality, is the implantation place. Guided Bone Regeneration (GBR) is a process that put membrane on bone defect and prevents fibrous tissue generation. The aim of this study is to evaluate the usage of stem cells in reconstruction of non space making bone defects.

*Material and Methods:* We used Wister race rats. After killing the rats and the fat tissue of the abdomen was collected. After isolation of Adipose Tissue Stem Cells (ADSCs) and expansion of them, twice passaged cells were seeded on blade type implants and membrane in culture plates. The specific design of flat titanium and the surface of membrane were used as a scaffold. Osteogenic induction of grafted cells started after attachment of cells and continued for 4 days then these scaffolds were implanted in femur by 2 different surgeries: (a) by usage of implant and (b) by usage nonresorbable membrane on implants. After 8 weeks the rats were killed and we excluded their femurs. Histological evaluations were done by light and scanning electronic microscope. The cell free surface of titanium was used as control site.

*Results:* We evaluated the osteogenesis by SEM: Presence of calcium which shows osteoblastic activity in upper site of titanium implantation in bone was evaluated & compared by the opposite site of the implant which was stem cell free. The results showed significant difference in mean of calcium density of control and treatment surfaces.

*Conclusion:* The treatment surface showed higher density. So adipocyte stem cell application can be a benefit way for regeneration of non spacing making bone defects. Key Words: Adipocyte Stem cells. Non space making bone defects, titanium Implant

## Ultrastructural Analysis of Dental Cavity Surface After MTAD solution Disinfection

ESC-ID **539**  
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 Institution **University of Nis, Faculty of Medicine**

*Introduction:* MTAD solution (Biopure, Tulsa Dentsply, Tulsa OK, USA) has been so far tested only as intracanal antiseptic in deletion of applied spread layer. The question remains, what effect MTAD solution has on spread film layer after treatment carious lesion in classical preparation technique.

*Aim:* Ultrastructural analysis of outlook of dental surface in the cavity after conventional preparation, by mechanical technique and cavity disinfection with MTAD solution.

*Methods and Material:* Ultrastructural analysis of dental surface took total of 40 mandibular and maxillary molars and premolars with evident carious lesion, extracted for

various reasons. In the first group (N = 10) – cavity preparation was done with classical mechanical technique with no application of any antiseptic (control group). In the second group (N = 30) – cavity preparation was performed with the same technique and disinfection by MTAD solution (duration of 60 seconds). For quantitative evaluation of superficial debris on the cavity walls H?lsmann criteria were applied. Samples were observed on scanning electronic microscope JEOL-JSM-5300.

*Results:* SEM outlook of dental surface after classical preparation shows “blurred” structure image. Presence of dental debris has been notes with large particula beneath which is filmsy layer present. Dental tubules can not be traced. SEM outlook of dentine after MTAD solution application offers entirely clean surface... Dentine debris and filmsy layer were completely deleted; dentine canals clearly open, regularly shapes and with even diameter; dental structure preserved.

*Conclusion:* MTAD solution as a tetracycline combination with weak organic acid and anionic active substances applied for 60 seconds results in complete deletion of filmsy layer from dentine surface

## Oral health indexes in young caucasian and non-caucasian population

ESC-ID **110**  
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 Country **Armenia**  
 Institution **Medical University**

A sample of Caucasians and non-Caucasian were asked to describe their beliefs regarding the causes and consequences of dental diseases. The study population consisted of 45 young Caucasian (average age 20.82 years) and 38 non-Caucasian (average age 20.97 years) living in hostels in Armenia. All the subjects completed a self-administered questionnaire on age, oral hygiene habits, previous dental examinations, and quantity and length of tobacco use. The periodontal examination consisted of the plaque index (PI); periodontal bleeding index (PBI); probing depth (PD); and clinical attachment level (CAL). Examinations were performed according to the World Health Organization's guidelines. The relation among dental beliefs, behaviors and oral health status was examined for each ethnic group. Caucasian reported fewer health behaviors and had poorer oral health than non-Caucasian. Non-Caucasian knew little about dental disease, but was motivated to maintain their teeth by a concern for esthetics, social acceptance and pain. The findings indicate that important cultural differences exist in dental behaviors, and are related to knowledge and motives.

*Background:* Higher social status is usually associated with better oral health.

*Objectives:* To examine the unique explanatory power of objective and subjective measures of social status in the number of missing teeth or teeth with filled or unfilled caries and other oral health index. This report presents additional information regarding the recommendations, briefly describes how the reviews were conducted, and provides information designed to help apply the strongly recommended interventions locally.

*Key words:* Oral health indexes, Young, Caucasian, non-Caucasian

### **Polymerase Chain Reaction (PCR) detection of Porphyromonas Gingivalis and Bacteroides Forsythus in chronic periodontal disease**

ESC-ID **741**  
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 Country **Iran**  
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*Aim:* Periodontitis is considered a multi factors based infectious disease in which bacteria play an essential role. In this study, the presence of putative periodontal organisms, Porphyromonas Gingivalis and Bacteriodes Forsythus were compared from untreated plaque samples taken from chronic periodontitis sufferers and control samples taken from healthy individuals in order to establish a link between the existence of the bacteria and chronic periodontal disease.

*Methods and Materials:* In the plaque samples 16s rRNA was used for detection of bacteria extracted using PCR. From 35 subjects diagnosed with chronic periodontitis and 30 healthy subjects, plaque samples were taken. DNA extraction was done using PCR and identification of bacteria was done through the 16s rRNA sequence.

*Results:* P. Gingivalis was present in 91 percent of chronic periodontitis sufferers as compared to 20 percent in healthy individuals. Similarly, B.Forsythus was detected in 71 and 23 percent of chronic periodontitis sufferers and healthy individuals, respectively.

*Conclusion:* Statistical analysis of our results indicated that these two species are highly correlated with periodontal disease while P. Gingivalis exhibited a stronger correlation. It should be noted that PCR has proven to be a highly effective tool in detection of bacteria relating to periodontal diseases.

### **Influence of cement type on separation of the crown from prosthetic abutment under exposure of temperature**

ESC-ID **467**  
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 Institution **Vilnius University**

*Introduction:* It is well known that cement-retained restorations on implants experience mechanical failures during years of service. Sometimes complications are so severe that veneering porcelain correction or remaking of the prosthesis becomes valid options. In that case restoration is usually retrieved together with prosthetic abutment from implant, if permanent cement was used for fixation of the prosthesis. Therefore, it is important to separate the crown from the abutment without the damage to both. Thus, the aims of the study were: 1) to determine the influence of cement type on separation of crown from abutment; 2) to establish mechanical factors having an impact on strength of restoration adherence to abutment; 3) to estimate the average separation temperature of each cement.

*Methods and materials:* 120 prosthetic implant abutments of various diameter, namely 40 units of 3.5 mm

(33.3%), 64 abutments of 4.0 mm (53.3%) and 16 units of 5.0 mm (13.3%) diameter were used in this study. The same amount of cobalt-chromium frameworks with occlusal openings were fabricated and placed on abutments with following cements - glass ionomer, modified with resins Fuji Plus (GC America, Alsip, IL), zinc phosphate (Harvard Dental International, Berlin, Germany) and dual cure resin luting cement Panavia F2.0 (Kuraray Medical, Osaka, Japan). All specimens were divided into 4 groups: 1) polished abutments and passive frameworks; 2) polished abutments and non-passive frameworks; 3) sand-blasted abutments and passive frameworks; 4) sand-blasted abutments and non-passive frameworks. Every specimen was placed in dental furnace (Vacumat 40T; Vita Zahnfabrik, Bad Sickingen, Germany) for 5 minutes in 300 C0 temperatures. The abutment and metal framework were tried to separate. If not successful, the specimen was put to oven for 5 min increasing temperature for 50 C0 till 800 C0. Statistical analysis was carried out using SPSS (v15).

*Results:* 1) Resin reinforced glass ionomer cement exhibited the lowest (p .05). 2) The passivity of the framework did not have statistically significant influence on the height of the separation temperature for any cement (p> .05). Sandblasting of the abutments correlated with the higher separation temperature for zinc phosphate and dual cure cements; 3) Average temperatures for glass ionomer were  $306 \pm 23$  C0, dual cure cement -  $363 \pm 71$  C0 and for zinc phosphate -  $420 \pm 150$  C0.

*Conclusions:* With the limitations of study, it can be concluded, that (1) if restoration is cemented with glass ionomer, modified with resins, it can be easily separated from abutment; (2) resin reinforced glass ionomer cement shows lowest mechanical properties; (3) it can be recommended to place restoration with the abutment for 5 min in the furnace with 300-350 C0 if glass ionomer was used for cementation, 350 – 400 C0 for dual cure resin luting cement. But no recommendations for zinc phosphate cement because of the big standard deviation and 8 unseparated specimens.

## *Dermatology*

### **Acute stress auguments cutaneous neurogenic inflammatory response**

ESC-ID **1031**  
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 Institution **Carol Davila University of Medicine and Pharmacy, Bucharest**

*Introduction:* Our study was designed to investigate whether acute stress influences the skin's defense response in neurogenic inflammation, a process by which inflammation is triggered by the nervous system, independent of the immune system. This type of inflammation can be simulated in research using capsaicin, the active component of chilli pepers and a powerful irritant for mammals, including humans.

*Materials and methods:* A number of 12 young adult male Wistar rats (200–300 g) were randomly divided into two groups (n=6): (i) control (rats were left undisturbed in their home cages), and (ii) acute stress (AS). Animals from



AS group were held in well ventilated Plexiglas restrainers for 2 h, a procedure meant to trigger psychological acute stress, through the animals' perception of confinement. Then they were released and anesthetized i.p. (0.5 mg CHL/10ml distilled H<sub>2</sub>O). We injected a 1% capsaicin solution in the rat's paw, subcutaneously. Blood flow was measured 20 minutes pre and 40 minutes post-capsaicin administration.

**Results:** We obtained an average normal cutaneous blood flow rate of 25 which was considered as the baseline level for this parameter. After capsaicin subcutaneous administration, measurements determined that in the control group the blood flow increased in average about 2 times more than baseline. In the acute stress group the blood flow showed an average increase about 3 times greater than the baseline.

**Conclusion:** These findings suggest that acute stress enhances cutaneous neurogenic inflammatory responses. Thus, our study supports a potential role of stress in the onset and exacerbation of several inflammatory skin diseases. Key words: neurogenic inflammation, stress, capsaicin

### **Optimization of treatment of patients with Lichen acuminatus through the use of systemic enzymotherapy, antioxidant complex and laser therapy**

ESC-ID **383**  
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 Institution **Bukovinian State Medical University**

**Aim:** The aim of the study is to increase the effectiveness of treatment of patients with lichen acuminatus through the use of systemic enzymotherapy, antioxidant complex and combined laser therapy.

**Material and methods:** We examined and treated 50 patients with lichen ruber planus from 18 to 76 years old. Control group included 20 healthy persons. Methods: clinical examination, biochemical (examination of oxidant-antioxidant homeostasis).

**Results:** In most observed patients with lichen acuminatus (68%) the pathological process was generalized. In 32% of patients the along with skin manifestations there appeared buccal mucosa rash. In biochemical blood study in patients with lichen acuminatus the changes of the parameters of the oxidant-antioxidant homeostasis proved true, which meant the activation of processes of lipid peroxidation on the background of insufficiency of factors of antioxidant protection. The most prominent changes of the oxidant-antioxidant homeostasis were observed in patients with generalized skin irritation. The patients were allocated to 2 groups: I (control) group (25 patients) was given basic medication, II (basic) group (25 patients) received complex treatment through the use of enzyme agent vobenzym, antioxidant complex Tri-V Plus and combined laser therapy (transcutaneous laser irradiation of the blood, differentiated laser phototherapy). The treatment analysis showed that the use of antioxidant complex Tri-V Plus, the means of systemic enzymotherapy vobenzym combined with laser photophoresis in II (basic) group of patients with lichen acuminatus, provides more quick regress of skin rash and shortens the treatment terms for 5-6 days as compared with the patients

of I (control) group, which received only means of basic dermatosis therapy.

**Conclusions:** In patients with lichen acuminatus observed the changes of the parameters of the oxidant-antioxidant homeostasis took place. Use of the means of systemic enzymotherapy (vobenzym), antioxidant complex (Tri-V Plus) and combined laser therapy (transcutaneous laser irradiation of the blood, differentiated laser phototherapy) in the complex treatment of patients with lichen acuminatus provides the increase in the effectiveness of the therapy of this dermatosis.

### **Appropriateness and clinical effectiveness of the using of chronodetermined antioxidant and laser therapy in complex treatment of allergic dermatoses**

ESC-ID **385**  
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**Aim:** To raise the efficacy of treating allergic dermatoses by means of using chronobiologically determined and differentiated methods of antioxidant and combined laser therapy on the basis of studying the parameters of the circadian rhythms of the patients' oxidant-antioxidant homeostasis.

**Material and methods:** We examined and treated 146 patients with allergic dermatoses (eczema, contact allergic dermatitis, atopic dermatitis) from 16 to 76 years old – inhabitants of the Chernivtsi region (Northern Bucovina). Control group included 26 healthy persons. Methods: clinical examination, biochemical (examination of oxidant-antioxidant homeostasis), chronobiological (cosinor-analysis).

**Results:** During of the acute condition of allergic dermatoses changes of circadian rhythms of the parameters of the oxidant-antioxidant homeostasis were possible to establish. It authenticates about an increase an activity of processes of free radical oxidation of lipids and proteins on a background of the tension and the decrease of the activity of antioxidant protect factors in the course of day and can be cause of the increase of endogenous intoxication. Interdependency between circadian rhythms of the oxidative modification of proteins and changes of clinical picture of allergic dermatoses during 24 hours has been established. Recommendation as to the necessity of prescribing chronodetermined antioxidant therapy for patients with allergic dermatoses have been proposed. Different modes of treatment of allergic dermatoses based on a chronodetermined (in the second half of the day) use of antioxidant agents (granulae quercetini, antioxidant complex Tri-V Plus) and combined laser therapy (transcutaneous laser irradiation of the blood, differentiated external laser therapy) have been elaborated and tested. It has been proved that a chronodetermined use a course of multimodality treatment of allergic dermatoses of antioxidant medicamental remedies and combined laser therapy brings about a normalizing and synchronizing effect on the state of oxidant-antioxidant homeostasis, as well as shortens the terms of patients' treatment, prolongs the duration of remission of allergic dermatoses.

**Conclusions:** Changes of the parameters of the circadian

rhythms of oxidation-reduction reactions have been ascertained in patients with allergic dermatoses that were manifested by an increase of intensity and desynchronization processes of lipid and protein oxidation against a background of the deficiency of factors of the body's antioxidant defense. The use of chronodetermined antioxidant and laser therapy in a course of multimodality treatment of allergic dermatoses exerts a normalizing effect on the parameters of circadian rhythms on the indices of patients' oxidation-reduction reactions.

### **Evaluation of light microscopic findings in prurient skin lesions in Sulfur Mustard veterans after and before treatment with betametazon and Hydroxyzine**

ESC-ID **460**  
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 Institution **Azad University**

*Background:* Sulfur mustard (SM) or mustard gas is one of the most important agents of chemical warfare its trait makes it readily available for both military and terrorist use. After absorption, sulfur mustard binds to active biological compounds and induces severe electrophilic tissue reactions through forming via carbonium ions and transient complexes with large molecules. Chronic purities, as one of the most prevalent complications in enormous Sulfur Mustard veterans, can lead to long term disability. With respect to unknown reason for chronic purities that can led to design new treatment, we planned this study.

*Material and Methods:* This randomized double-blind study was done on 30 veterans and 30 patients with chronic purities not due to chemical injury in Baqiyatallah hospital during 2005. They received pomade of betametazon and tablet Hydroxyzine for one month. In base of study and after one month, purities indexes and light microscopic changes were compared.

*Results:* Average age of patient was  $49.62 \pm 7.34$  years old with range of 29 from 37 to 66. This report describes the histologic features of developing and healing inflammatory lesions produced in the skin. In chemical veterans most changes was about epidermal inflammation and spongiosis and the less change was fibrotic changes. We considered less therapeutic effect in non chemical veterans; lesions were more extensive and had more resistancy to treatment. In chemical veterans the most diagnosis before treatment was chronic dermatitis (73% of cases) and after treatment was 63% of cases. In the control group it was 80% and 73% respectively.

*Conclusion:* Moderately severe pain and itching are common problems once blisters have developed and may be managed by the use of mild analgesics, antihistamines and small doses of diazepam. This study shows corticosteroids can be use for SM skin lesions in a period of time but long term usage may accompany with different side effects and also sedative effect of hydroxyzine should be considered  
 Key Words: chronic dermatitis - chemical veteran - light microscopy

### **Pathomorphism of clinical manifestations of cutaneous tuberculosis in Northern Bucovina**

ESC-ID **382**  
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*Aim:* To ascertain the clinical characteristics of cutaneous tuberculosis in inhabitants of the Chernivtsi region (Northern Bucovina) at present.

*Material and methods:* An analysis of the incidence rate of cutaneous tuberculosis among inhabitants of the Chernivtsi region (Northern Bucovina) in 2006-2008 in comparison with the 60s of the 20th century has been carried out. The author has ascertained the specific characteristics of the clinical course of cutaneous tuberculosis in patients who are under a regular medical check – up at the Chernivtsi TB dispensary. Methods: clinical and epidemiological examination.

*Results:* In 2001 an epidemic of tuberculosis was registered in Ukraine as stated by WHO. The tuberculous infection is detected especially often in patients with HIV/AIDS. At the same time the sickness rate of active forms of tuberculosis in Chernivtsi region (Northern Bucovina) has had a tendency to be on the decrease in recent years. There is a marked tendency towards a decrease in the region of the cutaneous tuberculosis morbidity: 1.22 cases per 100 thousand of the population in 2006 and 0.99 cases per 100 thousand of the population in 2008. Male patients (66.7%), persons over 50 years of age (54.5%) and inhabitants of the countryside (54.5%) predominate among patients afflicted with tuberculosis. A redistribution of the clinical forms of cutaneous tuberculosis has been established within the pattern of morbidity over the last years in the Chernivtsi region (Northern Bucovina) in comparison with the middle of the last century. Thus in 1963 in Northern Bucovina such forms as lupus vulgaris (37.0%), Bazin's disease (22.2%) and scrofuloderma (18.5%) predominated among the clinical forms of cutaneous tuberculosis, whereas in 2006-2008 disseminated forms: tuberculosis papulonecrotica (respectively: 54.5% and 55.6%) and Bazin's disease (respectively: 45.5% and 44.4%).

*Conclusion:* A prevalence of disseminated forms of cutaneous tuberculosis (tuberculosis papulonecrotica, Bazin's disease) has been marked in the Chernivtsi region (Northern Bucovina) in recent years, as well their primary detection in persons of elderly age, the latter substantiating a need of bringing up-to-date the activity concerning timely diagnostics of cutaneous tuberculosis, particularly among persons of young age, taking into account the specific features of their clinical course at the present stage.

## Different types of facial involvement in psoriatic patients, is it an evidence for the sever psoriasis?

ESC-ID 94  
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Psoriasis is a chronic disease that affects the skin and joints. It is estimated prevalence of psoriasis varies from 0.6% to 4.8%. Facial involvement gives rise to considerable concern among patients. Some authors have suggested that facial involvement may be a sign of severe psoriasis. The purpose of this study was to evaluate the prevalence and characteristics of different types of facial involvement and to compare the severity of psoriasis between patients among patients with different types of facial involvement and those without facial involvement. A total of 138 consecutive patients with psoriasis seen in Razi hospital between Jan 2006 and April 2007 were enrolled in this study. They were categorized into 2 main groups of patients with and without facial psoriasis. Then patients with facial involvement were classified into three subgroups based on the type of their facial lesion distribution as peripherofacial type (PF), centropacial type (CF), and mixed type (MF). The age of onset, duration of disease, family history, nail involvement, joint involvement, tongue involvement, pruritus, history if phototherapy or systemic therapy, admission history, relapse history, extent of involvement, and the effect of external factors were recorded. The severity of psoriasis on whole body and on the scalp was evaluated separately according to the Psoriasis Area and Severity Index (PASI). The prevalence of facial involvement was 47.1%. In particular with facial involvement subgroups, CF type facial psoriasis was significantly associated with more joint involvement while the mean PASI score on scalp was significantly higher in MF type and PF type facial psoriasis than that of the no facial involvement group. Within facial psoriasis subgroups MF type has a higher scalp PASI score in comparison to CF type facial involvement. Facial involvement was a marker of severe psoriasis in comparison with non facial involved patients. We categorized facial involved patients into a previously reported classification. Peripherofacial involvement might be an extension of severe scalp psoriasis, while centropacial involvement might be a marker of severe body psoriasis associated with more joint involvement. Consequently, during the treatment of patients with psoriasis, special attention should be taken place about different lesion distributions in patients, which may show different clinical characteristics of psoriatics.

## Investigation of the effect of Filaggrin mutations on skin barrier function and susceptibility to contact sensitisation

ESC-ID 337  
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 Institution University of Southampton

*Introduction:* Atopic Dermatitis (AD) is a complex multifactorial inflammatory skin condition. Loss of function mutations in FLG, which encodes filaggrin, are the most

powerful and widely replicated genetic linkage to AD and in particular, predispose to its early onset, persistence into adulthood and later presentation of asthma. Filaggrin is an important regulator of epidermal differentiation. It has been hypothesised that loss of function FLG mutations impair keratinocyte differentiation producing a dysfunctional epidermal barrier that permits the penetration of potential allergens leading to systemic sensitisation. Hypotheses: 1) Skin barrier function is impaired in individuals with AD, and in those expressing FLG loss of function variants. 2) Impaired skin barrier function correlates with enhanced elicitation of contact allergic dermatitis.

*Methods:* 59 individuals, 42 with currently active AD, were recruited from dermatology outpatient clinics at the Royal South Hants Hospital and patient databases. Restriction fragment length polymorphism assays were developed for the FLG polymorphisms 2282del4 and R501X. Extent and intensity of AD were assessed and objective SCORAD calculated. Transepidermal water loss (TEWL) was measured on the volar forearm at baseline and sequentially after multiples of 10 tape strips up to a maximum of 50. Subjects were sensitised to 2, 4-dinitrochlorobenzene (DNCB) (57mg) and challenged 4-6 weeks later with doses of 6.25, 8.8, 12.5 and 17.7mg. Responses were measured after 48 h as increase in skin fold thickness and erythema.

*Results:* The minor allele frequencies in AD were R501X 5.6%, 2282del4 22.8%, combined allele frequency 27.8%. AD severity was greater in FLG-related AD (FRAD) than in non FLG-related AD (NFRAD) as assessed through extent ( $p = 0.032$ ), intensity ( $p = 0.178$ ) and objective SCORAD ( $p = 0.098$ ). Baseline TEWL ( $p = 0.037$ ) and response of TEWL to tape stripping ( $p = 0.032$ ) were both greater in FRAD than in NFRAD. The number of tape strips required to produce the half maximal TEWL, TEWL50, was 17.9 in FRAD and 19.8 in NFRAD ( $p = 0.7366$ ). Clinical response to DNCB was lower in those with AD than in those without AD as assessed through both erythema ( $p = 0.121$ ) and skin fold thickness ( $p = 0.160$ ). FLG mutations were seen to confer reduced clinical response to DNCB as assessed by skin fold thickness ( $p = 0.486$ ).

*Conclusions:* Epidermal barrier function in AD is more severe in those with loss of function FLG mutations, however results suggested that other factors located either throughout, or below, the stratum corneum are also important. Impaired barrier function in AD did not determine a 'stronger' response to skin encountered allergens. FLG status conferred a further reduced clinical response to DNCB beyond that seen in AD as assessed through skin fold thickness, but not erythema, suggesting different pathomechanisms for the cutaneous infiltration/oedema and vascular inflammatory responses in AD.

## Identification of immunotherapeutic targets in melanoma

ESC-ID 1008  
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*Introduction:* Melanoma is a common malignant tumor of the skin, with a bad prognosis when disseminated disease

is present. Conventional therapies are of little benefit as tumor cells become chemoresistant. Lymphocyte infiltration of melanomas is associated with prolonged survival. Active immunotherapy has been shown to stimulate the immune response. Tumor antigenic peptides can specifically stimulate the immune response. By modifying peptides in a way that they bind stronger to HLA molecules, the immune response can be increased. Previously, the lab of D. Herlyn showed that peptides spanning the BRAFV600E mutation, a mutation in the Map-kinase pathway that is present in 70% of melanoma cells, were immunogenic. The goal of this study is to determine whether modified BRAFV600E peptides that bind stronger to HLA molecules, may induce a stronger immune response than unmodified BRAFV600E peptides in melanoma.

**Material and methods:** Twenty-three melanoma patients were included in this study. The HLA-A2 status of 14 patients was determined by flow cytometry. Genomic DNA isolated from tumor cells was used to determine the BRAFV600E mutation status. Polymerase Chain Reaction (PCR) was performed several times with different sets of primers. This was followed by sequencing of the amplified DNA fragment, using primers SP6 and reverse primer T7, and M13 forward and M13 reverse primer. A T2 peptide binding assay was performed to determine the binding ability of modified peptides to T2 cells to select the peptides with a high binding ability. A standard lymphocyte proliferation assay (3HT-thymidine incorporation assay) was used to determine the extent of proliferation of T-cells cultured with various peptides, so we could determine which peptides stimulated the T-lymphocytes best in vitro. Proliferating lymphocytes were phenotyped to determine whether it were CD4 (Th) or CD8 (CTL) cells. The proliferating cells were expanded further in the presence of IL-2 and used in a cytotoxicity assay, to determine whether they were efficient in killing tumor cells.

**Results:** Of the 23 patients, lymphocytes of 14 patients were tested for their HLA-A2 status. 4 (29%) Of those patients were HLA-A2 positive, and thus could be used for further experiments. Nine other patients were tested positive for HLA-A2 earlier and were also used in this study. Of the 11 patients tested for their BRAF status, 4 (36%) had the V600E mutation. The T2 binding assay showed a strong binding of modified peptide 4, which is altered in both anchor residues, compared to the unmodified peptide. The lymphocyte proliferation assay showed an increase in proliferation when some of the modified peptides were used for stimulation. The phenotyping of the proliferating lymphocytes showed mostly lymphocytes of the CD8 subtype. The proliferating lymphocytes are currently being tested in a cytotoxicity assay.

**Discussion and conclusion:** Modified peptides showed an increase in lymphocyte proliferation, mostly of the CD8 subtype. The cytotoxicity assay results, that will be presented at the congress, will show whether these lymphocytes are effective in specifically targeting tumor cells in vitro.

## Endocrinology

### Deficiency for Kinins Receptors Increases Insulin Resistance, Hyperglycaemia and Liver Abnormalities in Lep(ob) Mice

ESC-ID 945

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**Background:** Obesity and associated diseases such as type 2 diabetes affect over 300 million people worldwide. Kinins have been implicated in several aspects of metabolism, including the regulation of glucose homeostasis and the control of adiposity. However, due mainly to the functional redundancy of the kinin receptors and other components of the kallikrein-kinin system, the contributions of complete abrogation of kinin signaling to the pathogenesis of obesity and diabetes have not been described yet.

**Objective:** Here we use obese (ob/ob) mice deficient for both B1 and B2 receptor (ob/obB1B2KO) to tackle this question and investigate the consequences of lack of kinin signaling to the metabolic complications associated with obesity.

**Methods:** ob/obB1B2KO mice have been generated by backcrossing of B1B2KO mice with mice with a null mutation in the leptin gene (ob/ob). Body composition and food intake were monitored for 3 months in these mice. Glucose tolerance test (GTT) and insulin tolerance test (ITT) were performed with mice at two different ages (3 and 7 months). To explore hepatic function, gluconeogenesis was estimated by pyruvate challenge test and liver damage was measured by plasma transaminases AST and ALT, as well as by histopathological analysis.

**Results:** ob/obB1B2KO mice exhibited similar body weight and food intake than ob/ob mice, despite decreased epididymal adipose tissue mass (92 + 3 vs 110 + 5 mg/g body weight  $p < 0.01$ ). At the first 3 months of life, these animals also presented glucose levels, GTT and ITT similar to the ob/ob. However, a robust hyperinsulinemia accompanied by hyperglycemia was observed in ob/obB1B2KO at 7 months of age, supported also by decreased glucose tolerance and increased whole-body insulin resistance. Insulin resistance was also followed by hepatomegaly (57 + 4 vs 40 + 3 mg/g body weight,  $p < 0.05$ ), increased non-alcoholic hepatic steatosis and increased levels of AST (135 + 6 vs 83 + 4 u/ml,  $p < 0.05$ ) and ALT (128 + 3 vs 88 + 2 u/ml,  $p < 0.01$ ). In addition, a pyruvate challenge test suggested exacerbated gluconeogenesis in kinin receptors/leptin deficient mice. **Conclusion:** Kinins are necessary to preserve hepatic function and normal glucose levels in obese mice, suggesting a protective role for the kallikrein-kinin system against the complications associated with obesity.

## Improvement of endothelial function with testosterone replacement therapy in hypogonade men with metabolic syndrome

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*Objective:* Low testosterone levels in men are associated with metabolic syndrome. The hallmark of metabolic syndrome is insulin resistance. Several studies have confirmed a significant inverse relationship between blood testosterone levels and insulin resistance in men. Since insulin resistance contributes profoundly to endothelium dysfunction, it is righteous to claim an important role of androgen in vascular health. The aim of our study was to assess the effect of testosterone replacement therapy on endothelial function in androgen deficient men with metabolic syndrome.

*Methods:* For 7 months, 30 men aged  $62,7 \pm 10,0$  years, with diagnosed androgen deficiency and metabolic syndrome received testosterone replacement treatment with testosterone undecanoate. Patients were treated intramuscular depot injections of testosterone undecanoate 1000 mg. They received first injection at day 0, second after 6 weeks and third 12 weeks after the second. Endothelium-dependent flow-mediated dilation (FMD) and glyceryl trinitrate-induced endothelium-independent dilation at brachial artery were studied before the treatment began and 10 weeks after third injection of testosterone.

*Results:* With treatment, FMD of brachial artery improved significantly from 4,2 (SD = 4,6%) to 7,5 (SD = 4,9%) ( $p = 0.0012$ ). The endothelium-independent dilation did not change significantly.

*Conclusion:* A 7 month testosterone replacement therapy resulted in marked improvement of endothelial function in male patients with androgen deficiency and metabolic syndrome.

## Impaired IGF1-GH axis and new therapeutic options in Alstrom Syndrome patients: a case series

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*Background:* Defects of the primary cilium and its anchoring structure, the basal body, cause a number of human genetic disorders, collectively termed ciliopathies: primary ciliary dyskinesia, Bardet-Biedl syndrome, polycystic kidney and liver disease, nephronophthisis, Alström syndrome, Meckel-Gruber syndrome and some forms of retinal degeneration. Alström syndrome is an extremely rare, autosomal recessive genetic disorder characterized by a group of signs and symptoms including infantile onset dilated cardiomyopathy, blindness, hearing impairment/loss, obesity, diabetes, hepatic and renal dysfunction. Because adult growth hormone deficiency and Alström Syndrome share some clinical and metabolic fea-

tures, we studied the GH-IGF1 axis, using MRI techniques and dynamic tests in 3 unrelated patients with Alström syndrome. Case presentation The patients were hospitalized and the growth hormone stimulatory tests were made, as well as brain MRI. Insulin provocative test revealed a severe GH deficiency in these patients, defined by a peak response to insulin-induced hypoglycemia less than 3 ng/dl and IGF1 concentrations less than -2SDS. We didn't find multiple pituitary hormone deficiency and we noticed only a severe GH deficiency in all three patients. The MRI study of the diencephalic and pituitary region was suggestive for the diagnosis of empty sella in one patient. One patient received Recombinant-GH replacement for one year with very good results, one underwent a gastric sleeve with a satisfactory outcome, one patient died due to the progression of the cardiac myopathy.

*Conclusion:* Future studies are needed to assess if the substitution therapy with Recombinant Growth hormone is cost-effective and without risk in such patients with Alström Syndrome and severe insulin resistance, despite our good results in one patient. Also, careful clinical and genetic studies can contribute to a better understanding of the evolution after different therapeutical attempt in the complex disorders such as Alström Syndrome.

## Dependence of pituitary gland abnormalities on the cause of primary congenital hypothyroidism

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*Introduction:* Pituitary gland abnormalities are observed in adult patients with primary congenital hypothyroidism (PCH) when LT4-substitution treatment is incorrect. A thyroid stimulating hormone concentration is used to assess the effectiveness of the therapy. The aim of our study was to verify the dependence of pituitary gland changes on the cause of PCH and the serum TSH level.

*Material and methods:* 21 PCH patients, aged  $37 \pm 10$  were examined by means of magnetic resonance imaging (MRI) of pituitary, neck and lingual region ultrasonography, 131-I scintigraphy, serum thyreoglobulin level, skull X-ray and anterior pituitary gland hormones (except ACTH). Patients were divided into 3 groups according to the cause of PCH: the first group consisted of 6 patients with athyreosis, the second group – 11 patients with lingual ectopy and the third group – 4 patients with hormone synthesis defects.

*Results:* Hormonal evaluation did not show significant differences between the groups, although the MRI in the groups differed: patients with athyreosis had mostly (43%) hyperplasia, patients with ectopy – partially empty sella (50%) and in the dysmorphogenesis group there was no domination of any image. What is more, ectopy was relatively rarely related to any lesions in the pituitary (only 36% of individuals had a pathological finding on MRI), whereas athyreotic and dysmorphogenetic patients had abnormal pituitary MRI in most cases (71% and 75%, respectively).

*Conclusions:* The cause of PCH has a greater influence on pituitary abnormalities than TSH serum level at the time

of analysis. The previously established cause of PCH is a superior predictor of pituitary abnormalities, in comparison to TSH serum level and skull X-ray. It is necessary to take into consideration the cause of PCH in LT4-substitution treatment.

### Activation of mTOR and Erk/Akt pathways in pituitary adenomas concerning their endocrine activity

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*Introduction:* mTOR (mammalian Target Of Rapamycin), a serin/threonin protein kinase plays crucial role in regulation of cell growth by controlling ribosome biogenesis, protein translation and apoptosis. Alterations of the mTOR pathway often lead to uncontrolled cell proliferation resulting in tumor formation. Abnormal, excessive activation of mTOR occurs in breast-, prostate-, bladder- and kidney cancer. Rapamycin – an inhibitor of mTOR – has a documented anti-tumor activity. Promising results of several ongoing clinical trials on rapamycin derivatives, encouraged us to determine whether mTOR pathway is deregulated in other types of neoplasms. Pituitary tumors account for 15% of intracranial neoplasms and are classified into 6 types, according to their hormonal profile and clinical manifestation. Although lactotroph and somatotroph adenomas respond to pharmacological treatment, resection is often necessary since no effective pharmacologic alternative is available at the time. Our aim was to determine the activation of mTOR and interacting Erk and Akt pathways in a collection of 14 pituitary adenomas to verify if pituitary tumors can be considered as potential candidates for treatment with mTOR inhibitors.

*Materials and methods:* Activation of kinases: p90RSK, Akt, Erk1/2 and mTOR were examined by Western blot analysis of excised tumors using phosphospecific polyclonal antibodies.

*Results:* A total of 34 tumors were analyzed so far. We showed a tendency for the activation of mTOR in somatotropinomas (4 out of 10) and gonadotropinomas (3 out of 11). From the 7 null cell adenomas only one was active for mTOR. For the other types the number of specimen was limited (1 TSHoma, 2 prolactinomas and 2 corticotropinomas, with only one of the corticotropinomas active). 24 samples had strong activation of MEK/Erk cascade while 10 tumors had weak/undetectable phosphorylation of these proteins. It did not correlate with endocrine activity of pituitary tumor. Samples positive for mTOR activation were within Erk positive and Erk negative group of tumors.

*Conclusions:* Our results suggest a possible involvement of mTOR pathway in development of pituitary tumors. Activation of MEK/Erk pathways which was detected in the majority of these tumors offers a potential target for pharmacologic intervention in these adenomas.

### Orexin A regulates lipogenesis, glucose metabolism and adiponectin secretion and expression in isolated rat adipocytes and 3T3-L1 cells

ESC-ID **676**  
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Orexin A is neurohormone that regulates the sleep-wake cycle, cardiovascular functions and energy expenditure. Lack of orexin/orexin receptors is associated with increased body mass index and type 2 diabetes mellitus. Recently, we and others demonstrated that orexin receptors (orexin receptor 1 and 2) are expressed in the endocrine pancreatic islets and in adipose tissue in rodents. We therefore investigated the role of orexin in regulating the lipid and carbohydrate metabolism in adipocytes. We used isolated primary rat adipocytes and 3T3-L1-cells that were differentiated into adipocytes. We studied the short and long-term effects of orexin A (OXA) on lipogenesis, glucose uptake and ATP levels. Furthermore, we characterized the effects of OXA on adiponectin secretion and expression using RIA and Western blot analysis. We detected orexin receptor expression in both, differentiated 3T3-L1 cells and primary rat adipocytes, with OX1R, as the predominant receptor subtype. Isolated adipocytes showed higher rate of lipogenesis after a 2 hours exposure to OXA, as compared to non-treated cells. In addition, OXA potentiated the lipogenic effect of insulin. OXA increased intracellular triglyceride content after 24 and 48 hours of incubation and potentiated the effect of insulin. OXA increased glucose uptake by adipocytes and the intracellular ATP content. In addition, OXA increased the secretion and expression of adiponectin. In summary, the effects of orexin on adipose tissue may represent mechanisms, by which orexin lowers hyperglycemia, an effect previously reported in animal models of type 1 and type 2 diabetes mellitus

### Association of Microalbuminuria with Intima-medial thickness of Superficial femoral artery in Type 2 Diabetes Mellitus patients

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*Introduction:* The macrovascular angiopathy in Type 2 DM increases risk of amputations. An accurate and sensitive marker for measuring atherosclerotic changes predisposing to critical limb ischemia would be by assessing

the Intima-medial Thickness (IMT) of Superficial Femoral Artery (SFA) distal to its bifurcation from the common femoral artery, as collateralization occurs through this junction. However, measurement of the IMT is expensive and rarely practiced until the patient presented with symptoms of PAD, by which time the disease would have already progressed. Urine microalbuminuria is a valuable and well known early marker for microangiopathic atherosclerotic changes. Does microalbuminuria have a correlation with IMT of SFA? If so, then ascertaining the levels of microalbuminuria, would prove to be easier, earlier and less expensive for measuring macrovascular angiopathy, than measuring IMT.

**Aim:** 1. To measure the IMT of the Superficial Femoral Artery. 2. To associate the IMT with urine albumin-creatinine ratio. 3. To study hypertension, elevated blood glucose and dyslipidemia as additional factors aggravating atherosclerosis in patients with PAD.

**Materials and methods:** Cross sectional observational study Study period - 6 months Sample size - 30 known Type 2 DM patients aged between 40 and 65 years with duration of disease more than 5 years. Exclusion criteria: 1. History of physical injury to one/both limbs in past 15 days. 2. History of viral illness or streptococcal throat infections in past one month. 3. Pre-existent Foot Ulcers. 4. History of any previous renal pathology or dialysis 5. History of urinary tract infections and extreme exercise 6. Pregnancy, congestive heart failure or fever. The SFA IMT at the level of bifurcation from the femoral artery was ascertained by B mode ultrasonographic scan using Siemens G60. Microalbuminuria was measured using the TURBIDOMETRY. Urine creatinine was measured by Jaffe's method. Lipid profile was done by specific enzymatic methods in the auto analyzers. The atherogenic index, the ratio of total cholesterol to HDL was calculated. The patients were also screened for hypertension using the JNC 7 guidelines.

**Results:** Statistical analysis was done using Pearson's correlation method. A strong association ( $r = 0.946$ ,  $P < 0.0001$ ) was demonstrated between IMT and microalbuminuria, with the correlation being stronger in males than females ( $r = 0.964$  vs  $r = 0.894$ ). Significant associations were also found between IMT and SBP, age, and atherogenic index ( $P < 0.001$ ), but not with DBP ( $r = -0.017$ ,  $P = 0.928$ )

**Conclusion:** Using microalbuminuria as an index of macrovascular angiopathy, adverse changes in lower limb arterial structure predisposing to critical limb ischemia can be detected early. Being easier to perform and less expensive, this would help decrease the number of amputations and reduce health care costs in type 2 DM.

### Lymphocyte activation in type 1 diabetes: The altered function of potassium channels

ESC-ID **361**  
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Voltage-gated Kv1.3 and calcium-dependent IKCa1 potassium channels play a fundamental role in the activation of lymphocytes: their inhibition has immunomodulatory effects. However, there is only limited data available concerning their functional and clinical significance in the pathomechanism of type 1 diabetes mellitus. The aim of

our study was to determine the functional characteristics of both potassium channels in specified lymphocyte subsets (Th1, Th2, CD4, CD8 cells) in samples from healthy individuals through kinetic flow cytometry analysis, as well as to assess how these characteristics differ in type 1 diabetes. We also investigated whether the kinetics of calcium influx facilitated by cell activation is altered in lymphocytes of type 1 diabetes patients compared to healthy controls, and whether the different proportion of lymphocyte potassium channels has a role in the development of these changes. We took peripheral blood samples from five healthy volunteers and five type 1 diabetes patients. After marking the isolated mononuclear cells with specific antibodies for Th1, Th2, CD4, and CD8 cells, we stained them with fluorescent calcium binding dyes (Fluo-3 and Fura-Red), and activated them with phytohemagglutinin. We monitored intracellular free calcium levels with kinetic flow cytometry measurements, and detected the changes elicited by blockers of the Kv1.3 and IKCa1 channels (margatoxin, TRAM-34). We found that in healthy individuals Th1 cells are dominated by IKCa1 channels, whereas Th2 cells have a higher proportion of the Kv1.3 channel. The activation of CD4 lymphocytes was to be inhibited by the blocker of the Kv1.3 channel only, whereas CD8 lymphocytes were blocked effectively by specific inhibitors of both potassium channels. In type 1 diabetes, these findings are characteristic of Th1 and CD8 cells only – Kv1.3 channels in Th2 and CD4 cells were not to be inhibited significantly by their specific blocker in this disease in contrast to healthy individuals. We observed that an activating stimulus results in lower levels of calcium influx in lymphocytes, and hence a lower level of cell activation in type 1 diabetes compared to healthy controls. The altered function of Kv1.3 potassium channels might have a role in this finding in case of the Th2 and CD4 cell subsets. Our results help to better understand the role of lymphocyte potassium channels in the pathomechanism of type 1 diabetes, and also give an explanation for the lower level of lymphocyte activation present in diabetes. Nevertheless, further studies are needed to determine whether these results are also beneficial for the therapy of type 1 diabetes.

## Epidemiology

### Study upon dental students' attitude toward obese patients

ESC-ID **1026**  
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**Introduction:** Obesity is a medical condition obviously related to the reduction of life expectancy. We are now witnesses of the alarming worldwide extension of this disease. The serious associated pathology has assured the medical community of the necessity of implementing prophylactic programmes, aimed to reduce the prevalence of obesity. Precocious support and treatment are desired for obese patients and must be initiated by any doctor,

regardless of his speciality. However, obesity generates a heterogenous echo throughout medical society, as it is bounded to both organic and habitual aetiological factors. Therefore, students in medicine must be given the complete package of information about the exact meaning of obesity and how it can be treated in order to enable them to give medical support on a scientific basis.

*Aim:* This study was conducted with the aim of evaluating the perception on obese patients' medical and social condition and of identifying the attitude towards them (empathy or on the contrary, unprofessional judgemental attitude) among dental students, considering their medical defined point of view.

*Materials and methods:* We have surveyed a representative group of dental students from the University of Medicine and Pharmacy „Carol Davila”- Faculty of Dentistry, in the 3rd, 4th, 5th and 6th year of study. At this point, they have accomplished the Nutrition and Food Hygiene course, Internal Medicine and General Surgery courses and clerkships at „Sf. Ioan” hospital in Bucharest (the most prestigious clinic of bariatric surgery in Romania). The survey took place between the 5th and 21st of May. It consisted of 15 single choice questions and 3 „fill-in” tasks. Respondents were asked, on condition of anonymity, to mention personal data such as age, sex, weight, height, year of study. The valid answers have been interpreted on a statistical basis, which led to the construction of graphs revealing the most popular answers among the students.

*Results:* 386 questionnaires were filled by students, most of them – 150 – in the 3rd year of study. When it comes to the main cause in generating obesity, 58% of the students incriminated the loss of nutritional control and a sedentary life, fact emphasized by the high percentage -46%- of the students who believe that a balanced, well-conducted lifestyle would not lead to this severe pathology. The main benefit that should motivate an obese person to lose weight is considered to be life quality improvement – 78%. Although the surgical option for obesity treatment is likely to be efficient (54%), students seem confused in choosing the optimal surgical technique, probably because of their lack of information about each technique's indications. From a professional, social and emotional point of view students show sympathy, as they do not manifest any discrimination feelings towards obese persons. 74 % students expect themselves to be well trained at the moment of graduation so that they easily recognize clinical signs of general affections, obesity in this case. Moreover, most of them are willing to redirect an obese patient to a specialist (64%), considering this an appropriate reaction, for he is a doctor in the first place – 58%. The specialist that most students recommend is the specialist in nutrition and diabetes – 59%. When it comes to systemic complications generated by obesity, that could interfere with the treatment in the oro-maxillo-facial region, 44% of the students, as future dentists, consider precaution means necessary, and are willing to analyse before the beginning of treatment, para-clinical investigation results. On the contrary, 39% would skip this step.

*Conclusions:* Dental students should be well trained and prepared to recognize severe diseases and associated oral lesions in order to prevent both systemic and oro-maxillo-facial complications of obesity. In addition, they should be able to advise, conceal and direct obese patients to qualified specialists in order to stop the evolution of obesity and even start the remission of this affection. Future dentists are the most required specialists by most of

the people, considering the fact that when it comes to eating or fonation disfunctions, severe pain in the oro-maxillo-facial territory or the alteration of the aesthetic principles, patients don't hesitate appealing to the dentist.

*Key words:* obesity, dental students, empathy, judgemental attitude, survey

### Physiologic and psychological effects of placebo intervention on blood pressure

ESC-ID **1024**

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*Aim:* There is no consensus about the existence of physiologic response to placebo intervention. Many studies have been criticised not to measure the placebo effect itself; it was argued that rather spontaneous remission and regression artefacts would have accounted for the positive outcomes (positive outcomes mostly in regards to subjective but not to objective parameters). (1,2,3) Inspired by this criticism we aimed to investigate the short-term response of blood pressure (BP) and other autonomic as well as subjective measures to placebo intervention.

*Methods:* 92 healthy volunteers were randomly assigned to 3 groups. The application of an effective drug was suggested - to increase BP (condition 1), to decrease BP (condition 2). The control group obtained the information that placebo would be administered. In fact all groups received placebo. BP, heart rate, stroke volume, total peripheral resistance, heart rate variability, gastric and electrodermal activity were continuously recorded over 30 minutes before and after the administration of placebo. The volunteers rated their expectation about the drug effect and the subjectively perceived effect by means of visual analogue scale. Groups did not significantly differ with regards to socio-demographic characteristics. There was a trend towards a higher proportion of males in condition 1 ( $p = 0.06$ ).

*Results:* Subjects in condition 1 and 2 had higher expectations and subjective perception with regard to the drug effect compared to the control condition (all  $p < 0.05$ ). Mean BP increased by  $1.64 \pm 3.15$  mmHg in condition 1 ( $p = 0.01$ ), by  $1.21 \pm 3.37$  mmHg in condition 2 ( $p = 0.05$ ) and by  $1.05 \pm 3.91$  mmHg in the control condition ( $p = 0.15$ ). No significant time x condition interaction was found for any of the physiological parameters after adjustment for sex.

*Conclusion:* The data suggests that the placebo intervention induced mild autonomic arousal independently of the suggestion, which was possibly related to the anticipation of adverse drug effects. The lack of placebo effects was possibly due to the absence of disease and associated symptoms in these subjects. Future research should focus on clinical populations or use designs inducing symptoms.



## Prevalence of oral manifestations of Systemic Lupus Erythematosus (SLE)

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*Background:* Systemic Lupus Erythematosus (SLE) is an autoimmune disease that characterized by producing lots of antibodies. 5-25 percent of patients have oral lesions, however in some studies reported up to 80 percent.

*Objective:* The purpose of this study was to determine the oral manifestations prevalence of SLE patients.

*Methods:* In this study 70 SLE patients attending to Zahedan rheumatology clinic were included. SLE patient's mouths were examined under appropriate light by tongue blades after a rheumatologist confirmation. Regarding questionnaires were filled out. The questionnaires in these patients were recorded in the questionnaires after oral medicine specialist confirmation. Then necessary treatments were performed.

*Findings:* In this study 70 patients (63 male, 7 female) with the SLE disease were selected, with the age range of 15-70 years. 61.4% of patients had oral lesions. The most common lesions were red lesions (35.08%), white lesions (21.05%), pigmentations (19.29%), ulcers (10.52%), angular cheilitis (10.52%) and white and red lesions (3.52%). 51.4% of patients had xerostomia. Posterior area of hard palate and lower lip were the most involved sites. There was no significant difference between oral manifestations with age, sex and duration of disease activity ( $p > 0.05$ ).

*Conclusion:* As oral manifestations are one of the first SLE features, it shows the necessity of mouth follow up examination in order to early diagnosis and better treatment of oral lesions in the patients with SLE disease.

*Key words:* Systemic Lupus Erythematosus, oral lesion, Epidemiology

## Reforming the treatment of severe acute malnutrition in children under 5 years old in a rural Zambian hospital

ESC-ID **265**  
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*Introduction:* In children under 5 years old, Severe Acute Malnutrition (SAM) can be diagnosed as having a weight-for-height Francis Hospital (SFH), Zambia. SFH is a referral hospital whose catchment area extends to Mozambique and Malawi. SFH offers free services and receives funding from the US Government and other donors. This study concentrated on the WHO steps 5-10, which includes the prevention and treatment of micronutrient deficiencies; stabilisation; rehabilitation; loving care and stimulation; and preparation for follow up and discharge.

*Aims and Objectives:* • To determine the extent to which steps 5-10 of the WHO Ten Steps are implemented at SFH. • To identify challenges to implementing these steps. • To

help the staff find ways to overcome the bottlenecks. • To develop a monitoring and evaluation system that is not onerous.

*Methods:* Data were collected using three methods. First, an examination of medical notes of previous patients. Second, in-depth interviews with members of staff. Third, direct observations of procedures on the ward. The interventions included teaching sessions as well as practical solutions to organisational problems.

*Results:* Based on reported, rather than observed actions, there was a trend towards improved case management of SAM children.

*Conclusion:* This study highlighted that education alone does not necessarily change behaviour, and it is important to motivate staff to implement changes. A challenge is to ensure that any changes made are sustainable.

## Gastroenterology

### Colorectal Carcinoma in the kingdom of Saudi Arabia At King Abdullaziz Medical City

ESC-ID **225**  
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*Background:* Globally, the incidence of colorectal carcinoma (CRC) varies, with the highest incidence rates in North America, Australia, and Northern and Western Europe; whereas, developing countries have lower rates, particularly Africa and Asia. CRC represents 8.5% of all tumors in Saudi Arabia as reported from studies published in the literature in the past 30 years. The aim of this study was to assess the pattern of patients with colorectal cancer over a period of 5 years, in terms of various epidemiological and clinopathological features of the disease, as well as to identify any trends during the study period.

*Methods:* We carried out a retrospective chart review of patients diagnosed with colon cancer at King Abdulaziz Medical City, Riyadh – Saudi Arabia, during the period of January 2003 and December 2007. Data extracted from the charts included age, gender, nationality, clinical presentation and duration, cancer stage and tissue type, location site in the colon, associated malignancy, type of therapy received and outcome.

*Results:* Out of 243 patients included in the study, 89% were Saudis, 57% of whom were males. The mean (sd.) age of patients was 55.8 (14.2), where the peak age of onset was in the 4th, 5th, and 6th decade (with 16% under 40 years). About 10.9% of cases were diagnosed in 2003 and 28.9% of cases were diagnosed in 2007. Anemia was present in 55% of patients. The duration of the CRC symptoms varied from less than one month to 30 months with the majority of patients having symptoms for about 6 months. Majority of the cases (82%) had primary disease originating from rectum and the left colon, whereas 28% of patients had primary disease involving ascending and transverse colon. The prevalence of CRC of stage I and II was 28%, and the majority (63%) of cases was diagnosed at stage III and IV according to the TNM classification system. As for the histological characteristics, 97% of the patients were adenocarcinoma.

**Conclusion:** In our community, we found that CRC patients present at younger ages, as well as at more advanced stages compared to other populations, which affects the survival rates and response to treatments. Also we notice an increasing trend of CRC diagnosis over the last years. We stress to introduce public health education and national screening programs regarding CRC before the age of 40 especially for men, using flexible sigmoidoscopy or full colonoscopy.

### Overview of Helicobacter Pylori Infection in Saudi Arabia

ESC-ID **221**  
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 Institution **King Saud University**

**Background:** Helicobacter pylori (HP) is the most common chronic bacterial infection in humans; it has been demonstrated worldwide and in individuals of all ages. Conservative estimates suggest that 50% of the world's population is affected by HP, which is a spiral shaped, microaerophilic, gram negative bacterium. Little is known about the epidemiological characteristics of HP in Saudi Arabia. The aim of the current study was to evaluate the risk factors, complications and associated conditions of HP and response to therapy among Saudi Arabian population.

**Methods:** We carried out a chart review of all patients who had a gastric biopsy between January and December of 2007 in King Fahad hospital – National Guard, Riyadh, Saudi Arabia. Information collected included demographic data, clinical characteristics, endoscopic results, associated conditions, and response to treatment.

**Results:** Out of 704 gastric biopsies performed during the study period, 261 (37%) were positive for HP, whereas 443 patients were negative for HP (63%). The majority of the patients were Saudis (98.8%), 48% were males, with an age range of 1 to 104 years (64.4% between 10 and 40 years old). Major presenting symptom for (HP) positive patients was abdominal pain (90%), Gastro Esophageal Reflux Disease (61.7%), and Irritable Bowel Syndrome (46%). From the results of endoscopy, we found the majority had gastritis (92.7%), duodenal ulcer (6.5%), gastric ulcer (5.7%). Regarding the treatment, 73.6% received HP eradication therapy and 32.7% had more than one course of therapy. Symptoms improved in 40% of the patients, where 30% had mild improvement. 7 patients (1.6%) of (HP) negative group had gastric tumor whereas 13 (5.5%) of patients with (HP) positive group reported with gastric malignancy.

**Conclusion:** In a large group of patients the prevalence of infection with (HP) for patients who had gastric biopsy was its peak at the middle age and become less in elderly. HP eradication has moderate impact on the abdominal pain improvement. High prevalence of Gastritis among patients with (HP). Most HP infected patients received one course of therapy only. Gastric malignancy is more in patients infected with (HP). This descriptive overview (basic characteristics, complications, treatment and its response) of these patients would be useful in planning further both observational and experimental studies.

### The outcome of longterm surveillance of families with hereditary non-polyposis colorectal cancer

ESC-ID **866**  
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**Background:** The most common inherited colorectal cancer (CRC) syndrome is the Lynch syndrome (LS) that is responsible for 2-4% of all cases of CRC. About 1-3% of all CRC's is caused by familial CRC type X or non-lynch syndrome (non-LS). The Lynch syndrome is caused by germline mutations in one of the mismatch repair (MMR) genes whereas the underlying gene defect of non-LS is still unknown. Because of evidence of an accelerated colorectal carcinogenesis in inherited CRC, an intensive colonoscopic surveillance program with intervals of 2 years have been recommended. The aims of the study were to evaluate the effectiveness of this program in LS and non-LS families.

**Patients and Methods:** The database of the Dutch Hereditary CRC Registry was used. Lynch syndrome families with a known MMR mutation were selected for the study. Criteria for non-LS families included the presence of at least three relatives with CRC in two successive generations and the absence of microsatellite instability (MSI) in at least one colon tumour. The observation time was from 1-1-1995 until 1-6-2008. Endpoints of the study were CRC, death or the last colonoscopy. Kaplan-Meier analysis was used to calculate the risk of CRC.

**Results:** A total of 745 mutation carriers from 241 Lynch syndrome families were included. The mean follow up was 7.2 years. Thirty-three patients developed CRC under surveillance (83% Stage I and II). The cumulative risk of developing CRC was 6% at 10 yrs of follow-up. There was no difference between male and female mutation carriers and between carriers of mutations in the various genes. Forty-six families met the criteria for non-LS including 344 relatives. A total of six CRC's (all Stage I and II) were detected by the program, four at first screening, two during follow-up.

**Conclusions:** With surveillance intervals of 2 years, the risk of developing CRC in LS families under surveillance is substantially lower than reported for screening intervals of 3 years. Because of the low CRC risk in non-LS families, a less intensive protocol is recommended that is colonoscopy at 3-6 years intervals.

## Can Mucinous differentiation predict biological behavior of colorectal carcinomas?

ESC-ID 721  
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 Country Turkey  
 Institution Cerrahpasa Medical School

**Background:** Colorectal Carcinomas (CRC) are one of the leading causes of cancer deaths, but their clinicopathological characteristics are not well documented in Turkish population. We retrospectively analyzed 535 resection specimens of patients diagnosed with primary CRC between the years of 2000-2007, to refine the pathological criteria for the routine analyses of colorectal cancer specimens. There are many studies on the behavior of tumors with extracellular mucin secretion above 50% in the literature. But, we had the observation that tumors with extracellular mucin secretion lower than 50 % also had a poor prognosis. Therefore, in this study, we included the tumors with mucin secretion lower than 50%, too.

**Methods:** We retrospectively analysed the files of 535 consecutive patients diagnosed with primary CRC between the years of 2000-2007 and the specimens were obtained from archives of Cerrahpasa Medical Faculty, Pathology Department and were reevaluated. Clinicopathological features such as age; gender; size, location histological grade of the tumor; presence and percentage of extracellular mucin in the tumor; lymphatic vessel invasion (LVI); blood vessel invasion (BVI); perineural invasion (PNI); the number of total lymph node number dissected (TLN) and lymph node metastases (LNM) and tumor invasion level (pT) were analyzed. Cases were documented according to the amount of extracellular mucin secretion. All percentages of extracellular mucin secretion were included in the statistical analyses. And we subdivided the cases with extracellular mucin secretion below 10%.

**Results:** Mean age of patients was  $64,2 \pm 10,5$  (23-95) and female to male ratio was 0.8. Most common tumor locations were rectum and sigmoid colon in both females (28,4%; 22,3%) and males (29,1%; 24,1%), respectively. Only 3,5% of cases were younger than 40 years old. According to invasion level, 2,7% cases were T1 (n = 14), 25% T2 (n = 131); 36% T3 (n = 189); 36,4% T4 (n = 191). Invasion level was significantly correlated with tumor necrosis (p = 0,000), LVI (p = 0,000), BVI (p = 0,042), PNI (p = 0,000), LN metastases (p = 0,005). Mean value of TLN was 16,1 (0-68) and LNM was 2,36 (0-56). 43% of all cases had lymph node metastases. Mean number of LNM was associated with higher invasion levels; 1,70 for T2, 1,89 for T3 and 2,01 for T4. Increased mucin secretion was correlated with increased level of tumor invasion (p = 0,037); 29% for T2, 44% for T3 and 38% for T4. Tumors located more distally had higher extracellular mucin secretion. Tumors with mucin secretion above 10% were present in every part of the colon whereas tumors secreting less than 10 % were not present in the ascending colon and transverse colon. Tumors with mucin secretion below 10% were commonly seen in the cecum and sigmoid colon while tumors secreting more than 10 % extracellular mucin were predominantly found in the rectum.

**Conclusions:** Our results indicate that clinicopathologi-

cal characteristics of our series are similar to the other studies. The presence and amount of mucin secretion is related with the prognosis and therefore should be included in routine colorectal cancer pathology reports.

## Spreading and quality of diagnosis liver cirrhosis in Gomel region the Republic of Belarus

ESC-ID 166  
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 Institution Gomel State Medical University

**Aim:** The aim of our work to evaluate quality of vital diagnosis of liver cirrhosis in hospitals of Gomel region, and spreading of it in population. This disease is a very popular cause of death and also often seriously complicate course of non-liver pathology.

**Methods and materials:** We examined the samples of liver tissue was getting during the autopsy in dead patients. These samples were undergone painting of hematoxylin and eosin and examining by the microscope technique. We have determined the diagnosis of cirrhosis by the following signs: disruption of the normal liver architecture by fibrous scars and nodules of regenerative liver cells.

**Results:** The total number of patients is 260. The diagnosis of liver cirrhosis has been reported in 6 patient's cases histories (2.3%). But we confirmed it in 5 cases (there was a case of steatohepatosis once). 9 patients had no liver troubles in the cases histories, but we evaluate pathological changes in their specimens of liver tissues like cirrhosis.

**Conclusion:** Finally, the total number of cirrhosis suffered patients is 14 from 260 (5.4%). It level is in the average world borders – 3-9%. But the physicians made correct diagnosis only in 5 cases (35.7%). It illustrate deject low rate of quality of clinical diagnosis liver cirrhosis in our country.

## Characteristics of phagocytosis activity of leucocytes, lysozyme and ATPase activity in patients with liver disorders

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**Aim:** Estimation of influence of liver pathology on non-specific immune system on the grounds of lysozyme activity and phagocytosis activity of leucocytes. To evaluate diagnostic significance of the resulting changes of lysozyme activity and phagocytosis activity of leucocytes, as well as ATPase activity.

**Methods and Materials:** The first test-group consisted of 20 people with cholecystitis. Second group - 20 people with obstructive jaundice. Third group - 20 people with chronic hepatitis B. The fourth group -20 people with cirrhosis. The fifth, control group, was formed of 20 healthy people. All groups were formed of people in age from 19

to 64, both genders. Blood serum was used to evaluate lysozyme activity and whole capillary blood was taken for evaluation of phagocytosis activity of leucocytes. The hemolysate 1: 4 was used for ATPase evaluation. The phagocytosis number was measured using Nesterova and Svetlychna method. The ending index of phagocytosis was measured using Matusys and Pylaeva method. The lysozyme activity was measured using Yermolyeva and Kagramanova method. The ATPase activity was measured using Kazennov, Maslova and Shalabodov method.

**Results:** The intensity of phagocytosis was  $53.1 \pm 2.1\%$  in the control group,  $41.5 \pm 1.64\%$  in the first group,  $39.2 \pm 1.55\%$  in the second group,  $43.0 \pm 1.7\%$  in the third group, and  $21.6 \pm 0.9\%$  in the fourth group. The phagocytosis number was  $9.16 \pm 0.87$  in the control group,  $6.88 \pm 0.27$  in the first group,  $5.85 \pm 0.22$  in the second group,  $8.36 \pm 0.33$  in the third group, and  $3.15 \pm 0.12$  in the fourth group. The phagocytosis completion index was  $5.7 \pm 0.21$  in the control group,  $2.85 \pm 0.11$  in the first group,  $1.76 \pm 0.06$  in the second group,  $1.58 \pm 0.06$  in the third group, and  $0.78 \pm 0.03$  in the fourth group. The lysozyme activity was  $1.9 \pm 0.07$  mkg/min in the control group,  $1.1 \pm 0.05$  mkg/min in the first group,  $0.86 \pm 0.04$  mkg/min in the second group,  $0.72 \pm 0.03$  mkg/min in the third group, and  $0.5 \pm 0.02$  mkg/min in the fourth group. The ATPase activity was  $0.057 \pm 0.002$  mkM Pi /min in the control group,  $0.059 \pm 0.002$  mkM Pi /min in the first group,  $0.064 \pm 0.003$  mkM Pi /min in the second group,  $0.041 \pm 0.001$  mkM Pi /min in the third group, and  $0.023 \pm 0.0009$  mkM Pi /min in the fourth group.

**Conclusion:** In patients with liver pathology the non-specific immune system indicators reduce (in comparison to control group), which can lead to development of bacterial infection. The lowest levels of lysozyme and phagocytosis activity were in fourth group (people with cirrhosis). The ATPase activity was insignificantly increased in the first and second groups (people with cholecystitis and with obstructive jaundice) and reduced greatly in the third and fourth groups (with chronic hepatitis B and with cirrhosis). This can speak of connection with B12 deficiency anemia, which was observed in patients with chronic hepatitis B and with cirrhosis. We think that ATPase activity evaluation can be used as one of the methods for differential diagnosis between obstructive jaundice and hepatitis B.

### Estimation of the parameters of methabolic syndroma in patients with primary billiar cirrhosis

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**Introduction:** Metabolic syndrome is associated with abdominal fat, disorders of lipid metabolism, inflammation, insulin resistance or diabetes mellitus and increased risk of cardiovascular disease. Primary billiar cirrhosis is slow-progressive, autoimmune and chronic disease with unknown etiology, the most frequent in women. Beside high prevalence of hypercholesterolemia in patients with primary billiar cirrhosis are not noticed increased risk of cardiovascular disease in comparisone with general (healthy) population.

**Aim:** Estimate of the parametars of metabolic syndrome in patients with primary billiar cirrhosis.

**Material and methods:** In this cross study we involved 20 patients with primary billiar cirrhosis. We analyzed waist circumference, serum's glucose concentration, concentration of cholesterol (total, HDL and LDL), triacylglycerols and blood pressure. These data are compared with 20 healthy respondents (control group).

**Results:** There was significant difference in serum's concentration of cholesterol ( $p < 0.05$ ) and frequency of metabolic syndroma ( $p > 0.05$ ).

**Conclusions:** Patients with primary billiar cirrhosis despite high level of hypercholesterolemia do not have increased risk to spread metabolic syndroma or cardiovascular disease in comparison to healthy population.

**Key words:** primary billiar cirrhosis, metabolic syndrome, insuline resistance, cardiovascular disease

### Desacyl ghrelin inhibits the orexigenic effect of peripherally injected ghrelin in rats

ESC-ID **124**  
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 Country **Germany**  
 Institution **Charité Universitätsmedizin Berlin**

Studies showed that the metabolic unlike the neuroendocrine effects of ghrelin could be abrogated by co-administered unacylated ghrelin. The aim was to investigate the interaction between ghrelin and desacyl ghrelin administered intraperitoneally on food intake and neuronal activity (c-Fos) in the arcuate nucleus in non-fasted rats. Ghrelin ( $13 \mu\text{g}/\text{kg}$ ) significantly increased food intake within the first 30 min post injection. Desacyl ghrelin at  $64$  and  $127 \mu\text{g}/\text{kg}$  injected simultaneously with ghrelin abolished the stimulatory effect of ghrelin on food intake. Desacyl ghrelin alone at both doses did not alter food intake. Both doses of desacyl ghrelin injected separately in the light phase had no effects on food intake when rats were fasted for 12 h. Ghrelin and desacyl ghrelin ( $64 \mu\text{g}/\text{kg}$ ) injected alone increased the number of Fos positive neurons in the arcuate nucleus compared to vehicle. The effect on neuronal activity induced by ghrelin was significantly reduced when injected simultaneously with desacyl ghrelin. Double labeling revealed that nesfatin-1 immunoreactive neurons in the arcuate nucleus are activated by simultaneous injection of ghrelin and desacyl ghrelin. These results suggest that desacyl ghrelin suppresses ghrelin-induced food intake by curbing ghrelin-induced increased neuronal activity in the arcuate nucleus and recruiting nesfatin-1 immunopositive neurons. **Keywords:** Rat, desacyl ghrelin, ghrelin, food intake, c-Fos, ARC

## Follow up study of patients with alcoholic liver disease. Dependency risk factors and outcomes

ESC-ID 793  
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*Introduction:* The relationship between alcoholic liver disease (ALD) and dependency levels have been much debated. Studies show a proportion of ALD patients with the severest form of ALD (cirrhosis) do not exhibit a history of dependence, 1 therefore constitutional and environmental factors must contribute to dependency. 2 The impact of a patient's dependency level on their prognosis is also controversial. A select number of studies have suggested that subjects with alcohol dependence survive longer than non-dependent patients despite drinking more. 3 No study so far has looked at the effects of alcohol dependency on survival when comparing the popular dependency screening tools: 'Alcohol Use Disorders Identification' (AUDIT) and The Severity of Alcohol Dependence Questionnaire (SADQ).

*Aim:* To determine the survival of ALD patients with reference to their dependency levels. To determine the dependency risk factors that may contribute to alcohol dependency

*Method:* A cohort of 397 hepatology patients, with known dependency levels, were recruited at first presentation from the Southampton General Hospital. The follow-up period from initial presentation ranged from 1 to 6.5 years. The follow-up survey occurred between October 2008 and March 2009, during which time, a structured alcohol history with a dependency risk factor section was conducted with all suitable patients who were still alive. Patients who had died before the follow-up survey project, had a thorough clinical investigation and correspondence check, to confirm their most recent dependency level, and drinking status.

*Results:* Out of the original 397 patients with liver disease, 275 patients had the initial diagnosis of ALD. Of the 275 ALD patients, 170 patients had survived by the time of the follow-up survey. Using the Kaplan Meier Analysis I found there was no significant difference in survival between ALD patients who were classed as non-dependent and dependent by AUDIT. However, I found that survival of ALD patients with mild/ no dependency was significantly shorter than the survival of ALD patients with moderate or severe dependency, when dependency was assessed by SADQ. Additionally, dependency was found to be significantly associated with dependency risk factors such as marital and employment status. In conclusion AUDIT and SADQ assessed dependency yield unexpectedly different effects on ALD patients' survival. Therefore dependency is an unreliable marker of ALD patients' survival and therefore prognosis of disease. Most strikingly, our results show that the survival of ALD patients is not differentiated by AUDIT assessed dependency, bringing into question both the effectiveness of AUDIT and the World Health Organisation diagnostic criteria for dependency underpinning AUDIT. Dependency levels of ALD patients usually have important implications for their prognosis and treatment; therefore improved methods of assessing the dependency of ALD patients are necessary.

## Can mucinous differentiation predict biological behavior of colorectal carcinomas?

ESC-ID 735  
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 Country Turkey  
 Institution Cerrahpasa Medical School

*Background:* Colorectal Carcinomas (CRC) are one of the leading causes of cancer deaths, but their clinicopathological characteristics are not well documented in Turkish population. We retrospectively analyzed 535 resection specimens of patients diagnosed with primary CRC between the years of 2000-2007, to refine the pathological criteria for the routine analyses of colorectal cancer specimens. There are many studies on the behavior of tumors with extracellular mucin secretion above 50% in the literature. But, we had the observation that tumors with extracellular mucin secretion lower than 50% also had a poor prognosis. Therefore, in this study, we included the tumors with mucin secretion lower than 50%, too.

*Methods:* We retrospectively analysed the files of 535 consecutive patients diagnosed with primary CRC between the years of 2000-2007 and the specimens were obtained from archives of Cerrahpasa Medical Faculty, Pathology Department and were reevaluated. Clinicopathological features such as age; gender; size; location histological grade of the tumor; presence and percentage of extracellular mucin in the tumor; lymphatic vessel invasion (LVI); blood vessel invasion (BVI); perineural invasion (PNI); the number of total lymph node number dissected (TLN) and lymph node metastases (LNM) and tumor invasion level (pT) were analyzed. Cases were documented according to the amount of extracellular mucin secretion. All percentages of extracellular mucin secretion were included in the statistical analyses. And we subdivided the cases with extracellular mucin secretion below 10%.

*Results:* Mean age of patients was  $64,2 \pm 10,5$  (23-95) and female to male ratio was 0.8. Most common tumor locations were rectum and sigmoid colon in both females (28,4%; 22,3%) and males (29,1%; 24,1%), respectively. Only 3,5% of cases were younger than 40 years old. According to invasion level, 2,7% cases were T1 (n = 14), 25% T2 (n = 131); 36% T3 (n = 189); 36,4% T4 (n = 191). Invasion level was significantly correlated with tumor necrosis (p = 0,000), LVI (p = 0,000), BVI (p = 0,042), PNI (p = 0,000), LN metastases (p = 0,005). Mean value of TLN was 16,1 (0-68) and LNM was 2,36 (0-56). 43% of all cases had lymph node metastases. Mean number of LNM was associated with higher invasion levels; 1,70 for T2, 1,89 for T3 and 2,01 for T4. Increased mucin secretion was correlated with increased level of tumor invasion (p = 0,037); 29% for T2, 44% for T3 and 38% for T4. Tumors located more distally had higher extracellular mucin secretion. Tumors with mucin secretion above 10% were present in every part of the colon whereas tumors secreting less than 10% were not present in the ascending colon and transverse colon. Tumors with mucin secretion below 10% were commonly seen in the cecum and sigmoid colon while tumors secreting more than 10% extracellular mucin were predominantly found in the rectum.

**Conclusions:** Our results indicate that clinicopathological characteristics of our series are similar to the other studies. The presence and amount of mucin secretion is related with the prognosis and therefore should be included in routine colorectal cancer pathology reports.

### Establishment of humanized mice as a pre-clinical inflammatory bowel diseases drug testing model

ESC-ID **636**  
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**Introduction and aim:** Inflammatory bowel diseases are a group of chronic and relapsing inflammatory disorders of the gastrointestinal tract. Crohn's disease and ulcerative colitis represent the two main types of IBD. Currently, main therapeutic options for the treatment of IBD include 5-ASA derivatives, corticosteroids and immunosuppressants. Recent development of so called biologicals allowed us to influence the inflammatory processes with unprecedented precision. Due to lack of functional models of human immune system the pre-clinical testing of this human-specific class of drugs is very difficult. Intensified search of human-compatible animal models has led to the development of different types of humanized mice. The aim of our work was to assess usability of humanized mice as a human compatible IBD model. Additionally we modified common protocols by using newborn mice as host animals and additional stimulation of graft cells with retinoic acid which should lead to improvement in engraftment quality and gut homing capabilities of human cells.

**Material and methods:** Newborn NOD-SCID mice were injected i.p. with  $10 \times 10^6$  of human PBMCs. In some experiments cells were pre-treated with retinoic acid. After 8 weeks mice were sacrificed and analyzed with regard to the engraftment of human immune cells into the spleen and the gut by means of fluorescence microscopy and flow cytometry. Additionally endoscopical colitis score was assessed.

**Results:** Newborn NOD-SCID mice are able to reliably engraft human cells. Engrafted cells can be found mainly in the spleen and the results are comparable between RA and noRA groups. Gut engraftment is much more variable and the amount of cells found in the gut correlates slightly with endoscopical colitis score.

**Conclusions:** Adult NOD-SCID mice are not perfect host animals in establishment of humanized mice. Main problems include huge numbers of cells that have to be injected, variable engraftment quality and low numbers of cells that infiltrate gut mucosa. The use of newborn mice may help to overcome some of those problems. Newborn mice seem to engraft human cells more reliably and vast amounts of them can be found in the spleen. Unfortunately gut engraftment is still not perfect and there is no difference between cells pretreated with RA and those without stimulation. Mismatch of human and murine gut-homing molecules could be possibly responsible for such results.

**Key words:** humanized mice, IBD, biologicals

### Morphological changes of the stomach in diabetes mellitus type 1 and 2 patients, peculiarities of treatment

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**Introduction:** The investigation include a complex study of the endoscopic, histological features of diabetic gastropathy (DGP), gastric motor activity, main risk factors of DGP development – metabolic (hyperlipidemia, hyperglycemia, insulin resistance, intensification of oxidative, nitrositive stress), microvascular and rheological disorders, endothelium dysfunction (disorders of nitric oxide secretion). The research purpose is to show the endoscopic, histological features of diabetic gastropathy, motor function disorders of the stomach and prove the influence of lipoflavon (kvercetin) and rebamipid (mucogen) on patients of DGP.

**Material and methods:** We examined 110 patients with DGP, developed on background of type 1 (45) and 2 (45) diabetes in the dynamics of the treatment by lipoflavon (kvercetin) and rebamipid (mucogen). A control group was made 20 patients with diabetes mellitus without symptoms of DGP. The patients of the first group (control) (20) got only hypoglycemic medicine: metformin or insulin - depending on type of diabetes mellitus. Patients of second group (basic) (90) got metformin or insulin – depending on type of diabetes, and lipoflavon (550mg of lecitin and 15mg kvercetin) of one time per day during 10 days and rebamipid (mucogen) -100mg 3 times on day during 14 days.

**Results:** 67% of the patients had atrophic gastritis; 17% of the patients had erosive gastritis. 45% of the patients had delayed gastric emptying, 24% had accelerated motor function of the stomach. As a result of application of lipoflavon and rebamipid for the patients of the second group the removal of stomach disorders, strengthening gastric emptying, decreasing of diabetic microangiopathy, decreaseing of the oxidative, nitrositive stress intensity, normalizing the activity of inducible NO-synthase (iNOS) and secretion of nitric oxide was attained. Recommended scheme of the treatment influences on morphological changes of the tunic mucous of the stomach, blood lipid spectrum, endothelium dysfunctions and provides high efficiency in complex therapy of DGP in patients with diabetes mellitus of type 1 and 2.

### Biomarkers of Reflux into the Airways

ESC-ID **854**  
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**Background and Objectives:** Reflux of gastric and duodenal contents into the oesophagus and extra-oesophageal areas has been implicated in various disorders of the airways, including lung transplant rejection. Pepsin and bile acids have been implicated as damaging agents, and consequently been proposed as biomarkers of duodeno-gastric reflux for diagnostic purposes. This study aims to

determine if bile acids and pepsin are reliable reflux markers and to assess their lower limits of reliable detection.

**Materials and Methods:** Following ethical approval, gastric juice samples from 28 patients having undergone endoscopic investigation for a variety of upper GI diseases and 5 lung transplant subjects, were analysed for bile acid & pepsin content with a 3 $\beta$ -hydroxy steroid dehydrogenase (3 $\beta$ -HSD) assay kit (Biostat diagnostics U.K.), clinically used to measure serum bile acids, based on the principal of bile acid oxidation with concomitant Thio-NAD reduction, and an N-terminal assay for proteolytic pepsin activity, respectively. Statistical analysis was carried out with a two-tailed paired t-test.

**Results:** Bile acid levels ranged from 0 – 8000  $\mu$ M with a median of 70  $\mu$ M. Only five patients had levels above 300  $\mu$ M, and six had no detectable bile acids. The lung transplant patients had levels ranging from 0 – 1000, median 30  $\mu$ M. Active pepsin was detectable in 26 of 33 samples and levels ranged from 0 - 3039  $\mu$ g/ml, median 358  $\mu$ g/ml. Samples with no pepsin activity had pH levels of 7 and above which would irreversibly denature the enzyme. The pH optimum for pepsin activity lies at pH 2; above pH 7.0, pepsin becomes irreversibly inactivated. The lowest level for accurate quantitation of pepsin was 0.2  $\mu$ g/ml. Using the incubation times of 2 minutes as described in the 3 $\beta$ -HSD assay kit, the lower limit for bile acid quantitation was 10  $\mu$ M (5  $\mu$ g/ml) (p-value 0.01, 10 Vs 0  $\mu$ M, n = 12). However, sensitivity could be improved by a longer incubation time; at 4 minutes the quantification limit reached 6  $\mu$ M (p-value 0.04, 6 Vs 0  $\mu$ M, n = 12) and at 10 minutes 2  $\mu$ M (p-value 0.01, 5 Vs 7  $\mu$ M, n = 12).

**Conclusion:** If reflux into the oesophagus was being assessed by bile acids or pepsin activity alone, about 20 % of these patients would be negative for reflux. Gastric aspirates collected from the lung will be diluted at least 100-fold by oesophageal and salivary secretions and by collection methods. Therefore only 5 of the 33 samples contain high enough bile acid levels to be detected including 1 out of 5 of the transplant subjects, indicating poor validity of bile acids as a diagnostic tool for reflux. The N-terminal pepsin activity assay, on the other hand, would be sensitive enough in most cases to detect active pepsin if present. From this data pepsin has more potential as a biomarker of extra-oesophageal-reflux than bile acids. **Keywords** bile acids, pepsin, reflux, 3 $\beta$ -hydroxysteroid dehydrogenase assay

### Modelling Barrett's Esophagus in a novel organotypic model

ESC-ID **986**  
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**Aim:** To develop an organotypic in vitro model for Barrett's esophagus and use it to test the effect of Retinoic acid on Barrett's esophagus cell lines.

**Background:** Barrett's esophagus (BE) is a squamous-to columnar metaplasia and the prime risk factor for esophageal adenocarcinoma. Current in vitro models of BE lack a submucosal compartment which limits their generalizability. Here we describe the development of a novel organotypic in vitro model for BE in which we simulate the submucosa by seeding the BE cells on top of a

layer of collagen-embedded fibroblasts. Previous reports have showed that retinoic acid (RA) can induce columnar differentiation in squamous epithelium. We examined the effect of RA by investigating the expression of a panel of cytokeratin markers in our reconstructs.

**Methods:** Five cell lines derived from esophageal squamous and BE biopsies, including one from a patient with metaplasia (CP-A) and three from patients with high grade dysplasia (CP-B, CP-C, CP-D) were seeded into a transwell on top of collagen-embedded fibroblasts. Each cell line was cultured for three weeks under two conditions: with or without 0.33 $\mu$ M RA. We used immunohistochemistry to investigate the expression of squamous (CK13 and CK14) and columnar (CK8, CK19 and CK20) cytokeratins, MUC6, CDX2, Ki67 and Alcian Blue to stain for goblet cells.

**Results:** Epithelial cells continued to proliferate (Ki67 positive) after 3 weeks. The squamous control reconstructs expressed CK13 and CK14. The CP-A control reconstruct consisted of multilayered epithelium with goblet cells and patches of squamous epithelium. It expressed a mixture of squamous (CK13, CK14) and columnar (CK8, CK19) cytokeratins. The dysplastic CP-B, CP-C and CP-D control reconstructs expressed CK8, CK19 and CK20. In the squamous and metaplastic CP-A reconstructs addition of RA constrained the growth of the cells to a single layer and caused loss of expression of cytokeratins CK13 and CK14. RA had little effect on the dysplastic CP-B, CP-C and CP-D reconstructs. We found no expression of CDX2 or MUC6 in either of the reconstructs under either condition.

**Conclusion:** We have developed a viable and representative organotypic model for BE in which we were able to show that in squamous and metaplastic BE cell lines addition of RA can induce a more columnar phenotype.

### The influence of multiprobiotic symbiter on structural and functional state of gastric mucosa in omeprazole treated rats

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**Introduction:** The side effect of long-term use of proton pump inhibitors (PPI) is hypergastrinemia which leads to gastric mucosal hypertrophy and may provoke gastric tumors. From other side the resulting hypoacidity reducing the bactericidal effect of the gastric juice may lead to the colonization of the stomach by bacteria producing carcinogenic nitroso compounds.

**Objectives:** The aim of the study was to investigate the action of multiprobiotic Symbiter on modification of gastric acid secretion and mucosal hypertrophy in rats evoked by PPI omeprazole (OM).

**Methods:** The study was carried out on 44 white rats. They were divided into three groups. The animals of the control group were injected with 0.2mL H<sub>2</sub>O (i.p.). The rats of the second group were injected with OM (14 mg kg<sup>-1</sup>, i.p.). The rats of the third group were injected with same dose of OM and multiprobiotic Symbiter (the firm 'O.D.Prolisok'). All drugs were injected during 28 days. Symbiter consists of concentrated biomass of bioplasts of symbiosis of 14 microorganisms strains and their physi-

ology active metabolites. Symbiter was injected in dose 0.14 mL kg<sup>-1</sup> (per os) during the first 2 weeks and in dose 0.014 mL kg<sup>-1</sup> (per os) during the next 2 weeks. Functional state of parietal cells and gastric mucosal hypertrophy was evaluated by gastric acid output (BAO). BAO was determined after 24 h after last injection in acute experiments under urethane anesthesia (1.1 g Kg<sup>-1</sup>, i.p.) By method of isolated stomach perfusion by Ghosh and Schild. Tissue section of the stomach was fixed in 10% formalin, embedded in paraffin, stained with hematoxylin and eosin.

**Results:** It was established that in 28 days of OM injection BAO was increased by 328.0%. we concluded that hypergastronomia evoked by OM leads to the general hyperplasia and hyperplastic mucosa has an increased capacity to produce acid. The investigations of structural state of gastric mucosa in these rats confirmed our conclusion: depth of epithelium was increased. After 28 days of Symbiter and OM treatment BAO was diminished by 47.6% in comparison to group of rats after 28 days of OM injection. Symbiter removed the negative action of OM on depth of epithelium and area of nucleus section of endocrine cells in fundus. Also Symbiter diminished increase of area of nucleus section of parietal cells evoked by OM.

**Conclusion:** Multiprobiotic 'Symbiter' prevented mucosal hypertrophy in stomach during long-term use of OM. May be it is result of modifying stomach and gut pH and increasing the net production rate of short chain fatty acids (mainly acetate, propionate and butyrate), antagonizing pathogens through production of antimicrobial and antibacterial compounds (such as bacteriocins, cytokines and butyrate), stimulating immunomodulatory cells, or competing with pathogens for available nutrients, receptors and growth factors. Conflict of interest: None declared

### Morphological study of pancreas in monosodium glutamate neonatally treated rats

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**Introduction:** Monosodium-glutamate (MSG) is an additive used in food and pharmaceutical industry. Previous studies have shown its toxic effects on neurons, neuroendocrine regulation, retina, liver, kidneys, adrenal glands etc. There are numerous studies about morphological and functional MSG induced changes of pancreas in adult obese rats neonatally treated with MSG for elicitation of obesity. Most of these studies pursue and emphasize functional changes induced by neonatal MSG treatment. The morphological changes provoked by this treatment are given much lesser attention.

**Aim:** The aim of this study was to study the effect of the neonatal treatment with MSG on pancreas morphology in rats.

**Material and methods:** We used 40 Wistar rats of both genders. The rats were divided in 4 groups with 10 animals in each. Control (C) groups (one male group and one female group) were given 0,9% water solution of sodium chloride, while monosodium glutamate treated (MSG) groups

(one male group and one female group) were given MSG in dose of 4 mg/g BW on second, fourth, sixth, eighth and tenth postnatal day. Two months later, the animals were sacrificed, in pentobarbital sodium anesthesia and the organs were taken. Tissues were processed for histological examination and stained by HE, PAS, Jones, Van Gieson and Spicer methods.

**Results:** Parenchyme volume reduction of stroma was found in MSG treated rats. Exocrine pancreas had smaller acinii, consisting of smaller acinar cells, which have lighter hypogranular cytoplasm and euchromatic nuclei (with numerous nucleoli) presentations in both males and female rats. No signs of morphological changes were found in ducts of exocrine pancreas. Endocrine pancreas (islets of Langerhans) in both genders of these rats have presentations of amyloidosis, atrophy and smaller insulocytes, which are characterised by lighter cytoplasm and euchromatic nuclei in comparison to control groups. Lymphocytes have also been detected in endocrine pancreas (islets of Langerhans). Fenestrated capillaries of endocrine pancreas (islets of Langerhans) have wall morphology changes and show signs of blood stagnation and thrombosis. Stroma of pancreas present signs of fibrosis, lipomatosis (with adipocytes of changed morphology) and lymphocyte infiltration. Blood vessels present in stroma of pancreas show signs of thrombosis, hyalinosis and lumen diameter decrease in comparison to control groups in both genders. Ducts located in stroma suffered no significant and detectable morphological changes in MSG treated groups.

**Conclusion:** Tested dose of MSG leads to morphological changes in rat pancreas. Observed changes can be classified as changes in volume, cell morphology, blood vessels and cell types of parts of pancreas. These morphological changes confirm that MSG, administered neonatally for obesity provocation in rats, has toxic effects on this organ in rats. Keywords: monosodium-glutamate, pancreas, rats, morphological changes

### Hepatic differentiation of human embryonic stem cells under 3D dynamic perfusion culture conditions: a promising cell source for bioartificial liver systems

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**Background:** In this study we investigated the possibility of using hESC as a cell source for BAL devices. The advantage of this cell type is the abundant availability, though the major problem is the final differentiation of the hESC into mature functional hepatic cells. Many approaches have been made, including the formation of embryoid bodies, use of growth factors/cytokines, co-culture with hepatic cells or conditioned medium. Our hypothesis is that not only biochemical factors play a role in the process of differentiating hESC into hepatocytes, but also physical factors, such as optimal gas and fluid flow, shear stress and pressure as well as three-dimensional configuration, cell density and resulting cell-cell interactions.

**Methods:** We compared standard 2D culture protocol published in the literature (1) with the application of the same "cytokine-cocktail" in the 3D perfusion bioreactor cul-



ture. To evaluate the effect of 3D perfusion culture without growth factors, parallel bioreactors with a standard medium composition were run in a twin-system under identical conditions as the differentiation bioreactors. Hepatic differentiation and maturation of hESC was investigated using rt-PCR for liver hepatocyte specific gene expression, ELISA for typical liver proteins such as albumin and AlAT, colorimetric assay for urea production and NH<sub>4</sub>CL clearance as indicator for detoxification capability, flow cytometry analysis for the hepatocyte marker ASGPR as well as histology and immunohistochemistry for liver specific markers such as CYP3A4, HNF4a, albumin, CK18, CK8, CK19, CK7, ASGPR and AFP.

**Results:** High-density 3D perfusion culture showed the highest levels of RNA (up to 100fold compared w/ 2D) and protein (up to 20fold compared w/ 2D) expression of liver specific markers. The mature hepatocyte marker ASGPR was found on up to 30% of the cells using flow cytometry analysis. This was confirmed by immunofluorescence staining. Further investigation by immunofluorescence staining showed the presence of cells positive and double positive for the markers investigated (CYP3A4, HNF4a, albumin, CK18, CK8, CK19, CK7, and AFP). Within clusters of cells exhibiting hepatic parenchymal markers such as albumin we found structures lined by cells that express the bile duct marker CK19. Duct-like structures lined by CK7 and CK19-positive cells could be identified. Differentiated cells in the bioreactor show detoxification capabilities, demonstrated by the metabolism of NH<sub>4</sub>CL into urea. Also, successful induction of enzymes of the CYP3A family, important for the metabolism of many endogenous and exogenous agents, including many drugs, was achieved by stimulation of the cells with rifampicin.

**Conclusion:** The results show clearly that 3D perfusion culture using specific cytokines and growth factors is superior to standard 2D static culture approach. All parameters investigated specific for hepatic cells show the highest levels in the 3D perfusion culture using the cytokine cocktail. The results indicate that there is a synergetic effect of biochemical factors and physical parameters provided by the bioreactor system. It appears that the 3D perfusion culture enables effects that go beyond the mere effect of cytokines, either through physical parameters or optimized paracrine signaling of the differentiation cells, which enables in vivo-like neo-tissue formation.

### Polymorphism of the gene of endothelial nitric oxide synthase in patients with nonalcoholic steatohepatitis

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**Object:** The object of the research was to study the state of lipid metabolism in patients with nonalcoholic steatohepatitis depending on polymorphism in the gene of endothelial nitric oxide synthase.

**Material and methods:** Thirty patients with nonalcoholic steatohepatitis (30% of women and 70% of men, the average age being 36.5 ± 5.6 years) and 20 apparently healthy persons, that representative according to age and sex have been examined. The lipid profile was determined on the

basis of the patients' blood plasma content of total cholesterol, triacylglycerols, cholesterol of lipoproteins of low, very low and high densities. The alleles of polymorphic sites in the gene of endothelial nitric oxide synthase were studied by means of isolating genomic desoxyribonucleic acid from the leukocytes of the peripheral blood of subjects with further amplification of the polymorphic site by means of the polymerase chain reaction on the "Amply" amplifier (Moscow). The fragments of amplified desoxyribonucleic acid was separated by means of gel electrophoresis and stained with bromide ethidium. The fragments were visualized with the aid of an ultraviolet radiator.

**Results:** Among 30 investigated patients with nonalcoholic steatohepatitis Tyrosine-allele gene of endothelial nitric oxide synthase was determined in 80% of the patients, 83.3% of them being in the homozygous condition. Among the apparently healthy persons Tyrosine-allele gene of endothelial nitric oxide synthase was detected only in 25% of the subjects. The presence of Tyrosine-allele gene of endothelial nitric oxide synthase in patients with nonalcoholic steatohepatitis is accompanied by an increase of the plasma level of triacylglycerols by 38.2% (p = 0.006) (in carriers of the homozygous genotype – by 43.7% (p = 0.001)), by a low content of the cholesterol of high density lipoproteins (p = 0.008). Homozygous carriage of Tyrosine-allele of the gene endothelial nitric oxide synthase in female persons were accompanied by the highest plasma level of triacylglycerols (p = 0.001).

**Conclusions:** The carriage of Tyrosine-allele of the gene of endothelial nitric oxide synthase is an accessory independent predictor of impaired lipid metabolism in patients with nonalcoholic steatohepatitis, especially in persons of female gender with the homozygous Tyrosine-allele carriage.

### Immunohistochemical Investigation of Aurora Kinase B, a Novel Promising Target in Gastroenteropancreatic Neuroendocrine Tumor Disease

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**Aim:** Gastroenteropancreatic neuroendocrine tumors (GEP-NET's) are rare and heterogeneous in their tumor biology. The growth pattern ranges from well-differentiated to fast growing, undifferentiated aggressive types of carcinomas. The therapeutic options to prevent the growth and further dissemination of GEP-NETs are limited and still not satisfactory. Aurora kinase B (AKB) plays a role in cell cycle progression. Aberrant expression of these kinases occurs in different solid tumors. Little is known about the functional role of aurora kinases in GEP-NET's. The aim of this study was therefore to assess AKB expression in well-differentiated and undifferentiated human GEP- NET's.

**Material and Methods:** Tumor specimen from 81 patients treated at the Charité-Universitaetsmedizin Berlin, Campus Benjamin Franklin, at the Philipps University of Marburg and at the University Hospital of Freiburg (26 foregut, 43 midgut, 12 hindgut) were studied immunohistochemically with AIM-1 antibody for AKB expression (>5% = positive). The neuroendocrine feature of the tumors was proven by the positive immunohistochemical staining for chromogranin A and synaptophysin.

**Results:** The immunohistochemical analysis of AKB revealed an overexpression in a significant portion of tumors. In our patient population AKB is overexpressed in 8/72 (10%) well-differentiated tumors and in 8/9 (88%) undifferentiated highly malignant carcinomas.

**Conclusion:** To our knowledge this is the first study that shows a differentiated expression of AKB in GEP-NET disease. Moreover, this expression seems to correlate with the progression and the aggressiveness of the tumors. This fact makes AKB a valid target especially in undifferentiated GEP-NETs, since it is overexpressed in most of those tumors. In vitro, we already could demonstrate antiproliferative and proapoptotic effects of ZM447439 (Tocris™) a novel aurora kinase inhibitor, in GEP-NET disease 1. We therefore propose to incorporate the immunohistochemical expression of AKB into the routine diagnostics in order to individualize therapeutic strategies in this tumor entity in the future.

## Gynaecology

### The impact of increased body mass index (BMI) on pregnancy outcome

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**Introduction:** The rising prevalence of obesity among women of childbearing age is a major public health concern with 56% of all women being over the recommended BMI in the UK. The increase in obesity is associated with increased risks of adverse pregnancy outcomes, which have been documented by many studies. However few studies have quantified the degree of risk with the level of obesity.

**Aims:** This study investigates the maternal and neonatal complications associated with different levels of increased maternal BMI (Kg/m<sup>2</sup>) at booking.

**Methods and materials:** The study was a retrospective case note analysis carried out at St. George's Teaching Hospital, London. A total of 320 singleton pregnancies delivering between January 2007 and June 2008 were studied. 160 women were overweight (BMI >25.0 Kg/m<sup>2</sup>) and categorised into four BMI groups according to the World Health Organization (2004) classification. A control group of 160 women with normal BMI (BMI 20.0 - 24.9 Kg/m<sup>2</sup>), matched for age and parity with the overweight group were randomly selected over the same time period. The pregnancy outcomes of all overweight women were compared firstly with the control group and secondly across different levels of BMI to look for trends in the outcome data. The data are presented as raw frequencies and odds ratios with 95% confidence intervals calculated using binary logistic regression analysis. Chi-square test

was carried out to examine trends in outcome.

**Results:** The odds ratios (95% CI) in the first analysis showed a significant increase in the risks of total caesarian deliveries 2.97 (1.73 - 5.11); postpartum haemorrhage 6.30 (2.34 -16.80); fetal distress 2.93 (1.73 - 4.98), large for gestational age neonates 2.80 (1.51 - 5.20) and neonatal assisted ventilation 4.94 (1.82 -13.40) in all women with a BMI >25.0 Kg/m<sup>2</sup>. Analysis across sub-groups of overweight BMI demonstrated a statistically significant overall upward trend in the following outcomes: Induced labour (X<sup>2</sup> test for trend = 4.73, p = 0.03); total caesarian deliveries (χ<sup>2</sup> test for trend = 23.38, p <0.001); foetal distress (χ<sup>2</sup> test for trend = 15.57, p <0.001); large for gestational age neonates (χ<sup>2</sup> test for trend = 18.62, p <0.001) and admission to neonatal unit (χ<sup>2</sup> test for trend = 15.97, p <0.001).

**Conclusion:** Maternal obesity places a pregnancy at an increased risk of adverse outcomes. The level of obesity significantly influences the magnitude of risk.

### Progesterone and Estrogen Receptors during Implantation Window of infertile women and the relation to endometrial receptivity

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**Objective:** To investigate the expression of progesterone and estrogen receptors during the implantation window of infertile women who are suffering from tubo-peritoneal factor infertility and the relation to endometrial receptivity.

**Materials and Methods:** Endometrial biopsy was performed in 8 patients with tubo-peritoneal infertility during the implantation window period (mid-luteal phase). Three slides were prepared from the biopsy material of each patient, one stained with standard Hematoxylin and Eosin while another two with Immunohistochemistry on paraffin section with primary antibodies against Progesterone Receptors (PGR) and Estrogen Receptors (ER) [DAKA]. Samples were observed under standard light microscope. Morphology, amount of pinopodes, expression of PGR and ER according to their localizations are investigated. The results are compared with the outcome of in-vitro fertilization (IVF). Result(s): Implantation was successfully carried out in 5 patients with highly expression of matured pinopodes and progesterone receptors in the parenchyma (lumina surface epithelial and glandular epithelial). The 3 patients with negative IVF results have either regression form of pinopodes or low expressivity of progesterone receptors in the parenchyma.

**Conclusion:** The result suggests that the endometrial is related to the amount of pinopodes and hormonal action of progesterone but not tubo-peritoneal factors.

## Measured Cortisol Levels in Patients Diagnosed with PCOS

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The aim of the study was to assess the cortisol plasma levels in PCOS women Patients and methods: Cortisol measurements of 477 hospitalized patients from the Clinic of Gynecological Endocrinology, Krakow, Poland. The first group of 117 patients (A) had blood samples drawn during OGTT( Oral Glucose Tolerance Test) and were known to be diagnosed with PCOS (Polycystic Ovarian Syndrome). The second control group of 360 patients (B) did not have a history of PCOS. Blood samples were taken between the 5th and 8th day of the menstrual cycle at 7:00 and again at 17:00. The cortisol levels were then analyzed via ELISA using Elecsys by Roche®. Along with cortisol levels; LH (Leutenizing Hormone), FSH (Follicle Stimulating Hormone), estradiol, PRL (Prolatin), testosterone and DHEAS (Dehydroepiandrosterone ester) were also measured. A retrospective analysis was then performed on the data collected.

*Results:* Analysis revealed an elevated cortisol level ( $p < 0.05$ ) at 17:00 in the women that were confirmed to have PCOS (A) ( $11.8 \pm 4.1 \mu\text{g/dl}$ ) as compared to the control group (B) ( $7.4 \pm 5.0 \mu\text{g/dl}$ ). There was no statistical significance in the cortisol concentration at 7:00 in both the women with PCOS (A) ( $14.6 \pm 9.8 \mu\text{g/dl}$ ) and those of the control (B) ( $14.2 \pm 10.0 \mu\text{g/dl}$ ). In both groups cortical adrenal hyperactivity was ruled out.

*Outcome:* The results may suggest an involvement of the adrenal cortex in the development of PCOS.

## How does maternal diet during the pre-implantation period effect AMP-activated protein kinase localisation in the mouse embryo?

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*Background:* Numerous studies exploring the Developmental Origins of Health and Disease (DOHaD) hypothesis have shown that minor changes in the developmental environment can induce changes in developmental programming resulting in an altered phenotype in the offspring. Experimentally the maternal nutritional environment during the preimplantation period can be manipulated to demonstrate what happens to the intracellular localisation of 5'-AMP activated protein kinase (AMPK). AMPK is an energy sensing enzyme that is activated when the AMP/ATP ratio increases. Once activated, AMPK inhibits anabolic pathways and enhances catabolic pathways to increase cellular energy levels. Upon exposure to cellular stressors AMPK is known to shift its intracellular localisation to the nucleus. The ability of AMPK to respond to the intracellular energy status has made it a

potential therapeutic target for type II diabetes and other metabolic syndromes.

*Methods:* The maternal diet in mouse models was manipulated by varying the protein content of the diets during the preimplantation period. The effects of these dietary changes on intracellular AMPK localisation in the embryo were examined using immunofluorescence and confocal microscopy.

*Results:* This study shows that manipulating the maternal diet effects the intracellular localisation of AMPK, especially amongst th trophectoderm cells. A maternal high protein diet also results in reduced blastocyst cell numbers.

*Conclusion:* These findings highlight AMPK as a highly dynamic kinase that is able to change its intracellular localisation, suggesting a change in the cellular energy status of embryos. This could provide a potential therapeutic target for diseases characterised by altered energy metabolism, such as diabetes.

## Synchronous primary carcinomas of the endometrium and ovary

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*Aim:* Purpose of the study was to analyze the clinicopathologic features and survival outcome of patients with synchronous primary carcinomas of the endometrium and ovary.

*Material and method:* Between 1996. and 2005., 45 patients fulfilled the criteria of synchronous carcinomas of the endometrium and ovary and were included in the study. The medical records and the pathologic reports were retrieved. Kaplan-Meier survival analyses were performed and compared using the log rank test.

*Results:* The incidence of synchronous primary endometrial and ovarian carcinomas was 3,3% in patients with endometrial carcinoma and 7,2% in patients with ovarian carcinoma. Median age of patients with synchronous primary endometrial and ovarian carcinomas at diagnosis was 52 years (range: 37-70 years). The majority of patients ( 64,4% ) were premenopausal and ( 53.3% ) nulliparous. Twenty-nine patients ( 64,4% ) had similar (endometrioid/endometrioid) carcinomas in both the endometrium and ovary. There was no significant difference in survival outcome inpatients who had similar histopathology and those who had dissimilar histopathology (  $p > 0,05$  ).

*Conclusion:* The correct classification of synchronous primary carcinomas of the endometrium and ovary is often problematic because of the frequent confusion with their metastatic counterparts. The majority of patients with synchronous primary carcinomas of the endometrium and ovary belonged to concordant endometrioid histopathology in the endometrium and ovary.

## The results of Edinburgh Postnatal Depression Scale and influence of various factors on postnatal depression

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*Aim:* A delivery of a baby is a very important event in woman's life, so it can possibly produce a great impact on her psyche. The aim of this study was to assess if there are any factors that increase or decrease the probability of postnatal depression syndrome in postpartum women and to evaluate patients' psychological state.

*Methods and materials:* A group of 100 women admitted to the Department of Obstetrics of Medical University in Gdansk and the Department of Obstetrics of Pomeranian Center of Traumatology Hospital of Mikolaj Kopernik in Gdansk, Gdansk 2008-2009, were included in the study. The Edinburgh Postnatal Depression Scale (EPDS) was used as a mean method to assess the risk of appearance of depression. Questions including age, finished level of education, residence on basis of number of habitants, professional activity or its lack, number of deliveries in anamnesis, time after delivery in hours, subdivided into days; a way of delivery, presence of the father during delivery, a week of gestation in which delivery took place, time spent with a child after delivery were to investigate general situation of a patient. Subsequently, a survey on well-being followed. In this part, a patient had to assess how often she feels or thinks about a given affirmation in a scale from 0 (never) to five (always). The phrases were divided into following categories: well-being, positives thoughts about maternity, anxiety over taking care of a newborn child, willingness to take care of a newborn child, willingness to restart professional life, willingness to come back home, a level of mood, changes of mood, expected help from family, anxiety over next possible birth.

*Results:* In the study, 20% of patients acquired more than 10 points in EPDS, which indicates possible risk of postnatal depression. The influence of the different factors enlisted above were investigated in nonparametric tests U Mann-Whitney or Kruskal-Wallis. All of them were proved not to be statistically relevant, despite visible differences in means or percentages of possibly depressed patients between various groups. When compare women without great risk of depression, to possibly depressed patients (EPDS>10), those latter showed lower quality wellbeing (2,475 vs 3,275,  $p = 0,0019$ ), less positive thoughts about maternity (4,40 vs 4,025  $p = 0,047$ ), minor anxiety over care of child (3,55 vs 2,51  $p = 0,02$ ), lesser willingness to take care of a newborn child (2,51 vs 3,55  $p = 0,02$ ) and lower level of mood (3,425 vs 4,325  $p = 0,0001$ ).

*Conclusion:* Women's mental state may worsen after a delivery. Postpartum patients should be taken care of, not only from medical side but from psychological point of view as well.

## HPV infection of the female genital tract and its association with cervical cancer in Canton Tuzla, Bosnia and Herzegovina

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*Introduction:* Cervical cancer (CC) is caused by different types of human papillomavirus (HPV) and it represents the second most common cancer among Bosnian women. The primary risk factors for acquiring genital HPV are generally associated with sexual activity. HPV is recognized as one of the most common – if not the most common sexually transmitted infection. In this moment there are more than 40 genotypes of HPV that can infect the genital area of women.

*Aim:* Aim of our research is to determine the frequency of HPV infections among females and its association with cervical cancer, to determine the cytomorphological changes found in HPV positive women and as well to determine if there is a statistical significance of increased number of High Grade Squamous Intraepithelial Lesion (HGSIL) and CC in different age groups.

*Material and Methods:* This study has been conducted by a retrospective analysis, using all available information from Department of Pathology, UCC Tuzla. Samples of the cervical smear for the PAP test and HPV analysis were taken during routine gynecological examinations, by using brush and sticks with cotton, taken from the Digene Specimen Collection Kit, from the whole surface of a portion, and by mild rotating moves from the outer cervical entrance. Identification of the presence of HPV was carried out by the Digene Hybride Capture II test in all the cases. The cytomorphological changes found using light-microscope method were classified according to the Bethesda classification of cervical lesion.

*Results:* SPSS 16.0 (Statistical Package for Social Science) computer program is used for processing collected data, and further statistic methods are used: descriptive statistics and Chi<sup>2</sup> test ( $\chi^2$ ). In this study we included 2674 women from all age groups. These women were tested in the period of 2005-2007 in Tuzla Canton and the total number of women who tested positive was 992 (37%). According to critical value of  $\chi^2 = 3.84$   $p > 0.05$  there is a significant trend in reduction of positive HPV tests in last three years. There is statistical significance in increased number of cervical cancer comparing women born 1961-1979 with other age groups  $\chi^2 = 4.63$  and  $p > 0.05$ . The most common cytomorphological change was Benign Cellular Change (BCC) with 346 cases or (35%), followed by Low Grade Intraepithelial Lesion (LGSIL) with 199 cases or (20%) and CC with 187 cases (18%).

*Discussion:* As HPV infections are very common among Bosnian women it is important to inform and educate the public about its association with cervical cancer. We emphasize to improve the current prevention strategies and screening practices. HPV testing and vaccine could reduce incidence and mortality from CC in our region.

*Key words:* HPV infections, High Grade Squamous Intraepithelial lesion, Cervical Cancer

## Haematology/Oncology

### MDR-1 expression predicts molecular response in CML patients on nilotinib therapy after imatinib failure

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*Introduction:* Most CML patients generally remain in stable response under frontline treatment of imatinib mesylate. In case of disease progression, resistance or intolerance to imatinib, therapy is switched to novel second generation tyrosine kinase inhibitors, such as nilotinib or dasatinib. Apart from mutations in the kinase domain of BCR-ABL and heterogenous BCR-ABL-independent mechanisms, e.g. clonal evolution and pathways bypassing BCR-ABL, the efflux transporter protein multi-drug resistance (MDR-1) is postulated being involved in the biology of resistance to nilotinib (Mahon FX et al., Cancer Research 2008)

*Aim:* We therefore evaluated the predictive impact of MDR-1 expression levels from imatinib resistant CML patients on molecular responses during second line therapy with nilotinib.

*Methods:* A cohort of 192 imatinib resistant CML patients (98 females, 94 males; median age 67, range 25-89 years, 99 in chronic phase and 93 in advanced disease) were investigated. Median follow-up for achievement of MMR (BCR-ABL/ABL ratio equal to 0.1% or higher) was 164 days, for good response (BCR-ABL/ABL ratio equal to 1% or higher) 143 days. Baseline BCR-ABL mutations were detected by D-HPLC and direct sequencing. MDR-1 expression levels were measured by quantitative reverse transcription PCR (qRT-PCR) on LightCycler instrument. Resistant CML patients of both groups were compared and probability of achieving MMR or good response was analyzed through Log-rank (Mantel-Cox) Test and Kaplan-Meier analysis.

*Results:* A MDR-1/GUS ratio higher than 2.5 at baseline was significantly ( $p = 0.027$ ) associated with a higher probability of achieving MMR under treatment of nilotinib in chronic phase CML. From chronic phase patients, 24 achieved a MMR during nilotinib, whereas 75 did not. There was neither a significant difference in the probability of reaching MMR during advanced disease nor in achievement of good response to nilotinib in all phases due to MDR-1 expression at baseline. No difference was shown between patients harbouring BCR-ABL mutations 54% (P-Loop and A-Loop) and patients without mutations 46% ( $n = 192$ ) at baseline.

*Conclusion:* MDR-1/GUS ratio seems to be a predictive marker for MMR achievement within the first two years of treatment under nilotinib in chronic phase of CML.

### Electrolyte imbalances in patients admitted with chemotherapy induced febrile neutropenia: Patterns and impact on outcomes, a single centre study from Pakistan

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*Background:* Febrile neutropenia (FN) and myelosuppression remain challenging oncologic medical emergencies and dose limiting toxicities of chemotherapy. Various factors have been studied and found to be associated and affect outcome of patients admitted with FN. Electrolyte abnormalities have commonly been observed, but the real incidence and impact has been only scarcely studied. At the molecular level too, electrolyte imbalances can occur in these patients due to fluid shift mechanism from intracellular to extracellular compartments as a result of catabolic response sepsis[1].

*Objective:* This aim of this study was to observe trends of electrolyte abnormalities seen synchronously in patients admitted with chemotherapy induced febrile neutropenia and their possible influence on outcomes.

*Methods:* This was a retrospective, observational study of 215 patients admitted with FN between January 2007 and August 2008. Data was collected using Aga Khan University Hospital patients' record collection and database on files and computers. Data was entered and analyzed using SPSS 16.0. Toxicity profile was graded according to CTC version 3.0.

*Results:* Febrile neutropenia was seen to be almost equally distributed among patients with solid or hematological malignancies. Both genders were seen to be affected almost equally as well. A total of 83.5% patients demonstrated some degree of electrolyte abnormality. Hypokalemia, of any grade, was seen in 48% of patients, 51.4% having grade I, 33.3% grade III and 15.2% had grade IV hypokalemia. Hyponatremia of all grades was seen in 67.9% patients, 60.3% had Grade I, 33.3% grade III and 0.7% patients had grade IV hyponatremia. Hypomagnesaemia was seen in 54.3% patient, 94.7% having grade I decline. About 12% of patients with electrolyte deficit also had a co-existing vomiting or diarrhea or both. Overall 65% of our patients received intravenous electrolyte replacement therapy and disturbances in potassium levels were more critical as compared to disturbances of sodium and magnesium requiring significantly greater replacement of the former as compared to the latter ( $P = 0.022$ ). Average length of stay was 5.1 days in patients having coexisting electrolyte abnormalities, compared to 4.7 days in those who had not but the difference was not noted to be statistically significant ( $P = 0.69$ ). However, in those patients who were successfully discharged, average length of stay was 5.4 days whereas it was 11.4 days in patients who died. The two-tailed P value for this was found to be less than 0.0001. Similarly average length of stay in patients who received electrolyte replacement was 6.3 days compared to those who did not receive replacement in whom the average length of stay was 4.9 days. The difference was noted to be statistically significant ( $P = 0.016$ ).

*Conclusion:* The analysis, perhaps first of its kind, suggests that electrolyte abnormalities are frequently observed in patients presenting with FN, independent of

co-existing vomiting or diarrhea. They can have a negative affect on the outcome of such patients. Therefore the incidence and impact of electrolytes abnormalities on FN outcomes should be elucidated and the molecular mechanisms underlying this response be further researched and explained.

### Genome-Wide Screening of ERG Specific Target Genes in T-Lymphoblastic Leukemia by Chromatin Immunoprecipitation on Chip Analysis

ESC-ID **909**  
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The Ets related gene, ERG, encodes a transcription factor with a crucial role in hematopoiesis. Furthermore, ERG rearrangements correlate with subgroups of myeloid leukemia, Ewing sarcoma and prostate carcinoma. Also, high ERG expression predicts adverse outcome in patients with acute T-lymphoblastic and acute myeloid leukemia. Recently, ERG knock-out in mice caused failure in embryonic hematopoiesis and adult stem cell maintenance. The significance of ERG contributing to this disadvantageous phenotype in mice as well as in patients with acute T-lymphoblastic and acute myeloid leukemia still needs to be determined because downstream targets of ERG in leukemia remain unknown. Therefore, we performed a genome-wide analysis of ERG target genes in T-lymphoblastic leukemia. Chromatin Immunoprecipitation on chip (ChIP-on-chip) experiments were performed using two independent ERG specific antibodies for the enrichment of ERG-bound DNA templates in T-lymphoblastic leukemia cells (Jurkat) with input or IgG as controls. Enriched DNA templates and control DNA were differentially labelled and co-hybridized to high resolution promoter chip arrays with 50-75mer probes (770,000) representing 29,000 annotated human transcripts (NimbleGen). Based on two independent ChIP-on-chip assays, bioinformatic analysis (ACME) yielded statistically significant enriched peaks (using a sliding window of 1000 bp, and a p-value < 0.0001) identifying promoter regions of 365 potential ERG target genes. Bioinformatic analysis (DAVID server) categorized the 365 potential ERG target genes and genes belonging to leukemia and oncogenic transformation were selected for further validation by real-time PCR. The design of promoter primers included the highly conserved ETS GGAA DNA binding site. Genes with at least two-fold enrichment (ERG ChIP versus control) included WNT2 (17-fold), OLIG2 (14-fold), WNT11 (7-fold), UBAC2 (8-fold), CCND1 (5-fold), WNT9A (4-fold), DUSP4 (4-fold), CD7 (3-fold), EPO (3-fold), ERBB4 (3-fold), RPB1L (3-fold), TRADD (3-fold), PIWIL1 (2-fold), TNFRSF25 (2-fold), TWIST1 (2-fold), HDAC4 (2-fold). Genes correlated with the WNT-pathways (WNT2, WNT11, FZD7) and genes that have shown oncogenic potential (e.g. STAT5A) were

selected for additional validation by ERG knock-down and over-expression studies in different haematopoietic cell lines. siRNA knock-down of ERG in the myeloid cell line KG1, has shown a significant decrease in the expression of WNT11 suggesting that ERG acts upstream of this target gene. Further validation of putative target genes and disclosure of the effect ERG has on its downstream pathways may contribute to the development of potential drugs in the treatment of ERG deregulated leukemia.

### The role of platelets in chemotaxis of dendritic cells

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*Introduction:* The function of dendritic cells (DCs) is to present antigens to T-cells and to stimulate immune response.[1] Taking up antigens by immature DC (iDCs) takes place in peripheral tissues while T-cells activation by mature DC (mDCs) occurs in lymph nodes. Therefore, chemotaxis of mDCs from peripheral tissues to lymph nodes is required. The most important chemoattractant for mDCs is chemokine CCL19, acting through CCR7 receptor.[2] Other factors involved in DC maturation are CD40L and prostaglandin E2, which are abundantly produced by platelets [3].

*Aim:* The aims of this study were to determine whether platelets can replace CD40L in DCs maturation, so that they undergo chemotaxis toward CCL19 and whether this process can be inhibited by protein kinase inhibitors.

*Materials and Methods:* Peripheral blood was drawn from healthy donors in blood donation centre. Monocytes were isolated by immunomagnetic separation, while platelets by centrifugation. Monocytes were cultured with IL-4 and GM-CSF to become iDCs. Then they were matured to become mDCs in the presence of various combinations of CD40L, PGE2 and platelets. Protein kinase inhibitors (PP1, src-kinase inhibitor and SB203580, p38 mitogen-activated kinase inhibitor) were added 30 minutes before chemotaxis toward CCL19 conducted in Transwell® inserts. The percentage of cells undergoing chemotaxis was estimated microscopically.

*Results:* iDCs maturing with CD40L or platelets alone did not undergo chemotaxis, while with PGE2 alone 40 ± 7% of them did. Maturation with PGE2 and CD40L or PGE2 and platelets resulted in DCs chemotaxis of 51 ± 5% and 47 ± 7%, respectively. Addition of protein kinase inhibitors to colony maturing with PGE2 and platelets reduced percentage of DCs undergoing chemotaxis to 15 ± 5% for PP1 and 31 ± 5% for SB203580. These numbers are consistent with those obtained for DCs maturing with PGE2 and CD40L (16 ± 7% and 31 ± 6%, respectively).

*Conclusion:* Platelets can replace CD40L, but not PGE2, in the process of DCs maturation to chemotaxis. DCs maturing with platelets show similar sensibility to protein kinase inhibitors as do DCs maturing with CD40L.

## Virgin coconut oil inhibits SKBr-3 breast cancer cell proliferation and synergistically enhances the growth inhibitory effects of Trastuzumab (Herceptin™)

ESC-ID **117**  
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**Background:** Worldwide, breast cancer is the most common cause of cancer in women. The her-2/neu gene represents one of the most important oncogene in breast cancer as it is expressed in preneoplastic lesions, connected with metastasis, and associated with resistance to chemo- and endocrine therapies. The current treatment for this kind of breast cancer, Trastuzumab (Herceptin™), a humanized monoclonal antibody, is criticized for being too expensive and having only modest effects. Studies have shown that dietary fatty acids, like oleic acid, could downregulate the expression of the her-2/neu oncogene. Virgin coconut oil (VCO), which contains oleic acid and other fatty acids, was investigated for its potential to be an alternative or adjuvant therapy.

**Methods:** Using the MTT assay we characterized the effects of supplementation of VCO on the proliferation of her-2/neu overexpressing breast cancer cells. VCO was dissolved using 90% ethanol and bovine serum albumin. We also investigated the effects of VCO on the efficacy of Trastuzumab.

**Results:** MTT assay analysis revealed that increasing concentrations of VCO inhibit growth of SKBr-3 cells. It was observed that the optimal Trastuzumab concentration was 5 µg/ml and partial inhibition was observed with increased concentration. Varying volume ratios of VCO (2%v/v, 4% v/v, 6% v/v, 8%v/v and 10%v/v) were able to improve the ability of Trastuzumab to decrease the cell survival. No dose dependence was observed.

**Conclusion:** These findings demonstrate that VCO is able to inhibit the growth of her-2/neu overexpressing cell lines and has additive effects on Trastuzumab. This unrecognized property of VCO may open avenues for further studies on breast cancer treatment and prevention.

## Mechanisms of cell death by microtubule targeted anticancer drugs in Burkitt lymphoma

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Burkitt lymphoma is a fast-growing Non-Hodgkin's Lymphoma, that originates from mature B cells. It is generally associated with chromosomal translocations or Epstein-Barr virus infection leading to deregulated activation of the c-myc oncogene. Combination chemothera-

py with a vincristine containing regimen is the treatment of choice in Burkitt lymphoma, however, only 50% of the patients respond to treatment due to the high genetic instability, selection of resistant tumor cell subclones and development of clinical resistance to therapy. Apoptosis resistance is considered as the major cause of resistance in Burkitt lymphomas. To elucidate molecular abnormalities that are important for resistance, 19 different Burkitt lymphomas were investigated for apoptosis induction upon treatment with microtubule inhibitors taxol, nocodazole, and vincristine. Interestingly, cell lines which are highly resistant to apoptosis induction showed development of polyploidy (>4N cellular DNA content) and vice versa, displaying an inverse relationship between apoptosis and polyploidy induction upon treatment with taxol or nocodazole. Of note, the mitotic spindle toxin vincristine did not promote polyploidy. The underlying mechanism was characterized in three resistant and four sensitive prototypic cell lines. In sensitive cell lines, taxol- and nocodazole-induced apoptosis is accompanied by caspase activation, Bid cleavage and Mcl-1 downregulation. In contrast, most apoptotic resistant cell lines exhibited a loss of Bax and Bak and showed prolonged mitotic arrest with >4N DNA content upon treatment. Interestingly, inhibition of apoptosis in sensitive cells by caspase inhibition promoted polyploidy and subsequent death by a mitotic catastrophe confirming the inverse relationship between apoptosis and polyploidisation. Unlike taxol or nocodazole, vincristine treatment failed to induce polyploidy. Since polyploidy development of tumor cells occurs in consequence of chromosomal imbalances following disruption of the mitotic spindle machinery this might be a critical event leading to chromosomal imbalances in tumor cells. The failure of vincristine to induce such an undesirable event may explain why vincristine is a better treatment option as compared to taxanes in lymphoma therapy. Furthermore, drugs inducing caspase activity independently of Bax and Bak deficiency might represent an option to treat resistant Burkitt lymphoma.

## Increased phosphorylation of ribosomal S6 protein in mitotic human lymphoma cells

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Cell growth and division require precisely coordinated and regulated function of various proteins. Growth factors and mitogens stimulating protein synthesis can activate p70S6 kinase (S6K); this kinase in turn phosphorylates ribosomal S6 protein (rpS6). The localization and the upstream regulators of phosphorylated ribosomal S6 protein suggest that it can participate in the regulation of protein synthesis, cell growth (increasing cell size), cell proliferation and glucose homeostasis as well. However, despite an increasing amount of information on rpS6 regulating upstream signaling pathways, the exact role of its phosphorylation remains obscure. Previously we observed a surprisingly strong positivity in mitotic lymphoid cells in reactive human lymph nodes by phospho-S6 ribosomal protein (Ser235/236, p-rpS6) immunohis-

tochemistry. Our goal was to investigate the expression of this protein in cycling non-neoplastic lymphoid as well as lymphoma cells and to determine whether the observed high expression depends on mTOR activity and whether it is to observe in other cell- and tumortypes. The expression of p-rpS6 and pHH3 (a known mitotic marker) were studied by double immunocytochemistry labelling, fluorescent microscopy, flow cytometry and Western blot in different cell types (CEM, KM-H2, Jurkat, HT58, HT1080). Lymphoma cells were treated with nocodazole, rapamycin and staurosporine. Upstream regulators of rpS6 were analysed by using p-mTOR and p-S6K immunocytochemistry. p-rpS6 and pHH3 immunohistochemical stainings were carried out on various biopsy specimens of malignant and normal tissues (tissue microarray of other cancers and sarcoma biopsies). High extrachromosomal p-rpS6 expression was found on cytospin slides from different mitotic lymphoma cells by immunocytochemistry. Significant correlation between the number of p-rpS6 and pHH3 positive cells was observed in all samples after nocodazole treatment. Our Western blot and flow cytometry analyses also confirmed these observations. By performing double fluorescent immunocytochemistry we proved directly the co-expression of high p-rpS6 and pHH3 in dividing lymphoma cells. Enhanced p-mTOR and p-S6K immunocytochemical labelling were also found in mitotic lymphoma cells. However, high expression of p-rpS6 in mitotic cells was not a general hallmark of all tumor cell types as p-rpS6 negative mitotic cells were detected in epithelial tumors and sarcoma biopsies. Characteristic high expression of phosphorylated ribosomal S6 protein was proved in mitotic lymphoid and lymphoma cells as well. It was also confirmed that high expression of p-rpS6 in mitotic cells is cell type dependent. We also found that p-rpS6 expression in lymphoma cells might be dependent on mTOR activity, as rapamycin inhibited the expression of p-rpS6. Our findings suggest that the ribosomal S6 protein could be a promising target in therapeutical approaches of hematologic malignancies in the future. Further, challenging studies are needed to elucidate the exact role of p-rpS6 in mitotic lymphoid/lymphoma cells.

### **Thrombospondin – a tumor stroma constituent of diffuse gastric cancer potentially important for progression and invasion**

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Gastric cancer represents the fourth most common malignancy in the world. Despite declining incidence rates, the survival from this disease remains poor, making it the second most common cause of cancer-related death worldwide. By histology, two major subgroups can be distinguished – diffuse (undifferentiated/poorly differentiated) and intestinal (well/moderately differentiated) adenocarcinomas. Diffuse carcinomas are explicitly more invasive than intestinal ones, and affected patients possess extremely poor prognosis. In a whole human genome-microarray screen, I identified a signature of more than 1,000 transcripts to be significantly differentially expressed between the two types. Genes, upregulated in

intestinal type carcinomas are predominantly associated with cell proliferation and enhancement of cell growth (cell cycle, DNA replication, spindle assembly). In contrast, genes with high expression in diffuse carcinomas principally include genes for extracellular matrix proteins and proteins important for adhesion, morphogenic and developmental processes. Among these genes, thrombospondin (THBS) possesses the highest fold change of upregulation in diffuse carcinomas. Quantitative real-time PCR (TaqMan) revealed that intestinal carcinomas totally lack THBS expression, whereas it is expressed in different amounts within the diffuse carcinoma population. In situ, THBS protein could be localized to the tumor stroma of these carcinomas. Primarily, its expression was observed within the extracellular matrix surrounding the tumor cells. In some diffuse carcinomas, THBS staining was found in the cytosol of cancer associated fibroblasts (CAFs), suggesting that fibroblasts are the origin of THBS expression and secretion. In colocalization studies with markers for CAFs (ACTA2), tumor cells (pan-cytokeratin) and mesenchymal cells (VIM) I could confirm that THBS expression is restricted to CAFs in the immediate vicinity of tumor cells. In order to proof that increased THBS expression is a result of fibroblast activation by tumor cells, indirect coculture experiments using conditioned media from different gastric cancer cell lines were performed. Upon exposure to conditioned media of diffuse growing cancer cells, THBS expression was significantly increased in different fibroblast cells lines compared to control medium. Which factor/s released by the tumor cells is/are the driver/s of THBS expression needs to be investigated in the future. At the moment, functional analyses of THBS are being performed to elucidate its potential role for tumor progression, invasion or metastatic dissemination of gastric cancer. One obvious possibility could be that THBS and other extracellular matrix proteins serve as a scaffold for tumor cells, facilitating their motility and invasiveness.

### **Analysis of Tobacco Mosaic Virus(TMV) on Primary(COLO-320) and Metastatic(COLO-741) Human Colon Cancer Cells treated with $\alpha$ -Lactalbumin or Sulindac**

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*Background:* Colon cancer is the second most frequent reason in the cancer-related deaths in the world. It is reported that colon cancer is the 3rd most frequent cancer in males and the 4th most frequent cancer in female. During cancer therapy, the correct time and correct medicine is crucial for different patients. In addition, the primary and metastatic colon cancer therapy may also be different because of cancer cell behavior. We have investigated the TMV has efficiency on the medicine during treatment of cancer cells.

*Methods:* Colo-320 and Colo-741 lines were used in this study. The cells were cultured in RPMI-1640 media including %10 FCS, %1 L-glutamine and %1 penicillin-streptomycin. After 24 hours of culture, the cells were treated with either  $\alpha$ -lactalbumin or sulindac or  $\alpha$ -lactabu-



min+TMV or sulindac +TMV. Also the cells were cultured with TMV only. After 24 hours of treatment, culture mediums from all groups were collected for cytotoxicity analysis, the cells from all groups were fixed in 4% paraformaldehyde for 30 minutes for histochemical analysis. Cell cytotoxicity were evaluated with ELISA. Cell death was investigated using TUNNEL assay.

**Results:** The Colo-320 cells were semi-adhesive cells; the Colo-741 cells were attachment cells. After treatment with sulindac of Colo-320 cells, the number of alive cells was less when compared with other groups. In addition,  $\alpha$ -lactalbumin+TMV treated Colo-320 cells were also less than both only  $\alpha$ -lactalbumin and only TMV applied groups. It was also observed that the number cells in Colo-741 cells which were treated with only  $\alpha$ -lactalbumin or only sulindac groups had less cell amount than the other groups. The TUNEL positive cells were detected in all groups. However, there were more apoptotic cells in Colo-320 treated with both  $\alpha$ -lactalbumin and  $\alpha$ -lactalbumin+TMV applied groups. The higher number of apoptotic cells were detected in Colo-741 cells which were treated with  $\alpha$ -lactalbumin+TMV.

**Conclusion:** Our result suggests that both primary and metastatic cells were much more affected when TMV during treatment. However, the addition of TMV during treatment protocols may cause differences in drug interactions with cells. In further researchs, TMV can be used to recombine according to suitable receptor in cancer cells by gene therapy researchs. Simultaneously, TMV might be used to treat with different medicines of other cancer types. Researchs are still going on about this issue in our faculty.

### Chromosomal Engineering to Produce a Mouse Model of 5q- Syndrome

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**Aim:** The myelodysplastic syndromes (MDS) are haematological disorders characterised by ineffective haematopoiesis. 5q- syndrome is a distinct form of MDS characterised by partial deletion of the long arm of chromosome 5. This disease predominantly affects women and haematological features include refractory macrocytic anaemia and hypolobulated megakaryocytes. Critically the 5q-chromosome is the sole karyotypic abnormality; patients with additional chromosomal abnormalities usually have rapid leukaemic progression. The common deleted region has recently been narrowed to a critically deleted region of approximately 1.5 Mb in the 5q31- 5q32 interval which corresponds exactly with two syntenic regions on mouse chromosomes 11 and 18. To investigate the unknown mechanism(s) underlying this disease, various gene-targeting techniques were employed with the aim of producing an animal model of this syndrome. A single gene deletion or cumulative effects of multiple gene deletion may be the cause of this disease due to haploinsufficiency.

**Methods and Materials:** Deletions were generated on mouse chromosomes 11 and 18 in embryonic stem (ES)

cells using the Cre-loxP system, which allows excision of DNA between two correctly orientated loxP sites upon the action of Cre recombinase. Defined deletions were created on chromosomes 11 and 18 ranging from 150 kb to 700 kb. Deletions were confirmed by visual analysis of whole chromosomes using fluorescence in situ hybridization (FISH). In addition to these strategies, a traditional gene targeting approach was used to disrupt a candidate tumour suppressor gene, fat2, a homologue of the Drosophila fat tumour suppressor gene.

**Results:** Defined deletions ranging between 150 kb and 700 kb were successfully generated on mouse chromosomes 11 and 18 in ES cells. Novel mouse strains containing deletions on mouse chromosome 11 have been generated and analysed that are heterozygous for a deletion, although none have demonstrated significant disruption to haematopoiesis. Fat2-deficient mice have also been created and analysis thus far has demonstrated that the fat2 gene is not critical for normal haematopoiesis. However, mice containing a deletion of the region of synteny on mouse chromosome 18 demonstrate disruption of haematopoiesis similar to that found in 5q- syndrome.

**Conclusion:** Mice generated in this study have provided novel reagents with which to investigate the mechanisms of 5q- syndrome and allowed the elimination of important proposed candidate genes. Their continued use in conjunction with other reagents developed in the laboratory will prove useful in providing insight into the cellular and molecular controls that contribute to this disease and ultimately help highlight possible treatment interventions.

### The tumor suppressor protein p14ARF regulates cell cycle checkpoint control and induces cell death

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The tumor suppressor gene p14ARF is encoded by the human INK4A gene locus and has been shown to play a unique role in the regulation of cell cycle arrest and apoptosis following oncogenic stress. To dissect cell death signaling by p14ARF, we used MCF-7 breast carcinoma cells, which have lost the key executioner caspase-3, and MCF-7 cells that were stably transfected to re-express procaspase-3. P14ARF induces a strong G1 arrest in both cell transfectants. Cell cycle checkpoint abrogation by the kinase inhibitor caffeine was accompanied by loss of cell viability in p14ARF expressing cells. Caffeine interferes with ATM/ATR kinase activities and thereby blunts p53 activity resulting in downregulation of the CDK inhibitory p53 target gene p21CDKN1. Apoptosis was, however, executed only in caspase-3 proficient cells via the mitochondrial pathway as evidenced by breakdown of the mitochondrial membrane potential, cytochrome c release and caspase activation. MCF-7 cells, which have lost caspase-3 undergo a non-apoptotic cell death that involves autophagy. To visualize autophagy, we used GFP tagged light-chain 3 (LC3) protein, which is redistributed

to autophagosomes during the autophagic process. These studies revealed a punctuated fluorescence pattern of GFP-LC3 as well as processing of LC3-I to LC3-II in p14ARF expressing, caffeine treated cells. This was not the case in caspase-3 proficient cells. Thus, abrogation of p53-regulated cell cycle checkpoint control facilitates p14ARF-induced apoptosis in a caspase-3 dependent manner or triggers autophagy in the absence of a functional executioner caspase-3. These findings indicate that p53-mediated cell cycle arrest programs interfere with p14ARF-induced apoptosis that, in turn, strictly relies on caspase-3. Loss of the executioner caspase-3 does, however, not abrogate cell death but results in induction of alternative cell death via autophagy.

### Expression analysis of the ATPdependent chromatin remodeler CHD5

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In various types of human cancer the short arm of chromosome 1 is frequently deleted. Deletions of the 1p36 locus were first described in neuroblastoma. Later, such deletions were also observed in other neural-related tumors like glioblastomas as well as in hematopoietic and epithelial malignancies suggesting that this locus contains at least one tumor suppressor gene. Recently, in MEFs the CHD5 gene has been functionally identified as a tumor suppressor mapping to 1p36 [1]. CHD5 encodes the chromodomain helicase DNA binding domain 5 (Chd5) which controls proliferation, apoptosis and senescence presumably via the p19ARF/p53 pathway. Its expression is thought to be restricted to neuronal tissues based on RT-PCR data [2].

*Aim:* We carried out a detailed expression analysis of CHD5 to provide a basis for future functional studies.

*Materials and Methods:* RNA was extracted with Trizol according to the manufacturer's instructions and whole cell lysates with a lysis buffer (containing 50 mM Tris pH 8.0, 300 mM NaCl, 10 mM MgCl<sub>2</sub>, 0.4% NP-40 and protease inhibitors) from different cell lines (A172, U251, U373, U87, T98G, G1261, SH-EP, SH-SY-5Y, IMR-32, SMS-KCN, HEK293, HeLa, Pc12) as well as from a primary astrocyte culture, a neural stem cell culture and different tissue preparations from an adult Wistar rat and a C57/B16 mouse. RNA was reversely transcribed into cDNA using M-MLV Reverse Transcriptase (Invitrogen) and CHD5 expression was analysed by a SybrGreen-based quantitative RT-PCR. Protein lysates were subjected to western blotting using different polyclonal antisera generated by the lab and a commercial antibody (Santa Cruz) against CHD5.

*Results:* CHD5 protein was not detectable in all tested cell lines as well as in our primary astrocyte culture, rat lung, liver, heart and muscle. Lack of CHD5 expression was confirmed by the QPCR results. A high expression of CHD5 protein was restricted to whole brain lysates and rat testes as well as the neural stem cell culture.

*Conclusions:* We expected CHD5 expression levels in glioblastoma and neuroblastoma cell lines would be simi-

lar to expression levels observed in brain. Surprisingly, no CHD5 expression was detected in any of the cell lines tested. An influence of cell culture conditions seems unlikely because neural stem cells express high level in culture. Therefore, a tumor suppressive role in neuroblastoma and glioblastoma is feasible. The detection of CHD5 in rat testes is the first proof for the expression of CHD5 protein in non-neuronal cells. Its expression pattern can no longer assumed to be exclusively neural-related. This surprising finding necessitates further investigations on CHD5 function. Additionally, a detailed expression analysis in different cell types of the brain and testes will follow.

### Novel antiangiogenic compounds for innovative approaches in urologic tumor treatment

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*Aim:* For most metastatic urologic malignancies the previously established pharmacological therapies are still insufficient and therefore innovative therapeutic approaches are urgently needed. Angiogenesis is essential for tumor growth and metastasis. Vascular endothelial growth factor receptors (VEGFR) and their ligands are involved in increased angiogenic activity and disease progression of solid tumors. VEGFR-inhibition by specific tyrosine kinase inhibitors (TKI) has already become a clinically relevant strategy for innovative treatment of urological tumors. Searching for novel VEGFR-specific TKIs we here used an in silico screening approach and identified novel compounds with putative antiangiogenic properties. The antiangiogenic and antiproliferative potency of the most promising of these compounds (HP-2, HP-14) was studied.

*Methods and Results:* RT-PCR revealed a strong expression of VEGFR-2 in testicular germ cell tumor cells (Tera-1), whereas no expression could be detected in renal cell carcinoma cells (A498). Performing crystal violet assays the antiproliferative effects of HP-2 and HP-14 were examined in both cell lines. Corresponding to the observed VEGFR expression pattern, HP-14 exerted a marked growth inhibitory effect in Tera-1 cells in a time- and dose-dependent manner (IC<sub>50</sub> = 5,62 μM), while no appreciable growth inhibition was observed in VEGFR-lacking A498 cells. The effects of HP-2 and HP-14 were also evaluated on microvessel-forming endothelial cells (EA.hy926, HUVEC). Treatment of endothelial cells caused a pronounced time- and dose-dependent growth inhibition of up to 90%. Antimigratory effects were studied by performing scratch assays. HP-14 inhibited VEGF-induced migration of endothelial cells. Moreover, the influence of the novel compounds on capillary-like tube formation was investigated. Incubation with HP-14 for 6 h led to a dose-dependent inhibition of capillary-like tube formation. Unspecific cytotoxic effects of HP-14 and HP-2 were excluded by measurement of LDH release into the supernatant of incubated urologic tumor and endothelial cells. Additional Western blot analyses showed an induction of the cyclin-dependent kinase inhibitor p21Waf/CIP1, which was accompanied by a downregula-

tion of the cell cycle promotor cyclin D1. These data demonstrates that the mode of action of the HP-substances involves the induction of the cell cycle arrest at the G1/S>G2/M checkpoint. DNA-Microarray was used to explore changes in the expression pattern of various genes involved in angiogenesis. HP-14 treatment led to a reduction in several growth factor receptors (VEGFR-2, Tie1, Tie2) and corresponding growth factors (VEGF, ANGPT2) in endothelial and tumor cells. Finally, the chorioallantoic membrane assay (CAM assay) was used to evaluate antiangiogenic effects *in vivo*. 24h incubation of the CAM of fertilized chicken eggs with HP-2 led to an arrest in microvessel formation.

**Conclusion:** Our data shows that the investigated novel compounds potently inhibit the growth of VEGFR-expressing testicular germ cell tumor cells and decrease endothelial cell growth as well as microvessel formation *in vitro* and *in vivo*. The complex mode of action involves cell cycle arresting effects as well as a change in the expression pattern of several angiogenic genes. Thus, the identified new compounds appear to be very promising for innovative urologic tumor treatment and merit further elucidation.

### Activating Bak by targeting its inhibitors as a new chemotherapeutic approach for TRAIL resistant tumors

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**Aim:** TRAIL (TNF-related apoptosis inducing ligand) is a promising biological, apoptosis inducing anticancer agent in view of its preferential killing of tumor cells with limited cytotoxicity against non-malignant cells. In most tumors, the direct signaling from death receptor signals needs to be amplified via the mitochondrial pathway to induce apoptosis. Thus, in human carcinoma, TRAIL-induced cell death entirely depends on the pro-apoptotic Bcl-2 family member Bax that is often lost due to epigenetic inactivation or, in mismatch repair deficient cancers, due to mutations. Consequently, Bax deficiency confers resistance against TRAIL-induced cell death. Despite expression of Bak, Bax deficient cells were fully resistant to TRAIL-induced apoptosis. Here, we investigated which anti-apoptotic members of the Bcl-2 family are responsible for the inhibition of the Bak pathway in TRAIL resistance and how this dependency may be circumvented.

**Methods and Materials:** Bax pro- and deficient HCT116 and DU145 cells were treated with either siRNA or the kinase inhibitors R-roscovitine (Seliclib or CYC202), Sorafenib in the absence or presence of the death ligand TRAIL to induce apoptosis. Apoptotic cell death was measured by flow cytometry. Protein expression was detected by Western blot analysis.

**Results:** Targeted knock-down of Mcl-1, but not Bcl-xL, either by siRNA or drugs (R-roscovitine and Sorafenib) overcame TRAIL resistance in Bax-deficient cells and enabled TRAIL to activate Bak.

**Conclusion:** Our data show that Mcl-1 but not Bcl-xL is the main target to sensitize Bax-deficient tumors for

TRAIL-induced apoptosis via the Bak pathway. Therapy with drugs inhibiting Mcl-1, e.g. Nexavar (sorafenib) or Seliclib (R-roscovitine) in combination with TRAIL appears to be a promising strategy to overcome restraints in apoptosis signalling in tumor patients.

### Mechanism of Nbk-mediated Mcl-1 stabilization

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**Aim:** The Bcl-2 family member myeloid cell leukemia 1 (Mcl-1) plays a major role in the development and homeostasis of normal tissue. It is overexpressed in various human malignancies and involved in the resistance against conventional tumor therapies. Recently, we observed that the overexpression of the BH3 (Bcl-2 homology domain)-only protein Natural Born Killer (Nbk) results in an increased expression of Mcl-1. The molecular mechanism of this phenomenon remains enigmatic and is addressed in this work.

**Methods and materials:** To characterize the mechanism of the increased Mcl-1 expression, we used an adenoviral expression system and created, in addition, a stable conditional expression system for the DU145 prostate carcinoma cell line to overexpress Nbk and other BH3-only proteins. The expression of Mcl-1 was determined via quantitative real-time PCR and Western blot analysis. Furthermore, we applied siRNA-mediated knockdown studies and fluorescence microscopy.

**Results:** Here, we show that the expression of Mcl-1 correlates with an enhanced ectopic expression of Nbk. We further show that not only Nbk, but also the expression of other BH3-only proteins like Noxa, Puma, BimL and BimS increases Mcl-1 expression levels. The increased Mcl-1 expression upon Nbk-expression is not caused by an upregulation on the transcriptional level, but due to stabilization and, consequently, an increased half-life of the Mcl-1 protein. In contrast to Noxa, Puma, BimL and BimS, Nbk does, however, not physically interact with Mcl-1, which suggests a different mechanism of stabilization. Moreover we show that the Nbk-mediated stabilization is independent of the proapoptotic proteins Bax and Bak. In addition to the used DU145 cell line we observe the effect in HCT116 and MCF-7 cells. Furthermore we show that the downregulation of the E3 ubiquitin ligase Mule, which mediates the proteasomal degradation of Mcl-1, did not affect the Nbk-mediated stabilization of Mcl-1. To analyze the role of the BH3-domain, we tested the effect of the BH3-mimetic Gossypol, which also leads to an increased expression of Mcl-1.

**Conclusion:** We show that the proapoptotic protein Nbk mediates stabilization of the antiapoptotic protein Mcl-1. This seems to be a fail-safe mechanism to avoid accidental killing of BH3-only protein expressing cells and allows for a fine-tuning of the complex apoptosis machinery. The findings in this work have important implications for the design of novel anticancer therapies that target cell death signaling.

### Akt-inhibition as a new therapeutic option in gastroenteropancreatic neuroendocrine tumors

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*Aim:* It has been shown that in several types of cancer, upregulation of phosphatidylinositol 3-kinase (PI3K)-Akt signaling facilitates tumor cell growth and inhibits apoptosis. This pathway also plays an important role in chemotherapy resistance, especially in resistance of tumor cells to growth-factor blocking agents. The importance of Akt signaling has not yet been explored in gastroenteropancreatic neuroendocrine tumors (GEP-NET). In this study, our objective was to investigate the role of Akt in different cell lines of GEP-NET's as well as to find new therapeutic approaches by investigating the effect of Triciribine, a specific Akt-inhibitor, on these cells. Furthermore, we wanted to characterize the role of PTEN, an inhibitory protein of Akt, as a possible predictor of sensitivity to Akt-inhibitors in GEP-NET's. Finally, we evaluated a combinatory treatment of Akt-inhibition with an established mTORC-1-inhibitor, Everolimus, since Everolimus alone leads to an increased activation of Akt via a negative feedback loop.

*Methods and Materials:* Metastatic human pancreatic carcinoid tumor cell line BON, intestinal cholecystokinin secreting tumor cells (STC-1) and human insulinoma cells (CM) were treated with Triciribine (0 to 20  $\mu$ M) or with Triciribine and Everolimus. Crystal violet staining was used for the detection of cell number changes. Western blot analysis was performed for the expression of active, phosphorylated Akt (pAkt), total Akt, PTEN and Cyclin D1. RT-PCR was used for the detection of PTEN-mRNA.

*Results:* Treatment of CM- and STC-1 cells with Triciribine significantly reduced tumor cell growth (59% and 65% at 20  $\mu$ M). Growth of BON-cells was not affected (0% reduction at 20  $\mu$ M). Triciribine dose-dependent decreased activated Akt (p-AKT) as well as the expression of cell cycle-promoting Cyclin-D1, an effector of Akt. Akt and PTEN expression remained unchanged. mRNA of the regulatory protein PTEN were significantly greater in CM- and STC-1 cells compared to BON-cells. Combination of Triciribine with Everolimus leads to synergistic effects on tumor cell growth reduction.

*Conclusion:* The PI3K-Akt pathway plays an important role in tumor cell growth in CM- and STC-1 cells. Sensitivity to the inhibition of Akt was strongly correlated with the expression of the regulatory protein PTEN and could explain the resistance of BON-cells to Triciribine. Dual inhibition by everolimus and triciribine appears to be a promising approach to enhancing the effects of mTOR-AKT pathway-related growth inhibitor of GEP-NETs and merits further elucidation.

### Severity of Vaccination-induced Idiopathic Thrombocytopenic Purpura versus other causes of the disease in infants

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*Background:* Acute development of petechia and purpura in previously healthy children is mostly consistent with Idiopathic Thrombocytopenic Purpura (ITP) which can be induced following vaccination or infection. Patients present with mucocutaneous bleeding and platelet count less than 50000/mm<sup>3</sup> and severe cases with platelets less than 20000/mm<sup>3</sup> need urgent treatment. We sought to determine the relationship between vaccination and more severe cases of ITP.

*Methods:* In this 5-year retrospective study, we observed infants younger than 18 months who were admitted in our hospital with the primary diagnosis of ITP from 2002 to 2007. Patients were evaluated clinically for the causative factors and probable etiology of the disease (i.e. vaccination or infection) and were divided into severe and non-severe cases depending on their platelets count (cut point: 20000/mm<sup>3</sup>).

*Results:* We included 61 patients younger than 18 months (52 male and 29 female). Among which 29 patients (47.5%) had platelets <20000/mm<sup>3</sup>. Each group was divided into 2 subgroups based on the etiology of ITP (Vaccination vs. other causes). Surprisingly, there was a significant difference in platelets count between vaccinated and non-vaccinated groups (OR: 3.14; P value <0.0061). It was also noticed that younger infants (age <3 months) were more susceptible to severe ITP (OR: 1.47; P value <0.032).

*Conclusion:* Although thrombocytopenia is not mentioned as an adverse reaction following DTP & OPV vaccination, high prevalence of severe ITP following the first dose of the vaccine in this age group is more significant than others. Further research is needed to demonstrate the exact relationship of thrombocytopenia following vaccination and its effect on the severity of the disease.

### Expression of cytokeratine 5/6 in ductal infiltrating breast carcinoma

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*Aim:* The aim of this study was to determine the frequency of expression of basal cytokeratine 5/6 (CK 5/6) in infiltrative ductal breast carcinoma (non-specific type) and to establish the correlation between the expression of CK5/6 and histological grade of the tumor.

*Material and methods:* The research has been conducted on 121 randomly selected patients with infiltrative ductal breast carcinoma, grade II A (T1 N1 i T2 N0). Patients were operated on at the Institute of Oncology of Vojvodina.

*Results:* Positive expression of CK 5/6 was found in 29 cases (23,97%). In the sample of breast carcinoma, it was determined that a statistically significant correlation

existed between expression of CK5/6 and histological grade of the tumor ( $p = 0,011c2$  - test,  $c = 0,263$ ). CK 5/6 negative tumors had high and moderate differentiation grades (HG1, HG2), while CK5/6 positive tumors had low differentiation grades (HG3).

**Conclusion:** Basal CK 5/6 expression in the breast carcinoma could be an indicator of low grade differentiation. Furthermore, CK 5/6 could be a marker of basaloid tumor phenotype which is characterized by a very aggressive biological behavior. **Keywords:** Cytokeratine 5/6; Ductal infiltrating breast carcinoma

### Augmentation of aspirin effects by chocolate

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**Aim:** Researches have been done to elaborate the effects of flavonoids on platelets, but the role of chocolate as an augments of aspirin effects has not been brought into light in our part of the world. Our aim was to establish the anti-platelet or aspirin like effects of flavonoid rich foods i.e. Chocolate as an augments of aspirin functions.

**Subjects and Methods:** This was a non-randomized quasi experimental study in which, 65 healthy adult volunteers, including 27 males and 38 females participated. Subjects included were healthy, non-smoking, 20-30 years old adults, with no blood dyscrasias. Subjects with chronic liver disease, arterial hypertension, ischemic heart disease, taking hormonal, calcium and vitamin supplements and women who were taking estrogen or were pregnant were all excluded from the study. Health of subjects was evaluated by a questionnaire. Subjects gave written informed consent prior to admission in the study. Research protocol was approved by the college's, "Research and Ethical committee". Subjects were divided into two groups, a control group which consumed 100mgs of oral aspirin and an interventional group consuming 100mgs oral aspirin and 18.75gms of dark chocolate (flavonoid rich). The Bleeding time estimation of both the groups was done by trained lab professionals, who were blinded of the nature of the study; utilizing the Duke Method of bleeding time estimation before and after consuming the drug in case of the control group and drug along with chocolate in case of the interventional group. **Results:** Data was analyzed by SPSS 15.0. First bivariate analysis was done by comparing the means of both the pre-test groups i.e. before consumption of aspirin and also before consumption of chocolate and aspirin together through independent t-test in which we found a insignificant p- value ( $p = 0.798$ ) which showed that both the groups were similar before the intervention. The bleeding time of both post test groups i.e. groups after consumption of aspirin & also the group after consumption of aspirin and chocolate were again analyzed by the same procedure as described above. Independent t- test showed a highly significant p-value ( $p = 0.006$ ) which shows that our intervention i.e. chocolate has increased the effects of aspirin in comparison to when aspirin was used alone. Confidence interval and standard deviations

were also calculated. Further more multivariate regression models were also used which gave an  $R_$  value of .250.

**Conclusion:** Chocolate has a significant role in augmenting the anti-platelet effects of aspirin. However, study was conducted for a short period of time therefore it can only measure the short term effects of chocolate consumption on aspirin effects. For evaluating long term effects we recommend further studies.

## Immunology

### Correlation of TNF- $\alpha$ (-308) polymorphism with blood types in health people and patient with autoimmune disease

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**Introduction:** Blood type is the classification of blood based on the presence of antigens inherited substances. Combining 3 existing alleles on two homolog chromosome we obtain 6 genotypes which form 4 basic blood groups. TNF is pleiotropic cytokines whose role is to accumulate neutrophil and monocytes cells in parts where the infection occurs. Its role is also very important in autoimmune diseases and neoplasm. There is a great number of polymorphism in the TNF gene group, and one of the most important is TNF- $\alpha$ (-308).

**Aim:** The aim of study is to examine the correlation between TNF- $\alpha$ (-308)G/A polymorphism with blood groups in healthy subjects and in patients with rheumatoid arthritis and systemic lupus and to find out the correlation between blood types and autoimmune disease.

**Materials and methods:** The research was done on DNA of 41 of blood subjects, from patients with rheumatoid arthritis, systemic lupus and voluntary donors. Isolation of DNA has been carried out from fresh blood using proteinases K and commercial kit for isolation of DNA, after which on the derived sample by PCR-RFLP method, the TNF- $\alpha$ -308 polymorphism was examined.

**Results:** It was found that the largest number of examinees had O+ blood group, and that any significant difference in distribution G/G and G/A polymorphism was not noticed. Patients with B+ blood group equally had all genotypes, while the subjects with A+ blood group had no significant differences in the TNF-308 polymorphism for wild-type and heterozygote was noticed, but none of the examinees had A/A genotype. Distribution of all three genotype in subjects with AB+ blood group didn't show statistically significant difference.

**Conclusion:** The results showed that there had not been any clear correlation between blood groups and TNF-308 G/A polymorphism, and that the correlation between blood groups and risk of the rheumatoid arthritis was not prove, but the significant correlation between AB+ blood group and appearance of the lupus exists.

## The frequency of polymorphism of TNF- $\alpha$ - 308 G / A in patients with rheumatoid arthritis

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**Title:** The frequency of polymorphism of TNF- $\alpha$  -308 G / A in patients with rheumatoid arthritis  
**Introduction:** Rheumatoid arthritis (RA) is a systemic autoimmune disease in which inflammation and joint's damage tumor necrosis factor alpha (TNF- $\alpha$ ) plays a major role. Bearing in mind that the level of TNF- $\alpha$  in serum and synovial fluid of RA patients increased and the fact that the polymorphism -308 G / A is associated with increased transcription, the TNF- $\alpha$  gene may be considered as a genome marker for screening RA.  
**Aim:** The aim of this study is to determine the frequency of polymorphism (replacement between guanine and adenine) of promoting regions of TNF- $\alpha$  gene at the position -308 in patients with RA and to find out if that polymorphism is a risk factor for that autoimmune disease.

*Methods and material:* Research was carried out on material obtained from 79 patients, both genders, 12 men and 67 women, between 23 and 78 years old (average age 56.6  $\pm$  12.5 years), with RA from the Rheumatology Clinic, Clinical Center of Nis. The control group consisted of 34 healthy people between 23 and 78 years old. The polymorphism is examined on DNA isolated from full blood by phenol-chloroform method. The data were statistically processed by standard tests.

*Results:* There is a significant difference in the distribution of alleles between the patients with RA and the control group. Health people have statistically significant G allele in most unlike the patients with RA. In the group of patients with RA the most common genotype is G / A, and in the control group G / G genotype. G / G genotype is more frequent in women and A / A genotype in men.

*Conclusion:* The allele G and G / G genotype were statistically significantly less frequent in patients with RA compared with the control. Allele A and G / A genotype were more frequent in patients with RA, which points to the strong production of TNF- $\alpha$  in patients.

## Microglia induced regulatory T cells (Tregs) suppress EAE

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Microglial cells are the major immune competent cells of the brain. They are activated in various CNS affecting diseases and injury models including brain ischemia, entorhinal cortex lesion (ECL), or experimental allergic encephalomyelitis (EAE). Moreover, they play a crucial role in initiating innate and adaptive immune responses. The present study aims to elucidate the complex cross-talk between microglia and brain infiltrating immune cells,

specifically the direct microglia – T cell interaction. Here, we demonstrate for the first time that cultured microglia have the potential to induce CD4+CD25+FoxP3+, IL-10 and IL-13 secreting, regulatory T cells (Tregs). In detail, microglia cultured from C57BL/6-J mice were stimulated with 10U/ml IFN $\gamma$  for 24h to express intermediate levels of MHCII. Subsequently, cells were pulsed with low levels (1 $\mu$ g/ml) of an EAE eliciting peptide such as myelin oligodendrocyte glycoprotein (MOG35–55) and co-cultured with CD4+ T cells derived from T cell receptor (TCR) transgenic mice specific for MOG. Co-cultures of microglia and T cells were performed for further 7 days to induce Tregs. Interestingly, the induced relative frequency of Tregs versus activated effector cells depended directly on the amount of myelin antigen presented by microglia cells revealing only a tolerogenic phenotype of these cells when cultured with low doses of peptide. Stimulated and antigen primed microglia revealed intermediate expression of co-stimulatory molecule CD40 and iNOS. Moreover, after 7 days of co-culture CD40 expression on microglia was strongly downregulated whenever microglia induced a relatively high frequency of Tregs. In contrast, high levels of MHCII expression and high concentration of MOG peptide led to the induction of effector cells characterised by prolonged CD40 expression on microglia. We could also demonstrate neogenesis of microglia-induced Tregs by differentiating naïve and Treg depleted T cells into antigen-specific Tregs. Finally, to test the regulatory potential of microglia-induced MOG-specific Tregs in vivo, adoptive transfer of Tregs into EAE models was performed. Therefore C57BL/6-J mice were immunized with MOG35-55 peptide. Time point of disease onset and severity of EAE were diminished in recipient mice. In sum, our data demonstrate that activated microglia contribute to an antigen-dependent regulation of the adaptive immune response.

## Improved Inhibition of Adenovirus Infection by Multiple RNA-Interference Mediated Adenoviral Gene Silencing

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*Background:* Adenoviruses are very well known as pathogens in various infectious diseases, e.g. of the respiratory tract as well as in myocarditis. Gene silencing mediated by RNA interference (RNAi) is a promising new approach in the field of antiviral therapy. So far it has been shown that several viral infections as HIV, HBV and CVB can be inhibited by RNAi. We examined the effect of RNAi by use of small interfering (si) RNA on adenoviral infection and analyzed whether simultaneous silencing of different adenoviral target genes is more efficient in inhibition of adenovirus infection than single adenoviral target gene silencing. The siRNAs were directed against three different target genes within the adenoviral genome: E1A, hexon and IVa2. E1A is an immediate early gene and is as such the first mRNA/protein to be expressed. As a transcriptional regulatory factor the E1A protein is necessary for the transcriptional activation of other early

genes. Hexon is the major capsid protein of the adenovirus and IVa2 plays an important role in adenovirus packaging. All three proteins are essential for adenoviral replication. Deletion of these genes from the adenoviral genome abolishes adenoviral replication. First we investigated the effect of separately applied synthetic siRNAs against each target gene. By use of a luciferase reporter gene assay where the luciferase cDNA was linked with E1A, hexon and IVa2 siRNA target sequences, respectively, we were able to find siRNAs that showed similar silencing efficiency. Replication reduction assays carried out in HeLa cells revealed strong and siRNA dose-dependent inhibition of adenovirus replication. However, despite similar silencing efficiency of the siRNAs E1A silencing was of distinctly lower efficiency (70 %) than silencing of hexon and IVa2 (about 90 % each). In a next step we investigated the effect of a combined application of the siRNAs. The combination of two or three siRNAs when the total concentration equals the concentration of an individually applied siRNA, however, had no advantage regarding inhibition of adenoviral replication. Silencing efficiency of siHexon combined with siIVa was the same as of individual siHexon and siIVa, respectively. Surprisingly, in a finally conducted cell killing assay only in samples treated with siRNAs directed against E1A and hexon or E1A and IVa2 or the combination of all three siRNAs, respectively, adenovirus induced cytotoxicity was successfully inhibited, while the singular employment of each siRNA as well as the combination of hexon and IVa2 were inefficient. In summary, our investigations show highly efficient inhibition of adenovirus replication by use of RNAi and for the first time reveal that silencing of adenoviral genes involved in viral packaging is more efficient than inhibition of E1A. Moreover, adenovirus induced cytotoxicity is most efficiently inhibited when in addition to the packaging genes also E1A is silenced, indicating the critical function of the E1A gene in the adenoviral replication cycle.

### **Fate of monocytic cells in the CNS after injury**

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**Fate of monocytic cells in the CNS after injury** The brain was long believed to have an “immunologically privileged status”, based on two morphological peculiarities of the CNS: the absence of classical lymph vessels and the blood-brain barrier (BBB). In fact, the continuous influx of T cells and monocytes into the CNS has been demonstrated, and several routes of their entry have been characterized. Similarly, the idea that immunological cells are trapped within the skull as a result of the absence of lymph vessels has also been defeated by showing the drainage of soluble antigens and the traffic of CD4<sup>+</sup>-T-cells along the olfactory nerve to cervical lymph nodes. It has already been shown that circulating monocytic cells infiltrate layers of anterograde axonal degeneration where they transform into microglia. But 30 days after the lesion the total amount of monocytic cells in denervated layers is equal to the prelesioned state. So it was not clear whether monocytes also leave the CNS and which route

they use. To address this issue, I first isolated peripheral blood monocytes from transgenic mice expressing GFP (green fluorescent protein) by density-gradient centrifugation and magnetic cell sorting. The high purity, their viable and functional state was confirmed through a FACS- Analysis (= fluorescence activated cell sorting). The GFP-expressing CD11b positive monocytes fraction was then injected into the entorhinal cortex immediately after lesion C57/bl6 mice. The entorhinal cortex lesion imitates a central inflammatory process. To figure the time-frame the animals were then sacrificed 24h, 48h, 4days, 7d, 14d, 28d post lesion. Using an adapted protocol of decalcification, which allows us to cut whole head/neck crosssections and to preserve the fluorescence of GFP-expressing cells, we cut 50µm slices of the brains, cervical and inguinal lymph nodes. The migration route could then be monitored through fluorescence microscopy. The slices were stained by Mac-1 to affirm monocytes. In lesioned mice, GFP-expressing, activated monocytes were found at the injection site and attached to the ventricular surface and the choroid plexus. Although no quantification was performed, their distance to the entorhinal area (injection site) appeared to increase with time, and their distribution suggested migration along the alveus (a myelinated fiber tract) to the ventral horn of the ventricle (24hpl, 48hpl). At 7hpl (hours post lesion) some GFP cells resided in the cervical lymph nodes of individual animals and accumulated after 14hpl and 28 hpl. The population in inguinal nodes never reached the density observed in the cervical group. Our interpretation of this sequential distribution is that the injected monocytes in this study use the same route, which is known for the drainage of soluble antigens and CD4<sup>+</sup>-T-cells. Therefore it can be assumed that peripheral blood monocytes also contribute to an immune respond after a central inflammation, which has great importance in finding therapeutic strategies concerning e.g. central autoimmune diseases .

### **The effect of adipokines on polarization and functionality of human M1 and M2 macrophages**

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**Introduction and Aim:** While the incidence of inflammatory bowel disease (IBD) is increasing, its etiology is still not fully understood. However, two lines of evidence support the idea that the mesenteric fat tissue contributes to the pathogenesis of Crohn’s disease (CD): 1) in CD patients a phenomenon called creeping fat has been described to be pathognomic. Here the mesenteric fat tissue increases around the inflamed segments of the intestine. 2) The genome-wide association studies (GWAS) revealed a strong association between the autophagy gene ATG16L1 and CD. Remarkably, CD patients homozygous for the ATG16L1 CD risk allele expressed increased levels of leptin (Nature 2008; 456). Increased leptin concentration had already been described in the mesenteric fat tissue of CD patients. It has become well accepted that adipokines act as regulators of the immune system. Furthermore, from metabolic disorders an increase in pro-

inflammatory macrophages (M1) has been described to maintain the inflammatory condition. Activated macrophages play a key role in the pathogenesis of CD. Interestingly they represent a dominant cell population infiltrating the mesenteric fat tissue. Thus the aim of the present study was to evaluate the possible regulatory role of leptin and adiponectin on polarization and reactivity of M1 and M2 macrophages.

**Methods and Materials:** Monocytes were isolated from human blood via magnetic cell sorting and polarized for six days with either GM-CSF (M1) or M-CSF (M2). For each subtype there was an assay with and without leptin. The polarized macrophages were stimulated with the TLR4 ligand lipopolysaccharide (LPS) and/ or adipokines for 24 h. Supernatants were collected. Cells and supernatants were analyzed by flow cytometry using antibodies directed against surface markers and cytometric bead arrays to characterize cytokine profiles. TLR4 expression was evaluated by PCR.

**Results:** mRNA analysis revealed that the LPS receptor TLR4 was present in all cells, hence both subtypes can respond to LPS stimulation. The cultured cells presented two phenotypes. M1 cells showed "fried egg" morphology. They were CD14<sup>-</sup> but CD80<sup>+</sup> and MHCII<sup>+</sup> and produced high levels of IL-8, TNF $\alpha$  and IL-6 following LPS stimulation. In contrast M2 cells were more adherent and had a "stretched spindle-shaped" morphology. They were CD14<sup>+</sup>, CD80<sup>+</sup> and MHCII<sup>+</sup>. Upon LPS stimulation they produced high amounts of IL-10, IL-6 and IL-8. In both subpopulations stimulation with leptin resulted in an increased cytokine production. Simultaneous exposure to LPS and leptin led to reduced IL-8 but enhanced TNF $\alpha$  production in M1 macrophages, whereas adiponectin reduced the IL-1, IL-6, and IL 10 production following LPS stimulation in these cells. In M2 cells, adiponectin increased, but leptin decreased IL-8 and TNF $\alpha$  production in response to LPS stimulation.

**Conclusion:** Adipokines influence the phenotype and function of human macrophages and they exert different effects on M1 and M2 subpopulations. It is tempting to speculate that similar changes might happen in vivo when adipokine levels are altered. Therefore, the altered adipokine expression present in the mesenteric fat might be involved in the ongoing intestinal inflammation in CD patients.

## Elucidating the Molecular Clock in Macrophages

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Elucidating the Molecular Clock in Macrophages Many behavioural, physiological and immunological processes exhibit prominent daily variations even under constant environmental conditions, so called circadian rhythms. In mammals the master circadian pacemaker, the suprachiasmatic nucleus (SCN), is located in the ventral hypothalamus. It receives light signals by neuronal projections from the retina and synchronizes clocks in peripheral organs, which themselves regulate circadian rhythms locally. In the immune system various parameters and

immune functions, like blood levels of lymphocytes and cytokines are subjected to daily variations. At least some of these rhythms are likely of biological relevance since it has been shown that the mortality rate of mice dramatically depends on the time of day when LPS is injected, ranging from 10% at midnight up to 90% in the afternoon. Thus, it is assumed that the circadian system modulates immune functions. However, the molecular mechanisms that link the circadian clock and the immune system are so far unknown. To investigate the molecular mechanisms of circadian modulations in the immune system, we use mouse macrophages as a model system. We show that the mRNA levels of the canonical clock genes Per2 and Rev-Erba show circadian expression pattern in ex vivo isolated peritoneal macrophages. These rhythms persist even in in vitro cultured peritoneal cells from PER2::LUC reporter mice detected by bioluminescence recording. Additionally, we show that isolated splenic and peritoneal macrophages secrete TNF- $\alpha$ , one prominent LPS induced cytokine, in a circadian manner upon stimulation with LPS. These results indicate that a macrophage intrinsic clock modulates the response to LPS. To analyze the circadian regulation on a transcriptional level we performed a whole genome microarray analysis of isolated peritoneal macrophages, collected in a time course for two days. These data show that more than 10% of the macrophage transcriptome is rhythmically expressed. Besides core clock genes we could also identify circadian expression patterns for several key players involved in the LPS responsive pathway as well as in macrophage output function. Our data support the idea of a local circadian clockwork as a modulatory component in macrophage function and provide first insights into that regulation on transcriptional level.

## A new phenotype in asthma

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**Introduction:** Immune dysregulation in asthma have a few key elements, such as: mast cells, T cells, co-stimulatory molecules, eosinophiles, interleukins, cytokines and autoantibodies. IL-17 is a cytokine secreted by a newly described, special lineage of CD4 T cells (TH17) IL-17 depends on IL-1, IL-6 and TGF- $\beta$  for induction and also depends on nuclear transcription factor ROR $\gamma$ T and IL-23 for maintenance. IL-17 promotes inflammation and tissue destruction in both TH1 and TH2 type diseases. IL-17 was found to be increased in many human chronic inflammatory diseases such as rheumatoid arthritis, SLE, psoriasis or allograft rejection. In rheumatoid arthritis IL-17 was related to disease severity and joint destruction via excessive inflammation. IL-17 expression is increased in asthma but it's prognostic significance is unknown. Our aim is to find the prognostic value of increased serum IL-17 in asthma patients.

**Methods:** Our study has been done on 85 patients with asthma evaluated at inclusion for: serum IL-17 (ELISA), blood eosinophilia, BMI, FEV1 at baseline 20 pg/ml was tested as a risk factor for asthma severity using multiple regression analysis.



**Results:** From 85 asthma patients the mean age was  $49.99 \pm 14.1$  years, 61% females and mean asthma duration was  $12.64 \pm 1.19$  years. Also from our group 27% have mild persistent asthma, 30.6% moderate persistent asthma and 42.4% severe asthma. The serum IL-17 (medium values) were at mild asthma 14.21 pg/ml, moderate asthma 12.22 pg/ml and severe asthma 24.72 pg/ml We found IL-17 > 20 pg/ml in mild asthma at 3(13%) patients, in moderate asthma at 2(8%) patients and in severe asthma at 11(31%) patients IL-17 > 20 pg/ml was not correlated with other phenotypic features of asthma like eosinophilia, atopy, NSAID intolerance or Obesity. IL-17 > 20 pg/ml could depict a special asthma phenotype.

**Conclusion:** Serum IL-17 is increased in severe asthma compared to mild/moderate forms of the disease. Values of IL-17 > 20 pg/ml are an independent risk factor for asthma severity. Keywords: interleukins, asthma, multiple regression analysis.

### Increased serum IL-17 does not predict future risk and impairment in asthma

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**Background:** IL-17 is increased in sputum and bronchial biopsies of asthma patients (pts), and is correlated with airway neutrophilia and BHR. In a cross-sectional study serum IL-17 was increased in severe asthma and was an independent predictor for asthma severity. Prognostic value of increased serum IL-17 in asthma in a longitudinal study was not yet assessed. Design: 67 persistent asthma pts., (17 mild, 21 moderate, 29 severe), mean age  $44.35 \pm 10.2$  years, 58% females, were evaluated for serum IL-17 > 20 pg/ml, smoke, atopy, NSAID intolerance, obesity, chronic rhinosinusitis (CRS), blood eosinophilia, FEV1 5% compared to baseline and asthma control (GINA). Baseline risk factors were tested in the multiple regression analysis model (STATISTICA 7).

**Results:** For all asthma pts. group the following were independent risk factors: NSAID intolerance ( $p = 0.034$ ) and CRS ( $p = 0.0096$ ) for severe exacerbations; atopy ( $p = 0.019$ ), CRS ( $p = 0.005$ ), blood eosinophilia (0.036) and obesity ( $p = 0.029$ ) for fast FEV1 decline; NSAID intolerance ( $p = 0.007$ ) and CRS ( $p = 0.019$ ) for lack of asthma control for > 6 months. In the subgroup of severe asthma pts. the following were independent risk factors: CRS ( $p = 0.046$ ) for severe exacerbations; NSAID intolerance ( $p = 0.017$ ) and obesity ( $p = 0.033$ ) for fast FEV1 decline, NSAID intolerance ( $p = 0.048$ ) and CRS ( $p = 0.004$ ) for lack of asthma control. For none of the analysed end-points increased serum IL-17 was an independent risk factor, both for the whole asthma group and for the severe asthma pts.

**Conclusions:** Increased serum IL-17 does not predict future risk and impairment in asthma patients.

### NKT and $\gamma\delta$ T cells share their own molecules: A phenotypical dilemma

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**Introduction:** For the identification of lymphocyte subsets, standard cell surface markers are used in routine flow cytometric lymphocyte analysis. There are major markers used for defining lymphocytes such as CD3 for T cells, CD16/CD56 for Natural Killer cells and CD16/CD56/CD3 for Natural Killer T cells. NKT cells are cells which express both NK 1.1 and T cell receptor and they have antitumour and antimicrobial function.  $\gamma\delta$  T cells are a subset of T cells and they have TCR  $\gamma\delta$ . They are intraepithelial cells unrestricted to Major Histocompatibility Complex I-II. Heterogeneity is observed in all populations. The aim of this study is to decipher the relation between NKT cells and T cells by using flow cytometric analysis and to see whether CD3 over expression reflects any phenotypically different group.

**Materials and Methods:** Peripheral blood phenotypic analysis from 32 healthy adult volunteers (age range 19 to 45; 17 male, 15 female) was performed by three color flow cytometry. In this study fluorochrome conjugated monoclonal antibodies CD3-PC5, TCR  $\gamma\delta$  -FITC, and CD16/CD56-PE and Beckman Coulter Epics XL MCL flow cytometry analyzer were used. The data were analyzed using Expo 32 analysis programme. The statistical data were analyzed using SPSS v.13 software. Descriptive statistics were used to define the distributions of subsets; Mann Whitney U test and Pearson correlation analysis were performed.

**Results:** CD3 over expression on  $\gamma\delta$  T cells was observed in all subjects. The CD3 MFI of  $\gamma\delta$  T cells was found significantly higher than non- $\gamma\delta$  T cells ( $29.4\% \pm 5.8$  versus  $16.3\% \pm 3$ ;  $p < 0.001$ ). CD16/CD56 expression of  $\gamma\delta$  T cells was found  $29.3\% \pm 16.5$ ; TCR $\gamma\delta$  expression of NKT cells was found  $33.7\% \pm 20.8$ . CD8 positivity on NKT cells was observed significantly higher than CD4 ( $45.4\%$  versus  $7.6\%$ ;  $p < 0.05$ ). CD4-/CD8- double negative  $\gamma\delta$  T cells were observed extremely higher than CD4 or CD8 single positive  $\gamma\delta$  T cells (88.9% double positive; 10% CD8+; 1% CD4+).

**Conclusion:** This study reveals that  $\gamma\delta$  T cells express higher amounts of CD3 molecules than any other T cell subset. We observed a strong relation between  $\gamma\delta$  T and NKT cells and we showed that this relation is reciprocal. The  $\gamma\delta$  T cells are mostly double negative for CD4 and CD8. These conclusions are important because CD3 bright population might be used to identify  $\gamma\delta$  T cells. In addition, physiologically  $\gamma\delta$  T cells and NKT cells may have similar but unknown functions in addition to their conventional ones and our study confirms that NKT cells and  $\gamma\delta$  T cells have unconventional subsets.

## Combination of IL-10 promoter polymorphisms and HLA-DRB1 alleles in MS patients within Iranian population

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**Introduction:** Multiple Sclerosis (MS) is the most common demyelinating disorder among young people with inflammatory, autoimmune and neurodegenerative characteristics. It is widely accepted that susceptibility to multiple Sclerosis involves a complex interaction between many genes and the environment. Interleukin 10 (IL-10) is an anti-inflammatory cytokine mainly produced by macrophages and T Lymphocytes during autoimmune and infectious diseases. Progression or recovery of MS seems to have direct association with the amount of IL-10 production. Regarding of increasing incidence of multiple sclerosis in Iran and lack of data about genetic background of Iranian population, we have conducted a study to determine the genetic profile of MS patients and the impact of polymorphisms on susceptibility or resistance to MS in our population.

**Materials and Methods:** We screened 126 MS patients and 135 healthy controls for genetic variations in the promoter region of IL-10 gene and HLA-DRB1 alleles. Genomic DNA was extracted from 10 ml peripheral whole blood by standard protocol with some modifications. . SSP-PCR method was used for genotyping. The PCR reaction was performed in a Thermal Cycler. HLA-DRB1 alleles (23 alleles) were determined by using a low-resolution panel of allele-specific PCR primers along with a positive control primer according to manufactural protocol. Genotype and Allele frequencies were calculated and compared between groups by non-parametric tests.

**Results:** At position -1082 a significant association was found between G/G genotype and the risk of MS. Also G/A genotype had different frequency among DRB1\*16 negative subjects. In addition patients with IL-10 -1082 G/A genotype that were DRB1\*03 positive had more severe disease.

**Conclusion:** We have found that IL-10 SNPs might have an important role in the susceptibility to MS and its course at least in Iranian population, particularly when come together with specific HLA-DRB1 alleles

## Establishment of peptide-based allergy testing

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**Background:** Celery is a frequent cause of food allergy in pollen-sensitized patients and may induce severe allergic

reactions. Clinical symptoms cannot be predicted by skin prick tests or allergen-specific IgE determinations. By epitope-based specific recognition patterns it might be possible to improve the predictive value of specific IgE testing.

**Objective:** To determine IgE-binding patterns of Api g1, the major allergen of celery, and to establish a diagnostic tool for the discrimination between celery allergic and celery sensitized patients.

**Methods:** 21 patients with positive double-blind placebo-controlled food challenge to celery underwent skin tests along with determination of specific IgE. To identify and characterize IgE-binding patterns of Api g1 we used overlapping peptides which were prepared by SPOT-synthesis technology. Binding studies were performed on cellulose supported peptide arrays using sera from celery allergic patients and healthy controls. Binding peptides were detected by HRP-labeled anti-human IgE by chemiluminescence.

**Results:** For the detection of IgE-binding patterns we have developed an improved immunoassay protocol. Sera of celery allergic patients show binding to 12 amino acid short peptide sequences of Api g1. In contrast, no binding of one sequence was detected in sera of all 20 healthy controls. Application of our assay allows us to distinguish between celery allergic and healthy patients.

**Conclusion:** Our results indicate that the SPOT technology represents a valuable tool for allergy testing and identifying epitope-based sensitization profiles. In summary, the development of a peptide based diagnostic tool for the discrimination between celery allergic and healthy controls was proven. In the next step the discrimination between celery allergic and celery sensitized patients will be established.

## Monocyte cells in the CNS

ESC-ID **957**  
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The aim of our project is to identify the in vivo migration paths of blood monocytes in zones of axonal degeneration – induced by entorhinal cortex lesion (ECL) or spinal cord injury (SCI) in mice – and further in the draining peripheral lymph nodes. From the literature we already know that other leukocytes, like for example T-cells migrate out of an ECL to cervical lymph nodes via the cribroid plate and the nasal mucosa (Goldmann et al., 2006). Earlier investigations of the fate of blood derived monocytes after brain injury showed that they are able to differ into resident microglia cells (Bechmann, 2005). Using citrate coated iron oxide nanoparticles as Magnet resonance imaging (MRI) contrast agent, we want to label and track immune cells in vivo. First I had to adapt a purification procedure for blood derived monocytes. Therefore I took the blood of mice via cardiac perfusion, followed by density gradient centrifugation. After regeneration in a high FCS (fetal calf serum) concentration for one hour the highly adherent cells were purified by magnetic-labelled antibody cell sorting (MACS) for primary

cell culture. The sorting procedure is based on the CD11b surface antigen expressed on monocytes. The purity of the cells was checked via Fluorescence activated cell sorting (FACS) by the Ly6c surface antigen (CD115). By cell sorting via CD115 we are also able to split the monocytic population in two different groups, that are already known for their unequal migratory and differentiation fate (Geismann, 2008/Gordon 2005). For the validation of the labelling experiments MRI-studies were performed with cell-phantoms composed of different cell concentrations in 1% agar. These experiments were carried out with different types and concentrations of very small iron oxide particles (VSOPs) and variable incubation times. Thus we got some information about the best label conditions and particle type. In the next months I will perform some cell function tests to find out the best nanoparticle with the least interference on normal monocytic activity. A cytokine analysis of the monocytic cells during the labelling experiments was performed with a multiplex system: Multiplexing works like a soluble sandwich-ELISA-system with different sized Microbeads as a coating surface. The size differences can be analyzed using FACS, so that many different cytokines in the cell culture supernatant can be analyzed in one experimental set-up. Preliminary results indicate so far, that a synthesized nanoparticle fraction with a diameter of circa 7nm gives the best contrast for low cell concentrations. These results also show that the incubation period for labelling monocytic cells should be finished after 18 hours. Longer incubation times only show a slight increase in contrast and results in a strong differentiation and activation potential in the monocytes. Charite, Center of Anatomy, Institut of Cell Biology and Neurobiology Mentor: Prof. Dr. Robert Nitsch Project supervisor: Dr. Jana Glumm Co-Authors: Dipl. Bioengineer Martin Pohland, student Miriam Kaminski, Prof. Dr. Eyk Schellenberger and Dr. Jörg Schnorr Author: Dipl. Biologist Matthias Stöcker

## Infectiology

### Higher activation of TLR9 in plasmacytoid dendritic cells by microbial DNA compared to self DNA based on CpG specific recognition of phosphodiester DNA

ESC-ID **952**  
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Toll-like receptor 9 (TLR9) detects DNA in endo-lysosomal compartments of human B cells and plasmacytoid dendritic cells (PDC). Recently the concept of CpG motif specificity of TLR9-mediated detection, specifically of natural phosphodiester DNA, has been challenged. Unlike in human B cells, CpG specificity of natural phosphodiester DNA recognition in human PDC has not been analysed. Here we found that the induction of IFN- $\alpha$  and

TNF- $\alpha$  in human PDC by phosphodiester oligodeoxynucleotides containing one or two CG dinucleotides was reduced to a lower level when the CG dinucleotides were methylated, and was abolished if the CGs were switched to GCs. Consistent with a high frequency of unmethylated CG dinucleotides bacterial DNA induced high levels of IFN- $\alpha$  in PDC; IFN- $\alpha$  was reduced but not abolished upon methylation of bacterial DNA. Mammalian DNA containing low numbers of CG dinucleotides which frequently are methylated consistently induced IFN- $\alpha$  in PDC but on a much lower level than bacterial DNA. For activation of PDC both phosphodiester oligodeoxynucleotides and genomic DNA strictly required complexation with cationic molecules such as the keratinocyte-derived antimicrobial peptide LL37 or a scrambled derivative. In conclusion we demonstrate that self DNA complexed to cationic molecules activate PDC and thus indeed may function as damage-associated molecular pattern (DAMP); nevertheless, the preference of PDC for CpG containing DNA provides the basis for the discrimination of microbial from self DNA even if DNA is presented in the condensed form of a complex.

### Analysis of autopsies of HIV-infected died in Gomel region

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*Introduction:* The HIV-infection causes heavy defect of cellular immunity, preparing favorable circumstances for development of tumors and opportunistic infections which most often are the cause of death of these patients[1]. The largest number of HIV-infected in the Republic of Belarus is registered in Gomel region. The greatest number of people who died of HIV-infection in AIDS stage, is registered in Svetlogorsk and in Gomel.

*Aim:* In this research we determined and analyzed the structure of pathology among those died from a HIV-infection in the Gomel region for the period 2001-2008.

*Materials and methods:* In this research were used stuffs of the pathoanatomical conclusions and tissue specimens of the HIV-infected who died during the period of January, 1st 2001–December, 31st, 2008. The stuffs of the pathoanatomical conclusions and tissue specimens were taken from Svetlogorsk and Gomel departments of Gomel regional clinical bureau of pathology. The tissue specimens were imbued by a hematoxylin and eosine, on Van-Gizon, on Cill-Nilsen, on PAS.

*Results:* During the period of 2001–2008 there were 287 cases of death among the HIV-infected. And pulmonary tuberculosis was found on 219 (76,3 %) autopsies. The main clinico-anatomic forms of pulmonary tuberculosis that were detected were: fibro-cavernous tuberculosis and caseous pneumonia in 54 (24,7 %) cases, and hematogenically-disseminated forms in 165 (75,3 %) cases. Tuberculosis of intrathoracic, abdominal and peripheral lymph nodes has been noted in 111 (38,7 %) cases. Tubercular pleuritis has been found on 43 (15 %) autopsies. Tubercular meningoencephalitis has been detected 78 (27,2 %) persons. Tuberculosis of other localizations (a larynx, an intestine, a middle ear, kidneys) was detect-

ed 27 (9,4 %) patients. Candidosis infection contamination has been detected in 198 (68,9 %) persons. Onychomycosis was found in 17 (5,9 %) cases of death. Infection contaminations of a virus etiology have been found on 155 (54 %) autopsies. Most often, the virus infection contamination has been presented by HBV and HCV in 150 (96,8 %) cases. Cytomegalovirus infection has been detected at 3 (1,9 %) patients and had a form of interstitial pneumonia. Virus Epstein-Barr lesion has been revealed in 2 (1,3 %) cases. Cryptococcosis has been detected on 11 (3,8 %) autopsies. Pneumocystic pneumonia was found in 27 (9,4 %) cause of death. The oncological pathology has been revealed only at 2 patients (1 case of sarcoma Caposhi and 1 case of astrocitoma) in 2006. In 2007 there were detected 2 (0,6 %) cases of squamous cancer of the cervix. As of, 1st, 2008, bilateral multifocal primary lymphoma of the brain has been found only on one autopsy of the HIV-infected patient.

*Conclusion:* The presented stuff testifies that in the hematogenically-disseminated forms of the tuberculosis, with a high specific gravity of specific complications are the most frequentness in the pathology structure of the AIDS stage. There was detected the high frequency of lymph nodes lesions. Candidosis infection, HBV, HCV have the highest frequency.

### Metabolic syndrome in HIV-positive patients with antiretroviral therapy as a risk of heart disease?

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*Introduction:* In recent period morbidity and mortality of HIV-infection has dramatically increased in Ukraine. Accordingly to official statistics on September of 2008 in Ukraine in 137973 persons diagnosed HIV, and 14705 died of AIDS. The average age of patient with HIV is nearly 25-28 years. High active antiretroviral therapy (HAART) is a chance for this young people. Clinical and immune results of HAART cannot be disputed but there are many other problems with distant prognosis.

*Aim:* to research clinical and metabolic consequences of HAART in patients with HIV-infection.

*Material and Methods:* Retrospective investigation (2004-2008) of 100 patients with HIV-infection was performed. Therapy prescription, laboratory and clinical observation have been worked under WHO protocol. Laboratory investigation were performed 4 time in year.

*Results:* HAART was prescribed in 65% patients with III clinical stage, in 10% - IV stage of HIV (WHO guideline 2006). HAART was indicated in dependence of immunologic and virologic criteria for 5 % and 21% patients respectively. Patients with HIV had been co-infected by HCV, HBV (80%), more rarely and HDV in 5% cases. In 65% of these patients diagnosed HCV+HBV chronic hepatitis. Isolated elevation of cholesterol level was found in 5% cases, triglycerides - 35 %, and high-density lipoprotein - 15%. Increasing of high-density lipoprotein and cholesterol (>1-2 normal level, p 7 mmol/l. Mild arterial hypertension (140-145/95-100) was diagnosed in 5 %

patients with ART with more 2 years treatment. Average age of this group of patients is equal to 35+3,5 years. In 5 % patients diagnosed lipodystrophy. In 3% patients observed pancreatitis. Metabolic disturbances developed after 1 year therapy (1.5+0.4 year p2-4 normal level, p 45 mmol/l).

*Conclusion:* Metabolic disturbances in patients with HAART are one of common side effects which has influenced in life quality of this patients. The questions life prognosis, drug corrections and prediction have arisen behind medical researchers in condition of lifelong HAART. Metabolic syndrome in HIV patients with HAART may leads to heart disease.

### Acute viral hepatitis B - Clinico-evolutive profile

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Viral hepatitis B is a serious global infection in our country, being a continual medical and epidemiological problem. Objectives are to evaluate retrospectively the epidemiologic and clinical findings of viral hepatitis B on 316 patients, hospitalized in the Clinic of Infectious Diseases of Iasi, during 5 years (2003-2008). The majority of the patients had an urban origin (83,86%), with the predominance of the teenagers (58,86%) and young adults (23,73%). The mean incubation period was 4 months, 24% of patients had a prolonged form of disease and a fulminant evolution in 0.94% of cases. The mortality was 1.9% of cases.

*Key words:* Fulminant, Jaundice, Incubation

### Infectious disease diagnostic tools and African society

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The fight to improve global health needs, in addition to effective public health measures, requires rapid and efficient diagnostic tools; new vaccines and drugs, efficient delivery methods, and novel approaches to therapeutics; and low-cost restoration of water, soil, and other natural resources. Infectious diseases like Malaria, Tuberculosis, HIV/AIDS, Upper respiratory tract infections, Hepatitis and Helminthes infestation together with Diarrhea diseases in so many times go undetected. Diagnostic efforts are slow and expensive, and require specialized facilities and skilled technicians. The disease may be preventable or treatable, but without effective diagnostics, millions suffer. Yet the benefits of modern medicine have still not reached millions of people in developing countries like Tanzania. It is crucial to recognize that science and technology can be used very effectively in partnership with conventional public health practices in developing countries and can enhance their efficacy. Biotechnology has tremendous potential to address health and development

issues in developing countries if we rise to the challenge. Development of new biotechnology, especially in medicine, is essentially the domain of developed nations, and in Africa. In this study I discuss the risks and benefits of biotechnology especially regarding human health in developing countries. The emphasis will be on promoting translational research aimed at bringing basic knowledge through to clinical application, the integration of expertise from different disciplines will be essential in this area. The formation of partnerships between public and private institutions, as well as the involvement of research groups from developing countries to be a major goal. Thus this presentation deals with new diagnostic tools which are more accurate and easy to perform for attainment of the MDGs with example from an experience of Tanzania.

### **Pneumolysin activates the NLRP3 inflammasome**

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*S. pneumoniae* is a major cause of community-acquired pneumonia with high early mortality rates, and of bacterial meningitis. One of its key virulence factors is the pore-forming toxin pneumolysin (PLY). The host innate immune system detects invading pathogens by e.g. Toll-like receptors (TLRs) and NOD-like receptors (NLRs). Different NLRs form cytosolic multiprotein complexes called inflammasomes which mediate caspase-1-dependent processing of pro-IL-1 $\beta$ . Here we show that PBMCs infected with *S. pneumoniae* or stimulated with purified PLY produced IL-1 $\beta$ , whereas cells infected with *S. pneumoniae* lacking PLY or expressing a non-cytolytic PLY showed strongly impaired IL-1 $\beta$  response. RNAi experiments in human PBMCs as well as experiments in Nlrp3 KO BMM revealed that IL-1 $\beta$  production was dependent on NLRP3, whereas NLRP1, NLRP6 and NLRP12 appeared to be dispensable. Furthermore, experiments indicate that cathepsin B, ROS and K<sup>+</sup>-efflux were involved in *S. pneumoniae*-induced inflammasome activation. Overall, our results indicate that *S. pneumoniae*-infected host cells produced IL-1 $\beta$  depending on PLY and on NLRP3.

### **Evolutionary distances between mRNA of Homo sapience and Trichinella spiralis**

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Trichinosis, also called trichinellosis, or trichiniasis, is a parasitic disease caused by eating raw or undercooked pork infected with the larvae of a species of roundworm *Trichinella spiralis*, commonly called the trichina worm. Co-evolution of *T. spiralis* and human is well researched except of molecular co-evolution. Understanding of co-evolutionary mechanisms of trichina worm and human is

very impotent as fundamental knowledge and also lets us to prevent and explain resistance of *T. spiralis* to the antiparasirical drugs. Potential marker of co-evolution in "parasite-host" system is evolutionary distance [1] between a pair of sequences usually is measured by the number of nucleotide (or amino acid) substitutions occurring between them. Evolutionary distances are fundamental for the study of molecular evolution and are useful for phylogenetic reconstruction and estimating of divergence time. Evolution dictation consists of two compounds – synonymous and non-synonymous. Synonymous substitution in DNA chain does not cause a change of amino acid in a protein. Non-synonymous substitution in DNA chain causes a change of amino acid in protein. So, synonymous distance is the number of synonymous substations per synonymous site. Non-synonymous distance is the number of non-synonymous substations per non-synonymous site. The estimation of synonymous and non-synonymous distances is actually because ones are more sensitive markers of co-evolution. The aim of this research is estimation and analysis of evolutionary distances, synonymous and non-synonymous distances between mRNA coding 12 enzymes of the respiratory chain of *Homo sapiens*, *Trichinella spiralis* and *Caenorhabditis elegans* (control). *Caenorhabditis elegans* is a free-living nematode (roundworm), about 1 mm in length, which lives in temperate soil environments. We used 36 sequences from NCBI (National Center for Biotechnology Information [2]) of mitochondrial mRNA coding 12 enzymes respiratory chain (NADH 1, NADH 2, NADH 3, NADH 4, NADH 4L, NADH 5, NADH 6 dehydrogenases, cytochrome b, cytochrom-c 1-3 oxydase, ATP-synthetase 6) of *Homo sapiens*, *Trichinella spiralis* and *Caenorhabditis elegans* (control). Values of distances obtained with computer program MEGA 4 [3]. Values of evolutionary distances between *T. spiralis* and *H. sapiens* are statistically different and lower from one between *T. spiralis* and *C. elegans* in 8 out 12 (NADH 1, NADH 3 NADH 4, NADH 6 dehydrogenases, cytochrome b, cytochrom-c 1-2 and ATP-synthetase 6). Values of synonymous and non-synonymous distances have improved these results. From our point of view this difference is explained with bigger functional activity of these enzymes. The results of our research shows that co-evolutionary processes between *Trichinella spiralis* and *Homo sapience* really take place and we should pay more attention for molecular evolution in medical parasitology.

### **Pharmacoeconomics in treating urinary tract infections**

ESC-ID **264**  
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*Aim:* The aim of this study is to assess the therapeutic efficiency of the antibacterial drugs used in treating urinary tract infections and to assess the pharmacoeconomic justification of their use. Efficiency and cost price of the physicians' therapy of choice and the therapy according to the pharmacoeconomic guidelines in developed countries were compared.

*Material and methods:* A study was conducted at the Infectious Disease Clinic of the Vojvodina Clinical Center

which included 100 patients diagnosed for urinary tract infections divided into two groups of 50 patients. Control group patients were treated with the physicians' antibacterial therapy of choice and the efficiency and cost price of the therapy was determined. Study group patients were treated according to the developed countries pharmacologic guidelines and the efficiency and cost price of the therapy was determined.

**Results:** Based on the compared efficiency and cost price of urinary tract infection therapy before and after the introduction of the pharmacoeconomic guidelines, it was determined that there were no significant differences in the efficiency of the used therapies, while there were significant savings in cost price (35.67%) after the guidelines were introduced.

**Conclusion:** Efficiency of the physicians' antibacterial therapy of choice for urinary tract infections and the therapy according to the developed countries pharmacologic guidelines are equal. In terms of costs, the cost price of the therapy according to the guidelines used in countries with a developed pharmacological practice was more favorable.

## Microbiology

### Antibacterial activity of biocompatible polymer coatings with ciprofloxacin extended release

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 Institution **Gomel State Medical University**

Prevention of implant-associated infection is a major problem in developing of new biomaterials. Formation of bacterial biofilms on the polymer surfaces leads to persistent bacteraemia, fail of antibacterial therapy and selection of resistant strains. The aim of study is to estimate the antimicrobial activity of biocompatible polymer coatings with ciprofloxacin extended release in vitro, and to compare different coating bases – polyurethane and polyethylene by ability to form with ciprofloxacin a stable antibacterial composite.

**Materials and Methods:** Thin composite coat based on polymers (polyurethane and polyethylene) was applied on a gauze substrate in active gaseous phase generated by electron beam dispersion under vacuum. For estimation of antimicrobial activity 4 groups of samples of gauze 15x15 mm were made in aseptic conditions: 1- gauze with ciprofloxacin monocoating; 2- gauze with composite coating based on polyethylene (components ratio 1:1); 3- gauze with composite coating based on polyurethane (components ratio 1:1); 4- gauze without coating. All samples were underwent cycled washing in sterile saline warmed up to 37°C. Each of samples was exposed to 4 series of 5/10/20/40 cycles of washing. Then samples were shaken in 5 ml of Mueller-Hinton broth (MHB) and inoculated with 100 µl suspension of 24h cultures of *S.aureus* (ATCC 25913, Ciprofloxacin MIC 0,12-0,5 mg/L) and *E.coli* (ATCC 25922, Ciprofloxacin MIC 0,004-0,016 mg/L) with McFarland optical density 0,5 (1,5x10<sup>9</sup> CFU/ml). Samples and controls were incubated

in 37°C for 24 h. Results were accessed visually – absence/presence of bacterial growth.

**Results:** There was absence of growth of *S.aureus* and *E.coli* ATCC 25922 in any of samples and in positive control (1, 2, 3 sample and MHB with ciprofloxacin 0,5 mg/L), and presence of growth in negative controls (sample 4 and MHB without ciprofloxacin). It indicates of preserving antimicrobial activity of ciprofloxacin while coating the gauze with and without polymers using electron beam dispersion, suggesting of absence of any molecular changes in stages of dispersion and condensation. Monocoated samples had no resistance to leaching antimicrobial activity, it was not revealed as early as after 5 cycles of washing. Composite coating with polymers has much higher stability. Sample 2 preserved antimicrobial activity after 20 cycles, and sample 3 after 10 cycles of washing. Influence of polymer matrix on antimicrobial activity stability can be explained by morphological features of coating, leading to more effective absorption of ciprofloxacin with polyurethane.

**Conclusions:** Developed composite polymer coatings have a high antimicrobial activity to reference strains. The composite coats based on polyurethane and polyethylene were stable to ciprofloxacin leaching in isotonic sodium chloride solution. Polyurethane with ciprofloxacin forms more stable antibacterial composite than polyethylene with ciprofloxacin. Created polymer coatings can be effectively used for biofilms formation prevention on implant's surface.

### Resistance to carbapenems and metallo-beta-lactamases production of *P. aeruginosa* isolates in Belarus

ESC-ID **905**  
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*Pseudomonas aeruginosa* is the most common causative agent of nosocomial infections. Recent studies showed a rise of resistance of *P.aeruginosa* to almost all antibiotics. First-line treatment of severe infections caused by *P.aeruginosa* is carbapenems. Spread of metallo-beta-lactamases (MβL) in bacterial population leads to ineffectiveness of these antibiotics.

**Aim:** To estimate the prevalence of antimicrobial resistance among *P.aeruginosa* isolates in Gomel, Belarus, and to reveal MβL-producing multiresistant strains.

**Materials and methods:** About 197 *P.aeruginosa* isolated from different clinical samples (sputum, blood, wound secretion, exudates and urine). Repeated isolates from same person were excluded. All patients were undergone treatment in 9 in-patient and 11 out-patient clinics of Gomel. All samples were identified using routine microbiological tests. Antimicrobial resistance to ampicillin (Am), gentamycin (Ge), tobramycin (To), imipenem (Im), ciprofloxacin (Ci), cefoperazone (Cp), cefepim (Cm), ceftazidime (Cz) were evaluated using disc-diffusion method on Mueller-Hinton agar. Internal quality control performed with use of *P. aeruginosa* ATCC 27853 referent strain. For imipenem-resistant isolates phenotypical screening of MβL-producing was carried out using EDTA-double-disc synergy test. For MβL-positive isolates multiplex real-

time polymerase chain reaction analysis were performed to detect blaIMP and blaVIM genes.

**Results:** Imipenem were the most effective antibiotic (only 91.4% of sensitive stains). Sensitivity to cefoperason, ceftazidim and cefepim were significantly lower (15.7, 23.9 and 15.7% of resistant strains respectively). Aminoglycosides had apparently higher activity (amikacin – 41.6% of sensitive strains, gentamycin and tobramycin – 30.5 and 33.0% respectively). The most prevalent resistance profile was AmGeToCiCpCzCm (resistance to amikacin, gentamycin, tobramycin, ciprofloxacin, cefoperason, ceftazidim, cefepim). A total of 38.6% of stain has such profile. The second most prevalent resistance profile was AmGeToCiCpCzCmIm (resistance to all tested antibiotics). Such profile was observed in 13 (6.6%) strains. Only 4.1% of strains included in study has “zero” resistance phenotype (sensitivity to all tested antibiotics). All these stains were isolated from out-patients. In 3 of 13 multiresistant carbapenems resistant strains in EDTA-test production of MbL were revealed. These stains were isolated from surgical and intensive care unit’s patients of large multidisciplinary hospitals of Gomel. MbL-production were verified with multiplex PCR. All isolates were assigned to VIM-type (presence of blaVIM gene).

**Conclusion:** Isolation of poly- and multiresistant *P. aeruginosa* stains in certain hospitals of Gomel city requires introduction of epidemiological control of its circulation. Assuming easiness of MbL-producents spread, there is a necessity of introduction of infection control measures to prevent its drifting to another city hospitals.

### The importance of Immunoblot RIBA test in the differential diagnosis of acute and chronic HCV infection

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**Introduction:** Immunoblot RIBA test, that has a function of confirmatory assay in HCV infection, detects antibodies against recombinant HCV antigens ( C22-3, C33-c, C100-3 i 5-1-1).

**Aim:** Aim of the work was to determine the importance of Immunoblot RIBA test in the differential diagnosis of acute and chronic HCV infection.

**Methods and material:** Investigation included 60 patients with acute and 60 patients with chronic HCV infection. HCV infection diagnosis was made on the base of the increased transaminase values ( AST, ALT) and ELISA HCV positivity. All HCV infected patients were tested by RIBA test II generation.

**Results:** All the patients with acute HCV infection had anti C22-3, 95 % anti C33-c, 76,67 % anti C100-3 i 55 % diseased anti 5-1-1 antibodies. Anti C22-3 is the first marker of acute HCV infection. The level of anti C100-3 and anti 5-1-1 antibodies was lower (1+ do 2+) compared to the level of anti C22-3 and anti C33-c (3+ do 4+). Serological status of patients with chronic HCV infection is characterized by the presence of antibodies to all viral antigens.

The antibodies levels to all viral antigens were high (3+ do 4+).

**Conclusion:** Serological status of patients having acute and those having chronic HCV infection is different in type and level of antibodies. Immunoblot RIBA test can be usefil in making a differential diagnosis of acute and chronic HCV infection.

### Common Origin of Chloroquine Resistant Plasmodium falciparum and Subsequent Point Mutations in pfert1 Gene Associated with Chloroquine Resistance in Southern Pakistan

ESC-ID **645**  
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**Introduction:** Molecular studies show four different origins of Chloroquine-resistant *P. falciparum* and their subsequent spread. Flanking microsatellites for pfert1 gene remain conserved among geographical isolates. Furthermore, the pattern of critical mutation at 76th codon involving the K amino acid and the subsequent mutations at the 72nd-75th codon involving the C, V, M, and N amino acids also give the picture of relative-resistance and in addition the location of the isolate. The aim of this study was to find the origin and pattern of chloroquine-resistance in *P. falciparum* in southern Pakistan.

**Method:** In this study, we analyzed 227 chloroquine-resistant *P. falciparum* samples from the southern Pakistan region. We characterized point mutations in the pfert1 gene involved in Chloroquine resistance. We also assayed neutral microsatellite markers around the pfert1 gene to track the origin and spread of resistant parasite.

**Results:** Of the 227 samples, 97 showed positive results for genotyping of the 72nd, 73rd, 74th, 75th and 76th codon. 4 groups of genotypes were identified; SVMNT (83.5%), CVIET (1.0%), CVMNK (9.3%) and genotypes that did not correspond to known genotypes in the literatures(6.2%). 223 samples showed positive results for microsatellite assaying with majority of isolates having a single microsatellite haplotype which is similar to that found in India and Papa New Guinea. Our results indicate that Chloroquine-Resistant *P. falciparum* type that originated in Papa New Guinea and has spread to Indian region is the most common type found in southern Pakistan.

**Conclusion:** This is the first report of microsatellite analysis combined with characterization of pfert1 gene in the southern Pakistan region. This would be helpful in discerning the population genetics of Chloroquine-resistant *P. falciparum* in this region and in designing ‘Malaria Control Policies’ and principally in formulating drug policies.

### Analysis of the influence of attending clinical classes on the carrier-state of MRSA among students of the Medical University of Warsaw

ESC-ID **684**  
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 Institution **Medical University of Warsaw**

*Introduction:* Staphylococcus aureus is one of the most significant sources of human bacterial infections. Carrying of Methicillin-Resistant Staphylococcus aureus (MRSA) is a serious problem among surgical patients and hospital staff. Moreover, in accordance with the Polish law, MRSA strains are the "alert" organisms, because of its resistance to almost all beta-lactams except the latest, fifth generation cephalosporin – Ceftobiprole. The aim of our study was to assess whether attending clinical classes has an influence on the level of the carrier-state of the Staphylococcus aureus among students of Medical University of Warsaw.

*Material and methods:* The first group of our study included 90 third-year students before they begun visiting various wards. Two years later they have been examined once again - at their fifth year of study - after passing two years of clinical classes at hospitals. They were described as the second group, that included 74 students. The investigations were performed appropriately in 2007 and in 2009. They all have been examined for the carrier-state of MRSA. Smears from atrium of middle nasal meatus were taken with swabs, specimens were plated to Chapman medium and incubated for 48 hours. If any growth occurred, staphy-slide-test and the test for coagulase production were used for S. aureus identification. Strains recognized as S. aureus were screened for sensitivity to cefoxitin.

*Results:* In the first group of the third-year students there were 4 S. aureus carriers (4.4%). Among the fifth-year students we isolated 12 S. aureus carriers (16.2%). There were more MSSA (Methicillin-Sensitive Staphylococcus Aureus) carriers among students after attending clinical classes. We didn't isolate MRSA strains in the group of the third-year students neither in the second group.

*Conclusion:* Attending clinical classes increases level of carrier state of Staphylococcus aureus. However, it doesn't have influence on carrying of MRSA strains. Our study indicate that population of medical students should not be considered as a significant source of infection for patients.

## Nephrology

### Efficacy of subcutaneous terbutaline compared with inhaled albuterol for treatment of hyperkalemia

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*Introduction:* Hyperkalemia is a frequent and potentially lethal medical emergency in patients. Dialysis is the

definitive treatment of these patients. Beta2 adrenergic agonists cause potassium cellular shifting and lower in plasma potassium concentration.

*Material and Methods:* Study design: clinical trial (Randomized Controlled). Twenty patients between seven to seventeen years old with indication of dialysis were divided into two groups: terbutaline 7mg/kg, subcutaneously in ten patients and inhaled albuterol 2.5 mg/kg were administered in other patients. The level of potassium was measured q1h until 6h. We compared effects of terbutaline and albuterol on potassium responses and heart rate.

*Results:* We expect using albuterol is associated with fewer complications such as heart rate elevation, but lowering of potassium is significant in using terbutaline compared with albuterol.

### NPHS2 Mutations in Children With Steroid-Resistant Nephrotic Syndrome

ESC-ID **338**  
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*Introduction:* Idiopathic nephrotic syndrome (INS) has prevalence of 15.7 per 10 children (D). A growing number of genes have been identified that, when defective lead to inherited forms of INS. Podocin (NPHS2) mediated FSGS seems to be the most common inherited form of this set of disease. We evaluated the presence of a probable mutation in most common exons of podocin gene which is involved in steroid resistant nephrotic syndrome (SRNS). *Methods and Materials:* We studied 20 children with SRNS, which defined as the failure to respond to 4-8 weeks of daily prednisolone, from the onset of the disease. On first admission, blood pressure, serum creatinine concentration and hematuria were determined in these children. Genomic DNA was isolated from peripheral blood leukocytes. Mutation analysis was performed in fifth and seventh exons of the NPHS2 gene with the direct DNA sequencing method; and amplified by polymerase chain reaction (PCR). All PCR primers were designed from intronic sequences.

*Results:* Twenty children suffering from SRNS (10 girls and 10 boys) with mean age.  $5.8 \pm 3.8$  years. Ranges between 1 to 12 years. Of the 20 SRNS Iranian patients in our study, no mutations were found in fifth and seventh exons of NPHS2 gene.

*Conclusion:* Our study suggests that NPHS2 mutations exons 5 and 7 are not seen in our children. Therefore, we can not recommend NPHS2 (exons 5 and 7) mutation screening in Iranian children with resistant nephrotic syndrome. Additional studies are needed to confirm this finding.



## Cellular trafficking and phosphorylation of the bumetanide-sensitive Na<sup>+</sup>,K<sup>+</sup>,2Cl<sup>-</sup>-cotransporter NKCC2 are facilitated by Tamm-Horsfall protein

ESC-ID **123**  
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The bumetanide-sensitive Na<sup>+</sup>,K<sup>+</sup>,2Cl<sup>-</sup>-cotransporter (NKCC2) is the major salt transport pathway of the thick ascending limb of Henle's loop (TAL). Activity of NKCC2 is regulated by intracellular protein trafficking and phosphorylation which can be stimulated hormonally by vasopressin (AVP). Little is currently known on the proteins involved in these events. We have shown previously that lack of the co-expressed product of TAL, Tamm Horsfall protein (THP), impairs urine concentration in water-deprived mice (Bachmann et al., Am J Physiol 288: F559-F567, 2005). Here we hypothesize that THP modulates NKCC2-related functions of TAL. THP-deficient mice (THP<sup>-/-</sup>), wild-type controls (WT), and cultured TAL-cells transfected with THP were analyzed by morphological, cell biological, and functional methods. THP and NKCC2, which share their partial association with cholesterol-enriched membrane rafts, were intimately co-localized in immunoelectron microscopy. THP<sup>-/-</sup> mice demonstrated a significantly decreased baseline phosphorylation status of NKCC2 (-49% compared to WT). In cultured cells, transfection with THP markedly increased the baseline phosphorylation of NKCC2 (+38%). Lack of THP was further associated with intracellular accumulation of NKCC2 at steady state and blunted its apical sorting and phosphorylation after short term AVP treatment (1 μg/kg) in mice. AVP-induced shifts of immunoreactive NKCC2 to detergent-resistant low density fractions were also diminished in THP<sup>-/-</sup> mice. Functional and structural adaptation of the distal convoluted tubule, along with strongly enhanced phosphorylation of the Na<sup>+</sup>,Cl<sup>-</sup>-cotransporter (NCC), suggested impaired NaCl reabsorption in TAL in THP deficiency. These results suggest a permissive role for THP/uromodulin in the modulation of NKCC2-dependent TAL salt reabsorptive function.

## The Advantages of Bipolar Endoscopic Resection of Superficial Bladder Tumors

ESC-ID **480**  
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*Introduction:* The trans urethral resection of superficial bladder tumors using a bipolar resectoscope is an endourological technique introduced recently, which uses saline solution (instead of water), closing the electrical circuit inside the bladder, without the need for electricity to cross

the body. The objective of our study was to compare the clinical efficacy and safety of bipolar resection, compared with mono polar resection in superficial bladder tumors. *Material and methods:* We have investigated the patients with superficial bladder tumors that have been admitted in the Department of Urology from the Timisoara Clinical Emergency Hospital, from January 1 to March 30, 2009. The patients were divided in two groups, depending on the selected therapy: bipolar trans urethral resection was performed on 11 patients and mono polar trans urethral resection was performed on 13 patients. The primary end-point of the study was to quantify the intraoperative and postoperative bleeding and the secondary end-points were to measure the speed of transurethral resection, the time of continuous bladder irrigation, the urinary catheterization time, the eventual occurrence of obturatory reflex, and the histological quality of the tumor and bladder tissue obtained. The resections were performed with a Storz resectoscope, using mono- or bipolar energy. For monopolar resection sterile water was used and for bipolar resection NaCl 9 ‰ solution (saline solution) was used. *Results:* No significant difference was noted between bipolar and monopolar groups in terms of blood loss (the hemoglobin loss was 0.65 g/dL for bipolar vs. 0.81 g/dL for monopolar resection), of average operating time (17.9 min. vs. 20.3 min.), urinary catheterization time (2.1 days vs. 2.3 days). No significant obturatory reflex has occurred in the cases with bipolar technique, compared with 3 cases in the monopolar group. From the pathologist's view, the bipolar resected tissue samples permitted a more accurate staging, due to the fact that thermal injury of the submitted pathological specimens was significantly reduced.

*Conclusions:* Bipolar transurethral resection is safe and effective as a therapy for superficial bladder tumors, reducing the intraoperative bladder perforation rate and diminishing the incidence of the obturatory reflex. The bipolar resection can be used also in patients with cardiac pacemakers. The bladder tissue samples obtained by bipolar resection are of better histological quality than the samples obtained from monopolar resection.

## Demographic changes in patients with Balkan endemic nephropathy on the dialysis in north-eastern Bosnia and Herzegovina

ESC-ID **537**  
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*Introduction:* Balkan endemic nephropathy (BEN) is a chronic tubulointerstitial disease of unknown etiology. A high frequency of urothelial atypia, occasionally culminating in tumors of the renal pelvis and urethra, is associated with this disorder. Affected patients most commonly reside in southeastern Europe, including the areas traditionally consider to comprise the Balkans: Bosnia and Herzegovina (BiH), Serbia, Croatia, Romania and Bulgaria. A striking observation is that nearly all affected patients were farmers. In some regions in BiH, approximately 10 percent of the patients on dialysis have BEN as the cause of end-stage renal disease (ESRD).

*Aim:* The aim of this study was to examine potential

demographic changes in patients with BEN on the same dialysis centers in north-eastern BiH (Bijeljina, Samac, Brcko) before and after conflict in our country (1992-1995). Examinees and method In north-eastern BiH these are dialysis centers in endemic nephropathy area, in Bijeljina, Samac and Brcko. We prepared questionnaire for all the patients on dialysis in these centers, with special overview on patients with BEN. At the moment there are 273 patients on dialysis in these centers and we examined 206 (75,45%) patients. In our questionnaire we focused on the next data: age, gender, place of birth and place of residence, is BEN the cause of ESRD. We compared data from published data before war with our results.

**Results:** Of 206 examinees, 123 (59, 71 %) has BEN as cause of dialysis, 73 (35,44 %) are others disease and 10 (4,85 %) don't know what is their disease. There are 117 (56,80 %) males and 89 (43,20 %) females. We compared our results with results before conflict when in Bijeljina were 74 out of 89 patients with BEN (83,15 %) and now there are 71 out of 101 patients with BEN 70,30 %. In Samac and Brcko were 54 out of 109 patients with BEN on dialysis (49,54 %), and now there are 52 out of 115 patients (45,22 %). We also questioned all patients about their occupation and 62 patients (31,1 %) on dialysis are farmers and 43 (69,35 %) of them have BEN, 80 patients (38,83 %) are housewives and 50 of them (62,5 %) have BEN, rest 64 (31,07%) have other occupation and 30 of them have BEN (46,88%). We used descriptive statistic to compare our data including statistical significance (two-tailed).

**Conclusion:** Due to demographic changes in our country, many people change their place of residence and went to refugeism but there is still similar number of patients with BEN in dialysis centers in north-eastern BiH and that lead us to conclusion that even we don't know the real etiology for BEN we still can be sure that farmers and people from villages are more exposed to risk from BEN.

### **Obesity paradox in chronic hemodialysis patients and the effect of obesity on chronic renal failure**

ESC-ID **96**  
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**Introduction:** The obesity, which is determined as BMI > 30 kg/m<sup>2</sup> has an increasing prevalence whole the world. The obesity has fatal complications and the most frequent and fatal complications are DM, atherosclerosis and atherosclerotic cardiovascular diseases and chronic renal failure. The mortality rate of patients on hemodialysis is about 10-15% and the most frequent death reason is cardiovascular diseases. The latest studies have shown that mainly cardiovascular risk factors which are obesity, hypertension, and high plasma cholesterol provide paradoxwise better survival in patients on maintenance hemodialysis. However, because at which chronic renal failure stage this paradox starts and in whom it develops are not clear, we have aimed by our study to investigate the effect of obesity on stages of chronic renal failure and loss of kidney function.

**Methods:** We have investigated retrospectively the poly-

clinic files of patients, who had been admitted first time to I.U. Cerrahpasa Medical Faculty Nephrology Department within 1998-2004 years. According to National Kidney Foundation, the patients who had GFR0,6407 respectively). And 20% of non-obese and 12% of obese patients had been admitted to dialysis unit at the end of study while 25% of non-obese and 38% of obese patients had been still followed up.

**Conclusion:** In the present study, we have analyzed the effect of obesity on Chronic renal failure. We showed that the traditional cardiovascular risk factors ( blood pressure, serum cholesterol ) and CRP were lower by obese patients, albumine and Hb were higher in obese patients. However we could not find significant difference except for HDL. It can be concluded that initial higher GFR by obese patients has at the end reverse effect on kidneys and therefore a sharp slope occurs by loss of GFR. According to our results, we might say that obesity is a poor prognostic factor for progression of GFR and the obese patients might have a significant chance not to go to dialysis. If it can be clarified by prospective controlled studies, it can be claimed that there is also obesity paradox in patients with Chronic renal failure besides in Chronic hemodialysis patients.

## *Neurology*

### **An adenovirus expressing MyoD induces the myogenesis of human embryonic stem cells**

ESC-ID **476**  
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Human embryonic stem cells (hESC) have an endless self-renewal capacity and can differentiate into all types of cells found in the body. For this reason, they represent an unlimited source of cells for cell therapy of degenerative diseases, such as Duchenne Muscular Dystrophy (DMD). Previous studies have reported the derivation of skeletal muscle cells from hESC but the techniques used in these studies were time-consuming with relative low efficiency. Here, we report a new method to differentiate hESC into skeletal muscle cells using an adenovirus expressing the master gene MyoD under the CAG promoter (Ad.CAG-MyoD). The high efficiency of our adenoviral infection protocol was attested by almost 100% of the hESC staining positive for the transgene. Nearly 60% of the cells positively stained for desmin only five days after the infection with the Ad.CAG-MyoD, confirming their differentiation toward muscle precursor cells. Also, genes expression analysis indicated that the Ad.CAG-MyoD differentiated the hESC into a heterogeneous population of skeletal muscle cells, composed of early (satellite cells) and late (myoblasts) progenitor cells. Thereafter, the fusion potential of these cells was tested immunocytochemically in a low serum medium. Results revealed the presence of myosin heavy chain, a myotube specific marker, and several multinucleated myotubes were formed. These preliminary results indicate that Ad.CAG-MyoD infection is a promising technique to differentiate hESC into skeletal muscle cells that are functional in vitro.

## Oxytocin protects immature cultured hippocampal neurons against oxygen – glucose deprivation

ESC-ID **1027**  
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*Aim:*  $\gamma$ -aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the adult brain. However, in immature neurons, GABA has a depolarizing action and functions as the primary excitatory neurotransmitter. During delivery, maternal oxytocin triggers an excitatory-to-inhibitory switch in GABA signaling in the fetal brain, mediated by a change in intracellular chloride concentration. This transient switch was reported to increase the resistance of fetal neurons to insults during delivery. Our aim was to metabolically assess the protection offered by oxytocin against oxygen-glucose deprivation (OGD) in hippocampal cell cultures both immediately after the insult and during reoxygenation.

*Materials and Methods:* Primary hippocampal cell cultures were prepared from P0 (cin (1  $\mu$ M, Sigma) was blocked using the oxytocin receptor antagonist atosiban (1  $\mu$ M). Bumetanide 10 $\mu$ M (Sigma), which blocks the NKCC1 cotransporter, thus lowering intracellular chloride concentration, was also assessed for a neuroprotectant effect. Statistical significance was calculated using the two-tailed Student's t-test from at least three coverslips from three independent cultures.

*Results:* Cultured hippocampal neurons were excited by 1  $\mu$ M GABA at DIV 2, confirmed by a rise in intracellular calcium observed using Calcium Green imaging. One hour of OGD induced a decrease in metabolic activity to 75%  $\pm$  4 of control values (average  $\pm$  SEM, P ts. In addition, we bring new evidence that oxytocin also protects fetal neurons during the reoxygenation period, an effect shared with bumetanide. These results are significant for the pharmacotherapy of delivery and especially that of pre-term labor.

## Comparison of superficial touch sense - stereognosis - between the blind, amblyopic and people with correct eyesight

ESC-ID **357**  
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*Introduction:* It is commonly believed that the blind are able to recognize shapes and textures of objects better than people with correct eyesight. Even though this conviction is almost a dogma, publications referring to this subject are, at least, not comprehensive.

*Aim:* The purpose of this study was to determine whether the blind and amblyopic has better sense of superficial touch. We analyze statistic differences in stereognosis ability between study group (blind, amblyopic) and control group. We also investigate the influence of age, sex, current profession, everyday use of Braille alphabet, dom-

inant hand on stereognosis in the Study Group. And finally we check whether the time without vision has the correlation with stereognosis in Primary Blind, Secondary Blind or Amblyopic.

*Material and Methods:* Between January 2008 and April 2009 we have examined 65 disabled and 55 people with correct vision. According to study criteria 15 patients were excluded from the evaluation. There were 25 females and 25 males with average age 47,7 (18-60) in study group. Control group consist of 23 females and 28 males, average age 41,6 (18-60). Methods used to evaluate stereognosis: -Semmes-Weinstein Filament Test -Static and Dynamic Two Point Discrimination Test (TPD) - Shape/Texture Identification (STI) -Picking up objects test (Moberg Pick Up Test - MPUT) -ordinary objects recognition test (Moberg Object Recognition Test - MORT ) In addition, medical form including: sex, age, numbers of years without vision, reason of vision loss, using Braille alphabet and dominant hand was taken.

*Results:* This study proved that people with disabled vision have statistically significant ( $p < 0.05$ ) better stereognosis ability than people with correct vision according to STI and MORT. There was no correlation between years without vision and stereognosis in our research, but we find out that years without vision correlates with innervation density in blinds. Other interesting findings showed that females and physical workers recognize objects (MORT) faster and more correct and that everyday Braille using improves texture (STI) identification results.

*Discussion and conclusion:* Our study point out that the blind and amblyopic have better stereognosis ability than people with correct eyesight. What is more, the study proved that there were no differences in the cutaneous sensory and innervation density. These results give a conclusion that the adaptation of stereognosis takes place in central nervous system. Further investigation on a larger study groups are needed to confirm whether numbers of years without vision have any influence on the cortical areas responsible for stereognosis.

## Unruptured cerebral aneurysms - the incidence and localization

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*Introduction:* Cerebral aneurysm rupture is a main cause of subarachnoid haemorrhage, which is the life-threatening state. Most of the saccular aneurysms originate from the point of bifurcation of the main cerebral arteries. The prevalence of unruptured intracranial aneurysms in imaging based studies differs from the autopsy series.

*Aim:* The aim of our study was to evaluate the incidence and localization of cerebral aneurysms on autopsy series using microsurgical method of dissection.

*Material and methods:* The analysis was performed on vessels from 329 preserved brain specimens: 238 male and 91 female (male to female ratio: 2.62), aged from 2 to 94 years old (middle age of men: 49.46, middle age of women: 62.56). Blunt and sharp method of arachnoid mater dissection was used and then each major cerebral artery was examined in details using the surgical micro-

scope. All the found aneurysms were unruptured and of saccular type. The statistical analysis was made with Statistica 8.0.

**Results:** An aneurysm was detected in 32 cases (9.7%). We observed that the most common site of aneurysm occurrence was the anterior communicating artery (ACoA) which was found in 18 cases. That shows that 56% of aneurysms are placed on the ACoA. The second most often affected artery was the middle cerebral artery (MCA) - 6 cases, two times more often on the right side. The basilar artery bifurcation (BAB) was the localization of aneurysm in 3 cases and posterior communicating artery (PCoA) in 2 cases, on the left side. In 5 cases the aneurysm was located on the other brain arteries: left anterior choroidal artery - 2 cases, left posterior cerebral artery - 1 case. Interestingly, relation between number of male and number of female specimens with aneurysm was 3.57 while the same relation in non-aneurysm circle was 2.536, but we can say this is only a trend ( $p = 0.123$ ).

**Conclusion:** Our studies showed that the cerebral aneurysm occurred more often than it was presented in other researches (9.7% in our study to 1-5% in bibliography). They were located more frequently on ACoA than on other arteries. Aneurysms in the anterior part of the circle occurred significantly more frequently than in posterior part. There is a weak trend observed that aneurysms are more frequent in men than in women.

### Effect of Trolox on free-radicals formation in hippocampus of rat exposed to diurnal rhythm disturbance and alcohol

ESC-ID 377  
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**Background:** An insufficient antioxidant defense and excessive accumulation of free-radicals (FR) in the body result in an Oxidative stress (OS), which is involved in development and progress of the Alzheimer's Disease (AlD). The hippocampus is one of the first regions of the brain to suffer damage in AD. Diurnal rhythm disturbance (DRD) and alcohol (A) result in OS in the developing rat hippocampus. The effect of these factors together on the FR formation in the brain is still subject of investigation. Vitamin E reduces the OS level in the brain structures.

**Aim:** To investigate the effect of Trolox (TR, a water soluble Vitamin E) on the FR formation in the hippocampus of rats simultaneously exposed to DRD and alcohol.

**Materials and Methods:** Chemicals of finest grade (SIGMA) were used for preparation of all solutions for this experiment. Four groups of 5 male Wistar Albino rats ( $110 \pm 10$  g each) were used in this research. Two control groups (CT, C) lived at normal light/dark cycle and received tap water during three weeks. For the same period, two other groups (ADT, AD) were exposed to a constant light and were provided with 10% ethanol solution. Groups CT and ADT were treated with 200 mg/kg Trolox (p.o.) every day. All animals received standard rodent chow ad libitum. The hippocampus was removed after decapitation under anesthesia (ether) and immediately stored at  $-81^\circ\text{C}$ . All treatment protocols were in agreement

with the ethic standards in the Medical University of Sofia. Supernatanta was prepared by homogenizing the organs at 2000rpm, at  $0^\circ\text{C}$ , in PBR (pH of 7.5). The amount of proteins was determined, and the FR formation was measured spectrophotometrically, using transformation of MTT to MTT-formazan at  $25^\circ\text{C}$ , in a quartz cuvette. The supernatanta was omitted in the cuvette for the blank measurement. After subtracting the blank measurements, mean values and standard deviations of A(576), and the amount of MTT-formazan (in nmoles) formed in presence of one mg. proteins for 1 minute was calculated. The relative differences between data for the groups were statistically evaluated using the INSTAT program package.

**Results:** Exposure of rats to a combination of DRD and alcohol resulted in a very substantial increasing in the FR formation in the hippocampus, compared to the control group (5.7 times for group AD and 2.2 times for group ADT, compare with groups C and CT, respectively). Within groups living at same conditions, no effect of Trolox on the RF in the hippocampus of rats in groups C and CT was evident. At the same time, the RF formation for group ADT was 36% of this for the group AD.

**Conclusions:** The systemic ethanol intake substantially increases the free-radicals formation in the hippocampus of rat exposed to diurnal rhythm disturbance. This effect may be significantly diminished by treating of the animal with 200 mg/kg Trolox. **Keywords:** Diurnal Rhythm Disturbance, Systemic Alcohol Intake, Rat hippocampus, Oxidative Stress. **Acknowledgement:** This work was possible with the financial support of Grant 19/2008, scientific Board of the Medical University - Sofia, Sofia, Bulgaria.

### Fatigue, depression and sleep disturbances in patients with Multiple Sclerosis

ESC-ID 820  
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In order to evaluate fatigue, depression and sleep disturbances in patients with multiple sclerosis (MS), we asked 100 patients in Sina hospital outpatient MS clinic to complete BDI (Beck Depression Inventory), Pittsburg Sleep Quality Index (PSQI), Sleep disorder questionnaire, FIS (Fatigue Impact Scale) and ESS (Epworth Sleepiness Scale) questionnaires. Among patients, sixty four had fatigue complaint during day time and thirty six were not fatigued. BDI, PSQI, FIS and sleep disorder questionnaire scores were significantly higher in patients with fatigue than non fatigued group but there were no statistically significant differences in ESS, EDSS and duration of disease between fatigued and non fatigued cases. There were significant correlations between FIS and BDI scores ( $r = 0.49$   $p = 0.01$ ) and FIS and PSQI scores ( $r = 0.399$   $p = 0.01$ ). Our study confirms that fatigue in MS is not only associated with depression but also with sleep disorders, and this complaint should be evaluated and carefully managed to reduce fatigue in such patients.

## Diagnostic signs of Chiari I malformation for surgical treatment in child

ESC-ID **558**  
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*Introduction:* Chiari malformation (CM) is a disorder of central nervous system development which demands a surgical treatment. Purpose of investigation. The main purpose of this investigation was improvement of CM diagnosis in child in case of cerebral tonsil herniation to the level of foramen magnum or below one.

*Material and methods:* 43 children (15 girls and 28 boys) with CM (from 2 months to 18 years old) were investigated. 15 child had a clinical manifestation of CM I in combination with cerebral tonsil herniation only to the foramen magnum level. Magnetic Resonance Tomography (MRT) in vessel regime, Rentgenophotometry (RPHM), Emission Computer Tomography (ECT), Acoustic Bulb Induced Potentials Registration (ABIPR) where used as well as traditional methods of investigation. In order to define of hydrocephaly degree we marked of ventriculomegaly (VM) expression using of Evans index (EI) calculation (performed for 23 children).

*Results:* Type I of CM reviled in 26 (60,5%), type II – in 16 (37,2%) and type III – in 1 (2,3%) children. Spinal cord hernia diagnosed in 9 cases and fixated spinal cord syndrome – in 8 (18,6%) cases. Syringomyely presented in 2 (4,7%) child, hydrocephaly – in 24 (55,8%) child. EI was from 0,27 till 0,77 (in overage – 0,37; normally – less than 0,25). Degree I of VM presented in 14 (60,9%) cases, II – in 8 (34,8%) and III – in 1 (4,3%) case. Widening of Cavum Verga without VM reviled in 3 (7%) cases. We performed of surgical operation for 22 children. Multiple stage combined vice correction carried out for 8 (36,4%) children.

*Conclusion:* Combination positive results of MRT with RPHM, ECT and ABIPR is an indication to the surgical treatment of Chiari I malformation.

## Characterization of microglia activation in an in vitro model of primary brain tumour

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*Introduction and background:* Microglia, the resident macrophages of the brain, are known to be involved in cerebral inflammatory diseases. However their role in relation to brain tumours is poorly understood. Glioblastoma multiforme is the most common malignant primary brain tumour in adults. Due to its aggressive nature, treatment is palliative emphasising the need for new therapeutic agents. A better understanding of microglia function in the context of tumours could lead to possible immune-based treatments in the future.

*Objectives:* • To characterize the level of microglia activation in a rodent organotypic model. • To establish an organotypic glioblastoma model.

*Method:* A time-course of a rodent organotypic slice culture was established to characterise the level of microglia activation. At specific time point (day 0 to day 14), the slices were prepared for immunohistochemistry against the microglia lysosome CD68 and counterstained by haematoxylin. The CD68-positive cells were quantified and statistically analysed. The establishment of the organotypic glioblastoma model involved the addition of rodent C6 cell line to organotypic slice cultures. The slices were counterstained and CD68 immunohistochemistry was performed on the sections for assessment.

*Results:* The organotypic model demonstrates a reproducible pattern of microglia activation, with a significant increase at day 3, peak at day 5-7 and decrease at day 14. This activated profile was associated with morphological changes observed by cell loss followed by cell repair. The addition of the C6 tumour cells to the slices shows glioblastoma infiltration with loss of hippocampal architecture. Immunohistochemistry suggests that microglia CD68 expression might be decreased in the presence of glioblastoma cells.

*Conclusion:* Our study of microglia activation has shown that (i) the organotypic model is suitable for the characterization of microglia and (ii) some microglia function such as phagocytic activity, might be inhibited by the tumour. Therefore, this model appears suitable for the investigation of microglia-glioma interactions.

## Hexokinase II – A novel mediator of energy metabolism and cell survival

ESC-ID **177**  
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Hexokinase II (HKII) is one of 4 mammalian Hexokinase isoenzymes catalyzing the first obligatory step of glucose metabolism. In addition to their catalytical activity, HKII is also believed to play a role in regulating cell death. In the state of ischemic tolerance Hexokinase II is upregulated by Hypoxia inducible factor 1 (HIF-1). In order to investigate the role of Hexokinase II in neuroprotective signaling we used site directed mutagenesis to generate several murine HKII expression vectors with mutations in domains relevant for glucose metabolism and apoptosis regulation. The effect of HKII and its mutants on neuronal survival was studied using a sensitive technique based on co-transfecting embryonic rat primary cortical neurons with eGFP (green cells) and HKII or HKII mutant vectors. These co-transfected cells were co-cultivated with primary neurons expressing m-orange (orange cells) and subsequently submitted to an in vitro model of cerebral ischemia (oxygen-glucose-deprivation, OGD). The ratio of green vs. orange neurons before and after OGD was analysed. Thereby the effect of different HKII mutants on neuronal survival could be determined. Our data demonstrate that mitochondrial binding of HKII is a dynamic and adaptive response mediated by protein kinase B (PKB)/Akt kinase dependent phosphorylation that protects neurons from OGD induced apoptosis. We propose that HKII may serve as a molecular switch regulating in a glucose concentration depending fashion apoptosis and may those provide a link between cellular

metabolism and survival. In particular, our data might have a therapeutic relevance for stroke, since glucose hypometabolism and mitochondrial dysfunction are key features of cerebral ischemia.

### **A Comparative Study of Brainstem Evoked Auditory Potentials in Pre-Term and Full-Term Infants**

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*Aim/Objective:* To assess the difference in the maturation of the central nervous system of preterm and full term infants electro physiologically.

*Methods:* We conducted a retrospective study of Brainstem Auditory Evoked Potentials in 25 pre-term and 25 full-term infants. Babies having a history of birth trauma, metabolic disorders or intracranial infections were excluded. Waveforms 1-5 were analysed. Absolute and interpeak latencies were measured. 'Student T Test' was used to analyse the data.

*Results:* Data from 25 preterm and 25 full term infants revealed that there was a significant increase in latencies in pre-term infants in wave 2 (P-0.006), wave 5 (P-0.029) and interpeak latency of waves 1-3 (P-0.221) than that compared to full-term infants.

*Conclusion:* Central Nervous System maturation of pre-term infants was slower than the full term infants.

### **Prevalence of the circle of Willis variations based of the medico legal autopsy series during a 3 years observation period 2006-2008**

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*Introduction:* Brains were obtained from 200 medicolegal autopsy subjects of varying ages. The circle of Willis was examined at the base of the brain. The cerebral vessels were observed with regards to their origin, caliber and typical configuration. Variations were noted and grouped into different categories. Out of 200 specimens examined, xx (xx%) conformed to the typical pattern. In the rest of the specimens (xx%) there were variations in the circulus arteriosus.

*Methods and Materials:* patient: This study based on fresh cadaver autopsy in a 3 years period. All cadavers were candidate of autopsy for medico legal reasons in varying age and sex. The medico-legal office approved this study. The subjects with gross pathology of the brain excluded and 200 autopsies were included in the study. Brains were obtained from fresh cadavers via standard autopsy technique with following steps: 1. Cutting the Scalp started behind the ear, extended around the vertex,

and completed behind the other ear. 2. Lifting the Scalp and Galea aponeurotica from the periosteum of the bone. 3. Reflecting the Scalp back by using a scalpel to dissect the attached fibrous soft tissue. 4. Cutting the temporalis muscle to prepare the skull for sawing. 5. Sawing the skull circumferentially. 6. Removing of the calvarium 7. Removing of the Brain by cutting tentorium. After careful dissection with minimal manipulation, the circle of Willis was examined. The cerebral vessels were observed for caliber and typical configuration and variations were noted and grouped into 7 different categories. Vessel diameter less than 1 mm was defined as hypoplasia. The normal vascular pattern defined as type a. Type b, AcoA hypoplasia; type c, A1 hypoplasia; type d, unilateral PcoA aplasia; type e, bilateral PcoA aplasia; type f, unilateral PcoA hypoplasia; type g, bilateral PcoA hypoplasia; type h, other rare variations. The findings were classified in two tables and the final data were analyzed by using t-test and compared with the similar frequency of variations in other studies.

*Statistical analysis:* Data were analyzed using SPSS version 16.0 software (SPSS Inc, Chicago, IL, USA). All of the variables were compared using Student's t-test (for the quantitative variables) and the chi-square test (for the qualitative variables). Quantitative variables are provided as means (standard deviation [SD]). A p value less than 0.05 was regarded as being statistically significant.

*Results:* In total, 200 autopsy included in the study, 169(84/5%) males, 31(15/5%) females. The age range of subjects was between 16 and 71 years, mean 41/20 (SD = 14/41). Out of 200 specimens examined, 69 (34.5%) conformed to the typical pattern. In the rest of the specimens (65.5%) there were variations in the circulus arteriosus. Hypoplasia of AcoA in 28 cases (14%) and A1 in 9 cases (4.5%) were seen. Aplasia of unilateral PcoA in 6 cases (3%) and aplasia of bilateral PcoA in 2 cases (1%) were seen. In 37 cases (18%) one PcoA and in 48 cases (24%) bilateral PcoA were hypoplastic. Aplasia of P1 was seen in 1 case (0.5%), that defined as persistent fetal circulation. AcoA hypoplasia with unilateral PcoA hypoplasia in 14 cases (7%), AcoA hypoplasia with bilateral PcoA hypoplasia in 4 cases (2%), A1 hypoplasia with unilateral PcoA hypoplasia in 5 cases (2.5%) were seen. bilateral PcoA hypoplasia was the most common variation in our study. We did not see any case with aplasia of AcoA or A1 segment. Although this was not our primary objective, we found one aneurysm of the AcoA in a 43 years old man (case no. 81).

*Discussion:* In this study the anatomical variants of the entire circle of Willis were classified and compared with results of previous similar studies. The segmental variations were also compared with previous published data.

### **Visual evoked potentials as an additional diagnostic procedure in pediatric migraine in childhood and adolescence**

ESC-ID **661**  
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*Introduction:* Migraine is the most common primary headache in pediatric population. Migraineurs often show abnormalities in visual evoked potentials (PR-VEP).

These abnormalities are probably caused by, genetically determined, altered control of cortical excitability at the level of subcortico-cortical structures. Due to the lack of a specific diagnostic test in establishing the diagnosis of migraine some authors have decided to use PR-VEP as an additional tool in clarification of both pathophysiology and diagnosis of migraine.

*Aim:* Examination of PR-VEP characteristics in patients with migraine.

*Material and Methods:* The observed group consisted of 73 migraineurs (35 patients were boys (47.9%) and 38 patients were girls (52.1%), aged from 6 to 22 years) attending the Clinic of Neurology and Psychiatry for Children and Youth of Medical Faculty in Belgrade. The diagnosis of migraine was made according to the diagnostic criteria proposed by the International Headache Society. PR-VEP recordings were performed on all the patients, both binocularly (BE) and monocularly (LE-left eye, RE-right eye), using the standardized checkerboard pattern reversal technique, on a Medelec-Sapphire Premiere apparatus.

*Results:* Further abnormalities of PR-VEP were detected: BE – prolonged N2 latency in 27 patients (37%), increased N1-P100 amplitude in 53 patients (72.6%), increased P100-N2 latency in 43 patients (58.9%); LE – increased N1-P100 amplitude in 33 patients (45.2%), increased P100-N2 amplitude in 41 patients (56.2%); RE – prolonged N2 latency in 37 patients (50.7%), increased N1-P100 amplitude in 40 patients (54.8%), increased P100-N2 amplitude in 40 patients (54.8%).

*Conclusion:* Our study found increased amplitudes and prolonged latencies of PR-VEPs in children and adolescents suffering of migraine. These results suggest the use of PR-VEP as an additional diagnostic test for migraine. Key words: migraine, visual evoked potentials, PR-VEP, pediatric

### Neurophysiological aspects in migraine of childhood and adolescence

ESC-ID **157**  
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*Introduction:* Migraine is a paroxysmal hereditary disorder from the group of primary headaches. Headache is one of the most frequent somatic complaints among children and adolescents, but the estimated prevalence rates of primary headache disorders like migraine vary from 10% to 30% in different studies (1). The new studies show that migraine is caused by cortical hypersensitivity to different kind of stimuli (2). Migraine is diagnosed using anamnestic data, neurological examination and excluding other causes of headache. EEG and Pattern Reversal-Visual Evoked Potentials (PR-VEP) are useful in clinical evaluation of migraine.

*Aim:* Aim of the study is to determine the frequency of EEG abnormalities in children and adolescents with migraine, the correlation between EEG abnormalities and migraine with aura, the correlation between abnormalities in PR-VEP and EEG findings and to determine the prevalence of positive family history in patients with migraine.

*Material and Methods:* Our study evaluated medical history of 73 patients with migraine which were examined and

treated in the Clinic of Neurology and Psychiatry for Children and Youth, Medical Faculty University of Belgrade in the period 2003 - 2008. The diagnosis of migraine was made according to the classification of International Headache Society (3). PR-VEP and EEG were performed in all patients using standard procedures proposed by International Society of Clinic Neurophysiology on Apparatus Medelec Sapphire Premiere.

*Results:* We analyzed 73 patients, 35 males (47.9%), and 38 females (52.1%), aged 6 to 22 years (average age 14.2). Normal EEG findings were found in 14 (19.2%) patients, nonspecific changes were found in 36 (49.3%) patients and specific discharges were found in 23 (31.5%) patients. Migraine without aura was found in 51 patients (69.9%) and migraine with aura in 22 (30.1%) patients. Our study did not show statistically significant correlation between the presence of aura in patients with migraine and specific discharges in EEG. Increased amplitudes of following waves (N1-P100 binocular and monocular left and P100-N2 monocular on both eyes) correlated statistically significant with pathological EEG findings. Positive family history on migraine was found in 53 patients (73%).

*Conclusion:* Correlation between pathological EEG findings and higher PR-VEP amplitudes points out increased cortical excitability in patients with migraine and might be pathophysiological basis of migraine attacks. Further studies might be important in evaluation of influence of hereditary factors in increased cortical excitability. Keywords: migraine, childhood, adolescence, electroencephalography, visual evoked potentials

### The prevalence of patent foramen ovale in Chronic Obstructive Pulmonary Disease patients with increased pulmonary hypertension using Transcranial Doppler

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*Background and purpose:* Patent Foramen Ovale (PFO) can secondarily re-open due to pathologic cardio-respiratory conditions (e.g.: pulmonary hypertension) that tend to reverse the normal pressure gradient between left and right atria, causing transient valvular regurgitation. In this study we asked whether the increase in secondary pulmonary hypertension due to COPD is associated with PFO - which is a well recognized cause of stroke.

*Methods:* Transthoracic echo-cardiography (TTE) was performed on 90 COPD patients in order to measure their pulmonary artery pressure (PAP) and to rule-out cardiologic causes of possible right to left shunting (RLS). 90 patients with increased PAP and 20 patients with normal pulmonary pressure underwent transcranial Doppler (TCD) with Valsalva maneuver (VM) to detect the intracardiac RLS with the passage of at least 2 microembolic signals (MES) in the 40 second time window which is considered most likely to confirm the existence of RLS which in our cases is sustained by a PFO.

*Results:* During VM we detected PFO in 62 COPD patients with pulmonary hypertension and in 2 of the patients with normal pulmonary pressure. Comparison of pul-

monary pressure in PAP-raised COPD patients with or without PFO did not show any specific relation between the amount of increase in PAP and the prevalence of PFO ( $P = 0.2$ ). The same comparison between patients with raised PAP and control (normal pulmonary pressure) revealed an association between a proven pulmonary hypertension and the prevalence of PFO ( $P = 0.004$ ). On the other hand, the rise of pulmonary artery pressure was associated with more passage of MES ( $> 2$ ) on TCD with some of the first MES occurring later than the standard time window (Logistic Regression,  $p = 0.01$ ).

**Conclusion:** Our data does not show any significant relation between the amount of increase in the PAP and the occurrence of PFO suggesting that the probability of PFO re-occurrence may not increase with the progression of COPD leading to higher amounts of PAP. The raise in PAP, however, is associated with an increase in the possibility of significant MES - some of the TCD signals being considered as a PFO and some of it not- leaving a suspicion for the exact role of delayed first signal appearance (in time window  $> 40s$ ) on the TCD. While the presence of pulmonary hypertension alone can be considered as an independent predictor of occurrence of PFO during VM yet its clinical significance remains controversial and is not considered in the protocol for stroke preventive study.

### Does Endothelin-1 induce cortical spreading depolarization (CSD) via a direct effect on the vasculature?

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**Objectives:** Endothelin-1 (ET-1) has attracted increasing interest since its discovery by Yanagisawa in 1988. Recognized as a neuropeptide with neurotransmitter/neuromodulator functions, ET-1 is also a potent vasoconstrictor. Moreover, it potently induces CSD in vivo. ET-1 has been related to several pathophysiological states including subarachnoid hemorrhage, ischemic stroke and traumatic brain injury. The mechanism by which ET-1 induces CSD is not fully understood. Possibly ET-1-induced vasoconstriction leads to ischemia which causes CSD. Here we further analyzed whether ET-1-induced CSD is preceded by significant pial vasoconstriction and characterized pH changes and electrocorticogram (ECoG).

**Methods:** We used a two cranial window model in rats ( $n = 11$ ). ET-1 was brain topically applied in one window at 100nM and 1 $\mu$ M. A second window served as control. DC/AC-ECoG and laser-Doppler flowmetry were used to detect CSD. The pial arterioles were imaged onto a camera to assess whether significant vasoconstriction precedes ET-1-induced CSD. In the sequence of consecutive recorded images we marked our region of interest (ROI) at the right angle to the longitudinal axis of a pial arteriole. A single ROI is a line represented in MATLAB as a diagram with curves for the red, green and blue channels, where the x-axis represents length in pixel and the y-axis brightness. The blood vessel led to a clean valley with low noise in the green channel. The square root of absorbance between two inflection points was used to calculate the diameter value and diameter change over time. In another six experiments we recorded the pH changes associated

with ET-1-induced CSD using pH-sensitive microelectrodes (comparison with CSDs in five control experiments).

**Results:** We observed a cluster of recurrent CSDs starting from the ET-1 superfused window and propagating to the control window. ET-1 induced significant vasoconstriction in large, medium and small pial arterioles before the first CSD (Wilcoxon Signed Rank Test). Only in the presence of ET-1 a pH reduction to  $7.20 \pm 0.07$  preceded the sharp alkaline shift of the CSD in all experiments. The sharp alkaline shift coincided with the negative DC shift of CSD.

**Conclusion:** The pattern of the DC potential changes, the arteriolar constriction and pH reduction prior to the first cluster of CSDs support the notion that ET-1 induces CSDs due to its vasoconstrictive action. Our data may have implications for clinical conditions ranging from migraine to subarachnoid haemorrhage and ischemic stroke.

### Neurosurgical treatment of pharmacologically resistant epilepsy associated with subcortical heterotopia

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**Aim:** To improve the results of treatment in patients with subcortical heterotopia.

**Methods and materials:** 5 patients with subcortical heterotopia were observed. In 3 cases heterotopic foci were localized in posterior part of right frontal lobe and in 2 cases – in left frontal lobe. Average age of patients was 3,7 years old. In all cases according to EEG heterotopic foci coincide with foci of seizure activity. Single photon emission computed tomography (SPECT) was used to differentiate heterotopic focus from tumor. All patients had delay of motor and speech development. Pharmacologically resistant seizures (8-20 per 24 hours) were present in all cases. The intracranial operation with removing of epileptic focus was performed in all cases.

**Results:** Complete reduction of seizures was achieved during 4-6 months in all cases. Operation achieved increasing of muscle tone; patients got skills of staying and walking. In few months after the operation patients started to say some words. Changes of bioelectrical activity before the operation effected to normal brain development and showed pathological influence of heterotopic foci. The operation achieved general decreasing of bioelectrical activity of the brain; waves of ? and q diapasons decreased in 400-3000 mkV2; b-activity increased in 15-130 mkV2.

**Conclusion:** 1. MRI of the brain must be performed in patients with seizures to visualize malformations of the brain. 2. SPECT must be used to differentiate subcortical heterotopia focus from tumor. 3. Presence of subcortical heterotopia focus, which coincides with pharmacologically resistant epileptic focus, is the evidence for performing the operation. 4. Removing of subcortical heterotopia focus normalize bioelectric activity of the brain and release the brain from seizures.



## Acute Stress Effects on Spatial Contextual Memory on Healthy Young Men

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There is a variety of explicit tasks that are facilitated when the current context resembles the learning context. The neuroanatomy that underlies contextual implicit memory is the focus of a large body of recent research. fMRI studies and studies on patients with amnesia showed that the medial temporal lobe is functionally essential for this type of contextual facilitation, in spite of its implicit nature. On the other hand, these anatomical structures are susceptible to stress. Until this moment, the research results are controversial whether stress affects contextual spatial memory or not.

*Aim:* With this study we intended to: detect significant differences on the EEG maps between the stressed and control subjects during the contextual memory task; and to estimate the effect of acute stress administered prior to implicit contextual learning upon context-dependent performance.

*Methods and Materials:* The experiments were run on 12 young right-handed male subjects, that were equally spread in a Control Group and a Stress Group. Each subject underwent a computer version of the Chun&Jiang model of implicit contextual memory search task, that was split in two stages: Contextual Storage(administered on the first day) and Contextual Retrieval (administered on the second consecutive day). The time between the two stages allowed a period of consolidation. The Stress Group also performed a time-paced Stroop task, 5 minutes prior to the Storage Stage. EEG, pulse and skin conductance data acquisition was made using the Biopac MP100 system and the Acqknowledge 3.90® software. The source localization analysis of brain electric activity was computed in sLoreta®, one of the linear methods to compute 3D current density map from EEG data, that indicates the locations of the underlying source processes. Accuracy of responses and real time reaction times from the moment of stimulus presentation were evaluated with the SuperLab® software. Following the protocol used in earlier experiments, we considered the evaluation only for in the second part of the experiment (Contextual Retrieval). *Results:* According to the sLoreta mapping for the EEG data during the Retrieval Stage, there was a lower current generation in the temporal lobes in the stress group, as compared to the control group. This is consistent with previous results that the medial temporal lobe plays a major role in contextual memory function. All participants completed the memory task faster when the learning and retrieval contexts were congruent, but there was a significant decrease in performance for the Stress Group, where the contextual facilitation effect decreased with 20 milliseconds.

*Conclusions:* Acute stress administered prior to learning context for an explicit task decreases consequent performance and electric activity in the temporal lobe. Subsequent studies are needed in order to study the correlations that might exist between the contextual facilitation effect and the electric activity of the medial temporal lobe.

## Pioglitazone, a peroxisome proliferator-activated receptor gamma (PPAR-γ), protects sciatic nerve against ischemia/reperfusion injury in rats

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*Aim:* Ischemia/reperfusion (I/R) injury has an important role in the development of various neuropathies. Although reperfusion helps the maintenance of cells surviving ischemia, it may result in further tissue damage. According to clinical prominence of I/R injury, the quest for effective pharmacological agents seems relevant. Thiazolidinediones, such as pioglitazone, are peroxisome proliferator-activated receptor-gamma (PPAR-γ) ligands categorized in oral antidiabetic drugs. Their protective effects against I/R injury in different organs have been shown in several animal models. We hypothesized that pioglitazone ameliorates I/R injury in rat sciatic nerve and tested our hypothesis.

*Materials and Methods:* After approval of the ethical committee of animal research of Tehran University of Medical Sciences, we designed 10 experimental groups in each 6 rats. Treatment groups were given intraperitoneally 15 mg/kg pioglitazone dissolved in 0.5% dimethyl sulfoxide in phosphate buffered saline one hour before ischemia and subjected to either no reperfusion or 1-hour, 1-day, 4-day, and 7-day periods of reperfusion. The corresponding control groups were also designed where pioglitazone was excluded and only a vehicle (i.e.0.5% dimethyl sulfoxide in phosphate-buffered saline) was administered. Animals were anesthetized by intraperitoneal injection of ketamine (50 mg/kg) and xylazine (4 mg/kg). Rats were placed in supine position and after laparotomy and right inguinal incision, the right common iliac and femoral arteries just distal to the inguinal ligament were clamped for 3 hours using two Yasargil aneurysm clips.1,2 After certain time intervals of reperfusion, the function of the affected hind limb was evaluated using behavioral scores based on gait, grasp, paw position, and pinch sensitivity, while the score for each index varied between 0 (no function) and 3 (normal function) except for pinch sensitivity ranging from 0 to 2. At the end of the reperfusion times, blood samples for the malondialdehyde (MDA) assay were taken from the tail vein, and then the sciatic nerve was fixed in situ for 30 min using 4% formaldehyde in phosphate buffer, and subsequently the trifurcation of the sciatic nerve was removed. Sections were stained with hematoxylin/eosin and Gomori trichrome for light microscopy studies and graded for ischemic fiber degeneration (IFD) and edema. IFD was determined based on the percentage of fibers afflicted with IFD as follows: 0 = ~2%; 1 = 3–25%; 2 = 26–50%; 3 = 51–75%, and 4 = 1 75%. Moreover, sections were graded from 0 to 4 for edema based on severity and distribution. *Results:* Behavioral scores were significantly improved in the pioglitazone groups just on the 4th and 7th days of reperfusion. Pioglitazone treated animals had significant lower edema at 4 and 7 days. Although IFD decreased in the pioglitazone group at 7 days of reperfusion, it was not statistically significant. The MDA level was significantly lower in pioglitazone-treated animals.

*Conclusion:* The study showed that pioglitazone ameliorates I/R injury in rat sciatic nerve. The suggested mecha-

nisms are reduction of reactive oxygen species, regulating nitric oxide metabolism, and preventing the activation of transcription factors, e.g. NF- $\kappa$ B (nuclear factor kappa-light-chain-enhancer of activated B cells).

### T-Helper 17 Cells Expand in Multiple Sclerosis and Are Inhibited by Interferon- $\gamma$

ESC-ID **430**  
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*Objective:* T-helper 1 (Th1) and Th17 lymphocytes are involved in experimental autoimmune encephalomyelitis, the model of multiple sclerosis (MS). We characterized the Th1/Th17 cell populations in peripheral blood (PB), their interferon (IFN) receptor expression, and their sensitivity to IFN- $\beta$  in MS patients.

*Methods:* The study included untreated patients with active (AMS; n = 30) and inactive MS (IMS; n = 32), and healthy volunteers (n = 22). We measured intracellular cytokine expression, interleukin-17-producing myelin basic protein-stimulated peripheral blood lymphocytes, surface IFN type I receptor chain1 (IFN- $\beta$ R1) expression, IFN- $\beta$ -dependent signal transducer and activator of transcription 1 (STAT1) phosphorylation, and apoptosis of anti-CD3 monoclonal antibody-stimulated peripheral blood lymphocytes.

*Results:* The percentage of Th17 cells increased around sevenfold in AMS patients compared with IMS or healthy volunteers, but there was no change in Th1 cells. Th17 cells in AMS patients were myelin basic protein specific. The longitudinal follow-up of 18 MS patients shifting between AMS and IMS showed that the percentage of Th17 but not Th1 cells always increased in AMS. IFN- $\beta$ R1 expression, IFN- $\beta$ -induced STAT1 activation, and apoptosis were significantly greater in Th17 than Th1 cells. IFN- $\beta$ R1 expression and IFN- $\beta$ -dependent STAT1 activation progressively increased in vitro with a highly significant positive correlation developing only in Th17 but not in Th0 or Th1 cells. Interpretation: We present evidence of an expansion of peripheral Th17 cells, a Th subset that can infiltrate brain parenchyma and damage cells, associated with disease activity in MS. The greater IFN- $\beta$ R1 level expressed by Th17 compared with Th1 cells might make them a selective target for IFN- $\beta$  therapy.

### Epileptiform discharges are induced by Glutamine in superficial layers of the medial entorhinal cortex from pilocarpine-treated chronic epileptic rats in vitro

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Glutamine (GLN) being a precursor for synthesis of glutamate and gamma-aminobutyric acid (GABA), has been found in the cerebrospinal fluid (CSF) at mean concentrations of 0.6 mM. Usually, experiments on slices are performed in artificial CSF (aCSF) kept free of amino acids. Therefore, the role of glutamine, particularly in tissue of epileptic animals, remains elusive. We used extracellular recordings to study effects of GLN on field potentials and stimulus-evoked field responses in the medial entorhinal cortex (MEC) of combined entorhinal cortex hippocampal slices from pilocarpine-treated chronic epileptic rats and age-matched saline-injected control rats. In presence of GLN (0.5 and 2 mM) recurrent epileptiform discharges (REDs) were observed in slices from epileptic rats (64% and 80%, respectively), but not in slices from control rats. REDs were restricted to the superficial MEC, suppressed by the alpha-Amino-3-hydroxy-5-methyl-4-isoxazol-propionate (AMPA)/kainate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (30 microM), attenuated by the inhibitor of neuronal glutamine transporters methylamino-isobutyric acid (10 mM), and apparently augmented and prolonged by the GABA(A) receptor antagonist bicuculline-methiodide (5 microM). In contrast, amplitudes of stimulus evoked nonsynaptic and synaptic field responses increased in slices from control rats (+23% and +12% of the reference values) and insignificantly less or not in those of epileptic rats (+6.5% and -0.25%, respectively). Notably, stimulus-evoked slow negative transients confined to slices of epileptic animals were reduced in amplitude (-18%). In combined entorhinal hippocampal slices from chronic epileptic animals, GLN induces glutamatergic REDs via neuronal uptake in superficial layers of the MEC where inhibitory function seemed to be partially preserved.

### Silent strokes and cognitive impairment linked to metabolic syndrome in middle age adults

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*Background:* Several studies have demonstrated that metabolic syndrome (MetS) is a risk factor for stroke. The link between the MetS and silent ischemic brain lesions is not well settled yet. All the conditions above are known to be warning signs for future stroke and cognitive deterioration.

*Purpose:* To find a possible correlation between the presence of MetS in middle age adults and its correlation

with the presence of silent strokes and cognitive impairment.

**Material and method:** There were included 176 subjects, with the age between 45 and 65 years, with MetS and no past history of any neurological/psychiatric dysfunction, that presented in the ER for non-neurological/psychiatric symptoms. They were evaluated using MRI scan, Mini Mental Scale Evaluation, blood pressure, fasting glucose, blood lipids and body mass index (BMI).

**Results:** The male: female ratio was 1.31:1, and the mean age was  $54 \pm 6.7$  yrs. 33% of the subjects had MRI lesions of silent strokes, mostly in men (65.5%). In these individuals, the MMSE scores ranged from 16 to 30, but 62% of the subjects had MMSE score between 20 – 28, and only 10.3% had an MMSE of 29, or 30. The subjects with no MRI signs of silent strokes ( $n=118$ ) the MMSE scores varied from 14 to 30, most of the subjects (84.74%) having an MMSE of 29, or 30. In both groups the elevated BP, high blood lipids levels and high BMI significantly correlated with the severity of the MMSE score. A fasting glucose above 180 mg/dL correlated with the presence of silent strokes ( $p=180$  mmHg (pdiastolic blood pressure had no correlation with silent strokes).

**Conclusion:** The metabolic syndrome may be associated with silent strokes, and when this association occurs, these individuals are prone to develop cognitive impairment, especially in those with high blood pressure and high fasting glucose values.

### Comparison of the effect of interferon (IFN) beta-1b (Betaferon) and beta-1a (Rebif) on brain MRI lesions in patients with relapsing-remitting multiple sclerosis (RRMS)

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**Introduction:** Multiple sclerosis (MS) is an autoimmune inflammatory demyelinating disease of the central nervous system (CNS) which is a leading cause of disability in young adults. Relapsing-remitting MS (RRMS) is the most common clinical presentation of the disease. Recently worldwide prevalence of MS has been increasing noticeably. Within the last decade, three beta-interferon (IFN- $\beta$ ) formulations – Avonex, Rebif, and Betaferon – have emerged as first-line therapies for the treatment of RRMS, as disease-modifying treatment. The aim of this study was to compare the effect of disease-modifying treatment with interferon (IFN) beta-1b (Betaferon) and beta-1a (Rebif) on brain MRI findings in patients with RRMS.

**Method and material:** This semi-experimental study was conducted at Firoozgar University Hospital in Tehran, Iran; on 112 patients who had RRMS determined using McDonald criteria (Revision 2005). They were evaluated during a 24-month study period from January 2004 to January 2006. Patients were assigned in three groups: 37 patients under therapy with IFN beta-1a (Rebif) 44 mcg subcutaneously every other day (group A), 21 patients under therapy with IFN beta-1b (Betaferon) 250 mcg sub-

cutaneously every other day (group B) and 54 patients without disease-modifying treatment (group C). Changes of brain MRI findings at the beginning and the end of study were evaluated. All data were analyzed by SPSS 16.0 and Chi2 test, Mc Nemar, One Way ANOVA and paired T-test were used.

**Results:** Mc-Nemar test reveal that a significant amelioration is seen in juxta-cortical ( $P_A = 0.001$ ,  $P_B < 0.001$ ), cerebellar-brainstem ( $P_A = 0.015$ ,  $P_B < 0.001$ ) and periventricular ( $P_A < 0.001$ ,  $P_B < 0.001$ ) lesions in both groups A and B, whereas, these changes were not significant in group C ( $P > 0.05$ ). Spinal lesions deteriorated significantly in group C ( $P < 0.001$ ), ameliorated significantly in group B ( $p = 0.008$ ), but there was no significant amelioration in group A ( $P = 0.629$ ). Comparison of the effects of betaferon and rebif demonstrated that none of the patients in group B (betaferon) have experienced deterioration of spinal cord lesions, while, 7 patients (18%) of the patients in group A (Rebif) have more lesions in spinal cord at the end of follow-up ( $P = 0.041$ ). There was no significant difference in changes of juxta cortical ( $P = 0.148$ , Power = 53.59%) and cerebellar-brainstem lesions ( $P = 0.077$ , Power = 62.17%) between group A and B.

**Conclusion:** Based on literatures our study is one of the first that compared the effect of IFN beta-1a and beta-1b on the location of brain lesions in MS patients. Betaferon was more effective in improving brain lesions especially spinal plaques. Conclusively, it seems that the administration of disease modifying treatment (especially betaferon) in RRMS patients could potentially lead to a better prognosis.

**Key words:** Disease modifying treatment, Beta-interferon, Relapsing-Relapsing Multiple Sclerosis (RRMS), Brain MRI lesions

### Do rats play lottery?

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**Introduction:** Making decisions based on the probability of future events, e.g. assuming risks, is routine in our everyday life. However, its determinants are poorly understood and questions such as “How do we choose to take or not the risk?” or “How do we decide how much risk we are willing to take?” remain largely unanswered. In addition, little is known about the inter-individual differences in the willingness to take risks and its substrate. Rats perceive and respond to uncertain outcomes (1) and can also change their responses according to reward value (1,2) or task difficulty (2).

**Aim:** In this project, using the 5-hole apparatus, we aimed at creating a novel decision-making paradigm for the rat to assess risk-taking behaviour and its variation in view of immediate change of long-term gain.

**Methods:** A 5-hole apparatus has 5 holes in a curved wall (choice holes) and another in the opposite wall, connected to a pellet dispenser (reward hole), each hole having an infrared sensor to detect rat nose pokes on it and an associated light that can be used as a cue. This apparatus was transformed into a risky decision-making test by random-

ly assigning, in each trial, a safe hole (the choice of which delivers a small constant reward - 1 food pellet) and 4 risk holes (the choice of which has a 25% probability delivering a big, programmable, reward); light can be used to cue either hole and thus increase the discrimination of the task. The final protocol was run daily for 18 days, one 30-minute trial per day, and had two phases: in the first, risk choices are rewarded with 4 pellets, 25% of the times, resulting in no net long term gain compared to safe ones; in the second, immediately after the first one, risk choice reward is doubled, resulting in a long term profit for those who take it. All rats used were control Wistar males, with the same age (3 months, at the start of the experiment).

**Results and Conclusions:** Before we ran with the final 18 day protocol, we experimented with light associated with either risk or safe holes. We arrived at the following results: rats prefer safe choices when associated with light; however, when light was associated with risk, rats didn't display any preference. It would seem that safe choices and light are appetitive stimuli of similar strength that can antagonize each other. To rule out the fact that light could be a confounding stimulus we did a place preference test, in which light was eliminated from the test and the safe choice was always associated with the same hole. Once again, the rats went clearly for the safe choices in the same trend that was seen before when light was present. Therefore, light did not play a role. The final protocol used light and risk choices associated. After doubling the reward midway through, the rats went from not preferring anything to clearly prefer the risk choices. This model has solid foundations and has proved to work.

### Plasticity related gene-1 (PRG-1) expression in the developing mouse brain

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Plasticity related genes (PRGs) are integral membrane proteins and possess homology to lipid phosphate phosphatases (LPPs) which groups them to the superfamily of lipid phosphatases/phosphotransferases (LPT). Plasticity related gene one (PRG-1) is a brain-specific membrane protein, moreover PRG-1 knock-out mice show epileptic seizures. In order to understand the role of PRG-1 in neuronal outgrowth, excitability and synaptogenesis I described its expression patterns in the developing and in the adult mouse brain. I performed immunochemistry on mouse brain slices of different ages using an antibody against PRG-1 and the beta-Gal reporter which is expressed in the generated PRG-1 knock-out mouse. With this technique I described distinct PRG-1 expression patterns in the brain during development, morphological differences between wild type and knock-out mice and up-regulation of PRG-1 expression after lesion. Finally, I described the expression pattern of PRG-1 in adult and young human hippocampal slices confirming PRG-1 expression in glutamatergic neurons but not in inhibitory interneurons in humans. PRG-1 is a membrane protein exclusively expressed in mature, glutamatergic neurons, ultrastructurely localised in close proximity to the post-synaptic density of glutamatergic synapses, where it presumably modulates neuronal excitability.

### Does gamma knife radiosurgery stop vestibular schwannoma growth?

#### A prospective study

ESC-ID **105**  
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**Introduction:** Vestibular schwannoma (VS) is a benign tumor that arises in the eighth cranial nerve, giving symptoms of hearing loss, tinnitus, and vertigo. In general, it is a slow-growing tumor that in some cases even shows spontaneous regression. When and how to treat is debated, but in some centers, newly diagnosed small tumors are subject to conservative treatment, i.e. serial MRI scans without active treatment. In cases where the tumor has shown rapid growth in serial scans, or has become relatively large, treatment is usually offered. Two primary treatment methods prevail: microsurgery, where the tumor is physically removed; and gamma-knife radiosurgery (GKRS), where a gamma ray radiation dose is delivered to the tumor. This radiation dose, in theory, causes tumor growth arrest. In this study we wish to detail the success rate of GKRS, by comparing growth rates before and after GKRS treatment, and finding the proportion of tumors that get a successful treatment (defined as a negative or zero post-treatment growth rate).

**Material and methods:** Between 2000 and 2006, 347 patients were diagnosed with VS. From these, 159 (46%) received treatment by the end of 2007, while the remaining 190 (54%) were treated conservatively. A total of 41 (22%) of these conservatively treated patients later received GKRS treatment due to growth of the tumor, as detected by the serial MRI scans. These 41 patients were included in the study, and were followed for a minimum of two years after treatment. The tumor volume on both pre-treatment and post-treatment images were measured, and mixed effects models were used to analyze the growth rates before and after treatment. We also conducted a logistic regression analysis to determine whether the age of the patient or the growth rate before treatment are related to achieving successful treatment.

**Results and conclusion:** We found a lower success rate for GKRS treatment of VS than previously reported. This finding is likely because other studies have included patients that had no growth potential, while our study includes only patients who have had previously documented growth. Details will be presented at the conference.

### Opposite effects of Levodopa on conditioned behaviors

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**Objective:** To study the conditioning of inhibitory and executive actions in patients with Parkinson's disease (PD) on versus off Levodopa therapy.

**Background:** The basal ganglia (BG) have been implicated in the conditioning of behavior. Accordingly, it could be expected that in dopamine deficient Parkinsonian patients an impairment of this function will be restored by Levodopa treatment.

**Method:** Sixteen PD patients with Levodopa monotherapy and fourteen controls took part in the study. They engaged in a modified Go/NoGo task, the PD patients being tested twice in an on and off Levodopa state. In this, four equiprobable stimuli were repeatedly presented in pseudo-randomised order. One of the stimuli was defined as target to which a button press had to be performed (Go task) or withheld (NoGo task; here, all non-target stimuli were instructive for the button press). Both tasks comprised two conditions, one in which the target stimulus was always preceded by the same non-target stimulus (Conditioning), and another one in which the 'non-target to target sequence' was at random (Deconditioning). Go and NoGo task consisted of 800 stimuli in which Conditioning and Unconditioning were alternated within 4 blocks of 200 stimuli, avoiding a systematic vigilance effect in one of the conditions.

**Results:** In the NoGo task, controls and PD patients off Levodopa had a significant increase of errors from the coupled to the uncoupled condition (pushing the button although they had to withhold this response). In contrast, PD patients on Levodopa did not perform differently between conditions. In the Go task, controls and PD patients off Levodopa did not perform differently between coupled and uncoupled condition. However, PD patients on Levodopa showed a significant increase of errors from the coupled to the uncoupled condition (omitting the button press although they had to execute this response).

**Conclusions:** Levodopa has opposite effects on the conditioning of executive and inhibitory behavior: it hampers the conditioning of NoGo-stimuli as opposed to the deconditioning of Go-stimuli. This appears to indicate enhanced Levodopa-induced event coupling specifically under executive demands. As unmedicated PD patients followed the conditioning-deconditioning pattern of controls, this probably reflects a generic dopaminergic effect which might relate to complex dysfunctional behaviors in thus treated patients, as e. g. gambling and punting.

vision-related functioning by a valid questionnaire such as Ocular Surface Disease Index (OSDI), evaluation of ocular surface damage by special stains such as fluorescein and other methods. The aim of this study was to determine the correlation between 5-minute non-anesthetized closed eyes ST (STc) with OSDI questionnaire in the diagnosis of DTS.

**Methods:** In a prospective observational case series, 70 patients with rheumatoid arthritis completed the OSDI questionnaire (scored from 0-100) and then underwent a series of diagnostic tests including 5-minute STc, tear film breakup time (TBUT) and fluorescein staining test of the conjunctiva and corneal surface. The diagnosis of DTS was made according to the revised Japanese diagnostic criteria, based on the presence of ocular symptoms, presence of ocular surface damage and impaired tear break up time test. We categorized the patients as definite DTS group, probable DTS group and no DTS group and also a sum group (both definite and probable groups). Right eye data was used for statistical analysis. Those with incomplete data or the presence of other ocular surface disorders were excluded.

**Results:** Sixty-four eyes were included. There were 54 (84.4%) female. Mean age was 53.2 ( $\pm$  12.1) year. Twenty six (40.6%) had definite, 19 (29.7%) had probable, 19 (29.7%) had no DTS and 45 (70.3%) were in sum group. Mean OSDI score significantly increased from 12.5 ( $\pm$  12.9) in no DTS group to 43.7 ( $\pm$  21.1) in definite DTS group ( $P = 0.000$ ). Mean 5-minute STc significantly decreased from 13.8 ( $\pm$  8.1) in no DTS group to 10.5 ( $\pm$  8.9) in definite DTS group ( $P = 0.002$ ). A significant but weak positive correlation was noted between OSDI scores and STc in definite DTS group ( $R = 0.291, P = 0.001$ ) and probable group ( $R = 0.101, P = 0.016$ ). No such a correlation was found in no DTS group ( $R = 0.076, P = 0.093$ ).

**Conclusion:** This study showed a significant correlation between two important diagnostic tests in dysfunctional tear syndrome. However, weak correlation support the previous reports of discrepancies between symptoms and rate of aqueous tear secretion assessed by Schirmer's test.

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## Ophthalmology

### Correlation between Ocular Surface Disease Index (OSDI) questionnaire and Schirmer's test in Patients with Dysfunctional Tear Syndrome

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**Aim:** Currently the diagnosis of dysfunctional tear syndrome (DTS) relies on a set of diagnostic tests including assessment of the aqueous tear secretion by Schirmer's test (ST), assessment of the symptoms of ocular irritation consistent with dry eye disease and their impact on

### Role of systemic risk factors in diabetic retinopathy

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**Introduction:** Diabetes mellitus is a systemic metabolic disorder, with serious complications. Changes in the blood supply of the eye causes retinal damage called diabetic retinopathy. Diabetic retinopathy has two stages, non proliferative and proliferative retinopathy, which can be further divided into five clinical types. Diabetic retinopathy appears to be one of the major causes of blindness in adults.

**Aim:** Aim of the study was to determine the frequency of certain clinical types of diabetic retinopathy in population of adults with type 2 diabetes who have retinal diabetic changes, and to find out the possible correlation between severity of retinopathy and several systemic risk factors.

**Material and Methods:** This cross-sectional study analysed type 2 diabetic patients, who were referred from their local ophthalmologists due to diabetic retinopathy changes to Clinical Centre of Vojvodina for further investigation in period of November 1, 2008 to January 31, 2009. Patients underwent questionnaire and complete ophthalmologic examination. Then the data with emphasize on possible correlation between risk factors (such as duration of diabetes, glycaemic regulation, blood pressure) and severity of diabetic retinopathy were analysed. **Results:** There were 37 patients examined in this study the average age of the patients was 64,1 (youngest patient was 47, the oldest 85) the average duration of diabetes was 12,25 years. Glycaemic level was under the limit of 11,1 mmol/l in 84% of patients. The HbA1c level, was under the level of 7% in 30% of patients only. 54% patients had high blood pressure, extremely high blood pressure (>170/>90 mmHg) was found in 8% of the cases. All of the patients with extremely high blood pressure had proliferative diabetic retinopathy. The highest correlation was found between HbA1c level and severity of diabetic retinopathy ( $r = 0,39$ ). A bit smaller correlation was found between high blood pressure and diabetic retinopathy ( $r = 0,33$ ). The relationship between duration of diabetes and severity of retinal changes in our study was not so obvious ( $r = 0,20$ ).

**Conclusion:** Long term glycaemic level control and therapy of high blood pressure can save the eyes from developing a severe stage of diabetic retinopathy. While duration of diabetes has not much implication on severity of diabetic retinopathy.

## Orthopaedics

### Harris Hip Score in the Evaluation of Postoperative Results in Trochanteric Fractures

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During the last 50 years, the treatment of trochanteric femur fractures has evolved with improved understanding of both fracture biology and biomechanics. Previously, nonsurgical treatment of these fractures was associated not only with significant shortening and malrotation but also with the morbidity and mortality of prolonged immobilization.

**Objective:** The aim of the present paper is to compare the results obtained using two osteosynthesis methods developed for the surgical treatment of fractures of the trochanteric region of the femur: the Gamma nail and the Dynamic Hip Screw (DHS) using the Harris Hip Score.

**Material and method:** A retrospective study is presented of 73 cases of patients sustaining different types of trochanteric fractures treated surgically in the second Orthopedic Department at "Floreasca" Emergency Hospital, Bucharest, Romania between March 2007 and

September 2008. There were 48 females with an average age of 68 (46 – 93 years old) and 25 men with an average age of 63 (40 – 89 years old). Thirty-one trochanteric fractures of the femur were treated using the Gamma nail and forty-two were treated with the Dynamic Hip Screw (DHS) in our service with the prerequisite of a minimum follow-up of three months. **Main Outcome Measurements:** Radiographs were analyzed for fracture classification, evaluation of fracture reduction, implant positioning, later loss of reduction, and other complications. Postoperative functional status of the patients was recorded, with a minimum of three months follow-up using the Harris Hip Score.

**Results:** Nine percent of the patients in the DHS group and four percent in the Gamma group suffered significant secondary redisplacement during the three months follow-up, leading to a varus malunion, lag screw cutout, or excessive lag screw sliding with medialization of the distal fracture fragment. One patient (2.0 percent) in the DHS group suffered an implant-related femoral fracture below the plate and needed surgical reintervention, and one had a deep infection in the DHS group. All but one fracture in the Gamma group and two fractures in the DHS group healed within three months. After six weeks and then later at three months we evaluated the patients both radiologically and clinically using the Harris Hip Score Questionnaire. On the six weeks follow-up we obtained an 86 (66-97) medium Harris Hip Score for the Gamma nail group and a 77 (63-98) for the DHS group. On the three months follow-up we noticed that the medium Harris Hip Score for the Gamma nail group increased up to 91 (75-99), while for the DHS group was 89 (69-98).

**Conclusion:** The functional outcome of the surgical treatment in trochanteric fractures using the Gamma nail at six weeks evaluation seems to be above the results obtained using DHS, due to less exposure of the fracture, limited blood loss and early immobilization, however at the three months evaluation the Harris Hip Score leveled for the two groups. The use of close reduction, internal fixation with Gamma Nail has proven to be superior in the treatment of these fractures because intramedullary nails have a biomechanical and biological advantage over standard compression hip and less blood loss. Intramedullary nails seem to have some advantage in unstable fractures, especially fractures with reverse obliquity and subtrochanteric extension that cannot be treated easily with standard hip compression screws. However, at the three months follow-up the two osteosynthesis methods offered similar functional results.

### Differentiating between normal and torn rotator cuff tendons using Fourier transform infrared spectroscopy

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**Aim:** This study aimed to identify possible biomarkers of rotator cuff tendons (RCT) tear progression through the use of Fourier Transform Infrared Spectroscopy (FTIR).

**Introduction:** A challenge to medical diagnostics is to accurately and reliably identify pathology and monitor its progression. Rotator cuff tendon (RCT) tears are estimat-

ed to affect between 5-30% of adults [1] [2], placing a huge socioeconomic burden on ageing populations. The aim of this study was to identify biomarkers of RCT tear pathologies to aid accurate identification and monitoring of disease progression. FTIR provides unique biochemical fingerprints of tissue specimens. All molecules are excited to higher vibrational states at specific wavelengths, which can be used to identify the chemical composition of tissues. It was hypothesised that healthy and different RCT tears could be distinguished using attenuated total reflectance (ATR) FTIR spectroscopy.

**Methods and Materials:** The chemical composition of 50 formalin-fixed RCTs was measured from patients aged between 20 and 89. RCT tears were classified according to size as suggested by Post et al [3]; 10 each of small, medium, large and massive. These torn RCTs were compared to 10 uninjured RCTs. A diamond ATR accessory was used with a FTIR spectrometer to collect spectra for each sample. In order to correlate the unique FTIR spectra with chemical signals, the spectra were reduced and classified using standard multivariate analysis; principal component analysis (PCA), partial least square (PLS) and discriminant function analysis (DFA).

**Results:** Hierarchical cluster (HCA) demonstrated that normal and torn tendons could be clearly differentiated, and RCT could also be distinguished by their tear size. The spectral features accounting for the discrimination identified regions of importance: (i) 1030-1200cm<sup>-1</sup>: lipids/carbohydrates/phospholipids, (ii) 1300-1700, 3000-3350cm<sup>-1</sup>: collagen structural conformation, (iii) 2800-3000cm<sup>-1</sup>: lipids and (iv) 3400cm<sup>-1</sup>: hydroxyl group from formalin.

**Conclusion:** FTIR can clearly distinguish between normal and different sized RCT tears. This prospective non-randomized study indicates that the onset of RCT tear pathology is mainly due to an alteration of the collagen structural arrangements, with associated changes in lipids and carbohydrates. The approach described is rapid and has the potential to be used intra-operatively to determine the quality of the tendon and extent of disease, thus guiding surgical repairs.

### Which position causes higher spinal load – sitting or standing?

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**Aim:** Intradiscal pressure measurements of Nachemson et al. [1] showed 40% higher values for sitting than for standing. Therefore, spine patients were often not allowed to sit. Other groups (Althoff et al. [2]; Wilke et al. [3]) measured lower spinal loads for sitting than for standing. It is also unknown how the spinal shape correlates with the load. The aim of this study was to explore the reason for such contradictory results on spinal load and to determine the relationship between back shape and spinal load.

**Methods and materials:** A telemeterized vertebral body replacement (VBR) allows the in vivo measurement of the implant loads (Rohlmann et al. [4]). It was implanted in 5 patients suffering from a fractured lumbar vertebral body.

Implant loads were measured in several sessions during relaxed standing and during sitting on a stool. In the sitting position the arms were either hanging at the sides or placed on the thighs. Results were related to the value for relaxed standing of the same measuring session and the average of several trails was calculated. For sitting and standing, the shape of the back was determined by using raster stereography.

**Results:** Related to standing relaxed, the force on the VBR was increased for sitting by +10% for patient WP1, +32% (WP3), +37% (WP2), +51% (WP4) and +97% (WP5). For this same order of patients, the kyphosis angles of the thoracic spine were 41°, 48°, 55°, 59° and 67° and the distances between vertebra prominens and lordosis apex were 359 mm, 375 mm, 389 mm, 404 mm and 465 mm. Placing the arms on the thighs reduces the force on the VBR on the average by 15% (4% to 37%), when compared to sitting with arms hanging at the sides.

**Conclusion:** In conformity with Nachemson et al. [1], the forces on the VBR were higher for sitting on a stool than they were for standing. In contradictory studies (Althoff et al. [2]; Wilke et al. [3]), the arms of the volunteers were placed on the thighs, and this reduces spinal load. Thus, a direct comparison of these studies is problematic. The load differences between sitting and standing depend on several factors, including arm position and inclination of the upper body. This is reflected by the large variation of our results and those of other groups. The individual shape of the spine also affects the load during sitting. Patients with a strongly curved thoracic spine, and thereby a more caudally placed lordosis apex, have a higher spinal load while they are sitting.

### Rag-1 deficient mice have an accelerated fracture healing

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**Purpose:** Early phases of bone healing are dominated by immunological aspects. This project was aimed at further elucidating how the host immune system participates in the process of bone regeneration following fracture. The hypothesis of this study was that fracture healing would be delayed in the absence of the adaptive immune system. **Methods:** Complete absence of the adaptive immune system was modelled by using RAG1 (recombination activating gene 1) knockout mice lacking mature B and T lymphocytes. A standard closed femoral fracture was created in 8-10 weeks old wildtype (WT) and RAG1 knockout (RAG1) mice. For  $\mu$ CT analysis and biomechanical testing, animals were sacrificed after 14, 21 and 28 days (N = 8/time point). Statistical comparisons between the groups were performed using the Mann-Whitney U-test.

**Results:** Biomechanical testing demonstrated a significantly higher torsional moment at day 14 (77 (69/84) % vs. 60 (50/63) %; [median (25/75 percentile)], p = 0.005) and a trend to a higher torsional moment at day 21 (105 (95/122) % vs. 87 (77/91) %, p = 0.065) in the RAG1 in comparison to the WT group.  $\mu$ CT evaluation of RAG1 specimens showed a decrease in the total volume of the

callus (TV) from day 14 to day 21. In contrast, in the WT specimens the decrease of TV was seen one week later from day 21 to day 28. At day 21, TV was significantly lower in the RAG1 compared to the WT group (21 (16/33) mm<sub>3</sub> vs. 36 (25/46) mm<sub>3</sub>,  $p = 0.01$ ). At day 28 the bone volume fraction of the callus (BV/TV) was significantly higher in the RAG1 in comparison to the WT group (41 (33/44) vs. 30 (28/35),  $p = 0.02$ ).

**Conclusion:** The results of this study have shown a significant and unexpected phenotype of enhanced bone regeneration in RAG1 knockout mice compared to WT mice suggesting that mature lymphocytes may delay the early phase of fracture healing. The morphometric and biomechanical analyses provide strong evidence for this conclusion, but further analyses are necessary to identify the underlying molecular and cellular mechanisms.

### Evaluation of strength of titanium miniplates used for the mandibular angle fracture osteosynthesis according to different fixation techniques performed – an experimental study

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Evaluation of strength of titanium miniplates used for the mandibular angle fracture osteosynthesis according to different fixation techniques performed – an experimental study  
**Introduction** Mandibular angle fractures have been reported to comprise between 15 and 25% of all fractures of the mandible. One of the methods performed to stabilize fracture site is the use of titanium miniplates. Fixation must be able to resist the displacing forces acting on the mandible. There are few techniques of placing the miniplates in the angular region of mandible. Unfortunately in some cases under different factors the immobilizing element itself can be fractured resulting in malunion of the bone.

**Aim:** The aim of the study was to assess and to compare the strength of titanium miniplate setups depending on fixation technique performed to stabilize the angular fracture of mandible.

**Materials and methods:** In order to assess the strength of titanium miniplates acrylic resin models of the mandible with the fracture line on the angle were used. Fracture site was stabilized with 2.0 titanium miniplates with four holes and four 7mm screws in four ways. Prepared models were used to create by means of Finite Element Method (FEM) a computer model and stress simulation. The computer simulation findings were used as the preliminary results of the study as well as to design and prepare the experiment with use of QTest/10 strength-testing machine. Static rupture probing of miniplates in different osteosynthesis configurations was performed.

**Results:** The study has revealed the differences in the strength of the fixation depending on the osteosynthesis technique performed. The most durable miniplate setup appeared to be osteosynthesis technique with two miniplates on the lateral surface of mandible (average force

needed to damage the miniplates amounts 2050 N) and the weakest one with one twisted plate placed on the oblique line (860 N).

**Conclusions:** On the basis of FEM analysis and static rupture probing it has been revealed that material rupture is not significant factor when considering the fracture of titanium miniplates placed in the angular region of the mandible. Measured forces would destroy the bone before breaking the titanium miniplate.

### Fracture healing in the NF1flox/floxPrx1Cre mouse

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**Introduction:** Neurofibromatosis 1 (Morbus Recklinghausen, NF1) is a common genetic disease with an incidence of 1:3000. About 50 % of patients show skeletal lesions like bowing of the tibia which is used as a diagnostic criterion. In NF1, bone healing is affected and may lead to pseudarthrosis, which is very challenging to treat. Studies on tibial middiaphyseal fracture healing in NF1 ± heterozygous knock-out mice revealed no disturbed healing. Further studies of human tibial pseudarthroses in patients with NF1 could proof a loss of heterozygosity (LOH) as the cause for disturbed fracture healing. As animals with a homozygous knock-out of NF1 die during embryogenesis, studies of LOH are only possible with conditional knock-out. Aim of the current study is to analyse fracture healing and its disturbance in NF1 with LOH in the NF1flox/floxPrx1Cre knock-out mouse.

**Methods:** Male 8-10 weeks old C57B16/N mice (WT, N = 32) and knock-out mice with C57B16/N background (KO, N = 4) were included in this study. A femoral, transverse, middiaphyseal fracture was created and stabilized with an intramedullary nail. For  $\mu$ CT analysis, histological analysis and histomorphometry, femora of WT were harvested after 7, 10, 14 and 21 days (N = 8 each), femora of KO (N = 4) were harvested after 21 days. For histological analysis, the femora were embedded in paraffin, sectioned (5 $\mu$ m) and stained with Movat Pentachrome. Histomorphometry was performed with the Zeiss KS400 programme (Zeiss, Germany). Statistical evaluation was performed with the Mann-Whitney U test (SPSS) and the Bonferroni-Holm test procedure.

**Results:** At day 7 in the WT group, a cartilaginous callus was formed with cartilaginous hypertrophy over day 10. At day 14 slight mineralization of the callus followed. After 21 days, a bony bridged callus was formed. The total callus volume (TV) in the WT increased from day 7 (9.3 (7.2/10.3 mm<sup>3</sup>), [median (25% quartile/75% quartile)] over day 10 (18.6 (15.7/21.4 mm<sup>3</sup>);  $p = 0.001$ ) to day 14 (28.1 (25.3/39.9 mm<sup>3</sup>);  $p = 0.001$ ). Between day 14 and day 21, TV stayed nearly constant (30.7 (24.8/39.4 mm<sup>3</sup>);  $p = 0.8$ ). Bone volume (BV) increased during the whole time (day 7: 3.1 (2.5/3.2 mm<sup>3</sup>); day 10: 4.8 (3.7/5.1 mm<sup>3</sup>); day 14: 9.8 (7.6/10.8 mm<sup>3</sup>); day 21: 10.5 (9.7/14.6 mm<sup>3</sup>); all  $p < 0.05$ ). Compared to the WT, TV of the KO on day 21 (13.7 (8.4/17 mm<sup>3</sup>);  $p = 0.007$ ) as well as BV (6.3 (4/7 mm<sup>3</sup>);  $p = 0.007$ ) was decreased.



*Discussion:* The WT mice displayed regular, secondary bone healing with bony bridging. In contrast, fracture healing in the KO was delayed with less callus formation and lack of bony bridging by day 21. This delay might be based on a decreased differentiation capacity and mineralization defect of bone precursor cells due to loss of the ras-inhibitor neurofibromin (LOH) and activation of the Ras-MAPK-pathway.

### Characteristics of implants with thermic memory - Nitinol

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*Objective:* For more decades in the field of traumatology are widely used different systems of fixators that are made of stainless steel, titanium alloys, chromium and cobalt alloys. The main demands for this implants, along with biocompatibility, were considered the high mechanical and anticorrosive resistance. Only during the last few decades begun the study of mechanical compatibility of the implants used for restoring the integrity of different tissues such as bone, cartilage and ligaments. The matter is that the module of elasticity of the majority of constructional alloys is much higher, than at a bone, and furthermore of cartilaginous structures. At teamwork of the bone – implant, occurs non-uniform distribution of deformations and pressure which are maximal, as a rule, in places of implant fixation to a bone that causes danger of their destruction. At the same time is known a material, which mechanical behaviour mimics the behaviour of living tissues. These are alloys on the basis of nickel-titan or in another way "nitinol". Taking into account the mentioned above we have the objective to test the possibility of using the implants with thermic memory made of nitinol for the osteosynthesis of hand and foot bones as well as the proposal for an original implant made of nitinol for restoring the coracoclavicular ligaments in dislocations of the acromial part.

*Methods:* There were tested the properties of implants with memory made by Engineering-Medical Center (EMC "MATI-Medtech") for the osteosynthesis of the scaphoid bone at 4 patients and for osteosynthesis of the first metatarsal bone and of the basal phalanx in the surgical treatment of hallux valgus. Both for patients with scaphoid fracture (4) as well as for those with surgical treatment of hallux valgus, the bone consolidation after osteosynthesis with nitinol fixators evolved without complications in simple terms. Taking into account the main role of the coracoclavicular ligaments for the stabilization of the acromioclavicular joint and the absence till our days of an optimal fixator or of a surgical approach that is univocal accepted for these lesions, we want to develop an implant for dealing with acromioclavicular dislocations. The study was done together with the Engineering-Medical Center (EMC "MATI-Medtech") of "MATI"-Russian State Technological University named after Tsiolkovski under the lead of academician A. On 5 cadavers were performed anthropometrical studies of the clavicle, conoid and trapezoid ligaments, as well as of the coracoid process. The obtained data are recently used

for the creation of a mathematical model and computer simulation of the new model for the restoring of the coracoclavicular ligaments.

*Conclusions:* The preliminary results obtained both in clinic and experimentally, are optimistic and motivate further studies in the usage of implants with thermic memory for the restoring of different structures of the osteotendinous apparatus.

### Fracture Healing is Accelerated in the Absence of the Adaptive Immune System

ESC-ID **692**  
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*Introduction:* In the early phase of fracture healing, the immune system plays an important role. The immune system and the skeleton closely interact, due to shared anatomical compartments, cell precursors and molecular mediators such as cytokines. Studies show that an intact cellular immune response is important for a normal outcome of tissue repair. This project was aimed at further elucidating how the host immune system participates in the process of bone regeneration following fracture. The hypothesis of the study was that fracture healing would be delayed in the absence of mature B and T lymphocytes.

*Methods:* The RAG1 (recombination activating gene 1) knockout mouse was used to model the complete absence of the adaptive immune system. C57Bl6/N wildtype (WT) mice act as control. A standard transverse closed femoral fracture was created in the 8-10 weeks old mice. The fracture was stabilized by an intramedullary nail. For histological and histomorphometrical analysis, femora were harvested after 3, 7, 14, 21 and 28 days (N=6). The femora were embedded in paraffin and slices of 4µm were taken. The sections of the callus region were stained with Movat Pentachrom. Statistical comparisons between the groups were performed by using the Mann-Whitney-U-test.

*Results:* At day 7, a cartilaginous callus was formed in the WT mice which began to mineralize at day 14. The histomorphometrical analysis showed a significantly higher fraction of bone (12 (6/17)%; [median (25% percentile/75% percentile] vs, 4 (1/6)%, p = 0.015) and a significantly lower fraction of cartilage (40 (33/46)% vs. 50 (44/60)%, p = 0.041) in the callus of the RAG1 in comparison to the WT mice. During the remodelling phase of fracture healing at day 28, both mouse strains seem to have equal amounts of bone.

*Conclusion:* This study showed enhanced bone regeneration in the RAG1 knockout mice compared to the WT mice. This unexpected and significant result suggests that mature B and T lymphocytes and their secretion of mediators and cytokines delay the early phase of fracture healing. It is necessary to identify the underlying mechanisms by immunohistochemistry molecular analyses and to get to know which subpopulation of lymphocytes is responsible for this positive effect an adoptive transfer is planned.

## Bone Bridge Formation and Vascularisation after Transphyseal Drill Hole Lesion in Young Rat Tibiae

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*Aim:* Physeal lesions are caused by traumas (directly or by fractures crossing the growth plate) or by advancing implants through the growth plate during surgery in order to stabilize fractures. These lesions can initiate the formation of reversible bone-bridges resulting in bone length discrepancy, axis deviation or joint deformity. Our aim was to show what happens within the lesion and the physis when an equivalent trauma is admitted to the bone. We suspected a formation of bone bridging the growth plate, as known from body-imaging in children, and an uptake of vascularisation factors such as VEGF and its receptors R1 and R2 due to the formation of new bone tissue.

*Material and Methods:* 35 male Sprague Dawley rats at an average age of 4 weeks and a weight of 100g ( $\pm$  5g) were narcotized and an axial hole was drilled through the proximal Tibia growth plate. The animals were euthanized on days 1, 3, 7, 14, 28, 42 and 82 and their left and right tibiae were extracted. Sections of the growth plate at the level of the lesion on the left side were stained using hematoxylin and eosine. Immunohistochemically anti-VEGF, anti VEGF-R1 and anti VEGF-R2 antibodies were used to show the presence of VEGF, R1 and R2. Sections of the growth plate of the right leg were used as controls and were stained and examined the same way.

*Results:* Histological examination of the physeal lesion showed a hematoma on days 1 and 3 containing displaced bone fragments. On day 7, reorganization of those fragments was observed prior to bone bridge formation on day 14. By day 82, this bone bridge was replaced by lamellar bone. Immunohistochemical staining of the bone showed clear positivity for VEGF, VEGF-R1 and VEGF-R2 of the displaced bone fragments on days 1 to 7. The bone bridge remained VEGF positive, but the positivity decreased from the proximal to the distal part on day 82. VEGF-R1 and -R2 were absent in the temporary bone bridge but VEGF-R2 was positive in the early lamellar bone formation on day 42. The border between the lesion and the physis was VEGF, VEGF-R1 and -R2 positive throughout the days, showing maximums of R1 on day 14 and of R2 on days 14 to 42. VEGF was not expressed in the first 2 to 4 physeal columns adjoining the lesion, while R1 showed increasing positivity in this region on days 1 to 7. R2 remained negative in the physis, except for the mineralizing zone but showed no change in the area surrounding the defect.

*Conclusion:* This is the first paper to show the progress and development of a bone bridge leading to length growth lesions. The fragments are positive for VEGF, R1 and R2 on days 1 to 7 which shows the importance of those three factors in the early stage. The temporary bone bridge does not depend on R1 while VEGF is needed throughout the examined days for formation of lamellar bone until it also starts to decrease on day 82 from the proximal to the distal part. R2 seems to be necessary for

a switch to lamellar bone. The border between the lesion and the growth plate plays an important role in reorganization and reformation of bone tissue. The adjoining region of the growth plate shows a changing expression of VEGF and R1 in days 1 to 7, this may be a direct reaction to the suffered trauma.

## Long-term functional outcome following antegrade versus retrograde nailing of femoral shaft fractures

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*Introduction:* Femoral shaft fractures are frequently seen fractures of the long bones. These are generally caused by high energy trauma, i.e. motor vehicle accidents or falls from heights. The management of femoral shaft fractures using antegrade or retrograde intramedullary nailing is a popular method. These procedures involve insertion of a titanium nail into the bone marrow, resp. through the hip and knee, to obtain a rigid fixation of the bone fragments. Antegrade nailing has been the standard procedure, but retrograde nailing has become increasingly popular over the years. The purpose of this study is to assess the long-term functional outcome after antegrade versus retrograde intramedullary nailing of traumatic femoral shaft fractures.

*Methods:* In a retrospective study, patients with no other injuries to the lower limbs or pelvis than a femoral shaft fracture were included. 62 Patients met the inclusion criteria: 43 patients were treated using antegrade nailing and 19 patients using retrograde nailing. Functional outcome scores (Short Musculoskeletal Functional Assessment (SMFA), Western Ontario and McMaster University Osteoarthritis (WOMAC) index, Harris Hip Score (HHS) and the Lysholm knee function scoring scale) were measured at a mean of 7.8 years ( $\pm$  3.8 yrs) postoperatively. The visual analog score (VAS) was used to determine pain complaints of the lower limbs. The range of motion (ROM) of the hip and knee joint were assessed according to the neutral-0 method. Chi-squared analysis was performed to evaluate differences between groups for dichotomous variables, and t-tests were used for continuous variables.

*Results:* The mean injury severity score (ISS) was equal in both groups. The ROM of the hip and knee joints did not differ. Although the mean VAS was greater in the antegrade group (2.5) compared to the retrograde group (1.9), this difference was not significant. The mean SMFA in the antegrade group was 16 and in the retrograde group 15. The joint-related outcome scores (WOMAC, HHS and Lysholm scoring scale) did not differ between the two groups either.

*Conclusion:* This study shows that the ROM of the hip and knee joints are equal on the long term after antegrade compared to retrograde nailing of femoral shaft fractures. Furthermore, there are no differences in the long-term functional outcome scores between the groups.

## In vivo Measurement of shoulder joint loads during activities of daily living

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*Introduction:* Loads of the shoulder joint must be known to understand its normal and pathologic functions. Calculations of these loads are not validated for simple movements like abduction and even more uncertain for activities of daily living (ADL) [1].

*Methods:* For this study (approved by our Ethics Committee) clinically proven BIOMET Biomodular shoulder implants were equipped with 6 strain gages and inductive powered 9-Channel transmitter [2]. Measured are 3 force components (reported here) and 3 moments.

*Results:* Results of four patients with arthrosis (2 male, 2 female; Age 63-80y) and data from 5 ADL are reported here, measured 3 to 12 months after implantation. COMBING: The patients were not advised how to comb their hair. NAILING: Patient is standing with horizontal forearm and vertical upper arm and nails with a hammer (500g) against the wall. HOLDING: The Patient holding a weight of 10 kg beside the body with hanging arm LIFTING: Starting from the Holding position, the patient lifts up the weight by 30cm SHELF: The Patient places a weight of 2 kg on a shelf in waist height in a distance of 30cm and takes it out again RESULTS COMBING: Combing lead to a maximum force of 81% bodyweight (BW) although no markedly external weight is moved. NAILING: During the nailing task values between 83,5 und 117%BW were reached. In absolute values the male patients had higher forces (976N & 858N) than the female ones (590N & 540N). HOLDING: Passive holding lead to surprisingly low contact forces in the shoulder joint between 9.2 and 17.9%BW. LIFTING: At lifting the 10 kg weight the contact loads went up to 87 - 95%BW SHELF: the forces for placing an item in the shelf varied strongly between 54 and 137%BW. At all patients the forces were lower when they removed the item compared to placing it.

*Discussion:* The measurements showed that some ADL cause very high shoulder contact forces. Shoulder loading can even be higher than the own bodyweight. In the first weeks after shoulder arthroplasty or fracture fixation in the shoulder region patients should avoid to lift objects with stretched out arm. Even the simple elevation or abduction of the straight arm without additional weight causes high shoulder contact forces. Surprisingly we found that placing a weight causes markedly higher shoulder contact forces than removing it. We assume that the control of height and horizontal position when laying down a weight requires stabilization of the joint by high antagonist muscle activities. When picking up the weight less navigation and stabilization effort is needed. Furthermore the passive holding of the weight caused low forces compared to calculated values in the literature [1]. These interesting effects will be investigated in more detail. Data from more patients and activities will be published.

*Remark:* The presented results were previously partially published at the german orthopaedic congress and the ISB congress.

## Otorhinolaryngoscopy

### Anatomical research on the discomalleolar ligament

ESC-ID 702  
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*Introduction:* The discomalleolar ligament (Pinto ligament) is a structure of fibrous tissue connecting the malleus through the petrotympanic fissure with the medioposterosuperior part of the temporomandibular joint (TMJ). Although it is not described in classic anatomical textbooks it is supposed to be one of the explanations for the aural symptoms associated with TMJ dysfunction, trauma and should be taken under consideration during diagnosing such symptoms as tinnitus, vertigo or otalgia. What is more, knowledge of the anatomy of this structure is important during surgical procedures performed on TMJ such as arthroscopy or condyle exfoliation.

*Aim:* The aim of our study was to investigate the frequency of occurrence, attachments and topography of the discomalleolar ligament (DML) by means of dissection and microtomographic imaging. The ligament was also studied during arthroscopy of the TMJ. Received visualisation allowed us to examine the impact of movements in TMJ on the malleus threw the DML to verify, whether damage or extended tension applied to the DML can cause aural symptoms in TMJ pathology or after TMJ surgery.

*Materials and Methods:* The study was conducted on 40 en bloc specimens of the temporal bone with undamaged TMJ and on 2 fresh frozen heads collected from adult cadaver bodies. Imaging was performed by SkyScan 1017 microtomograph (30  $\mu$ m resolution) and elaborated in 3D technique. After arthroscopy specimens were dissected with the use of the surgical microscope and microsurgical instruments. Dissection was performed from the superior approach through the middle cranial fossa to expose TMJ, auditory ossicles and the petrotympanic fissure. Afterwards investigated structures were measured and movement of the TMJ was inducted to examine the malleus dyslocation.

*Results:* Dissections exposed the DML in 40 (91%) specimens. It was either longitudinal or triangular in shape with the average length of 7 mm what correlated with results obtained during microtomographic and arthroscopic examination. We observed the movement of the malleus resulting from the tension applied to the TMJ structures.

*Conclusions:* Our investigation confirmed that there is an evident connection between the malleus and medioposterosuperior part of the TMJ and that DML should be considered as an intrinsic ligament of the TMJ. Clinical implications are of notice as the induction of tension from TMJ structures on malleus was noticed in the significant amount of cases and therefore need further investigation.

### Pure-tone audiometry and DPOAEs registration in patients with diabetes mellitus and hearing loss: diagnostic and psychological aspect

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**Introduction:** Evaluation of the functional status of the inner ear can be provided with the otoacoustic emission (OAE). Cochlear hair cells produce a weak acoustic signals, and the essence of otoacoustic emission is registration of these signals. OAE reflects active micromechanical process on the outer hair cells in the organ of Corti. Method of the otoacoustic emission is used for diagnostics of damage of the inner ear (perceptive auditory organ).

**Aim:** Comparison of the diagnostic value and stressor's possibility of the pure-tone audiogram and DP-gram of the distortion products otoacoustic emission (DPOAEs) registration in patients with diabetes mellitus and sensorineural hearing loss.

**Methods and Materials:** We examined 37 patients with diabetes mellitus and sensorineural hearing loss. Investigation was provided in the following way: anamnesis, examination of the LOR-organs, impedance audiometry, initial pulse measurement, pure tone audiometry, DPOAE. After every procedure of pure tone audiometry and DPOAE had finished every patient had pulse measurement again. The age of patients was 25 to 58 years, 21 males and 16 females. The hearing thresholds of patients were up to 55 dB. All subjects had normal (type A) tympanograms.

**Results:** Comparing the pure-tone audiogram and DP-gram of our patients we recognize the similar form of the pure tone audiogram and DP-gram curves on frequencies from 125 to 10000 Hz. The disturbance of average level of interval between graphics of tinnitus and otoacoustic emission in majority of patients was corresponding to the hearing loss level in pure tone audiogram. The patients' pulse rate after audiometry increased by 11 beats in a minute in average comparing with their initial pulse rate. No changes in pulse rate were observed when DPOAE had been used.

**Conclusion:** In this study we compared indices of pure tone audiometry and distortion products otoacoustic emission in diabetes patients with hearing disorders. In response to sufficient great percentage of correlation in this indices, objectivity, rapidity and easiness, using DPOAE for evaluation the hearing function, especially in dynamics in patients with diabetes mellitus, is expedient and advisable. In addition, pulse rate's increasing after pure-tone audiometry may indicate anxiety arises in patients due to efforts and mistakes they make. DPOAE thus seems to be less traumatic for patients' state of mind.

### Paediatrics

#### A case report of successful interferon therapy in a neonate with Kasabach-Merritt syndrome

ESC-ID 250  
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We report a case of 10-day-old boy presenting with a giant hemangioma in his left hand complicated by Kasabach-Merritt syndrome with thrombocytopenia. After poor response to corticosteroid therapy we began interferon alfa. This patient showed a dramatic response to interferon alfa.

#### Lipid nutrition and susceptibility to Chronic Lung Disease: The role of changes to the composition of lung surfactant phospholipids in preterm babies

ESC-ID 235  
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Studies have shown that lipid nutrition is able to modify surfactant phospholipid composition in preterm infants. Further studies have shown that phospholipid molecular species are able to differentially influence inflammatory cell function. It is currently unknown whether exposure to exogenous lipids with different fatty acid compositions will produce marked differences in surfactant phospholipid composition and what the immunologic consequences of this will be.

**Objectives:** Identify whether different lipid emulsions used in total parenteral nutrition (either Intralipid or ClinOleic) influence the composition of phospholipids in the lung surfactant of premature babies. Investigate whether any resultant variation in phospholipid composition correlates with rises in inflammatory markers such as CRP, WCC and neutrophil count. Identify whether there are advantages using one type of nutrition over the other in terms of immunological consequences.

**Methodology:** Tracheal aspirate samples were collected from babies on the NNU who were born <34 weeks gestation or <2000g, ventilated and receiving either Intralipid or ClinOleic lipid emulsion within the TPN. Surfactant was isolated by density ultracentrifugation techniques and lipids extracted with chloroform and methanol. Mass spectrometry was performed to analyse phospholipid composition.

**Results:** There were small but statistically significant differences in the surfactant phospholipid molecular species composition between infants receiving ClinOleic versus Intralipid. However there was no consistent common theme of change across the three classes of phospholipid. The main results from this study demonstrate a significant (P<0.01) enrichment in the monounsaturated PC molecular species with administration of ClinOleic.

**Conclusions:** This research has provided provisional evidence that lung surfactant phospholipid composition of

preterm infants can be modified by administration of different lipid emulsions. The immunologic effects and clinical outcomes produced as a result of this still needs to be established.

### Total anomalous drainage of pulmonary veins in the structure of inborn heart defect

ESC-ID 717  
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*Aim:* To define the place of Total anomalous drainage of pulmonary veins TADPV in the structure of inborn heart defects in L'viv region during 1999-2008. To select the basic criteria of early diagnostics of TADPV and optimize the algorithm of examination of patients.

*Materials and methods:* The statistical analysis of medical documentation (protocols of echocardiogram diagnostics and case records of children with TADPV) of L'viv Regional Children's Clinic from 1999 to 2008 has been conducted.

*Results:* Annually about 25 thousand of children are born in L'viv region, about 200 of them have inborn defects of heart. During the period from 1999 to 2008 2054 children with inborn heart defects were born in L'viv region, 9 of which had TADPV (0.44%). Protocols of echo cardiogram diagnostics and case records of these 9 children - five boys and four girls at the age of five days to one month have been analyzed. None of the examined children had genetic or concomitant pathology. Different anatomic variants of TADPV were diagnosed with children, two of which had infracardial form, two had intracardial and five - supracardial form. A clinical picture was characterized by considerable variability. The most characteristic symptoms were: marked cardiac insufficiency and speeded-up palpitation; shortness of breath and noisy breathing; a presence of moist wheezes in lungs; marked cyanosis which can increase after having a meal; absence of cardiac noise or presence of permanent soft noise in an untypical place (for example the area of jugular cut, the area of liver). Such changes as dilatation of right auricle and right ventricle; presence of pulmonary veins collector; expansion of pulmonary artery and its branches; presence of defect of between- auricle partition; diminishing in the sizes of left ventricle and left auricle have been found out with the help of echocardiogram. Configuration of the heart in the form of a «snowman» has been found out with the help of roentgenogram. Echogram signs: hypertrophy of right ventricle and right auricle; a high indent of R in taken AVR; presence of blockade of right foot of GIs's fascicle.

*Conclusions:* Specific gravity of TADPV in the structure of inborn heart defects in L'viv region is 0.44% in comparison with 1-3% world statistics and 1.2% in Ukraine. The algorithm of examination of babies with TADPV includes the clinical methods of examination, echocardiography, electrocardiography and roentgenography. Because of absence of clinical pathognomonic signs of TADPV - echocardiogram remains the basic method of diagnostics.

### Advanced echocardiographic imaging of right ventricular function in post operative tetralogy of Fallot

ESC-ID 844  
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*Background:* Tetralogy of Fallot (TOF) is the most common cyanotic congenital heart disease and is characterized by right ventricular outflow tract obstruction due to antero-cephalad deviation of the outlet septum, a ventricular septal defect which the aorta over-rides and right ventricular (RV) hypertrophy[1]. These patients undergo complete repair in the first year of life which involves a trans-annular patch being placed across the pulmonary valve (PV) annulus to overcome the outflow tract obstruction. This unfortunately disrupts the integrity of the PV resulting in pulmonary regurgitation which places a significant burden on the RV. It is therefore common practice to replace the pulmonary valve later in life[1,2]. Cardiac Magnetic Resonance (CMR) Imaging is the gold standard for assessing Right Ventricular (RV) Function in Tetralogy of Fallot (TOF) patients. Speckle Tracking Echocardiography (STE) is a novel technique for assessing regional myocardial function with the advantage over other echocardiographic imaging modalities of being able to produce objective and angle-independent measurements[3]. This study will look at STE as a possible alternative to the more costly and time consuming CMR Imaging.

*Methods:* 18 TOF patients and 17 controls were enrolled. Images were acquired using Philips iE33 and S5 probe. Apical 4-Chamber views, mid-level short-axis and short-axis oblique views were obtained for both groups. Offline analysis was done with QLab 6.0. Tissue Motion Annular Descent (TMAD) was used as a surrogate marker for assessing the longitudinal myocyte function. Tissue traction points were assigned to the hinge points of the anterior-superior and septal leaflets of the tricuspid valve and aortic and mural leaflets of the mitral valve and the corresponding ventricular apices. In the short-axis view with superimposed colour kinesis, circumferential contraction was assessed from end-diastole to end-systole. Time-to-peak contraction was assessed in the short axis oblique view. Multiple measurements were taken and analyzed with SPSS17.

*Results:* Longitudinal shortening was greater in the RV than the left ventricle (LV) ( $23.2 \pm 3.3\%$  vs.  $17.0 \pm 3.3\%$   $p = 0.0001$ ) in the normal heart, being most pronounced in the free wall ( $16.5 \pm 6.1\text{mm/m}$  vs.  $11.1 \pm 3.8\text{mm/m}$   $p = 0.0001$ ). Longitudinal function of both ventricles decreased with age ( $p = 0.0001$ ). No change in circumferential function was seen. Longitudinal shortening was reduced in the RV of TOF patients ( $16.1 \pm 4.9\%$  vs.  $23.1 \pm 6.1\%$   $p = 0.0001$ ), due to reduced shortening of the RV free wall ( $11.7 \pm 4.9\text{mm/m}$  vs.  $16.5 \pm 6.1\text{mm/m}$   $p = 0.02$ ). LV function was spared. Time-to-peak contraction was shorter in the area of the pulmonary than the tricuspid valve. The intra-observer reliability co-efficient was 0.98 (0.96, 0.99).

*Conclusion:* RV function can be reliably quantified using STE. Longitudinal shortening in the normal heart is greater in the RV than the LV which can be accounted to

the greater longitudinal shortening of the RV free wall. Longitudinal function of the LV and RV of the normal heart decreases with age. This is also seen in TOF patients. Impaired longitudinal shortening of the RV seen in TOF predominantly affects the RV free wall. LV longitudinal and circumferential function is relatively preserved. The peristaltic mechanism of the RV is disrupted in TOF patients.

### **Determination of dialysis adequacy in children hemodialysis population and its associated factors in Iran**

ESC-ID 791  
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*Aim:* Dialysis is the passage of molecules in solution by diffusion across a semi-permeable membrane. Large proportion of children with End-Stage Renal Disease (ESRD) require a prolong period of treatment with long-term hemodialysis before or between renal transplantations. For measuring the effectiveness of hemodialysis treatment protocol, dialysis adequacy is used. One of the ways for evaluating dialysis adequacy is the calculation of KT/V formula, which is measured by Pre and post-dialysis blood urea nitrogen (BUN) levels, duration of dialysis and ultrafiltration. Due to the lack of enough data of dialysis adequacy in pediatric patients in Iran, we aimed to measure dialysis adequacy according to KT/V formula in Ali-Asghar hospital and Children Medical Center and evaluated its effects on renal and cardiac function factors between 2008 and 2009.

*Methods and materials:* In this cross-sectional study, all children and young adults who were received chronic hemodialysis, in two pediatric hemodialysis centers from May 2008 until March 2009, were selected by convenient sampling. BUN, blood creatinine concentration and weight before and after the dialysis were measured. Using echocardiography, left ventricle ejection fraction (LVEF) was determined. Dialysis duration was recorded for all patients as well. Based on dialysis adequacy which was measured by KT/V calculation with a computer software, patients were categorized in two groups: Low dialysis adequacy (KT/V less than 1.2), and High dialysis adequacy (KT/V more than 1.2). Data analysis was performed using SPSS v.16 via Independent-samples T-test and Non-parametric Correlation tests.

*Results:* 31 patients were included with the mean age of  $14.65 \pm 5.3$  years (20 male, 11 female) and the mean dialysis session of  $2.62 \pm 0.62$  times/wk. seventeen percent of patients (n = 5) had low KT/V and 83% (n = 25) had high KT/V. According to analysis, in high-adequate dialysis group the mean concentration of post-dialysis blood creatinine, was significantly lower (P = 0.000) and the mean of the LVEF was significantly higher (p = 0.004) than the other group. Duration of dialysis was significantly different between two groups of KT/V (P = 0.000); Based on Independent-samples T-test, the highest duration was in the high KT/V group (mean  $\pm$  SD:  $233.3 \pm 19.4$  minutes) and the lowest duration was in the low KT/V group (mean  $\pm$  SD:  $180 \pm 69.2$  minutes). A moderate positive correlation was observed between duration of dialysis and LVEF (P = 0.020, r = 0.581). Post-dialysis BUN had significant

negative correlation with LVEF (P = 0.027, r = -0.520).

*Conclusion:* This study showed that dialysis adequacy, more than 1.2 (according to calculating of KT/V) can decrease post-dialysis blood creatinin and increase the LVEF of the children undergone chronic hemodialysis. This may be associated with the time of dialysis which affected on removal BUN. Although, low frequency of children had low dialysis adequacy, these patients were at increased risk for cardiac dysfunction. It seems more attention must be paid on dialysis adequacy by increasing the time of dialysis sessions in pediatric hemodialysis population.

### **The assessment of the incidence and risk factors of retinopathy in preterm babies admitted to Paediatric Intensive Care Unit in Zabrze between 2006 and 2007**

ESC-ID 587  
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 Institution **Medical University of Silesia**

*Purpose:* To analyze the frequency of occurrence and risk factors for retinopathy of prematurity (ROP) among preterm infants.

*Materials and Methods:* 139 infants at risk for ROP admitted to the Paediatric Intensive Care Unit in Zabrze at the Medical University of Silesia in Katowice between 2006 and 2007 were included in this study. The International Classification of Retinopathy of Prematurity (ICROP) was used for describing the findings of active ROP. The Stages describe the ophthalmoscopic findings at the junction between the vascularized and avascular retina. Stage 1 is a faint demarcation line. Stage 2 is an elevated ridge. Stage 3 is extraretinal fibrovascular tissue. Stage 4 is subtotal retinal detachment. Stage 5 is total retinal detachment.

*Results:* Of the 139 neonates screened, ROP affected 18 (12,9%) babies among them 7 (38,9%) developed the stage I of disease, 4 (22,2%) the stage II and 7 (38,9%) the stage III. 11 babies (61,1%) with stage II i III were treated with laser photocoagulation or vitrectomy. ROP was strongly associated with smaller (p = 0.0001), more immature (p = 0.0001) babies and with Apgar Score at 1st minute (p = 0.003).

*Conclusion:* The stronger risk factors for ROP in premature seriously ill infants are birth weight and gestation and Apgar Score at 1st and about 13% of studied infants developed ROP

### **High SNAPPE-score is associated with a high cerebral tissue oxygenation and a low oxygen extraction in preterm infants**

ESC-ID 884  
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*Introduction:* The Score of Neonatal Acute Physiology Perinatal Extension (SNAPPE) provides an indication of clinical condition during the first 24 hours after birth.

SNAPPE-score ranges from 0-125. A high SNAPPE-score reflects a worse clinical condition and this might affect brain function. A non-invasive method to assess cerebral oxygenation is Near Infrared Spectroscopy (NIRS). With NIRS, the regional cerebral tissue oxygen saturation (rcSO<sub>2</sub>) is measured. With rcSO<sub>2</sub> and the transcutaneous arterial oxygen saturation (tcSaO<sub>2</sub>), the fractional tissue oxygen extraction (FTOE) is calculated as follows:  $FTOE = (tcSaO_2 - rcSO_2) / tcSaO_2$ . The FTOE reflects the balance between oxygen supply and oxygen consumption. Currently, little is known about the relation between SNAPPE and NIRS-values. Therefore, the aim of this study was to determine associations between SNAPPE, rcSO<sub>2</sub> and FTOE.

**Materials and methods:** In this study, 14 preterm infants (median birth weight 1220 gram, range 560-1975 gram; median gestational age 29.1 weeks, range 25.4-31.7 weeks) were included. The tcSaO<sub>2</sub>, SNAPPE, rcSO<sub>2</sub> and FTOE in the first 24 hours were estimated. The transcutaneous arterial oxygenation was measured by a pulseoxymeter. The SNAPPE-score was determined by 31 variables, e.g. blood pressure, pCO<sub>2</sub> and Apgar scores. The remaining variables were measured with NIRS. The Spearman Rank Order Correlation test was used to determine correlations between the SNAPPE-score, rcSO<sub>2</sub> and FTOE.

**Results:** A high SNAPPE-score correlated with a high rcSO<sub>2</sub> and a low FTOE (Spearman's  $r = 0.323$ ,  $p = 0.042$ , respectively  $r = -0.355$ ,  $p = 0.025$ ). The SNAPPE-score in this group ranged from 6 to 40. No correlation was found between tcSaO<sub>2</sub> and SNAPPE-score, so a change in FTOE will reflect changes in cerebral oxygenation only.

**Conclusions:** This study demonstrates that a high SNAPPE-score is associated with a high rcSO<sub>2</sub> and a low FTOE. A worse clinical condition goes along with a lower oxygen extraction in preterm infants during the first 24 hours after birth. Possibly cerebral metabolism is lower in these preterm infants.

**Key-words:** Score of Neonatal Acute Physiology Perinatal Extension, Near Infrared Spectroscopy, preterm infants, cerebral oxygenation Note: Currently, this study is in progress, so the final results will be based on a larger study population.

### Cerebral autoregulation in preterm newborns is mostly but not always intact

ESC-ID **877**  
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**Introduction:** Preterm newborns are at risk of developing intraventricular haemorrhage (IVH) or periventricular leukomalacia (PVL). The pathogenesis of these disorders is not completely understood. Cerebral ischemia and reperfusion are presumably involved. Hypotension and blood pressure fluctuations are possible causes of diminished cerebral blood flow, in case of disturbed cerebral autoregulation. A way to monitor cerebral blood flow continuously and non-invasively in the neonatal period is by Near Infrared Spectroscopy (NIRS). NIRS measures the regional cerebral tissue oxygenation (rcSO<sub>2</sub>). By combining rcSO<sub>2</sub> with the arterial oxygenation (SaO<sub>2</sub>) the Fractional

Tissue Oxygen Extraction (FTOE) can be calculated. FTOE represents the balance between oxygen delivery and oxygen consumption. The aim of our study was to investigate the relationship between Mean Arterial Pressure (MAP) and FTOE in preterm newborns. We hypothesized that we would not find any correlation in most infants, reflecting an intact cerebral autoregulation. Because extraction of oxygen will be higher in case of diminished blood flow, we expect a negative correlation between MAP and FTOE in the absence of autoregulation.

**Patients and Methods:** This was a prospective observational study of preterm infants (present in preterm infants with their MAP between discrete lower and upper limits. It might be that most data on FTOE in this study were obtained with the MAP values within these limits. Further assessments will be done in the near future on factors influencing the presence of cerebral autoregulation in preterm infants.

### Acute central effects of neonatal Dexamethasone treatment in rats

ESC-ID **1025**  
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Dexamethasone (DEX) is a synthetic glucocorticoid, often given to premature children to treat severe respiratory distress syndrome. Despite the potential short-term benefits on lung function, there has been growing concerns about the long-term neurodevelopmental outcome of this treatment, since follow-up studies of premature infants have shown persistent adverse neurodevelopmental effects. Rodent studies further indicated that neonatal treatment with DEX results in a reduction in lifespan of 25%, which is accompanied by reduced growth and motor performance as well as impaired cognitive performance and hippocampal function. In a rat model, we have previously shown that neonatal DEX treatment exerts lasting effects on hippocampal cell proliferation and results in a reduction in glial activity one week post-treatment in the corpus callosum as well as in the hippocampus. These adverse effects were greatly attenuated by the glucocorticoid receptor (GR) antagonist RU486. The goal of this project is to elaborate on the results of our previous work and investigate the acute effects of DEX treatment on brain development. We hypothesize that neonatal DEX treatment will also acutely affect glial activity and neuronal proliferation, i.e. immediately after treatment. We also aim to antagonize specifically the central effects of DEX treatment, while leaving the peripheral action intact by intracerebroventricular (ICV) administration of RU486. Rat pups were injected with tapering doses of DEX (or vehicle (VEH)) on postnatal days (PNDs) 1, 2 and 3. On PND4, all the pups were sacrificed by transcardial perfusion and brains were collected for analysis of a marker for astroglial activity (GFAP) in the corpus callosum while other brain areas (i.e. hippocampus) are also currently under investigation. Glial activity from these animals were compared to that of animals which had received an ICV injection of RU486 to specifically block brain GR prior to DEX/VEH peripheral administration. We report a

trend (although not statistically significant (ns)) towards a decrease in GFAP activity in the corpus callosum of animals treated with DEX. There was also a trend (ns) for a reversal of this effect by RU486. These results are in line with our previous findings at PND10 and support the proof of concept that GR antagonism could serve as an efficient therapeutic intervention to counteract the detrimental effects of DEX exposure in the immature brain. Supported by EU LifeSpan (FP6 036894) (SC), KNAW (ERdK), and SmartMix (DC)

### Screening for isolated sulfite oxidase/molibden cofactor deficiencies among the pediatric patients with encephalopathy and mental-motor retardation

ESC-ID **1010**  
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*Aim:* Inborn errors of metabolism are caused by the genetic deficiencies of the enzymes in the metabolic pathways. The prevalence inborn errors of metabolism are significantly high in Turkey probably due to the high rate of consanguineous marriages which is around 25%. The active Molybdenum cofactor (MoCo) is essential for the function of the enzymes sulfite oxidase, xanthine dehydrogenase and aldehyde oxidase in mammals. In patients with MoCo deficiency, disease-causing mutations have been identified in MOCS1, MOCS2 and GEPH genes which are all inherited in an autosomal-recessive trait. In the overwhelming majority of cases, the condition presents with a severe and progressive neurological manifestation, including brain abnormalities, mental retardation and intractable seizures. The phenotype is indistinguishable from the one observed in isolated cases of sulphite oxidase deficiency due to the mutant with the exception that xanthinuria is absent. In this study, Turkish pediatric patients with encephalopathy and/or mental and motor retardation were screened for MoCo/isolated sulfite oxidase deficiencies.

*Materials and Methods:* A total of 504 Pediatric patients aged 0-16 years, referred to the Department of Pediatric Metabolism, Gazi University Hospital with encephalopathy and/or mental and motor retardation were initially screened with urine-test strips for sulfite. In the cases with positive urine sulfite strip test, sulfite oxidase, xanthine dehydrogenase and aldehyde oxidase activities were determined.

*Results:* One case aged a 4- month -old girl with intractable seizures and feeding difficulty was diagnosed with isolated sulfite oxidase deficiency. Consciousness was clear. Physical examination revealed signs of spasticity in lower an upper extremities with increased deep tendon reflexes. Ophthalmologic examination at this time revealed horizontal nistagmus. She presented with multifocal myoclonic seizures and progressed rapidly to profound psychomotor retardation, encephalopathy. The study still continues.

*Conclusions:* MoCo/isolated sulfite oxidase deficiencies should be always considered among the etiologic factors of childhood encephalopathy/ and/or mental and motor retardation.

### The immune status of children with rotaviral mixed infection

ESC-ID **811**  
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During the last years rotaviral infection has been attracting increasing attention of scientists. The part of rotaviral infection in general structure of intestinal infections worldwide, according to different authors, is 35-75 % and has constant tendency to grow. Some authors show, that in 60-70% of cases rotaviral infection occurs not as mono-, but mixed infectious pathology of gastrointestinal system caused more often by combination with conditionally pathogenic and pathogenic bacteria. The aims of the research is to improve features of reaction of blood interleukins and the immune response in children with rotaviral mixed infection. Complex immunological investigation of 64 children from 1 month to 3 years old with rotaviral mixed infection has been carried out. Levels of interleukin -1, -4, -6, tumor necrosis factor  $\alpha$  (the hard-phase immune-enzyme analysis), subpopulations of lymphocytes CD3+, CD4+, CD8+, CD14+, CD19+ (the method of monoclonal antibodies) and immunoglobulins of basic classes (A, M, G) (the method of simple radial immunodiffusion) in blood were determined. Investigation shows that acute period of rotaviral mixed infection accompanied by reliable increase of interleukines -1, -4, -6 and tumor necrosis factor  $\alpha$ . The clinical manifestation depends on the level of interleukines. In children with high level of interleukine-1 frequent vomiting was reported; in children with high level of interleukine-4 severe diarrhea and catarrhal syndrome was reported; in children with high level of interleukin-6 temperature increased more than 38,50C and frequency of stool increased more than 8 times a day; in children with high level of tumor necrosis factor  $\alpha$  frequency of stool increased more than 8 times a day and frequent vomiting. In early convalescence period the level of these interleukines stays increased. The immune response of rotaviral mixed infection is characterized by decreased level of CD3+, CD4+, CD8+ lymphocytes at the background of physiological level of CD14+, increased level of CD19+ during the whole period of the disease, physiological level of immunoglobulins A, M, G in acute period and increased level of antibody G in early convalescence period. Mathematically proven fact is children with rotaviral mixed infection has hypocompensatory variant of operation of immunity. Thus, analysis of functioning of immune system shows that immunity operation in hypocompensatory variant, leads to interleukine-1-4, il-6, tumor necrosis factor  $\alpha$  CD19+ cell increase and CD3+, CD4+, CD8+ cell decrease, level of immunoglobulins does not change. The weakest link of immune system is T-system.



### The assessment of the correlation between pathogens isolated from blood and endotracheal tube aspirates in very sick neonates admitted to the Intensive Care Unit

ESC-ID **586**  
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*Aim:* To assess the correlation between pathogens isolated from blood and endotracheal tube aspirates and evaluate the risk of hospital acquired infection.

*Materials and Methods:* 58 neonates who were admitted to the Paediatric Intensive Care Unit in Zabrze at the Silesian Medical School in Katowice (2005 – 2007) due to respiratory failure. They were intubated and had been ventilated for longer period of time. Serial cultures were carried out at 2nd, 14th and 18th day of admission.

*Results:* 1. 17 (29,3%) babies had a positive blood culture and 20 (34,5%) had a positive tracheal aspirate culture (mostly Gram positive flora) on the 2nd day of admission. 65% of the aspirates and 70,7% of the blood cultures were sterile. There was no agreement between pathogens isolated from blood and from endotracheal tube isolates 2. On the 14th day of admission 19 (32,75%) babies had a positive blood culture and 18 (31%) had a positive tracheal aspirate culture. Pathogens isolated from blood were not correlated with those from tracheal aspirates. 3. At the third measurement on the 18th day of admission only 14 (24,1%) infants had a positive blood culture and 7 (12%) tracheal aspirate culture with no agreement between them. 4. Among 58 studied infants the risk of developing hospital acquired infection was found to be X%. 5. Only 2 of cultures of endotracheal tube aspirates obtained from nonsurvivors (7 babies – 12%) grew pathogens.

*Conclusions:* 1. The results of cultures of endotracheal tube aspirates were not useful in predicting systemic infection in our population of critically ill babies. 2. The lack of correlations between results of the tracheal aspirate cultures and the blood cultures do not allow to administer antibiotics on the basis of pathogens isolated from endotracheal tube aspirates only. 3. We found a significant increase of the risk of developing hospital acquired infection at the end of the second week of endotracheal intubation and mechanical ventilation.

### Peripubertal growth retardation of female rats after neonatal treatment with monosodium glutamate

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The aim of our research was to examine toxic effects of monosodium glutamate (MSG) on the dynamics of growth and development of rats in the period from birth to rats adults period following somatometric characteristics. In the experiment we used female Wistar rats, who was divided into two groups. The experimental group (group M) were subcutaneously treated with MSG in a dose of 4mg/g body weight during the first ten days of each other on. The

control group (group C) were treated with normal saline solution in the same dose on the same manner. Animals were sacrificed after 90 days from the last application of MSG and normal saline solution. We measured values of body mass, bone length and mass, relative mass, naso-anal and caudal length of the body rats every 15th days. The results were statistically processed using the average value and statistically significant difference is expressed through the Student t-test. The results showed that there is statistically significant difference in reducing body mass and length of tails of rats between groups M age sixth and eighth weeks compared to the rats in group C the same age. There is statistically significant difference in the reduced length of the body of rats group M age fourth, sixth and eighth weeks compared to the rats in group C the same age. Relative weight of rats group M is reduced and statistically significant in comparison to group C. Our results showed that statistically significant difference between M i C group there is in the peripubertal period of rats, so that these changes associated with a reduced concentration of growth hormone and gonadotropins rats in group M. Based on the findings of other researchers as well as our we can assume that the reduced levels of growth hormone and gonadotropins resulted in lesions caused by the influence of MSG in adenohipophysis, which is manifested in slow growth and development of puberty in rats.

### Haptoglobin polymorphism in children with autoimmune hepatitis and Wilson-disease

ESC-ID **236**  
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*Background:* Haptoglobin (Hp) is a haembinding glycoprotein with important antioxidant, scavenger and immunomodulant activity. Hp is expressed by a genetic polymorphism as three major phenotypes: 1-1, 2-1, and 2-2, which are different in structure and function. Antioxidant activity is more related to Hp type 1-1 than to type 2-2. The role of the Hp polymorphism in immune-mediated liver diseases (e.g primary sclerosing cholangitis) was investigated in previous studies.

*Aim:* In this study we investigated Hp polymorphism in paediatric patients with autoimmune hepatitis (AIH) type I. and with Wilson-disease.

*Patients and methods:* Hp phenotypes were determined by sodium-dodecyl-sulphate-polyacrylamid gel electrophoresis (SDS-PAGE) and immunoblotting of the sera for 10 children with AIH and 10 with Wilson-disease in comparison with 103 adult patients with AIH and 384 healthy controls.

*Results:* The frequency of Hp 1-1 was higher in paediatric patients with AIH (20%) than in adults AIH patients (12,6%) or in healthy individuals (11,5%), at the same time the frequency of Hp 2-2 was lower in AIH paediatric patients (30%) compared with the control populations (39,8% and 42,4%). In Wilson-disease the frequency of Hp 2-2 was dominant (70%) in comparison with the healthy population (42,4%), however the frequency of Hp 2-1 was lower (20% vs. 44,1%).

*Conclusion:* We found different pattern of Haptoglobin

polymorphism by the children with AIH type I. in comparison with adult patients. The higher frequency of the Hp 2-1 phenotype may refer to, that the oxidative processes may not have important role in the pathomechanism of AIH in children as in adult patients. In Wilson-disease we found the dominance of the Hp 2-2 phenotype, which antioxidant activity is the lowest. It may influence the toxic effect of copper and may have a role in the process of the disease.

### Placental pathology is associated with a high SNAPPE score in preterm infants

ESC-ID **962**  
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*Introduction:* Placental pathology consistent with maternal vascular underperfusion is associated with neurological impairment in infants older than 20 months. The Score of Neonatal Acute Physiology Perinatal Extension (SNAPPE) provides an indication of clinical condition during the first 24 hours after birth. SNAPPE-score ranges from 0-125. A high SNAPPE-score reflects a worse clinical condition. The aim of this study was to determine whether pathological evidence of maternal vascular underperfusion affects the SNAPPE-score in preterm infants born <32 weeks gestational age.

*Materials and methods:* In this cohort study 14 preterm infants (median birth weight 1220 gram, range 560-1975 gram; median gestational age 29.1 weeks, range 25.4-31.7 weeks) were included. In all of them the placenta was available for histopathological examination. Placental pathology consistent with maternal vascular underperfusion was defined as retroplacental hematoma, hypoplasia, villous hypoplasia or placental infarction. The SNAPPE-score was determined by 31 variables which provide an overall impression of the clinical condition during the first 24 hours after birth, e.g. blood pressure, pCO<sub>2</sub> and Apgar scores. The Mann-Whitney U test was used to compare the results of pathological review of the placentas and the SNAPPE-score.

*Results:* In 5 placentas, pathology consistent with maternal vascular underperfusion was present and correlated with a high SNAPPE-score (Mann-Whitney U, p = 0.006). The SNAPPE-score in this group ranged from 6 to 40. Two placentas showed hypoplasia and villous hypoplasia, one placenta showed an infarction. Two placentas showed all three lesions stated above. Placental pathology subgroup analysis showed no correlation with the SNAPPE-score.

*Conclusion:* Placental pathology consistent with maternal vascular underperfusion is associated with a higher SNAPPE-score. This reflects a poor clinical condition in preterm infants during the first 24 hours after birth. These data suggest that when there are placental lesions present during pregnancy, preterm infants will experience an ongoing effect of it at least during the first 24 hours after birth.

*Key-words:* Maternal vascular underperfusion, preterm infants, Score of Neonatal Acute Physiology Perinatal Extension (SNAPPE) Note: We are currently conducting this study with a larger study population, the final results will be based on these outcomes.

### Efficacy of Recombinant Human Growth hormone in children with short stature

ESC-ID **516**  
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*Introduction:* Growth hormone deficiency (GHD) is the most common endocrine cause of short stature in children. It is a disorder in the hypothalamic control of GH secretion or an inability of the pituitary itself to secrete GH. Also, children with Turner Syndrome and chronic renal insufficiency (CRI) are presented with growth retardation. Recombinant Human Growth hormone (rhGH) which is identical to the human's, is readily available and standardized treatment.

*Aim:* The aim of this study is to evaluate efficacy and safety of the rhGH therapy in different conditions presented by short stature.

*Patients and Methods:* The study group included 35 children and adolescents, 18 male (51, 43%), 17 female (48, 57%), aged 5,32 – 17,91 and were observed at the Children's Hospital, Clinical Center Nis, Serbia during 2008. The diagnosis of GHD was based on auxological criteria and provocation tests for GH secretion: insulin tolerance test, clonidine test and serum concentration of IGF-1 and IGFBP-3. Karyotyping confirms the diagnosis of Turner Syndrome. The patients were divided into 3 groups: Group 1: GHD idiopathic (27/35): - Without the history of perinatal trauma 68,57% (24/35); - With the history of perinatal trauma 3,57% (1/35); - perinatal history unknown 7,14% (2/35). Group 2: Turner's syndrome 11, 43% (4/35). Group 3: Other causes (4/35): - GHD due to irradiation 3,57% (1/35), - GHD due to trauma 7,14 % (2/35), - CRI 3, 57% (1/35). All patients were treated with 0.035 mg/kg rhGH daily evening subcutaneous injections and 0.045 mg/kg in Turner's syndrome.

*Results:* After one year of the rhGH therapy we found out that height velocity in groups were: Group 1: GHD idiopathic: - Without history of perinatal trauma 9,94 cm/year; - With history of perinatal trauma 7,46 cm/year; - perinatal history unknown 8,13 cm/year. The average for this group was 8,51cm/year. Group 2: Turner's syndrome - average was 11, 3 cm/year. Group 3: Other causes - average was 9, 68 cm/year.

*Conclusion:* Individual response can be variable and influenced by factors such as aetiology of short stature, DH dose, age at start of therapy and response to a provocative tests. In our study group mean "Catch up growth" in the first year of the therapy was 9,83 cm/year. Significant side effects of the rhGH treatment in children were not identified.

### If we can see effective volemia?

ESC-ID **845**  
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*Introduction:* Important element of treatment of diabetes decompensation in children is proper hydration. Effective volemia is a volume of circulating blood which is moni-

tored by baroreceptors and chemoreceptors in vessels and it is dependent on an efficient heart and kidney function. In clinical practice it is not simple to estimate effective volemia. Our study is the first trial of connecting effective volemia and measuring IVC/Ao ratio.

*Aim:* Presentation of 3 cases of patient with diabetes mellitus admitted with hyperglycemia in wich trial measurement of effective volemia was made.

*Material and methods:* 3 children with hyperglycemia admitted to Department of Pediatric Diabetology were examined with ultrasound. Patients were 4 and 9 years old girls and 13 years old boy. 4 year old girl was admitted in hyperosmolar coma state, the others in keton coma state. Diameter of inferior vena cava (IVC) and aorta (Ao) were measured and IVC/Ao ratio was calculated on admission and after treatment. Results were compared with clinical examination and laboratory results.

*Results:* In these cases we found correlation between IVC/Ao ratio and effective volemia.

*Conclusions:* Our studies suggest that IVC/ao may be useful in estimation of effective volemia in patient with diabetes mellitus and other groups of patients who need estimation of effective volemia to active treatment. Results suggest further studies on bigger group of patients.

### The Evaluation of the Risk Factors in Recurrent Wheezing after Hospital Admission for Acute Bronchiolitis

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Our aim was to make a retrospective study, based on the questionnaire method upon infants that came to the Intensive Therapy Unit of the IOMC Hospital, Bucharest, Romania with the diagnosis of acute bronchiolitis from 15 November to 31 st December 2005 (83 patients). Severity score was calculated according to the Wainwright C et al. N Engl J Med 2003;349:27-35 criteria in BMJ. The overall severity score was moderate (2 or 3) and severe (> than 3).

*Materials and methods:* The variables taken into consideration included the main risk factors associated with Viral Induced Asthma(exposure to cigarette smoke during pregnancy and after birth, lack of breast-feed, a family history of asthma or atopy, a personal history of atopy),the number for wheezing episodes that needed to be admitted in the hospital and the total number of wheezing episodes even without need hospital admission.

*Results:* Recurrent wheezing (RW)(> 3 admissions to hospital in 1.5 years) was over 22% -Passive smoking- the risk of RW in infants with smoking mothers was increased by 4 times (33% vs. 8%). -A family history of asthma increased the risk by 3 times (29 % compared to 9%). -Lack of breast feeding increased increased the risk by 2.66 times (50% vs. 19%)

*Conclusions:* The study represents the first epidemiological study regarding the risk factors of developing recurring wheezing after hospital admission for acute bronchiolitis aimed at children from the southern part of Romania. Due to the low number of patients, it is manda-

tory that further studies be undertaken in order to validate this first epidemiological enquiry.

### Acute Otitis Media- Clinical-evolutive and therapeutically aspects

ESC-ID **283**  
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*Introduction:* Otitis media represents an acute or chronic inflammatory process with or without liquid accumulation , of the middle ear. The past fifteen years have shown a notable increase in the number of children identified as having otitis media.

*Aim:* The aims of this study are the following: -evaluation of acute otitis media's incidence among hospitalized children, evaluation of the relation between risk factors and age, the management of the main clinical-biological parameters, evaluation of the gravity and also of the complications and the therapeutically aspects.

*Material and methods:* This retrospective study was conducted in the Pediatrics Department II of the Emergency District Clinic Hospital Constanta (Romania) and its purpose is to evaluate the clinical-evolutive aspects of the acute otitis media cases. The study was done between 1 january 2008 and 1 january 2009 on a group of 91 patients hospitalized during this interval and diagnosticated with acute otitis media. The patients included in the study had ages between 0-16 years. The data was taken from record medical files.

*Results and conclusions:* Distribution of the cases regarding clinical-evolutive form in our group was the following: 38,46% of the cases were congestive otitis media, 49,45%- suppurative otitis media, 4,39% serous otitis media and 7,69%- acutization of a chronic serous otitis media. The aetiology of suppurative acute otitis media is dominated by Stafilococcus Aureus (45,71%), urmat de Streptococcus pneumoniae ( 22,85%), Klebsiella (14,28%) and Escherichia Coli (8,57%). The medium interval of treatment for suppurated acute serous otitis media was 6-10 days- 52,32% of the patients. The evolution was favorable (86,81%).

## Pharmacology

### Correlation between therapeutic and protective efficacy of SP-AQ (sulphadoxine-pyrimethamine - amodiaquine) - IPTp(Intermittent preventive treatment in pregnancy) and the clinically important parameters( on 3 IDPcamps in Sudan)

ESC-ID **144**  
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*Background:* correlation between therapeutic and protective efficacy of SP-IPTp and the clinically important

parameters, namely low birth weight, maternal anemia, and clinical episodes of malaria and their relationship to peripheral and placental parasitaemia, needs to be validated and accurately calibrated. This calibration will inform the use of future in vivo efficacy studies to determine when SP should be replaced with a more effective antimalarial.

**Methods:** During the malaria transmission seasons of 2007&2008, 450 children – between six and 59 months of age, with uncomplicated malaria in Wad albashir, Mayo and Bantio IDP(internal displaced people) camps at Khartoum state, Sudan, were randomly assigned to one of two treatment arms. In vivo outcomes were assessed using WHO standard protocols.

**Results:** Day 28 adequate clinical and parasitological responses (ACPR) were 42.3% and 68.9% in 2007, 40% and 65.2% in 2008 for sulphadoxine-pyrimethamine and SP-amodiaquine combination respectively. After molecular correction, ACPRs (cACPR) were 68.5% and 78.0% in 2007, 65.2 and 76.6% in 2008 for SP and SP-AQ respectively. Effects on fever were 41.5% 62.2% on 2007 and 43%, 69.3 % in the 2008 for the SP and SP-amodiaquine combination respectively.

**Conclusion:** In this study, treatment with sulphadoxine-pyrimethamine-amodiaquine combination emerged as the more efficacious on uncomplicated falciparum malaria as SP. The study demonstrated that sulphadoxine-pyrimethamine and amodiaquine were appropriate partner drugs that could be associated with artemisinin derivatives in an artemisinin-based combination therapy.

### **Forced Swimming test, an experimental model for depression; effect of hypertension and gender differences**

ESC-ID **492**  
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**Introduction:** Depression is a treatable common but serious illness that afflicts millions of people all over the world. Discovery of new drugs for the treatment of depression requires screening of potential compounds in experimental animals using animal models of depression. These include the forced swimming test. The test has yielded useful results in screening novel compounds that can be therapeutically useful in human patients<sup>1</sup>. It is not known whether there is a gender difference in the response of rats in this test and whether hypertensive rats would respond differently in this test. The aim of this research is to compare values obtained from male and female rats in forced swimming test and to compare values obtained from normotensive and hypertensive strains in forced swimming test.

**Methods and material:** Male and female Wister, Sprague-Dawley (SD) and Spontaneously Hypertensive Rats (SHR) were used for the forced swimming test. Ten males and 10 females were experimented from each strain (total rats = 60) weighing within (180-250 g). A standard Porslot forced swimming test was carried out as described<sup>2</sup>. Comparison between different strains and genders immobility time was carried out using SPSS program.

**Results:** The results of forced swimming test showed no significant difference between Wister, SD and SHR strains

in immobility time means ( $p>0.05$ ) (194 sec, 155.2 sec, 172.8 sec) respectively. Also, there was no significant difference between means of male and female rats within the different strains including Wister (males = 179.2, females = 209.1), SD (males = 165.3, females = 145.0) and SHR (males = 183.8, females = 161.7) ( $p>0.05$ ).

**Conclusion:** Although Published reports found a 3-fold higher frequency of major depression in patients treated for hypertension<sup>3</sup>, there was no effect of hypertension on forced swimming test in rats. On the other hand, in humans there is a significantly higher incidence of depression in female gender with a ratio of 2:14. In FST there was no effect of gender on the immobility time. Results indicate that with such factors isolated the noted differences in humans could be attributed to other causes or to the interaction of multiple causes. These could be based on models of differences in socialization, genetics, hormonal variations throughout a woman's reproductive cycle, and neurotransmitter deficits<sup>5</sup>.

### **The investigation of the oxidative stress parameters in hyperhomocysteinemia induced in rats**

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**Introduction:** Hyperhomocysteinemia is a pathological condition characterized by high blood levels of homocysteine. It is known the fact that increased plasmatic levels of homocysteine affect the epithelial function and are associated with cardiovascular diseases. The mechanism through which homocysteine produces these effects is not discovered yet but it is believed that in this process are involved reactive species generators of free radicals.

**Aim:** In this paper we studied the influence of hyperhomocysteinemia experimentally induced on systems involved in antioxidant defence at lab animals (rats).

**Method and Materials:** There were determined the plasmatic concentrations of homocysteine and the intracellular activity of superoxide dismutase (SOD), glutathione peroxidase (GPx) and the total serum antioxidant capacity (CAT).

**Results:** The results show an increase of the SOD activity which means an increase of reactive species. At the same time there is a decrease in the activity of both GPx and CAT as result of the reduced detoxification capacity of reactive species. **CONCLUSION** We can say that high plasmatic levels of homocysteine are associated with a diminution of the antioxidant capacity.

## Antitrichomonal activity of plant extracts from family Lamiaceae (*Coleus Blumei*, *Origanum vulgare*, and *Vitex Negundo*):

### In vitro studies

ESC-ID **210**  
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*Introduction:* Trichomoniasis, caused by the protozoan *Trichomonas vaginalis*, is the most common curable sexually transmitted disease in the world. A recent Philippine study conducted on the prevalence of the disease showed that 37% of Filipino women are affected. Long been regarded as an infection of minor importance, trichomoniasis is now being implicated in a variety of adverse outcomes in both men and women, such as preterm labor and delivery and amplified transmission and infection of HIV. Oral metronidazole is the recommended drug of choice for trichomoniasis. However, some *T. vaginalis* strains have now developed resistance to the drug, leading to the prescription of higher and even toxic doses for patients with resistant disease. A search for an alternative treatment may well prove to be beneficial, especially in the Philippine setting, whose abundant ecological niche has not yet been fully explored for alternative therapies. These herbal treatments are likely to be cheaper and more readily available for Filipinos; in addition, they may even be safer and more effective than synthetic drugs.

*Aim:* The study was conducted to determine the antitrichomonal activities of three crude ethanolic plant extracts (*Coleus blumei*, *Vitex negundo*, and *Origanum vulgare*). The study also aimed to characterize the biochemical components of the most potent plant extract and determine its toxicity in mice.

*Methods and Materials:* Crude ethanolic extracts of the three plants were obtained, and a minimum inhibitory concentration (MIC) assay was done. Fourteen different set-ups were used: three different concentrations of *C. blumei*, *O. vulgare* and *V. negundo* extracts, metronidazole as the positive control, and two negative controls. *T. vaginalis* was inoculated in each tube and incubated at 37°C. Growth after 24, 48, and 72 hours of incubation was measured using a hemocytometer, and the rough MIC of each plant extract was determined. A more accurate MIC of *C. blumei* was then obtained. Since the MIC of *C. blumei* from the preliminary survey was 10 mg/mL and *T. vaginalis* growth was not inhibited at 1 mg/mL, extracts of 2, 3, 4, 5, 6, 7, 8, 9, and 10 mg/mL concentrations were used, with the same positive and negative controls. Phytochemical screening of *C. blumei* was performed by the College of Pharmacy, UP Manila. A mice toxicity assay of *C. blumei* was also done. Measures were taken to ensure that the least amount of pain was inflicted on the animals, and proper decapitation and disposal procedures were utilized.

*Results:* *C. blumei* exhibited the greatest inhibitory concentration against *T. vaginalis*, with an MIC of 2 mg/mL,

and an activity not significantly different from metronidazole ( $p = 0.809$ ). Phytochemical analysis of the *C. blumei* extract indicated the presence of glycosides, plant acids, reducing agents, and alkaloids. The mice toxicity assay revealed minimal side effects even at several magnitudes of concentration (250 - 500x) higher than the MIC. *Conclusions:* These initial findings warrant further studies to isolate, purify, and elucidate the active antitrichomonal product in the *C. blumei* extract, and to explore its potential as an alternative STD medication for human use.

## Histopathological changes in the spleen and thymus of rats treated with monosodium glutamate

ESC-ID **290**  
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*Aim:* The aim of this paper was to investigate the effects of neonatal treatment of Monosodium glutamate (MSG) on rat's spleen and thymus.

*Material and methods:* White Wister rats were used in the experiment. Experimental and control group of animals included 10 rats each. Experimental group animals were treated with monosodium glutamate in dose of 4 mg MSG/g BW on the 2nd, 4th, 6th, 8th and 10th day of their postnatal life. Control group animals were treated with equivalent dose of physiological solution. Five animals from experimental and control groups were sacrificed 7 day after they received the last dose of MSG for the purpose of studying histopathological changes on the thymus. The remaining animals were sacrificed 4 months after MSG treatment in order to study histopathological changes in rat's spleen. Paraffin sections of the thymus and spleen were stained with hematoxylin-eosin (HE).

*Results:* Macroscopically, the treated animals demonstrate skeletal development arrest and cushingoid type of obesity („buffalo type“). In the experimental group of animals pathohistological changes of both spleen and thymus were noticed while spleen and thymus in the control group of animals demonstrated normal constitution. Macroscopically the thymus of experimental group animals shows atrophy. Depletion of thymocytes with the increased presence of macrophages was observed in the cortex. The thymic medulla showed the presence of extravasation of blood elements, followed by destruction of normal tissue cytoarchitecture. Macroscopically the spleen of experimental group animals shows atrophy. Pathohistological changes in the spleen manifested as the atrophy of the white pulp. Majority of follicles of white pulp show the absence of germinal centres while follicles with preserved germinal centres were rare. In the red pulp of the spleen there were morphologic elements of chronic delay, abundance of hemosiderophages and cell elements hematopoiesis, especially megakaryocytes.

*Conclusion:* According to the results of our study we can firmly say that in the spleen and thymus of White Wister rats occur the following pathohistological changes, as a consequences of neonatal MSG treatment: 1. Macroscopic atrophy of thymus. 2. Depletion of thymocytes with the

increased presence of macrophages in cortex and the presence of extravasation of blood elements, followed by the destruction of normal tissue cytoarchitecture in medulla of thymus. 3. Macroscopic atrophy of spleen. 4. White pulp of the spleen shows atrophy and the majority of follicles show the absence of germinative centres. Red pulp of spleen displays signs of chronic delay, abundance of hemosiderophages and cell elements hematopoiesis, especially megakaryocytes.

### Determination of hard metals in Beverages of tea fungus using potentiometric stripping analysis

ESC-ID 488  
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*Introduction:* Tea fungus or Kombucha is a traditional refreshing beverage manufactured at home by the fermentation of sugared black or green tea with a symbiotic culture of acetic bacteria and fungi, consumed for its beneficial effects on human health. In addition to a large number of biological important compounds, in the Kombucha beverage have been found some heavy metals (Pb and Cd), which in higher concentrations may be harmful to human health.

*Aim:* The purpose of the investigation presented in this paper was to determine the content of heavy metals in beverages of tea fungus stored during a certain period of time in containers of different materials by using potentiometric stripping analysis (PSA).

*Methods and materials:* As samples in this study, the solutions of tea fungus grown during the definite fermentation time in black and green tea are used. The content of heavy metals in the samples is determined by using PSA known as a high sensitive microanalytical technique.

*Results:* As a parameter of fermentation in the Kombucha beverage, the pH decreased with time. Due to acidity increasing, the content of Pb was higher in the beverage stored in the glass container compared to the same stored in the plastic container. By comparing results with black and green tea, the beverage of tea fungus cultivated in green tea contained more lead. Cadmium was only detected in the beverage obtained from the green tea.

*Conclusion:* In order to avoid possible harmful effects of heavy metals, it is necessary to cultivate and use the tea fungus beverage properly.

### Protective Effect of Nagella Sativa (Black seed) Against Doxorubicin dose Dependent Cardiotoxicity

ESC-ID 794  
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*Introduction:* Doxorubicin, DOX (Andriamycin) belong to the family of Anthracyclines, widely used for the treatment of Sarcomas and variety of Carcinomas, including

breast and lung cancers. It is also employed in the treatment of Lymphomas and acute Lymphocytic Leukemia. DXR therapeutic use, however, is limited by its adverse, sometimes irreversible dose-dependent cardiotoxicity and bone marrow suppression, triggered through release of semiquinone free radicals, which steals electrons from molecular O<sub>2</sub>, producing superoxide ions and hydrogen peroxide that mediate single strand scission of the DNA. Cardiac cells and tumors are low in ample superoxide dismutase, SOD, or glutathione peroxidase that mops up reactive oxygen species, ROS, making the former vulnerable to toxicity. Many compounds like garlic, mistletoe, et cetra believed to possess anti-oxidant properties have been studied for their potential protective agents against DOX induced cardiotoxicity. Black Seed, BSD, an annual herbaceous plant, native to the Mediterranean but cultivated also in the Middle East and Africa have been noted for its anti-tumor, antioxidants, antibiotic, hepatoprotective, anti-inflammatory and immune strengthening properties.

*Aim:* Would these documented antioxidant properties of BSD, prove a useful therapeutic adjuvant against DOX treatment-generated free radicals induced myocardial toxicity?

*Materials and Methods:* Albino rats were obtained and divided into 4-groups (n = 5): the DOX treated group was acclimatized to oral daily dose of 5ml/kg/b.wt distilled water for 2wks, followed by single dose intraperitoneal injection of 10mg/kg/b.wt DOX after 7dys. The 2nd group was fed BSD liquid extract of 500mg/5ml liquid extract for 2wks, then given the same single dose injection of DOX after 7dys, preceding BSD treatment. The 3rd group received BSD extract for 2wks, then injected with one dose of 5ml/kg/b.wt after 7dys of BSD treatment. The control group received 5ml/kg/b.wt distilled water for 14 days, and then injected single dose saline after 7dys of water treatment. In the end, the animal were anesthetized with diethyl ether and blood and heart tissues were collected from all the groups for further procedures. Levels of Calcium (Ca), Catalase (CAT), Malondialdehyde (MDA), Protein Carbonyl (P.Carbonyl), Myeloperoxidase (MPO), and Total Antioxidant Capacity (TAC) were determined.

*Results:* Serum marker of heart damage, AST, significantly increased (p<0.001) in DOX treated group compared to control. Group administered DOX with BSD showed slight increase in AST. TUNNEL-positive cells and Bcl-2 protein expression, were significantly elevated in DOX treated group compared to control, so also were MDA, P.Carbonyl and MPO levels, with depletion of SOD and Total Antioxidant activities and markedly increase in Calcium levels. Those increases were however, attenuated in BSD-DOX treated group. Histological studies also revealed several histopathological lesions associated with DXR treated group but moderate to normal preserved myocardial cells in DOX-BSD treated group.

*Conclusion:* Cardiotoxicity induced by DOX administration may be ameliorated with co-administration with BSD through acting as a sort of Interferon, with an immune booster and antioxidant efficacy.

## Effects of vigabatrin on thermoregulation in rats

ESC-ID **420**  
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*Introduction:* Thermoregulation in mammals maintains the core temperature at a certain level. The thermoregulatory centre is situated in the preoptic area of the anterior hypothalamus (PO/AH). Opioids like morphine or other  $\mu$ -receptor agonists are known to cause hyperthermia in low doses, while GABA-receptor agonists produce hypothermia. Vigabatrin is a GABA-derivative ( $\delta$ -vinyl-GABA) which inhibits irreversibly the GABA-T enzyme and thus increases indirectly GABA's concentration.

*Aim:* The aim of our study was to investigate the effects of vigabatrin on thermoregulation in intact rats and following a morphine-induced hyperthermia, as well as to examine its effects on neuronal activity and temperature sensitivity (TC) of warm-sensitive neurons in rat PO/AH slice preparation.

*Materials and Methods:* The study was conducted on male Wistar rats ( $220 \pm 10$  g), divided into groups of six, at ambient temperature of  $22^\circ\text{C}$ . The substances used were Vigabatrin (Sigma, Germany) administered intracerebroventricularly (i.c.v.) and Morphine (Sigma, Germany) administered intraperitoneally (i.p.). The International Guiding Principles for Biomedical Research Involving Animals and the guidelines by the Research Ethics Commission in Medical University-Sofia were strictly observed. In vivo experiments included implanting of cannules into the right lateral ventricle of rats: coordinates L 1,5; P 1,2; H 3,5 (Paxinos & Watson, 1997). We monitored the core body temperature (rectal thermistor probes TX8 and multichannel thermorecorder Thermex 16) after administration of vigabatrin i.c.v. in doses  $100 \mu\text{g}/5 \mu\text{l}/\text{rat}$  and  $400 \mu\text{g}/5 \mu\text{l}/\text{rat}$ . Effects on hyperthermia were studied through application of a combination of  $10 \text{ mg}/\text{kg}$  morphine i.p. and vigabatrin i.c.v. in doses  $100 \mu\text{g}/5 \mu\text{l}/\text{rat}$  and  $400 \mu\text{g}/5 \mu\text{l}/\text{rat}$ . In vitro experiments were performed on  $400 \mu\text{m}$  slices from the PO/AH as extracellular recordings of the neuronal activity (firing rate) and temperature sensitivity (TC) in the conditions of perfusion with artificial cerebrospinal fluid (ACSF) and of superfusion with  $10 \mu\text{M}$  vigabatrin. All data was statistically analyzed using one-way ANOVA, Student's t-test and paired t-test.

*Results:* Vigabatrin administered i.c.v. in vivo did not result in statistically significant change of core body temperature in dose  $100 \mu\text{g}/5 \mu\text{l}/\text{rat}$ , while in  $400 \mu\text{g}/5 \mu\text{l}/\text{rat}$  it caused a significant hypothermia developing between 90th and 210th min post administration with a maximum response reached at the 150th min. The combination of  $10 \text{ mg}/\text{kg}$  morphine i.p. and vigabatrin i.c.v. in doses  $100 \mu\text{g}/5 \mu\text{l}/\text{rat}$  and  $400 \mu\text{g}/5 \mu\text{l}/\text{rat}$  both resulted in statistically significant decrease of the morphine-induced hyperthermic effect from the 90th until the 150th min following vigabatrin administration. During in vitro experiments the superfusion of  $10 \mu\text{M}$  vigabatrin led to a statistically significant increase of the thermosensitivity (TC) in warm-sensitive PO/AH neurons.

*Conclusions:* Our results show that vigabatrin causes in

vivo a dose-dependent decrease in core body temperature in rats and significantly attenuated morphine-induced hyperthermia. Its effect on the thermosensitivity (TC) of warm-sensitive neurons situated in PO/AH is further proved in vitro.

*Discussion:* Vigabatrin is a GABA-mimetic which increases GABA's concentration indirectly. Our results suggest that GABA-mimetics like vigabatrin cause a dose-dependent hypothermia by affecting the thermoregulatory neurons in PO/AH. The influence of vigabatrin on morphine-induced hyperthermia suggests an interaction between the opioid and GABA-ergic system.

## Correlation of methotrexate efficacy and toxicity with AICART T28G polymorphism of the amino imidazole carboxiamide ribonucleotide gene in patients with rheumatoid arthritis

ESC-ID **275**  
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*Aim:* Methotrexate (MTX) is an antifolate agent that inhibits 5-amino-4-imidazole carboxiamide ribonucleotide transformilase (AICART), and promotes intracellular accumulation of AICAR, resulting in increase release of the anti-inflammatory agent adenosine. This study investigated whether AICART T28G polymorphism in gene contributing to anti-inflammatory adenosine release is associated with efficacy or toxicity of MTX in patients with rheumatoid arthritis (RA).

*Methods:* A total of 76 RA patients who were received MTX were evaluated. Genotype analysis of the AICART T28G polymorphism was performed by polymerase chain reaction – restriction fragments lengths polymorphism (PCR-RFLP). MTX efficacy was evaluated as the change in the number of tender and swollen joints 6 month after initiation of therapy, using Disease Activity Score (DAS 28) and compared among genotypes. MTX related toxicity was defined as presence of side effects.

*Results:* Among 76 patients enrolled in the study, 33 had TT, 35 TG and 8 GG genotype. A total of 23 patients (30%) presented with a side effects. The side effects were more common in patients bearing TT and TG genotype, compared to patients bearing GG ( $p < 0,05$ ).

*Conclusion:* There is correlation between MTX toxicity and AICART T28G polymorphism, while there is no correlation with MTX efficacy. Our findings suggest that patients bearing TT and TG genotypes carry increased risk of presence of adverse effects.

### Matrix metalloprotease inhibitor Ilomastat protect against cell damage during hypoxia/reoxygenation in neonatal rat cardiomyocyte cultures

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*Introduction:* It is known from previous studies, that matrix metalloproteases (MMPs) are activated during ischemia and released by cardiomyocytes during early reperfusion. They play a crucial role in the formation of ischemia/reperfusion injury.

*Aim:* The aim of this study was the examination of the effect of the MMP inhibitor Ilomastat on cell damage used in a concentration dependent manner during hypoxia/reoxygenation.

*Materials and methods:* Cell cultures containing more than 90% of cardiomyocytes were prepared from hearts of neonatal rats. Cardiomyocytes were exposed to hypoxia for 150 minutes following with 120 min reoxygenation, while 0, 0.5, 5, 50 and 500 nM of Ilomastat was administered. Trypan blue staining was used for the determination of cell viability.

*Results:* The rate of cell death was  $49.7 \pm 3.2\%$  in the hypoxic control. Ilomastat, used in 50 nM concentration seemed to decrease significantly the rate of cell death (to  $30.5 \pm 2.9\%$ , p is less than 0.05). However, 0.5, 5 and 500 nM of Ilomastat did not have this protective effect. (Percentages of cell death were:  $38.6 \pm 3.6\%$ ,  $42.4 \pm 2.7\%$  and  $41.2 \pm 3.5\%$ ).

*Conclusion:* According to our results, Ilomastat is supposed to have protective effect against hypoxia/reperfusion injury hence pharmacological inhibition of matrix metalloproteases might provide therapeutical potential in the future.

## Physiology

### Negative inotropic effect of angiotensin 1-7 and its modulation by the endothelial endothelium

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Among the new functional bioactive peptides of the renin-angiotensin system, the heptapeptide angiotensin 1-7 has received an increasing attention for its critical role in the counter-regulation of angiotensin II effects in the cardiovascular system (Ferrario et al., 2005). Considering that the heart was identified as one of angiotensin 1-7 main targets and that ACE2 (Angiotensin converting enzyme II) and Mas receptor have a high expression in this organ, this new renin-angiotensin system pathway may have a key role on cardiac renin-angiotensin system actions (Castro et al.,

2005). There are still many questions regarding angiotensin 1-7 function in cardiovascular homeostasis. In particular, its actions on the acute modulation of myocardial function are not yet completely understood. Thus, in the present study we aimed to evaluate the acute inotropic and lusitropic effects of angiotensin 1-7. Right ventricular rabbit papillary muscles were immersed in a modified Krebs-Ringer solution (1.8mM Ca<sup>2+</sup>; 35°C) and electrically stimulated (0.6Hz). Increasing concentrations of angiotensin 1-7 (10<sup>-9</sup> to 10<sup>-5</sup>M) were added in the following conditions: (1) in baseline conditions with intact endothelial endothelium (EE); (2) after selective removal of the EE with Triton X-100 (1s, 0.01%); (3) with intact EE in the presence of the Mas receptor antagonist A-779, the AT<sub>1</sub> receptor antagonist ZD-7155, the AT<sub>2</sub> receptor antagonist PD-123,319 or the nitric oxide synthesis inhibitor NG-nitro-L-arginine (L-NA). Evaluated parameters: active tension (AT), maximal velocity of increasing and decreasing tension (dT/dt<sub>max</sub> and dT/dt<sub>min</sub>, respectively), muscle peak shortening (PS), velocity of contraction (dL/dt<sub>max</sub>), relaxation velocity (dL/dt<sub>min</sub>) and time to half relaxation (tHR). The results are presented as mean  $\pm$  standard error (p < 0.05). Concerning the effects on contractility, we observed a significant decrease in active tension, dT/dt<sub>max</sub>, peak shortening and dL/dt<sub>max</sub> of  $-10.5 \pm 3.6\%$ ,  $-8.0 \pm 3.0\%$ ,  $-5.3 \pm 2.6\%$  and  $-5.7 \pm 2.3\%$ , respectively. There was no change on relaxation parameters, namely on dT/dt<sub>min</sub> or dL/dt<sub>min</sub>. Time to half relaxation was significantly decreased. The presence of ZD-7155 or PD-123,319 did not change these effects. However, angiotensin 1-7 effects on myocardial properties were abolished after selective EE removal and in the presence of A-779 or L-NA. In conclusion, in this animal species, angiotensin 1-7 through its binding to Mas receptor induces a negative inotropic effect modulated by the EE and nitric oxide and independent of AT<sub>1</sub> or AT<sub>2</sub> receptors activation. As the effects described in the present work were influenced by the endothelial endothelium, they may be disrupted in situations associated to endothelial dysfunction, as in heart failure or myocardial ischemia.

### Leptin regulates blood pressure by ACE changes in ob/ob mice

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Leptin is a hormone linked to food intake control and metabolism. It also influences blood pressure, but the mechanisms triggered in this process are not yet elucidated. Angiotensin-I converting enzyme (ACE) is a transmembrane metalloproteinase which regulates cardiovascular functions and recently has been associated with metabolism control and obesity. In this work we tried to answer the question whether ACE could be a link between blood pressure regulation and obesity in ob/ob mice, a model lacking leptin. These mice are obese and diabetic, but present normal 24h mean arterial pressure. We measured ACE activity in plasma and in lung membrane prepara-



tions from ob/ob mice using the fluorescence resonance energy transfer peptide AbzFRK(Dnp)P-OH. Our results show that plasma and lung ACE activities as well as ACE mRNA expression were significantly decreased in this model. In agreement with these findings, the ACE inhibitor enalapril provoked an attenuated blood pressure decrease in ob/ob mice. Renin, angiotensin I, bradykinin and angiotensin 1-7 in plasma were increased in obese mice, but plasma angiotensin II concentration was unchanged. After leptin infusion, renin activity and angiotensin II concentration increased in both groups, whereas in ob/ob mice leptin replacement normalized ACE activity but not blood pressure levels. Moreover, the effect of ACE inhibitor on blood pressure after leptin treatment was not changed in lean, but increased four times in obese mice. In summary, our findings show that the renin-angiotensin system is altered in ob/ob mice, with markedly reduced ACE activity, which suggests a possible correlation between BP regulation and leptin. These results point to an important interplay between the angiotensinergic and the leptinergic systems and may contribute to clarify the role played these systems in the pathogenesis of obesity, hypertension and metabolic syndrome.

### **Benefits of early reperfusion and ischemic preconditioning upon the myocardial response to acute hemodynamic overload during ischemia**

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*Aim:* The systolic response to hemodynamic overload is already well characterized. However, its diastolic counterpart still remains largely unknown. In this way, our objective was to evaluate diastolic response to acute mechanical overload in the normal cardiac muscle, in the setting of myocardial ischemia and after ischemic preconditioning.

*Methods:* Rabbit papillary muscles (modified Krebs solution, 0.2Hz, 1.8mM Ca<sup>2+</sup>, 30°C) were acutely stretched from 92% L<sub>max</sub> to 100% L<sub>max</sub> (length at which maximal force is developed) under non-ischemic conditions (control: normoxia and presence of glucose; n = 9), during an ischemia/reperfusion insult (IR: stretch during 15 minutes of ischemia followed by reperfusion; n = 7) and after an ischemic preconditioning event (IP: stretch in muscles previously subjected to 2 cycles of 5 minutes ischemia followed by 10 minutes of reperfusion; n = 8). Immediate and delayed responses to muscle stretch were evaluated. Results presented as mean ± standard error (pime-dependent decrease in myocardial stiffness. Moreover, its inhibition under ischemic conditions, as well as its enhancement upon myocardial reperfusion and ischemic preconditioning, highlights the possibility of its active modulation in order to prevent further hemodynamic decompensation especially during myocardial ischemia.

### **Hydrogen-ventilation decreases functional and morphological damage of the neurovascular unit caused by asphyxia/reventilation in newborn piglet**

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*Introduction:* Perinatal asphyxia is the major cause of serious brain damage in newborns. The oxidative stress following the hypoxic events is considered as one of the mechanisms that play an essential role in provoking the impairment of central nervous system. The lesions can be aggravated by increased expression of cyclooxygenase-2 (COX-2) the major COX isoform expressed in nervous system of the newborn. According to recent data, hydrogen (H<sub>2</sub>) in low concentrations (2-4%) has an antioxidant potential by neutralizing the hydroxyl radicals selectively resulting in reduced oxidative stress.

*Aims:* This study aimed to examine the short-term functional and morphological effects of H<sub>2</sub> supplemented ventilation in a piglet model of perinatal asphyxia/reventilation (A/R).

*Materials and methods:* For the study newborn (and the blood gas values were determined at definite time points. Standard histopathological examination and COX-2 immunohistochemistry were performed on formaldehyde fixed and paraffin embedded brain samples.

*Results:* MABP and HR showed dramatic increases after asphyxia in the room air-ventilated group; however, these changes were markedly reduced in the H<sub>2</sub>-ventilated group. In contrast to room air-ventilation, arterial pCO<sub>2</sub> tension was within the normal range in the H<sub>2</sub>-ventilated group. In addition, H<sub>2</sub>-ventilation preserved the dilation of pial arterioles to hypercapnia after asphyxia, whereas NMDA-induced vasodilation was not influenced by asphyxia in any types of ventilation used. Histopathological examinations showed that there was a pronounced decrease in early neural damage of all examined cortical and subcortical brain areas in the H<sub>2</sub>-ventilated group. The COX-2 immunoreactivity exhibited marked region specific differences in all groups but the profile of the expression was independent of the ventilation types applied.

*Conclusions:* The administration of H<sub>2</sub> during reventilation could be very a simple way of elimination of hydroxyl radicals. According to the findings of this study H<sub>2</sub>-administration may be a promising therapeutic approach in reducing the perinatal hypoxic injuries of the central nervous system.

### **Influence of D,L homocystein thiolacton on electroencefalografic characteristics and glucose concentration in adult rats**

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*Introduction:* Accumulating evidence suggests that homocysteine metabolite, D, L homocysteine thiolactone,

plays an important role in dementias, stroke, Alzheimer disease and seizure. Homocysteine and its derivatives are structurally similar to glutamate and seem to express direct excitatory effects on N-methyl-D-aspartate ionotropic and metabotropic glutamate receptors and induce convulsions in adult, as well as in immature rats.

**Aim:** The aim of the present study was to determine the effects of D, L homocysteine thiolactone on glucose concentration as well as on electroencephalographic (EEG) characteristics of seizure activity in adult rats.

**Materials and methods:** Adult Wistar rats were divided into two groups: 1. Control (C) (0.9% NaCl; n = 14); 2. Homocysteine (H) group (D, L homocysteine thiolactone, 8 mmol/kg; n = 14). Substances were administered intraperitoneally. Concentrations of homocysteine and glucose were measured 25 min after administration of D, L homocysteine thiolactone. For the EEG to be chronically registered three gold-plated electrodes were implanted over frontal, parietal, and occipital cortex.

**Results:** In group H, concentration of homocysteine was significantly decreased while glucose concentration was significantly increased in comparing to the control. We observed that D, L homocysteine thiolactone induced two types of seizures, the coexistence of convulsive and non-convulsive epilepsy. EEGs recordings in convulsive animals showed a high-voltage spike-wave and polyspikes complexes. The second, absence-like, nonconvulsive seizures were accompanied by the EEGs mostly with 6–8 Hz spikes-and-wave discharges (SWD). Maximal median number of the SWD were registered in a period 35–40 min and it was significantly increased in comparison to period 0–15 min.

**Conclusion:** The results indicate that D, L homocysteine thiolactone activates mechanisms responsible for hyperglycemia and induces two types of seizure, convulsive and nonconvulsive. These facts indicate that Hct may have important role in epileptogenesis in hyperhomocysteinemia. **Key words:** EEG, D, L homocysteine thiolactone, glucose, SWD, rats

### Role of glyated haemoglobin and microalbuminuria levels in type 1 diabetic patients

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Nephropathy is one of the major complications of Type I diabetes mellitus that could lead to end stage renal disease. Microalbuminuria is the best predictor of developing diabetic nephropathy. The risk of microalbuminuria in patients with type I increases with increase in glyated haemoglobin (HbA1c) levels, suggesting that efforts to reduce the frequency of diabetic nephropathy should be focused on reducing glyated haemoglobin levels.

**Aim:** Considering the high prevalence of diabetic nephropathy, the present study was carried out to determine microalbuminuria and glyated haemoglobin in adults with type I diabetics and compare with healthy controls and to find correlation between them.

**Materials and Methods:** Twenty clinically diagnosed type 1 diabetics and twenty healthy controls in the age group of 17–30 years constituted the study population.

Microalbuminuria was measured by turbidometry method. Glycated haemoglobin was measured by HPLC. Statistical analysis was done by student's t test and Pearson's correlation.

**Results:** Statistically significant increase in glyated haemoglobin, serum urea, serum creatinine and microalbuminuria in type I diabetics when compared to healthy controls. Statistically significant positive correlation was found between microalbuminuria and HbA1c levels in cases.

**Conclusion:** The correlation of microalbuminuria levels with poor glycemic control in type 1 diabetics indicates progression towards diabetic nephropathy. The study reveals that there is an increased risk of nephropathy in type 1 DM patients due to increased levels of glyated haemoglobin. Due to the obvious hazards of nephropathy, it is recommended that diabetic patients optimize glucose control to prevent unprecedented complications of diabetes mellitus.

### The Protective Effect of Chitosan on Gastric Ulcer Experimentally Induced by NSAIDs

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**Introduction:** The gastrointestinal tract is the main target of NSAIDs toxicity which causes ulcerations, severe hemorrhage and perforations. Conventional NSAIDs determine non-selective inhibition of cyclooxygenases (COX-1, COX-2), decreasing the protective prostaglandins and mucus secretion, inducing vasoconstriction and, consequently, ischemia. These processes are mediated by the reactive oxygen species (ROS) with oxidative effects on membrane lipids and cellular proteins. Chitosan is a polysaccharide polymer derived from chitin extracted from crustacean shells, having reepithelialisation, haemostatic and antioxidant qualities.

**Aims:** To assess the preventive effect and the healing promotion of Chitosan on gastric ulcer as well as oxidative stress parameters and to provide a novel approach to the prophylaxis and treatment of NSAID-induced gastropathy.

**Materials and Methods:** For this experiment we used 45 adult female Wistar rats (250 g), divided into 3 equal groups. Through gavage, they were administered 200 mg/kg b.w. of Ibuprofen and 0.15 mg/kg b.w. of Chitosan, as follows: control group - Ibuprofen and at one hour distilled water; 2nd group - Chitosan one hour before Ibuprofen; 3rd group - Ibuprofen and after one hour Chitosan. The animals were sacrificed at 6 hours, 24 hours and 7 days. The gastric mucosa was examined macroscopically and microscopically to estimate the lesional severity score. From the homogenates and serum were determined the malondialdehyde (MDA), glutathione (GSH) values and total concentration of thiol groups.

**Results:** The macroscopic lesional scores after Ibuprofen treatment were  $13.25 \pm 3.06$  at 6 hours and  $3.22 \pm 2.06$  at 24 hours. The microscopic lesional score confirmed that Chitosan had a protective effect on gastric mucosa in pre-administration and that it reduced the injurious capacity at first post-administration. Ibuprofen was an inductor of

oxidative stress suggested by the MDA values of  $0.203 \pm 0.06$  at 6 hours from administration. In serum the -SH groups increased at 24 hours and 7 days in the control group consequently to extension of the oxidative stress as an adjusting measure to counteract the effect of ROS. Also, the pre-administration of Chitosan raised the levels of GSH ( $41.86 \pm 17.53$  at 24 hours and  $1.43 \pm 0.62$  at 7 days) comparing to the first group. In the post-treatment with Chitosan, the GSH levels decreased at 24 hours and 7 days comparing to the values in the control group at the same intervals. Regarding NO we observed a slight significant increase in its value comparing the second and the third groups, but only at 6 hours.

**Conclusions:** Ibuprofen produces severe long term gastric injury and induces oxidative stress both in the stomach and serum. Chitosan exhibits a prolonged preserving effect on gastric mucosa and serum in NSAIDs pre-administration, diminishes the harmful action of NSAIDs on first post-administration and brings a mild increase in NO values.

### The condition of nitric oxide system under inveterate hyper immune complexity and its correction by corvistine

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The undeviating growth of immune pathologic diseases over the last decades along with adverse prognostication of their further development have resulted in the problem of allergy as one of the most relevant on the today's socio-medical agenda. Within the structure of allergic diseases, the ranking place is set by the illnesses which pathogenesis is dominated by hyper immune complexity. Many researches focus their efforts on the examination of illnesses primarily caused by the intensified concentration of circulating immune complexes. Taking this into account, it is relevant to examine immune complex pathogenesis and seek opportunities of its correction. In due respect, specimen that involve antioxidant and membrane-protective attributes are of great concern; these include bioflavonoids, among which quercetin (sephoretin, meletin) is the most active.

**Aim:** The aim of this research study consists in the examination of the impact of corvistine on fermentative activity of nitric oxide synthases and the level of stabile metabolites, i.e. nitrite-and nitrate-anions in blood serum under inveterate hyper immune complexity.

**Materials and methods:** Experiments were carried out with 20 sexually mature scrub white male rats weighing 200-250 g. each, which were fed by standard diet of animal quarters (retainer). Inveterate hyper immune complexity was recreated in accordance with the method forwarded by C Cochrane and D Koffer. Rodents featured by simulated inveterate hyper immune complexity process were injected with corvistine. The activity of nitric oxide synthases and the level of stabile metabolites were determined in blood serum by spectrophotometric method.

**Results:** The development of inveterate hyper immune complexity is accompanied by the changes of indicators of nitric oxide system. There is an evident increase in the activity of inducible synthase of nitric oxide (iNOS) by

2.3 times compared to intact rodents. The activity of constitutive synthase of nitric oxide (cNOS) is inhibited almost by 1-2 times. Secondary to such changes is the increase in the level of nitrite-anion by 5.5 times, and nitrate-anion – by 1.4 times, accordingly. The application of corvistine in rodents featured by hyper immune complex syndrome caused the decrease in iNOS activity by 1.4 times and increase in cNOS level by 3 times. Instead, the contents of stabile metabolites in serum decreased, as well as the level of nitrite-anion – by 2 times, and nitrate-anion – by 1.6 times, accordingly.

**Conclusion:** Hence, corvistine reduces the activity of inducible NO-synthase and the level of nitrite-anion, which provides grounds for further experimental research aimed at the correction of inveterate hyper immune complex syndrome, as well as the probability of its further successful application in case of allergic and immune complex diseases.

### H-currents in human neocortical neurons in epilepsy surgery tissues

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The cellular mechanisms of hyperexcitability in epileptic disorders are poorly understood. Classical concepts favour an imbalance between excitatory and inhibitory synaptic transmission to account for hyperexcitability. In addition, altered ionic currents of individual neurons may cause changes of excitability at the network level (see ref. 1 for review). In this context hyperpolarization-activated inward currents (H-currents, I-H) have attracted considerable attention. In neocortical neurons I-H stabilize the resting membrane potential and dampen the temporal summation of excitatory postsynaptic potentials. Reductions of I-H have been reported from different animal models of epilepsy (e.g. 2) and some antiepileptic drugs augment I-H (e.g. 3). Our previous data (4) indicated a comparatively small I-H in the human epileptogenic neocortex. We further investigated I-H of neocortical neurons in epilepsy surgery tissues from pharmacoresistant patients (4). All patients provided informed consent to use their tissue for research purposes, according to ethical requirements (Declaration of Helsinki). We used whole-cell patch-clamp recordings with IR-videomicroscopy to characterize the properties of I-H. The I-H was evoked by hyperpolarizing command potentials (2s, from -60 to -140 mV) and ascertained by the established I-H blocker ZD7288 (n = 7). The current densities of I-H (estimated from the amplitudes of the fast component at -140 mV and the membrane capacitance) averaged  $2.8 \pm 1.7$  pA/pF (mean  $\pm$  s.d.; n = 323) in neurons from temporal lobe epilepsy (TLE) tissues and  $4.7 \pm 2.3$  pA/pF (n = 28) in neurons from frontal lobe epilepsy (FLE) tissues (P15 grand mal seizures (GM);  $2.2 \pm 0.6$  pA/pF, 35 patients) compared to those with fewer GM ( $2.9 \pm 1.0$  pA/pF, 18 patients; P = 0.0037). These data indicate a marked reduction of I-H in the human epileptogenic neocortex, presumably due to a reduced expression of HCN-channels, which may increase excitability and probability of seizure activity. A less pronounced downregulation in the

frontal lobe tissues might account for the lower incidence of FLE compared to TLE.

### Role of the Glutathion Peroxidase 4 in the vascular system

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Glutathione Peroxidase 4 (GPx4) belongs to the family of selenium dependent glutathione peroxidases. Besides protecting cells from free radicals and regulating the cellular oxidative tone, it plays an important role in many cellular signaling processes (e.g. induction of apoptosis and mitosis). Due to its ability to directly reduce hydroperoxides bound to phospholipid complexes in cell membranes, GPx4 also interacts with fatty acid metabolism, especially metabolism of arachidonic and linolic acid. Although hypoxia and reactive oxygen species (ROS) are well known to play a crucial role in angiogenesis, the function of the GPx4 in this context is largely unknown. In order to explore the role of the GPx4 in the vascular system we used an inducible knockout mouse model in which the expression of GPx4 was solely disrupted in endothelial cells by means of "Cre Lox" technique. Subcutaneous implantation of Lewis Lung Carcinoma cells (LLC1) resulted in tumors of similar volume and size in knockout as well as in wildtype mice. Tumor tissue was analyzed by immunohistochemical methods. Evaluation of tumor vascularization by CD 31 staining showed no differences in vessel number or caliber in both types of animals. For in vitro studies we are currently performing aortic ring assays. Small rings of the mouse aorta are embedded in Matrigel and will be analyzed by the length of upcoming sprouts and the number of branches. In order to examine the functional integrity of GPx4 deficient endothelium, we plan to analyze endothelial permeability by intravital microscopy either in the dorsal skinfold chamber or in the Musculus cremaster model using fluorescein-coupled plasma markers. Based on previous experiments which demonstrated that tumors derives from Gpx4 deficient tumor cells displayed a denser vascular network, but a reduction in the overall vessel diameter, GPx4 might play a distinct role in vessel wall maturation of newly formed vessels. Since endothelial-cell derived GPx4 knockout mice showed no obvious phenotype in tumor vascularization, we hypothesize that GPx4 might play its important role not in endothelial cells but in pericytes/vascular smooth muscle cells. Therefore we are planning to perform similar experiments in pericyte-specific GPx4 knockout mice.

## Pneumology

### Influence of ozone on the microscopic morphology and physiology

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Ozone is an allotropic form of oxygen with a high reactivity and having a strong oxidant action. Regardless of the fact that it presents antiseptic properties and that it is used in some forms of treatment for some diseases, this study is meant to be an alarm signal regarding the harmful effects of this gas on the respiratory system and on the hepatic tissue. The aim of this article is to present the damaging consequences of the lungs and liver obtained after long exposure to ozone. The material used was represented by two groups of 10 Wistar female rats each, that were daily exposed to ozone for 10 minutes at 0.5 ppm O<sub>3</sub>, and a third group represented by the witness batch. From the first group blood was collected after 2 weeks to determine the indicators of oxidative stress and samples of lung and liver were drawn for histological studies. Tissue changes were highlighted using hematoxylin eosin and red Sirius. In addition, the lung samples taken from study subjects were turned into homogeneous preparations in order to determine the intensity of oxidative stress occurred in these organs compared with the witness group. The second batch was exposed for a further two weeks, after which the same sampling techniques and determining methods as for the first group were applied. The results show a correlation between the values of malondialdehyde (MDA) and glutathione (GSH) -obtained both in blood and in the homogeneous preparations, and the microscopic changes that implicate a pathological state. The necrosis elements that were discovered were accompanied by a dramatic decrease of glutathione(GSH) in the lungs. Infiltrates of lymphocyte occur after severe damage of the immune system. Furthermore, the bronchial epithelial cells, macrophages and epithelial cells of type I. were all affected together with strong vascular changes. The study also reveals an inflammation of the airways. The liver indicated hepatocellular necrosis, extended from the port area to the centrolobular vein (bridge necrosis). To conclude with, the presence of oxidative stress has a strong impact on the respiratory system. Also, the increased number of free radicals in the blood indicated on a circulatory path to necrosis in the hepatic tissue.

*Key words:* ozone, oxidative stress, lung, liver, necrosis, lymphocyte, free radicals

## Transforming growth factor- $\beta$ and resistin-like molecule- $\alpha$ are upregulated in asthmatic airways following both repeated allergen and methacholine inhalation

ESC-ID **658**  
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Transforming growth factor- $\beta$  and resistin-like molecule- $\alpha$  are upregulated in asthmatic airways following both repeated allergen and methacholine inhalation. Valdeep Singh Dulay, Chris Grainge and Peter Howarth University of Southampton School of Medicine, Southampton, UK. Transforming growth factor- $\beta$  (TGF $\beta$ ) is a potent profibrogenic factor that is fundamental to airway remodelling. Resistin like molecule beta (RELMA) is a soluble protein that has been shown to directly induce collagen deposition in mice. We aimed to test the hypothesis that TGF $\beta$  and RELMA were present in human airways, upregulated by allergen and/or methacholine and associated with remodelling. Bronchial biopsies were obtained from 21 mild asthmatics before and four days after challenge with either house dust mite allergen (n = 13) or methacholine (n = 8). We demonstrated using immunohistochemistry that TGF $\beta$  is upregulated following repeated allergen challenge (p = 0.021) and, for the first time and to a greater extent, following repeated methacholine challenge (p = 0.018). We report the first documented identification of RELMA in human airways. RELMA, like TGF $\beta$ , was upregulated following repeated allergen challenge (p = 0.007) and trended towards increase following repeated methacholine challenge (p = 0.063). Basement membrane thickness also increased post allergen (p = 0.016) and post methacholine (p = 0.043). We propose a novel theory whereby bronchoconstriction, that occurs from disease onset and underpins characteristic hyperresponsiveness, represents the key determinant of airway remodelling in asthma which is mediated by TGF $\beta$  and/or RELMA.

## Comparison of the serum concentration of zinc in patients with bronchiectasis and the control group

ESC-ID **896**  
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Comparison of the serum concentration of zinc in patients with bronchiectasis and the control group  
*Background:* Bronchiectasis is an abnormal and permanent dilatation of bronchi. Infection plays a major role in causing and perpetuating bronchiectasis, reducing the microbial load and attendant mediators is a cornerstone of therapy. Zinc, as an integral micronutrient, is involved in the immune response and the response to infection. Zinc deficiency is associated with impaired phagocytic function, lymphocyte depletion. Mild zinc deficiency has

been described in many infectious diseases such as abscess, cellulitis, chronic diarrhea, pneumonia, TB, ... in several studies.

*Study objectives:* The purpose of this study was to determine the serum zinc levels in a series of patients suffering from bronchiectasis and compare it with the control group.

*Methods and Patients:* This analytical cross-sectional study was performed on 34 patients with proven bronchiectasis and 29 healthy control subjects, who were referred to Rasul Akram hospital, Iran, Tehran between "March 2005 to March 2007". The exact serum concentration of the zinc was demanded for all of the subjects under this study and other information was completed according to their medical records. Both groups (case and control) were frequently matched regarding their age and sex. All the data was analyzed with SPSS v.16 using chi<sup>2</sup> and independent T test.

*Results:* The case group included 11 males (32.4%) and 23 females (67.6%) with the average age of 55.03 (SD = 17.06) years and the control group included 17 males (58.62%) and 12 females (41.38%) with the mean age of 57.86 (SD = 14.16) years. The mean serum zinc levels in the case and control group were 94.06 (SD = 20/96) mcg/dl and 103.7 (SD = 11.96) mcg/dl, respectively. Independent T-test analysis showed that the serum zinc concentration in the case group was significantly lower than control group (p = 0.02).

*Conclusion:* The results of our study show that serum zinc level in the patients was lower than the control group and this relation was significant. According to our findings, the use of zinc supplement with its role of improving the immune system and some unknown mechanisms can reduce the progression of the infectious disease. Therefore, prophylactic and therapeutic use of zinc can be beneficial and must be evaluated in further trials. Key words: bronchiectasis, zinc, immune system

## Effect of Zafirlukast on lung function improvement in patients with Chronic obstructive pulmonary disease (COPD)

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*Introduction:* Chronic obstructive pulmonary disease (COPD) is a progressive airflow limitation caused by chronic inflammation of the airways and lung parenchyma. It is estimated that COPD is now the fourth leading cause of death worldwide. Cysteinyl leukotrienes (Cys-LTs) are proinflammatory mediators which may cause bronchial smooth muscle contraction, stimulation of mucous production, enhancement of vascular permeability, and recruitment of eosinophils which lead to an accelerated decline in the pulmonary volumes. Zafirlukast is a highly selective LTD<sub>4</sub> antagonist but there is few evidence of a role of these mediators in COPD. A reduced need for short-acting rescue  $\beta_2$  agonists, fewer exacerbations of asthma, and an increased quality of life have been previously noted as the possible benefits of Zafirlukast in asthma. The aim of our study was to evaluate the effects of

Zafirlukast on lung function improvement in patients with COPD.

**Patients and Methods:** Twenty four patients with moderate to severe COPD, in the stable phase of the disease, participated in this interventional, quasi-experimental study after giving their informed consent. The patients had to fulfill the American Thoracic Society (ATS) criteria for the diagnosis of COPD. Inclusion criteria were: age more than 40, history of more than 10 pack-year smoking, moderate severity of COPD; whereas patients with unstable respiratory disease requiring oral/parenteral corticosteroids within the 4 weeks before the study, upper or lower respiratory tract infection within 4 weeks of the screening were excluded. All patients were received 40mg oral Zafirlukast per day for 2 weeks. Pulmonary function Test was performed both at the baseline and at the end of the study. Data were analyzed using SPSS v.16 and Paired T-test and Wilcoxon test were performed.

**Results:** The mean age of the patients was 67.29 (SD = 5.56) years with the mean baseline FEV1 was 41.79% (SD = 14.96) of predicted value. After 2 weeks, the mean improvements in FVC, FEV1 and FEV1/FVC were 4.75% (SD = 13.18), 3.71% (SD = 9.19) and 9.33 (SD = 27.08), respectively. Wilcoxon paired test showed that the mean ratio of FEV1/FVC was significantly improve after two weeks of treatment [58.96% (SD = 14.39) vs. 68.29% (30.14), P = 0.033]; while other changes were not statistically significant (P>0.05).

**Conclusion:** Our results showed that zafirlukast has no considerable bronchodilatory effect in COPD except for FEV1/FVC. Demonstrated effects of zafirlukast were mostly observed in asthma; and its results in COPD is still controversial. There is a few studies that show zafirlukast has bronchodilator effect in COPD and cysteinyl leukotrienes might contribute to the bronchospasm in these patients. Present study consisted of a very short treatment period and it is possible that the extension of this period could possibly have more effects. Additional larger studies are needed to verify the impact of leukotriene receptor antagonists on the lung function improvement in COPD patients. **Keywords:** Chronic obstructive pulmonary disease (COPD), Pulmonary function, Zafirlukast

### Value of C-reactive protein in patients with community acquired pneumonia

ESC-ID 411  
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**Introduction:** Pneumonia is a lung inflammation often caused by infection with bacteria, viruses, or other organisms. Community acquired pneumonia (CAP) is the most common type of pneumonia. People with this type of pneumonia contracted the infection outside a hospital setting. The aim of study: To assess whether C-reactive protein (CRP) is a sensitive marker of community acquired pneumonia and to evaluate whether it may be used as an index of treatment response.

**Methods and material:** After admitting to hospital patients past standard clinical procedures, blood tests, chest radiography, microbiology testing in order to

set the diagnosis of disease. We studied 20 patients diagnosed with CAP. Patients were treated and examined at the Clinic for lung diseases and tuberculosis, Knez Selo. Serum CRP levels, were measured in all patients on the first and seventh day of hospitalization. Patients with diagnosed CAP were divided in groups according to: 1. flow of disease and complications during the treatment, 2. the use of antibiotic therapy before the treatment, 3. presence of associated diseases.

**Results:** In 17/20 (85%) of patients with CAP disease was a favorable clinical course. The first day of hospitalization measured CRP values were  $187.58 \pm 83.58$  mg/L with a median of 194.0 mg/L. The seventh day of hospitalization values were significantly fallen, under 50 mg/L. In patients with unfavorable clinical course measured CRP values were  $204.66 \pm 100.25$  mg/L with median of 200.5 mg/L. Although it was used antibiotic therapy treatment, values of CRP measured on seventh day were still very increased and amounted  $141.57 \pm 44.18$  mg/L with a median of 144.2 mg/L. Fourteen out of twenty (70%) patients were using antibiotic therapy before hospitalisation. In this group the value of CRP measured on the first day of hospitalization was almost half below than the values of CRP in patients who did not use the antibiotic therapy. That value was  $153.91 \pm 69.03$  mg/L with a median of 152.6 mg/L. On the seventh day values were under 50 mg/L. In the group of patients who didn't use antibiotics before hospitalization CRP values on the first day of hospitalization were  $276 \pm 53.48$  mg/L, with a median of 279.85 mg/L. On the seventh day of treatment measured values of CRP were around 150 mg/L. High CRP values despite the antibiotic therapy may indicate to complications or incorrect therapy. In 17/20 patients (85%) with CAP was registered the presence of one or more associated diseases. Measured value of CRP on the first day of hospitalization was  $178.73 \pm 82.22$  mg/L. In patients with CAP and no associated diseases, values of CRP were higher and amounted to  $257.68 \pm 78.36$  mg/L.

**Conclusion:** CRP is a sensitive laboratory parameter in the diagnosis of CAP and the excellent biochemical marker of disease activity and the intensity of infection.

### Intrabronchial foreign bodies – an emergency to diagnose and treat in current pneumological practice

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**Aim:** To reveal most significant diagnosis and treatment particularities of intrabronchial foreign bodies, in our experience.

**Material and method:** A retrospective analysis of 106 cases of intrabronchial foreign bodies found in the past 40 years in the bronchoscopy department of Clinic of Pulmonary Diseases of Iasi was done. Diagnosis was based on anamnesis, clinical, radiologic and endoscopic criteria. Treatment outcomes were reviewed for each case. **Results:** Study group was made of 84% males, predominant from rural areas (76%) and adults 41-50 years old age-group was most exposed to this condition. We found a wide spectrum of inhaled foreign bodies: 31,3% with

metallic origin, 52,3% were organic and also various structures (plastic, fibers, glass piece of sail, pen top, beads, buttons) of foreign bodies were identified. Foreign bodies were more frequently located in the lower right bronchus. Interval between foreign bodies' aspiration in the airways and foreign body removal ranged between 10 hours and 4 years. Relevant or even spectacular aspects of diagnosis, together with those cases which confronted our department with special technical difficulties, such as dentist needle extraction and 12 months ignored inhaled tooth, are shown. Foreign bodies were extracted by rigid or flexible bronchoscopy, except 3 cases, which underwent surgery.

**Conclusions:** Intrabronchial foreign bodies represent an emergency condition to diagnose and treat. Such cases require qualified personnel and specific equipment. In our experience, 92,5% of cases were successfully removed bronchoscopically, while only 2,8% underwent surgery and 4,7% were lost in follow-up.

## Psychiatry

### Correlates of insight in patients with schizophrenia admitted in a psychiatric hospital in Romania

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**Background:** Insight is defined as one's capacity to recognize his mental illness and to specifically attribute symptoms to the pathology of the illness. Different studies of insight in schizophrenia have led to inconsistent findings and conflicting results alternatively stating that acknowledgement of one's mental illness is a detriment and a key to successful adaptation.

**Objective:** This cross-sectional study investigates the relationship between the amount of insight in schizophrenia and its influence on frequency of hospitalization and relapse, as well as the way insight relates to other important variables, such as depression, quality of life and optimism. Patient's social context and available support system in Romania were also carefully taken into consideration.

**Materials and Methods:** All patients diagnosed with chronic schizophrenia (DSM-IV-R), and having a history of relapse (more than six hospital admissions in one year in a psychiatric hospital in Bucharest, Romania) and admitted in Department No X of Hospital "Prof. Dr. Alexandru Obregia, Bucharest, Romania" in a three month period were asked to participate in the study. Patients who gave consent (45 patients) were evaluated using the Quality of Life Enjoyment and Satisfaction Questionnaire (QLESQ-18), The Life Orientation Test-Revised (LOT-R) - in order to assess individual differences in generalized optimism versus pessimism, and the Perceived Stress Scale (PSS), which evaluates the degree to which situations in one's life are appraised as stressful. Results to PANSS (Positive and Negative Syndrome Scale) item G12 were used for the assessment of insight. Admission charts

and interview were used for the assessment of social and familial support.

**Results:** The patients who had moderate or good insight had also reduced optimism, a high rate of perceived stress, these being associated with a high frequency of hospital admissions and with a lower quality of life. 25 subjects, or 71%, had severe insight deficits or even lack of insight. These findings were associated with higher optimism, and a lower rate of perceived stress. The frequency of hospital admissions for these patients was not significantly different from the one of the patients with higher insight. Patients who benefited of support system and had an adequate social context had fewer hospital admissions.

**Conclusion:** What is interesting and needs to be mentioned is the fact that even though the two categories of patients had different scores in the scales regarding optimism, quality of life and perceived stress (limited insight into illness appearing to have protective functions) their record shows no significant differences concerning hospital admissions. Taking into consideration the fact that better insight and awareness of illness has been previously advanced as a key to adaptation, this conclusion might appear as strange. We could advance the premise that the incapacity to benefit from this higher insight is due to the poor quality of the supporting system in our country. It is vital to be able to connect the capacity of "being aware" that the patient has, with a proper supporting system and social context, in order to obtain a maximum of efficiency.

### An application of structural and functional neuroimaging in diagnostics of different depression types

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**Introduction:** Depression is one of the most common mental health problem and it grows greater every year around the world (Tiganov AS, 1999; Sadock BJ, 2000)

**Aim:** The main aim was to analyze the possibility of using functional and structural neuroimaging methods in diagnostics of different depression types.

**Materials and Methods:** 40 patients with depression syndrome were distributed into 3 groups: 1) Neurotic Depression (Diagnosis: mixed anxiety-depressive disorder, Adjustment disorders) – ND group; 2) Psychotic Depression (Diagnosis: Recurrent Depression, Bipolar Affective Disorder – current depressive episode, Schizoaffective disorder, depressive type) – PD group, 3) Depression due to organic pathology (Diagnosis: Organic depressive disorder, Organic mixed affective disorder) – OD group. Controls were 16 age- and gender-matched healthy participants. We used several methods of functional (positron-emission tomography, functional magnetic resonance imaging) and structural (voxel-based morphometry, diffusion-tensor imaging) neuroimaging.

**Results:** We found several functional and structural abnormalities in limbic structures within all three groups. Some of them were same, some were different.

**Conclusions:** We found several functional and structural abnormalities into all three depressive groups. Almost all of them are parts of so-called frontal-subcortical circuits (Cummings J.L., 1993), disfunction of which, according

to the present knowledge, could play crucial role in depression pathogenesis. Based on the results and analyzing the data of our colleagues, we propose a theory of pathogenesis of different types of depressive disorders. Summarizing our own results and analyzing the data of our colleagues, we complement a theory of depression pathogenesis and propose an original point of view for neurobiological basis of different types of depressive disorders.

## Radiology

### Accuracy of Pulmonary CT Angiography for the Assessment of Right Ventricular Dysfunction in Patients with Acute Pulmonary Embolism

ESC-ID 633  
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*Clinical relevance:* Knowledge of right ventricular dysfunction (RV) is the most important factor to manage clinical risks in patients with acute pulmonary embolism (PE). Purpose: Pulmonary computed tomography angiography (CTA) is the first line diagnostic tool to rule out acute PE. Until now, echocardiography has been the accepted gold standard for diagnosing RV-dysfunction in patients with PE. However, several independent studies demonstrated the prognostic value of an increased right ventricular/left ventricular (RV/LV) diameter ratio assessed by CTA. So far, the diagnostic value of CTA for the assessment of RV-dysfunction is unknown if compared to echocardiography.

*Method and Materials:* CTA RV/LV diameter ratio measured in transverse sections (RV/LVtrans), four-chamber view (RV/LV4ch), and RV/LV volume ratio (RV/LVvol) were analyzed in 51 patients (female = 29, male = 22, mean age 63 years  $\pm$  16) with acute PE (central = 20, peripheral = 31). All Patients also underwent echocardiography for the assessment of RV-dysfunction within 48h. Based on the echocardiography findings patients were grouped in a consensus reading by two observers as having none, low to moderate and high right ventricular overload. Receiver operating characteristic [ROC] analysis was used to compare RV/LVtrans, RV/LV4ch and RV/LVvol to positive findings in echocardiography for PE-related RV-dysfunction.

*Results:* Based on echocardiography, sixteen (31%) PE patients were diagnosed with RV-dysfunction of which nine cases had greatly reduced RV-Function. PE patients with RV-Dysfunction had significant higher ratios in RV/LVvol (1.87 vs 1.09), RV/LVtrans (1.39 vs 1.03) and RV/LV4ch (1.32 vs 1.01) if compared to PE patients with normal RV-Function ( $p = 0.001$ ). RV/LVvol was significantly more reliable as a predictor of RV-dysfunction than

RV/LVtrans or RV/LV4ch (area under the curve [AUC] 0.88 vs 0.73 and 0.75,  $p = 0,009$ ). The same was true for the subgroup of patients with highly decreased RV-Function (AUC 0.91 vs 0.74 and 0.76,  $p = 0.02$ ). Using a cut-off for RV/LVvol of  $>1.5$  the sensitivity, specificity, positive predictive value, and negative predictive value for the prediction of RV-Dysfunction were 0.75 (95% confidence interval [CI]: 0.50 - 0.90), 0.88 (95% CI: 0.73 - 0.96), 0.75 and 0.89, respectively. For the prediction of greatly reduced RV-Function a cut-off value for RV/LVvol of  $> 1.8$  achieved a sensitivity, specificity, positive predictive value, and negative predictive value of 0.78 (95% CI: 0.44 - 0.94), 0.93 (95% CI: 0.80 - 0.98), 0.70, and 0.95, respectively.

*Conclusion:* The RV/LV volume diameter ratio is more accurate than the four-chamber RV/LV and axial diameters ratio of CTA if used for assessment of RV-Dysfunction in patients with PE. Due to its negative predictive value of 0.89 it should be considered as an alternative to echocardiographic assessment in order to rule out RV-Dysfunction.

### Diagnostic of intracranial aneurysm with magnetic resonance angiography

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*Aim:* to determine possibility of diagnosis of intracranial aneurysm by the help of MRA and to compare findings of TOF MRA with digital digital cerebral subtraction angiography (DSA).

*Material and methods:* By retrospective study, 42 persons without symptoms SAH were taken in the period from January to December 2008. according to the protocol, those patients who are suspect to have intracranial aneurysm, TOF-MRA is being done. The patients are middle-age,  $52,5 \pm 23,5$  years of age. Those who were found out to have aneurysm, while having done TOF MRA, digital subtractional angiography was being done (DSA).

*Results:* MRA came a cross aneurysm at 42 patients. 13 patients who were found to have aneurysm, while having done TOF MRA, DSA cerebral angiography was being done. The most common localization of aneurysm, determined by the method of MRA, was at the bifurcation MCA (14/42 or 33,33%) and was not statistically and significantly different from the frequency of the same localization of aneurysm found by DSA (3/13, 23,07%): Fisher:  $p = 0,732$ . The biggest aneurysm was dimension 18x11mm, 15x9mm 4x3mm and the least was 2mm. 13 patients, having been tested by both methods, didn't have statistically significant difference at the size of aneurysm ( $p > 0,005$ ).

*Conclusion:* Rupture aneurysm of arterial blood vessels of brain is the most common cause of spontaneous subarachnoidal bleeding. SAH (up to 80%) so as to determine the diagnosis intracranial aneurysms as it is still used conventional cerebral angiography ("golden standard") MRA enabled quicker and univasive cerebral angiography ("golden standard"). MRA is a contemporary, accurate and univasive method for the determination of asymptomatic intracranial aneurysms. The results of our study shows the match between MRA findings, localization and



size of aneurysm in comparison to “golden standard” – cerebral DSA.

**Key words:** intracranial aneurysm, MRA, DSA

### Clinical functional MRI at 3 Tesla – establishing optimal protocols

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**Aims:** While functional MRI (fMRI) methods are routinely used in pre-surgical brain mapping nowadays, no standardized protocols have yet been established across research centers. The vast majority of clinical fMRI studies have been validated on 1.5T; however, the better signal-to-noise ratio and spatio-temporal resolution of higher field scanners can yield different maps for similar paradigms. Therefore, we aimed to evaluate a set of paradigms used for mapping of language production and comprehension, the motor cortex and the hippocampus, as well as to define optimal acquisition strategies.

**Material and Method:** We performed functional MRI on 17 healthy volunteers. The evaluated paradigms – picture naming, grammatical decision, auditory comprehension, auditory grammatical decision, cued motor response and spatial memory testing tasks – were presented in a block design format. Statistical significance versus paradigm length was tested using parametric methods by modifying the number of paradigm blocks processed in order to find the shortest paradigm length providing solid results. The configuration of functional activations yielded by the different language mapping paradigms were also compared using group analysis.

**Results:** All of the investigated language, motor and memory mapping paradigms provided solid and consistent activation maps corresponding with results from the literature. The auditory grammatical decision paradigm was capable of mapping language production and comprehension networks in one step. The configuration of language-related activation maps differed across paradigms within the same functional areas depending on the task and the number of stimulus blocks. Four active blocks would already serve for defining the centre of gravity of activations; however, processing more blocks increases the complexity of the obtained maps. Interestingly, the variability of the maps starts to decrease when using more than 9 blocks. Lateralization indices also were dependent on the number of blocks: lateralization tended to decrease if more than 6 blocks were included in the model. The cued motor response task yielded activations as expected from the literature. The investigated spatial memory paradigm activated the anterior and posterior hippocampus in a different manner.

**Conclusion:** Using multiple paradigms for language mapping improves clinical utility of fMRI; in addition, with tackling different aspects of language it also provides an opportunity for a finer grain identification of processing steps. However, it is possible to map language production and comprehension in one step. Based on our preliminary results, six repetitions of the active block seem optimal for language mapping, having the smallest impact on lateralization calculations, and resulting in a relatively low

variability in the maps. The tested memory paradigm needs to be re-evaluated and reworked.

## Surgery

### Sevoflurane decreases the ischaemia-reperfusion lung injury in an experimental auto-transplant animal model

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**Aim:** Sevoflurane has proven to protect several organs from ischaemia-reperfusion injury (IR); however this has been poorly studied in the lung. IR is characterized, among other events, by oxidative stress and inflammation. Release of proinflammatory mediators and leukocyte activation have been established as important markers of inflammation, while lipidic peroxidation markers are used for measuring the presence of oxidative stress. The aim of this study was to determine the effect of Sevoflurane on oxidative stress and inflammation during the IR.

**Methods:** 14 large-white pigs were divided into two groups: sevoflurane preconditioning (SEVO) and control (C). Both groups were submitted to a lung auto-transplant (pneumonectomy, ex-situ superior lobectomy and lower lobe reimplantation). All of them received the same anesthetic induction, while maintenance was performed with sevoflurane 4% (group SEVO) or propofol (group C), until one-lung ventilation started, before the pneumonectomy, then propofol was used for both groups. Lung's samples were taken at three different moments: 1) pre-pneumonectomy, 2) pre-implantation of lower lung lobe, 3) post-reperfusion of the implant. The biomarkers gauged were: Malondialdehyde (MDA) and lipid hydroperoxidase (LPO), both metabolites of lipidic peroxidation, to measure the oxidative stress, and myeloperoxidase (MPO), interleukin-1 (IL1) and tumor necrosis factor (TNF) for inflammation. U Mann-Whitney was used as non-parametric test to find statistical meaning.

**Results:** We found a statistically significant decrease ( $p < 0.05$ ) in all the markers gauged at the post-reperfusion moment in the SEVO group compared to C group (results are expressed as average with its standard deviation; SEVO vs C): LPO (2,87 (0,09) vs 3,50 (0,2)), MDA (3,5 (0,3) vs 4,93 (0,1)), MPO (0,09 (0,01) vs 0,17 (0,008)), IL-1 (0,986 (0,13) vs 1,57 (0,11)) and TNF (0,629 (0,06) vs 0,848 (0,04)). A significant decrease in LPO (2,8 (0,1) vs 3,54 (0,09)), IL1 (1,113 (0,11) vs 1,813 (0,05)) and TNF (0,588 (0,03) vs 0,808 (0,06)) was also described at the pre-implantation moment.

**Conclusions:** It was demonstrated that Sevoflurane preconditioning decreases oxidative stress and inflammatory markers, which may indicate a protective effect over ischaemia-reperfusion injury in our lung auto-transplant model.

## Comparative histological analysis of alternative methods of lower eyelid reconstruction using two different types of ear cartilage graft: an experimental study in rabbits

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*Background and purpose:* After aggressive resection of malignant tumors of head and neck, burns or complex traumas, the functional and aesthetic reconstruction of the involved areas has been difficult and complex. Various methods have been reported for reconstructing the lower eyelid, the main purpose being the re-establishment of protective and supportive functions of the eyelid for the globe. To achieve this, a rigid structure such as cartilage is needed to accomplish the function of the tarsus and also the skin deficiency of the eyelid and periorbital region needs to be solved for the success of the reconstruction. Another important issue is the adaptation of the flap to the new local environment and the effects of the flap on the globe and especially the cornea. The aim of the present study is to assess and compare the morphostructural changes between an autologous auricular flap consisting of both cartilage and skin and an autologous cartilage graft, used for eyelid reconstruction in rabbits.

*Methods:* The posterior lamellae of 18 lower eyelids from 9 rabbits were reconstructed by using autogenous grafts of ear cartilage. In the left eyelid, the graft consisting of both cartilage and skin was placed with the skin in direct contact with the eyeball, whereas the right eyelids were reconstructed in a similar manner but using only cartilage grafts. The skin and cartilage graft was introduced as a novel experimental method of eyelid reconstruction. The grafts were then retrieved in the second, fourth and sixth week. The samples were analyzed macro- and microscopically and the histological change of each graft were observed.

*Results and conclusions:* The data obtained from the histological analysis describes the different adaptation manner of the two grafts: conjunctival epithelization in the cartilage graft versus metaplasia from pavementous epithelium to mucosal membrane in the cartilage and skin graft. The extent of the transformation in the graft surface as well as the effects on the eyeball were compared. The significance of the study resides in proving the validity of the experimental graft through its transformation in the surface epithelium determining a high anatomical and functional similarity to the tarsus and conjunctiva of the natural eyelid, excluding therefore the need to use a chondromucosal graft which is usually more difficult to obtain and has a higher morbidity.

## Bloodless cardiac Surgery: A possibility?

ESC-ID 447  
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*Introduction:* With the increasing number of Coronary Artery Bypass Grafts being performed on patients there has been a parallel rise in the amount of blood products

being transfused in these procedures. Despite a dramatic decrease in mortality associated with CABG procedures, questions remain concerning the safety of blood transfusion and the effect on patient condition before, during, and after surgery. Standardized guidelines governing blood transfusion needs in such cases do not exist, and therefore, much is left up to individual discretion. The aim of this study is to prepare a clinically sound and applicable predictive model for determining the need to transfuse various blood products in association with coronary bypass procedures.

*Methods:* It is a prospective observational study, based in a tertiary care hospital, where we examined and followed approximately 500 patients admitted for CABG for a period of 1 year starting 1st of July 2008. We assessed independent pre-, intra- and post-operative predictors of blood product transfusion based on our retrospective study, and for whose validation is this being done. The patients were all who had undergone first time CABG and who were transfused blood. The controls underwent the same procedure but no blood was transfused. The data was analysed using univariate and multivariate analysis where appropriate; and the Hosmer and Lemeshow goodness-of-fit test.

*Results (expected results based on retrospective study: actual results to be made available in July):* Based on our retrospective study and the current data we expect to have a mean age of  $58 \pm 9$  years of our patients with a male preponderance, pre-operative Hb of  $26.6 \pm 4.5$  kg/m<sup>2</sup>; INR of  $1.02 \pm 0.13$ . Among the intra-operative variables we expect to have CPB time of  $99.1 \pm 31.9$  min with On pump procedures carried out 97.7% of the time; and hemorrhage being the most prevalent complication. Among the comorbidities analysed, the ones most significantly associated were hypertension = 70.7%, diabetes = 43.1%, dyslipidemia = 39.0% and renal disease = 4.5%. We expect to have blood or blood products transfused in at least ? 40% based on our observation (actual results to follow late). The presence of diabetes would make a significant difference between the patients who require transfusion and who do not.

*Discussion:* The differences in transfusion rates have been attributed to adherence to different blood saving techniques and adherence to different guidelines, availability of products and quality of surgical care to the patients. However, we expect to find quite a significant number of factors to be associated differently with the variables as the literature cites. This lack of association in our data could be attributed to different transfusion related practices in our institution. The prediction rule comprises of presence of pulmonary disease, diabetes mellitus, ongoing/recent myocardial infarction and a low ejection fraction. This rule is being validated to assess its applicability within the institution and possibly on a global aspect.

## In vivo characterization of a murine syngenic glioma model in the chronic cranial window

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Tumor angiogenesis represent a promising target for anti-glioma therapy. To detect the complexity of tumor angio-

genesis it is necessary to visualize vascular, cellular and molecular mechanisms ideally in an orthotopic tumor model to closely mimic the human pathology. The chronic cranial window (CCW) is a versatile model for a direct, continuous and noninvasive approach to visualize these mechanisms. However, the use of immunodeficient animals like athymic nude mice to avoid rejection of tumor cells remains an important experimental drawback. Therefore, we aimed at facilitating and characterizing a high quality orthotopic in-vivo-model which maximizes the similarity to human glioma disease by establishing a syngeneic glioma model in immunocompetent animals. Using GL261 cells, we generated spheroids out of  $1 \times 10^3$  cells, fluorescently labeled with DiI, and implanted them into CCW preparations in wildtype C57/BL6 mice ( $n = 8$ ), followed by intravital fluorescence microscopy (IFM) on day 3, 6, 9, 12, 15, 18, 21, 24, after implantation. We distinguished between a peripheral compartment and a central compartment. Host vasculature in control animals without tumor implantation served as control vasculature. Using FITC-Dextran and Rhodamine 6G microvessels and leukocytes respectively were visualized. The microvessel diameter increased steadily during tumor development (tumor group: day 6:  $19,5 \mu\text{m}$ , day 24:  $23,1 \mu\text{m}$ ; control group:  $12,6 \mu\text{m}$ ). Total vessel density of the tumor increased up to day 9 and remained on a plateau for the rest of the experiment. Functional vessel density in the peripheral compartment was significantly higher compared to the central compartment. A decreasing microvascular perfusion index was observed in tumor vasculature over the course of the experiment (tumor group: day 6: 82,8%, day 15: 71,4%, day 24: 63,7%; control group: 97,7%). Perfusion index was significantly lower in the central compartment compared to the peripheral compartment and significantly reduced compared to control vasculature. Furthermore the permeability index was significantly decreased in tumor vessels and leukocyte rolling in tumor microvessels was significantly enhanced compared to controls. Our data clearly demonstrate glioma-typical microvascular features with facilitated angiogenesis in tumor periphery, dysfunctional tumor microvasculature especially in the tumor center and increased leukocyte endothelial interactions. This model may represent the experimental basis to study tumor angiogenesis and related molecular mechanisms in a syngeneic, repetitive and in vivo experimental approach.

### **Surgical treatment of postoperative giant incisional hernia**

ESC-ID **240**  
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*Introduction:* Postoperative giant incisional hernia is a frequent complication of the abdominal surgery and, by definition, it measures over 10 cm in width or length. Its' treatment presumes two steps: the eradication of the sack and the closure of parietal defect. The repair method of the abdominal wall can follow one of the three surgical techniques: direct suture, auto-grafting or allo-grafting with synthetic prosthesis. Our main purpose was to establish which is the appropriate surgical attitude.

*Material and Method:* A 5-year (January 2003- January 2008) retrospective study was performed: 310 cases operated in the Second Surgical Clinic Cluj-Napoca, Romania. Inclusion criteria were: (i) abdominal wall defect larger than 10 cm; (ii) age less than 70 years; (iii) appearance at least 6 months after a surgical intervention, excluding this way a possible medical error.

*Results:* Out of the total number of patients, 58,13% presented giant incisional hernias. The topographical localisation of the hernias was: median 41,95% cases, pararectal 15,51%, right iliac fossa 22,41%, right subcostal region 13,79% and lumbar 6,32%. The type of preferred surgical repair method consisted in: 36,20% cases substitution grafting and in 63,79% cases using muscle and aponeurosis plain non-resorbable suture. The substitution mesh was placed intraperitoneal in 26,98% cases and extraperitoneal in 73,01% cases. The postoperative evolution was favourable in 81,60% cases. Postoperative complications that appeared were wound infection (10,34% cases), seroma (6,89% cases), haematoma (5,17% cases) and cutaneous necrosis (2,87% cases). Most incisional hernias were encountered after hysterectomy interventions and were located on the linea alba especially in the lower umbilical region; other cases revealed incisional hernias occurred after opened-cholecystectomy, hernias situated above the umbilical region.

*Conclusions:* None of the used methods for incisional hernia repair can consist of a valid technique for all patients; the intervention itself was adapted to each patient's history and comorbidities. We prefer using the allo-grafting method with synthetical materials such as polypropylene. Placing the mesh in a correct and suitable manner and furthermore, the use of a patch with minimal foreign-body proprieties, coupled with its' tensile strength, may improve the prognosis of the intervention. Open giant incisional hernia or recurrent incisional hernia should be performed by trained surgeons to reduce the risk of relapses.

### **Restoration of intestinal continuity – failure analysis**

ESC-ID **712**  
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*Introduction:* Fecal diversion is often used as a surgical procedure in the acute disorders of left side colon, injures of rectum and perineal region and in case of very low anastomosis after rectum resection.

*Aim:* The aim of our study was the failure analysis among patients who were primary qualified for restoration of intestinal continuity.

*Methods and materials:* Retrospective analysis allowed to include 61 patients into the study (46% female, 54% male, average age 67 years, range 24- 89) who were operated in the 3rd Department of General Surgery between 2006 and 2008. We analysed influence of sex, age, main illness, comorbidities, type of surgery, type of stoma, elective/acute indication for surgery, results of anorectal manometry on failure of restoration of intestinal continuity.

*Results:* The intestinal continuity was restored in 32.7%. 55% of the patients were operated due to a malignant neo-

plasm of the left side colon. The analysis showed that age, co-morbidities, type of surgery, type of stoma and results of anorectal manometry are statistically significant risk factors for not performing the restoration of the intestinal continuity. The analysis showed that the reasons for not performing the intestinal continuity were: death (34%), internal contraindications (24%), the recurrence of malignant process (16%) and lack of the patient's consent (13%).

*Conclusion:* Preoperative analysis of the risk factors can allow not only for a more conscious consent for the surgery but also for a better management of the patient (including psychological counselling) in the postoperative period.

*Key Words:* stoma, restoration of intestinal continuity.

### The importance of early determination of haemostasis system parameters for acute pancreatitis severity prediction

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*Aim:* Acute pancreatitis (AP) is not just a local inflammatory disease limited to pancreatic and peripancreatic tissues; it can present in its most serious form as the systemic inflammatory response syndrome (SIRS), multiple organ failure, sepsis, and death. Studies on the clinical value of parameters of haemostasis in predicting pancreatitis-associated complications are still scarce. Aim of this prospective study was to identify the useful haemostatic markers for prediction of severity of AP.

*Material and Methods:* In 91 consecutive primarily admitted patients with AP, prothrombin time (PT), activated partial thromboplastin time, fibrinogen, antithrombin III (AT III), protein C, D-dimer, and plasminogen were measured in plasma within the first 24 hours of admission and 24 hours thereafter. Two study groups comprising 33 patients with mild form of acute pancreatitis and 58 with severe acute pancreatitis (SAP) were compared.

*Results:* Levels of PT, AT III, protein C, INR, d-dimer and activated partial thromboplastin time were significantly different at first and second day. The level of d-dimer was out of physiological limits. A middle value of d-dimera at first day in group with mild form of acute pancreatitis was  $258 \pm 182 \mu\text{g/l}$ , and in group with SAP was  $681 \pm 459 \mu\text{g/l}$ , which was significant positive correlation. An d-dimer value of  $414.00 \mu\text{g/l}$  on admission was the best cut-off value to predict development of SAP with sensitivity value of 73% and specificity value of 91%.

*Conclusions:* Measurement of plasma levels of d-dimer on the admission is an accurate method for identification of patients who will develop severe acute pancreatitis (SAP).

### Interventional radiology in treatment of splenic artery aneurysms

ESC-ID 1036  
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*Introduction:* Vessels anomaly, artery-venous fistulas and splenic artery aneurysms (SAA) are rare causes of gastrointestinal haemorrhage, though on progress of diagnostic methods are difficult task. High lethality in case of SAA rupture demand clear diagnostic and treatment.

*Materials and methods:* We have an experience angiography diagnostic of 23 patients with SAA who were treated at IGUS AMSU from 1995 to 2009 years. Among the reasons leading to the formation SAA, the 21 patients had portal hypertension (PG), complicated splenomegaly and bleeding from esophageal varicose veins and in 2 patients blunt abdominal trauma. Men were 6 and women - 17 in age from 16 to 71 years. Angiographic investigation (AI) was conducted to clarify the diagnosis and selection of future surgical tactics. Was performed splenic artery embolization with an aim to reducing the pressure in the portal vein and reduce the risk of rupture of SAA. At the same time sought to establish a spiral in the cavity of the aneurysm and in the lumen of splenic artery (SA) to the aneurysm. In 2 patients on different dates after embolization performed splenectomy with resection SAA.

*Results:* Thus 165 patients surveyed of the PG, in 21 patients after the AI has revealed SAA. Of these aneurysm intraorganic segmental branches of the SA from 4 to 15 mm in diameter was detected in 14 patients; aneurysm proximal SA from 3.0 to 7.5 cm in diameter - in 6 patients and 1 patient was identified arteriovenous SAA. One observation was further revealed splenic vein aneurysm. If aneurysm was off-organic location used two-staged surgical treatment. In the postoperative period of relapse, 1 patient died of bleeding in patients with localization of SAA in the proximal part. There are not complications require surgical treatment.

*Conclusions:* We believe that with the localization of SAA intraorganic more justified embolization of SA. If aneurysm was off-organic location used two-staged surgical treatment. The first stage is carried out the reduction of splenic blood flow to reduce pressure in the portal-vein, the second stage - splenectomy with ligation of SA proximal SAA, in our opinion, the best, because resection with SAA plastic SA can be displayed while maintaining the spleen.

### Morphofunctional aspects in modelling diabetic foot syndrome

ESC-ID 720  
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Objectives of the investigation is to establish morphological changes from the system of microhaemodynamics in the experimental model of suppurative wound on the background of hyperglycemia.

**Materials and methods:** 28 mature white rats of Wistar line with modeled diabetic foot syndrome. Control group – animals without hyperglycemia. Material was taken every 14 and 26 day, preparations were prepared according to the standard methods.

**Results and discussion:** Early stages were characterized by practically identical changes in the condition of wound process: all the micropreparations revealed the picture of a purulent inflammation with diffuse exudates of the hypoderma, focal destruction of the adipose tissue, significant infiltration by polymorphonuclear leukocytes; the wounds were covered by fibrin layers and contained detritus. The main group was marked by a substantial slowness of a reparative process: the signs of microcirculatory disorders, macrophage reaction insufficiency, inhibition of proliferation and dystrophy of fibroblasts, disturbed collagen synthesis. Focal infiltrations are located mostly on the wound surface, exudates reaction was sharply reduced in the hypoderma. The development of vessels both vertical and horizontal is mostly expressed as compared with the main group and clinical prototype.

**Conclusion:** Application of the suggested method of modeling allows to form adequate pathomorphologic changes in animals. The drawback of this model is inadequate consideration of neuropathological changes in the tissues. The most substantial changes were observed in hyperglycemic animals.

### Coronary artery bypass grafting as the treatment of choice in a case of absent left main

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The instance of coronary obstructive disease in the context of an anomalous coronary tree is a rare, yet a possible scenario. Herein we present the case of a patient with myocardial ischemia and absent left main stem. An anomalous arterial trunk originated from the mid-right coronary artery to supply the left anterior descending and the circumflex arteries. The proximal right coronary artery was 80% stenotic before the bifurcation with the trunk supplying the left coronary system. Superimposed fixed coronary obstruction in the setting of an isolated coronary anomaly is possible, whilst it has been suggested that coronary segments with aberrant course are no more prone to obstructive disease than are normal segments in the same patient. The coexistence of obstructive coronary lesions may justify a proportion of the cases of sudden death associated with course anomalies of the coronary arteries. Nevertheless, the strategy of treatment for a patient with myocardial ischemia and a combination of isolated coronary anomaly and obstructive disease remains to be customized in each case. The variability of anatomical, functional and clinical pictures has made the development of sound guidelines for treatment unfeasible. Few cases are reported in the literature over the treatment of coronary atherosclerotic disease in patients with anomalous coronary anatomy similar to that reported herein. Percutaneous coronary intervention (PCI) with stent deployment has been reported for particular conditions which could be essentially compared to single-vessel disease settings,

and in the absence of further causes of myocardial ischemia PCI can actually be considered as the best curative option. One case is reported of PCI treatment for severe obstructive disease of a common main stem originating from the right anterior sinus of Valsalva (a situation much more similar to ours), with emergency stent deployment above the level of bifurcation with the vessels pertaining to the left coronary system: the patient suddenly died due to stent occlusion after discontinuation of aspirin therapy. The entire myocardium is virtually at risk in the presence of an obstructive lesion within the context of anomalous coronary anatomy as depicted in this and in our case. Coronary bypass surgery with venous grafts was reported in 1980 to successfully treat a patient with absent LM and obstructive disease of the RCA, the LAD and the Cx. Thus, we managed our patients as an 'extreme' form of critical LM disease. Coronary bypass surgery was then indicated according to guidelines. We believe that the surgical indication should be seriously considered in a similar setting, even though the common main stem lesion is apparently stable and well-suitable for PCI. Such approach may allow to inscribe these protean cases into a recognized category of recommendations for treatment.

### A new method for motion analysis of the aortic arch as a risk stratification after supra-aortic rerouting and endovascular stent-graft placement

ESC-ID **477**  
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**Objective:** Total supra-aortic rerouting as well as double vessel transposition followed by endovascular stent graft placement are now an established tool for the treatment of various aortic arch pathologies. However, details about the motion of the aortic arch after this procedure remain unknown. Moreover, no perfectly fitting risk stratification score exists for outcome prediction of this specific patients.

**Materials and Methods:** We applied a fully automated method to quantify the deformation patterns of the aortic arch in a gated CT sequence. The aorta is detected and segmented by an active surface approach, that accurately identifies the vessel wall in all frames. The correspondences of landmarks on the vessel wall are established by tracking the deformation during the cardiac cycle, resulting in a dynamic deformation model of the structure.

**Results:** With help of this model, global and local deformation properties like stretching and bending were measured. After registering the models acquired pre-treatment, post-transposition, and post-stent-graft-placement we compared these local properties and were able to quantify the change caused to the aortic arch motion.

**Conclusion:** This new method of automated computational motion analysis of the aortic arch may establish a risk stratification score for outcome prediction after supra-aortic rerouting followed by endovascular stent-graft placement.

## Cells Dedifferentiation of BHK – fibrosarcoma in repeated transplantations at hamsters

ESC-ID 278

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*Introduction:* BHK-fibrosarcoma, used experimental, obtained from kidney cells culture of newborn Syrian golden hamster. Preliminary results showed BHK 21-C 13 cell culture can be inoculate at hamsters in which grows like tumor node, and also that this way obtained tumor can be transplant from one to another individual. Nucleolar organization regions (NOR) are DNA parts in nucleoli where's running rRNA transcription. Size and number of nucleoli depends of cell type, approves activity grade of some cell and number in the nucleus increases with growing mitotic activity – especially at embryonal and cells of malignant tumors. Dexamethasone is a fluoride glucocorticoid with the strongest antiinflammatory and immunosuppressive effect amongst the corticoid drugs. Very often they are also given to patients with malignant disease together with citostatic therapy. The fact that is at patients with malignant diseases the immune system suppressive and through this stimulate the growth of the tumor, has not yet been proven.

*Aim:* Investigation of biological conduction and histological features of tumor induced by cell culture BHK 21-C 13 in series of successive transplantations at hamsters and effect of dexamethasone on BHK-fibrosarcoma by cytological merits of BHK-tumors with NOR silver-staining method (AgNOR).

*Material and methods:* Tumors induced at hamsters in the first and tenth passage and dexamethasone treated group were analyzed histological (fixed in 10% formalin, histological preparations provided with classical paraffin technique and cut on sections of 3 micrometers), and determined nuclear volume, mitotic index, nucleolar organization regions (AgNOR), as reticulin fiber in tumor tissue. These parameters were compared between the first and tenth passage, some with initial cell culture earlier prepared as staining preparation and also dexamethasone treated tumor. Statistical significance of obtained results analyzed by  $\chi^2$  test in compared groups with cells of normal hamsters' dermis.

*Results:* It was found large difference of cytological features in tumor cells between the first and tenth passage in meaning increasing nuclear volume, mitotic index and AgNORs, but decreasing reticulin fibers around tumor cells. Based to these parameters, in the first passage tumor cells haven't statistical significant difference from fibrocytes and fibroblasts of normal dermis, and tumor correspond to fibroma. In the tenth passage all parameters are statistical significant increased compared with dermis and the first passage, and cells morphological correspond to fibrosarcoma. At all passages there was no metastasis, only local infiltrative grow. Results of effect of dexamethasone by AgNOR analysis of experimental fibrosarcoma showed statistic significant difference between controlled hamster tumor and group treated with dexamethasone. Also measuring of the tumor volume thirty days later, discovered significant difference - the average tumor volume at treated group was 7.22 cm, and in the control group 184.98 cm.

*Conclusion:* Series transplantations of BHK-tumor at hamsters leads to significant dedifferentiation of tumor cells. In the first passage tumor have cytological features

of fibroma, and in the tenth of fibrosarcoma. AgNOR staining method also showed antiproliferative effect of dexamethasone on BHK-fibrosarcoma.

*Key words:* BHK21-C 13 cells, fibroma, fibrosarcoma, transplantation, dexamethasone, hamster

## Treatment of non-ruptured symptomatic (in acute expansion) abdominal aortic aneurysm

ESC-ID 398

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*Introduction:* The non-ruptured symptomatic abdominal aortic aneurysm (AAA) is a form of clinical manifestation of AAA related to a lot of controversies including the algorithm of diagnostic and therapeutic procedures, especially of those related to the timing of (endo)surgical eradication of the disease called aneurysm.

*Aim:* The aim of this study is to define an entity of non-ruptured symptomatic AAA, to examine the influence of the timing of the surgical treatment and to analyze the results of its treatment.

*Material and methodology:* The material contains the retrospective analysis of 390 operatively treated patients during the last five years at the Clinics of Vascular Surgery in Novi Sad. All of the patients are grouped into four categories: elective operative surgical treatment, surgical treatment after 24 hours since the reception through the Ward of Urgent Surgery with the immediate CT diagnostics (within first 2 hours), surgical treatment within first 24 hours, and immediate surgical treatment.

*Results:* During the last 5 years, 390 patients with AAA were operated on. 89 patients had ruptured AAA, 52 were operated on after 24 hours since they were urgently admitted to hospital, 18 patients were operated on within the first 24 hours, and 231 patients were planned for the elective surgery. Comparing the groups, rates of mortality are: elective surgery-5,1%, patients that were operated on after 24 hours -7,2 %, patients operated on within the first 24 hours -23%, and patients who had ruptured AAA-34 %.

*Conclusions:* Considering the results we have got, further conclusion could be made; the treatment of non-ruptured symptomatic AAA is related to the higher risk of postoperative mortality in relation to an elective surgery, so we can infer that the early (semi) elective surgery is a method that should be chosen for the treatment of non-ruptured symptomatic AAA in acute expansion.

*Key words:* aneurysm, surgical treatment, timing.

## Repair of Common Bile Duct Stricture by Auto-venous Transplant

ESC-ID 425

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Common bile duct (CBD) repair is a complicated procedure with a high rate of postoperative morbidity/mortality. The aim of our study was to obtain a good

working model which can help to understand the pathological processes of iatrogenic strictures of the common bile duct and devise a method of stricture repair. 20 rats weighing 250-300g were divided into 2 groups: 5 in the control group and 15 in the experimental group. For the control group the CBD was cut and repaired by simple anastomosis. The experimental group was first subjected to the creation of CBD stricture by method of ligation which was before hand impregnated in 5% iodine solution, then the formed stricture was excised and the femoral vein grafted. The experimental main group was further subdivided into 3 minor groups, which were examined during 1, 3, and 5 week intervals. After the first week post-operation signs of inflammation were present within the venous walls. The vascular endothelium common to the femoral vein was gradually replaced by columnar epithelium typical to that of the common bile duct. 11 of the 15 experimental subjects survived, with no signs of abnormalities on x-ray investigation and liver biochemical tests. Our conclusion is that femoral vein grafting may be a successful method of treating common bile duct strictures.

### Transplantation of small kidneys to adult recipients

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*Background/Purpose:* The purpose of this presentation is to examine whether the kidneys which come from donors under the age of eighteen, can be successfully transplanted in adult recipients and whether these kidneys offer a good life quality to the recipients.

*Methods:* The donors were divided in two groups, infants and children. In infants we checked also whether the en bloc kidney transplant technique is better than the single kidney.

*Results:* Small kidneys do fit in adult recipients, offering a good quality of life. Additionally the en bloc kidney technique is better than the single kidney technique in the transplantation of kidneys from infants.

*Summary/Conclusion:* We are allowed to transplant kidneys from young cadaveral donors, as they offer an excellent life quality to the recipients.

### Gastric bypass as one of the methods of surgical treatment of morbid obesity and metabolic syndrome

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WHO named obesity epidemics of XXI century. It is proved that patients with obesity suffer accompanying diseases such coronary heart disease, type II diabetes, impaired reproductive function in both men and women, osteoporosis, sleep obstructive apnoea and others; have impaired glucose, lipid metabolism. G. Reaven joined hyperinsulinemia, impaired glucose tolerance, hyper-

triglyceridemia, high level of low density lipoproteins (LDL) and arterial hypertension in one syndrome – metabolic syndrome.

*Purpose:* To investigate dynamics of changes of lipid and glucose metabolism in patients with morbid obesity (MO) and metabolic syndrome (MS) before and after gastric bypass (GB).

*Materials and Methods:* 48 patients with MO (body mass index (BMI) > 40 kg/m<sup>2</sup>) and MS were included in this study. Among them 35 women, 13 men; age – 42,8 ± 6,4 years old; body weight – 164,7 ± 39,8 kg; BMI 55,1 ± 17,7 kg/m<sup>2</sup>. All patients underwent GB during 2002 – 2006. Observation period 1 year. Before GB all patients had arterial hypertension, arterial pressure 168/12/105/6; cholesterol levels reached 6,5±0,8 mmole/l. 26 (55%) patients had high levels of triglycerides - 1,7±0,6 mmole/l; in 25 (54%) patients LDL - 4,9±0,8 mmole/l, in 14 (30%) very low density lipoproteins (VLDL) - 0,5±0,1 mmole/l, 17 (37%) had decreased level of high density lipoprotein (HDL)- 0,8±0,1 mmole/l. Atherogenic degree was high in 41 (86%) patients. Before GB 6 (12,5 %) patients had normal glucose tolerance, 31 (66 %) – impaired glucose tolerance, 11 (21,5 %) – type II diabetes. All patients in pre and post operative period underwent: measuring of arterial pressure, levels of cholesterol, triglycerides, LDL, VLDL, HDL; glucose tolerance test was performed.

*Results:* After GB all patients had decrease of body weight: after 3 months – 145,4 ± 36,6 kg, after 6 mon. – 113 ± 27,2 kg, after 12 mon. – 91,14 ± 26,64 kg. Excess body weight loss after 3 mon. – 11,7 %, after 6 mon. – 30,9%, after 12 mon. – 44,6%. BMI after 3 mon. – 51,1 ± 12,1 kg/m<sup>2</sup>, after 6 mon. – 38,6 ± 8,2 kg/m<sup>2</sup>, after 12 mon. – 32,4 ± 8,3 kg/m<sup>2</sup>. After GB all patients had decrease of levels of arterial pressure till 140/90. Cholesterol levels decreased to 4,5±0,8 mmole/l; levels of triglycerides decreased till 1,1±0,5 mmole/l; LDL – 3,3±0,4 mmole/l, VLDL – 0,2±0,1 mmole/l, HDL – 1,0±0,1 mmole/l. Also improved glucose metabolism after GB was installed. Normal glucose tolerance had 14 (31%) patients, impaired glucose tolerance – 30 (62,5 %), type II diabetes – 2 (6,5%) patients.

*Conclusion:* Gastric bypass is one of the methods of surgical treatment of morbid obesity, which provides constant and considerable weight loss. In postoperative period patients show impaired arterial pressure, lipid and glucose metabolism, that is why the operation gastric bypass can be one of methods of surgical treatment patients with MO and MS.

### The double access for the endoscopic submucosal dissection: a new technique

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*Background:* Endoscopic Submucosal Dissection (ESD) has an high en bloc resection rate and complete resection rate, regardless in tumor size although it leads to more complications, such as bleeding or perforation, whose incidence is increased for lesions or situated in “cumbersome” positions, or very (>30mm) large or for submucosal lesion because of the deeper layer. Classical ESD is limited by the

absence of a "second hand" to provide tissue countertraction and an adequate exposure of the submucosa.

**Objective:** To report a new technique, percutaneously-assisted endoscopic surgery with a gastric access for advanced ESD, to perform easier and more rapid procedures in treating lesions of the stomach.

**Patients and methods:** 2 patients with Submucosal Tumor (SMT), the one located in the gastric body, the other in the gastric fundus, both >20 mm in diameter and confirmed on classical endoscopic and EUS criteria, were enrolled. The procedure was performed with the patient under general anesthesia in an operating theatre by using a single channel videoendoscope. After marking the lesion, submucosal injection of a saline solution of epinephrine was performed. Then, a small initial incision was made with a needle-knife just outside the line of the marking dots. After that, the incision around the lesion was completed by using an triangle-tip knife (TT-knife) and the submucosal layer below the lesion was directly dissected with the same TT-knife. It was impossible to proceed due to the insufficient exposure of the submucosa and of the bottom of the lesion, so that a gastric access was performed as follows. The abdominal wall was sutured to the anterior gastric wall by using a double lumen gastropexy device. Under endoscopic vision a suture was inserted in one channel and taken out by a snare that captured the suture from the second channel. The gastropexy was applied. Other two gastropexy sutures were applied delimitating a triangular area. After an incision in the middle of this area, the stomach was punctured by using a 5mm trocar. After a laparoscopic forceps was inserted through the trocar, the lesion was grasped, pulled away from the muscle layer, and then the dissection was completed, using the same TT-knife. Endoclips were placed to close the small hole made in the stomach by the gastric port.

**Results:** The technique was performed successfully. The lesion was resected en bloc, with free lateral and vertical margins. The patient was refed after 24 hours, discharged after 48 hours and after 5 days gastropexy sutures were removed. An endoscopic control after 60 days showed absence of relapse and complete cicatrization of the treated area. Limitations: Single endoscopist, small patients number.

**Conclusions:** The double access to ESD is an easy and safe procedure that allows an excellent visualization of the submucosa layer, and is useful in treatment of gastric lesions located in various regions of the stomach. It is easy intuitive that, if this procedure was so effective in treating SMT, it should be even better if applied to mucosal lesions. Further studies are required.

### **Sirolimus therapy in liver transplantation for HIV-coinfected patients**

ESC-ID **686**  
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**Background:** Sirolimus (SRL) is a macrolide lactone which has proven antifungal, antitumoral and immunosuppres-

sive properties. A number of isolated reports and papers including small series of patients (pts) undergoing liver transplantation (LT) have recently been published with encouraging results, although worse results are reported in human immunodeficiency virus and hepatitis C virus (HIV-HCV) co-infected liver transplant recipients. We present a retrospective study regarding HIV co-infected patients receiving SRL monotherapy after LT.

**Methods:** Since June 2003, 18 HIV patients have received cadaveric-donor LT due to end-stage liver disease (ESLD) at Transplant Center of Modena. Moreover, 12 of these patients were also affected by Hepatocellular Carcinoma (HCC), 14 affected by HCV. Mean Model for End-Stage Liver Disease (MELD) score was 22 (range 9-32). Patients were assessed using the specific HIV-related inclusion criteria: CD4 T-cell-count over 200/mL and plasma HIV viral load levels lower than 50 copies/mL in pts undergoing highly active antiretroviral therapy (HAART); CD4 T-cell-count over 100/mL in pts intolerant to HAART, but with clinical and HIV-resistance mutation test prediction of efficacy of the antiretroviral regimen in the post-transplant period. Mean primary immunosuppression was based on Calcineurin Inhibitors (CNIs), but 8 patients were switched to SRL monotherapy due to CNIs adverse effects, like post-transplant renal failure (RF), or Kaposi's Sarcoma. SRL was the primary immunosuppressant in 4 patients. RF has been defined by detection of a serum Creatinine (sCr) over 1.6 mg/dl in three consecutive weekly controls and with estimated Glomerular Filtration Rate (eGFR) lower than 60ml/min/1.73 mq. SRL has been administrated with a loading dose of 0.1mg/Kg/die, then the dose was adjusted to obtain blood levels of 10ng/ml  $\pm$  2 in the first 6 months post-transplant; 8ng/ml  $\pm$  2 until 12 month and then 7ng/ml  $\pm$  2.

**Results:** The retrospective study evaluated 16 males (88.8%) and 2 females (11.2%). Mean age at the time of transplant was 42.6 years (range 32-50). Cellular rejection occurred in 3 patient under CNIs and in 1 patient under SRL. HCV recurrence arose in 7 patients under CNIs; two patients, after conversion to SRL, developed HCV-RNA clearance. Mean switch period from CNIs to SRL was 67 days (range: 10-840). Significant better control of HCV replication was found among patients taking SRL monotherapy, moreover SRL seems to improve the HIV-1 replication. Mean sCr level before switch was 1.9 mg/dl (range: 1.8-2.7 mg/dl). After three months from switch, mean sCr level was 1.2 mg/dl (range: 0.6-2.2 mg/dl). Mean eGFR before switch was 44.8 ml/min/1.73 mq (range: 18.8-82.4 ml/min/1.73 mq), while mean eGFR after three months from switch was 64.9 ml/min/1.73 mq (range: 18.8-175.6 ml/min/1.73 mq). The mean duration of hospital stay was 24.8 days (range 9-54).

**Conclusions:** HAART has significantly improved HIV management; today the major complications of HIV pts are represented by HCC and HIV-related nephropathy. LT is an excellent treatment in pts with ESLD and HCC. According to our series, we believe that HAART with concomitantly SRL can be an alternative treatment in HIV (with or without HCV) co-infected liver transplant recipients with RF.



## Methods and Periods of Surgical Treatment of Acute Destructive Pancreatitis

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*Objective:* the treatment of acute pancreatitis and its late complications is the subject of great discussions and contradictions in defining the methods and periods of treatment of such patients. The aim of our study is to define the optimum tactics of acute destructive pancreatitis treatment by carrying out the comparative analysis of efficiency of different surgical methods.

*Materials and methods:* Case records of 77 patients aged 23 to 89 suffering acute destructive pancreatitis have been studied. 73 patients were treated surgically, 4 – conservatively. Videoscopic interventions were performed to 36 patients, who were divided into 2 equal groups of 18: patients from the 1st group were operated in 72 hours from the beginning of the disease, from the 2nd – in 3 to 14 days. Percutaneous drainage under the control of supersonic scanning (PCD) was performed to 30 patients by one or repeated punctures with installations of constant drains. The indications for PCD were accumulation of liquid in retroperitoneal fat or bursa mentalis (according to the results of ultrasonic scanning or computer tomography), which was kept or increased in 7-10 days from the beginning of the disease, or the suspicion of contamination. Fistulography was performed to all patients after PCD to specify the sizes and localization of pathological cavity, presence of sequesters. 18 patients managed to reach steadfast recovery with the help of PCD only. Inefficiency of PCD, sequesters more than 2 sm in diameter determined the necessity of opened methods of treatment. Laparotomy as an independent method of treatment was performed to the last 7 patients of the group suffering peritonitis caused by pancreatitis.

*Results:* Videolaparoscopic interventions with drainage of the abdomen cavity were more effective among patients from the 1st group (89% to 56%). Percutaneous drainage under the control of supersonic scanning was performed to 30 patients, as a final method of treatment to 18 of them. Indications for laparotomy performed to 15 patients were retroperitoneal phlegmon, absence of effect from conservative or minimally invasive treatment, haemorrhages or purulent peritonitis.

*Conclusions:* videolaparoscopic interventions allow to verify the diagnosis of acute pancreatitis with high accuracy and drain the abdomen in case of diffusive enzymatic peritonitis. They are more effective in early period of disease (during the first 72 hours). Percutaneous drainage under the control of supersonic scanning should be performed to all patients suffering pancreatic accumulation of liquid caused by acute pancreatitis and can be the final method of treatment, used in different periods of disease. Laparotomy should be performed in different periods of disease in case of significant prevalence of pathological process on parapancreatic and retroperitoneal fat, or presence of big sequesters (more than 2 sm in diameter) in the abscess cavity, or absence of positive effect from minimally invasive interventions.

## Soft tissues fixation improvement in plastic surgery

ESC-ID 201  
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Discussions and researches in modern plastic surgery mostly refer to operations and techniques and do not take into consideration the fixation aspects. Incomplete fixation leads to the relapse of the deformity and gravity ptosis postoperatively, especially in follow-up period. The aim of the research was to compare traditional soft tissues fixation (polypropylene thread) with modified – using polypropylene mesh paraligaturally for providing larger joining surface and, therefore, the best fixing/lifting long-term result in plastic surgery operations (face-lift, mastopexy and otoplasty)

*Materials and methods:* Experimental part of the research was carried out on 50 rabbits “Chinchilla” breed. During the experiment we modeled such fixation systems: SMAS-polypropylene thread-SMAS vs. SMAS-polypropylene thread-mesh-SMAS dermoglandular flap-polypropylene thread-muscular fascia vs. dermoglandular flap-polypropylene thread + mesh-muscular fascia auricular cartilage-polypropylene thread-auricular cartilage vs. auricular cartilage-polypropylene thread+mesh-auricular cartilage Clinical part included 495 patients. 816 operations were performed. Control group included 171 patients with traditional fixation: facelift-52; mastopexy-86; otoplasty-33 patients. Totally –277 operations Researched group included 314 patients with traditional fixation: facelift-74; mastopexy-192; otoplasty-48 patients. Totally –529 operations. For morpho-hystological investigation we took biopsy rabbit tissue examples. The results were evaluated on 180 day postoperative-ly: after traditional fixation there were two functional layers around the thread – inner (rich-cytoplasm, functionally active cells), outer – collagen fibers and small amount of fibrocytes. We found that connective tissue around the thread is thin, with tendency to paraligaturally limitation. Moderate surface of contact between polypropylene thread and autostructures (fascia, SMAS, cartilage) doesn't provide firm and effective connective tissue lock. In control group there was gradual connective tissue growth involving mesh cells and bordering soft tissues lots of fibroblasts and macrophages infiltration, that evidence of connective tissue maturing. On 180 day- mature connective tissue that ingrowths the mesh and both flaps connected, adjacently there are collagen fibers mass, oriented circularly around the mesh. Results of operations with modified fixation evaluated in early postoperative and follow-up period. Proposed fixation method allows to increase good long-term results of facelift – per 53.4%, mastopexy – per 55,5%, otoplasty – per 30%.

*Conclusion:* Morpho-hystological and clinical research showed formation of stronger and long-term fixation using polypropylene mesh during facelift, mastopexy and otoplasty operations in comparison to traditional thread fixation.

## Intraparenchymal inflammatory response after subarachnoidal hemorrhage – cause or consequence of early brain damage?

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**Objective:** The mechanisms of early brain damage (EBD) following aneurysmal subarachnoidal hemorrhage (aSAH) are still a matter of debate. Within the last years theories about the influence of inflammatory processes in different cerebral pathologies have evolved. Our study was conducted to observe if the extraparenchymal pathology of aSAH would be succeeded by an intraparenchymal inflammatory response. To quantify the inflammatory response after aSAH we evaluated human and murine brain slices.

**Methods:** C57Bl/6 mice underwent a filament perforation procedure to induce SAH. The brains were harvested on days 2, 4, 7, 14 and 28. Immunohistochemical stainings were performed for ionized calcium-binding adaptor molecule 1 (Iba-1), Glial fibrillary acidic protein (GFAP) as well as Amyloid precursor protein (APP) to evaluate astro- and microglial activity as well as axonal damage respectively. Sections on different brain levels were evaluated. Special emphasis was set on regions like thalamus, hypothalamus, hippocampus, corpus callosum and different cortical areas. In addition 12 human brains of patients who died from aSAH at the respective time points were analyzed in a similar manner. The number of Iba-1-positive cells and APP-reactivity were observed per high power field (HPF) in different areas of the brain, namely at the close and at far site of the bleeding as well as in the hippocampus.

**Results:** In the murine brain sections a strong, focal inflammatory response measured in terms of GFAP-, APP- and IBA1-reactivity was observed in association to the site of bleeding over a period of 14 days (day 2: 1.5/mm<sup>2</sup>, 0.4/mm<sup>2</sup>, 1.7/mm<sup>2</sup>; day 4: 2/mm<sup>2</sup>, 2.3/mm<sup>2</sup>, 4/mm<sup>2</sup>; day 14: 12.8/mm<sup>2</sup>, 5.5/mm<sup>2</sup>, 16.6/mm<sup>2</sup>). After 28 days a less dense but more global reactivity across both hemispheres could be observed. As to the human brain sections a likewise strong inflammatory response in terms of Iba-1-positive cells and APP-reactivity, was detected with a peak on day 10-14 (day 1-2: 6.5/HPF, 0/HPF; day 3-4: 14/HPF, 0.3/HPF; day 5-8: 46/HPF, 1/HPF; day 9-14: 74/HPF, 1.7/HPF; day 15-22: 57.7/HPF, 2/HPF; day 23-28: 6.5/HPF, 13.8/HPF).

**Conclusion:** To the best of our knowledge our study is the first one to demonstrate an intraparenchymal inflammatory reaction following the extraparenchymal pathology of SAH. A time-dependent reaction could be characterised in murine as well as in human brain sections with a peak on day 10-14. This activation of Microglia (Iba-1) and Astrocytes (GFAP) as well as extracellular APP accumulation might well contribute to early brain damage mechanisms after SAH and therefore yield potential in further scientific as well as therapeutic aims.

## Clinical assessment of joined effects of peptic rebleeding's risk factors

ESC-ID **475**  
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**Aim:** The aim of our study was to establish an impact of risk factors in occurrence of ulcer rebleeding in urgently hospitalized patients.

**Material and methods:** Our study included 127 patients with symptoms of ulcer disease. Control group was composed of 75 patients with gastrointestinal bleeding, while examination group was composed of 52 patients with ulcer rebleeding. Patients' data obtained by clinical interview, clinical examination of the patients and their clinical records. X2 test and Student t-test were used for statistical data processing. Eleven elements were analyzed: age, gender, size and location of the ulcer change, co-morbidities, Forrest score, length of hospitalization, hemoglobin, hematocrit, presence of hypovolemic shock, and mortality.

**Results:** Considering that ulcer rebleeding presents urgent condition, it is important to determine risk factors, and their combination. The examined group was consisted of 30 (61.54%) patients over 60 years, and by the relation of chances (odds ratio) it is proved the ulcer rebleeding occurs 3.2 times more frequently in the examination group than in the control group. There were 32 (63.46%) males in the examination group, and in the control group there were 52 (69.33%) males, although, by statistical data processing, it was proved that the difference is not statistically significant for gender. Results of our study have shown that rebleeding frequently appeared with duodenal ulcer in 40 (76.92%) patients, and the diameter was larger than 1cm in 30 (57.69%) patients of the examination group, for 1.21 times. Co-morbidity in the examination group was 6.3 times more than in the control group. High risk features (Ia, Ib, IIa) were established in 44 (84.62%) patients in the examination group, concerning 4.81 times more frequently than in the control group, and thereby longer hospital stay for 13.62 days. Persons with rebleeding demand longer medical treatment. Levels of hemoglobin in the examination and control group were  $79.99 \pm 13.94$ g/dl,  $94.7 \pm 28.29$ g/dl, and of hematocrit were  $0.245 \pm 0.047$ ,  $0.20 \pm 0.087$ . Results of our study have shown that rebleeding was 22.22 times more frequently in patients with hypovolemic shock and low levels of laboratory analyses (hemoglobin, hematocrit). Mortality also was higher in the examination group, in which were 8 (15.38%) deceased patients. Combination of three factors bears risk of 50% for the beginning of rebleeding, and presence of five factors bears risk of 100% for occurrence of bleeding.

**Conclusion:** Our study has shown that age, size and location of the ulcer change, high risk features, and low levels of laboratory analyses (hemoglobin, hematocrit), hypovolemic shock and co-morbidities are risk factors for ulcer rebleeding, and also length of hospital stay and mortality are higher in the study group.

## Celiac axis compression syndrome – clinical manifestations, surgical treatment and post-operative quality of life. Preliminary report

ESC-ID 779  
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*Introduction:* Celiac axis compression syndrome (CACS) is a rare cause of recurrent, nonspecific upper abdominal pain and was first described independently by Harjola and Dunbar in 1960s. The pain results from compression of the celiac trunk by the median arcuate ligament of the diaphragm and celiac plexus at the aortic hiatus. Symptoms such as weight loss, diarrhea, postprandial emesis and loss of consciousness may also be present. Decompression of the celiac artery by division of the median arcuate ligament is thought to reestablish hemodynamics in this vessel. Although many reports have since been published, due to uncertain pathophysiological mechanism of CACS and its clinical implications many authors question the necessity and effectiveness of surgical treatment, especially in patients with low and mild grade stenoses.

*Aim:* The aim of this ongoing study was to evaluate the most frequent clinical presentation of CACS and to assess the feasibility as well as effectiveness of surgical treatment in this syndrome.

*Materials and methods:* Study involved 61 patients (19 males, mean age  $48.5 \pm 20.1$ ) admitted at the Department of General, Vascular and Transplant Surgery, The Medical University of Warsaw, who underwent surgical decompression of the celiac artery by division of the median arcuate ligament and has already responded to postoperative checking (mean follow up was 54 months). Data was obtained through a detailed questionnaire including peri- and postoperative assessment, clinical evaluation and patient's medical history. For statistical significance McNemara's and chi square tests were performed, using Statistica 8.0 PL software.

*Results:* Patients involved in the study suffered mainly from abdominal pain (90,16%), nausea (57,37%), diarrhea (37,7%), and emesis (37,7%). Complete and maintained relief of main symptoms at four and a half years follow-up was noted in 59%. Remaining 36% of patients reported decreased intensity of symptoms. In cases with persistent abdominal pain, nausea or diarrhea, statistically significant decrease in frequency, intensity and time of episodes was noted. 21,3% of patients reported weight loss, of whom 54% increased weight during the first year (mean 6 months) of postoperative period. 24,6% of patients experienced at least one episode of loss of consciousness. During the 4,5 year follow-up loss of consciousness occurred in 1 case (1,6%).

*Conclusions:* These satisfactory outcomes of decompression of celiac axis suggest that a strong evidence can be made for the existence of the syndrome. Moreover, excellent long-term results prove this relatively simple operation to be safe and effective method of treatment.

## Abdominal sepsis: Hyperimmune serum efficacy in experiment

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*Background/Objectives:* A review of the literature on sepsis results in conclusion that it is difficult to diagnose and treat but incidence is steadily increasing, as is the number of deaths resulting from sepsis each year. It is reported that in United States the incidence of sepsis increased by over 300% in last two decades resulting in responsibility of sepsis for at least 40% of intensive care units. We consider passive immune therapy to be a method of choice for the correction of immune disorders in the first days period of disease, what probably can prevent from unwanted complications of the Systemic Inflammatory Response Syndrome (SIRS) like irreversible shock and/or Multiple Organ Dysfunction Syndrome. The aim of study was to experience changes of immune status in rats and to evaluate the efficacy of hyperimmune serum of previously immunized ones.

*Methods:* The study was conducted experimentally on 60 Wistar line rats. Serum concentrations (ELISA) of major antibodies were determined against most significant pathogens (E.coli, Staphylococcus spp., S.aureus, Bacteroides spp, K. pneumoniae, P. aeruginosa).

*Results:* Changes of serum antibodies concentrations were time dependent and fluctuating during the current of AS forming the waveform curves. Most remarkable decreases were found during 1-3 day of AS. The highest levels of antibodies were found in rats which received vaccine against main pathogens 2 weeks prior to investigation. Their plasma was used in treatment of other 2 research groups of rats. Administration of 2-3 ml of hyperimmune serum had evidently better results than in control group which received ordinary serum and almost the same results in the research group where the antibiotics were also used.

*Conclusion:* There is an exact evidence of hyperimmune serum-based passive immunotherapy in rats with artificially modeled AS; leaning on accordance of the experimental model of AS to clinical prototype we can assert that the kind of passive immunotherapy we used can be effective enough also in clinical conditions and at least the proposed method will be cheaper.

## Effect of shunt catheter on the systemic immune response: evaluation of neutrophil count, function, and rate of chemotaxis

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*Introduction:* The localized impairment of the host defense mechanism due to the presence of a shunt apparatus has been suggested as a risk factor for shunt infection.

The purpose of this study was to evaluate the probable systemic effect of a shunt catheter on neutrophil phagocytosis and chemotaxis *in vivo*.

**Methods and Materials:** Twenty-four children with hydrocephalus who were referred to the Children's Hospital Medical Center in Tehran for ventriculoperitoneal shunt placement were included in this study. Neutrophil count, chemotaxis, and nitroblue tetrazolium (NBT) tests were performed before and 2 months after the operation. Factors such as neutrophil count, NBT percentage, and chemotaxis (with and without the addition of a chemoattractant factor) were evaluated preoperatively and postoperatively and compared to each other.

**Results:** In comparing the preoperative neutrophil count, NBT percentage, and chemotaxis (with and without the addition of a chemoattractant factor) with these same factors postoperatively, the authors found no statistically significant differences. In four children, shunt infections developed during the follow-up period. There were no significant differences between the aforementioned parameters in children with infected shunts and those with uninfected shunts.

**Conclusions:** The results of this study do not support the idea of systemic impairment of neutrophils after shunt insertion. Further studies with more specific methods are required to elaborate on this issue.

**Key words:** • hydrocephalus • shunt infection • neutrophil • chemotaxis • nitroblue tetrazolium • pediatric neurosurgery

### Direct Epicardial Shock Wave Therapy for Regeneration of Ischemic Myocardium

ESC-ID **468**  
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**Introduction:** Recently shock waves at low energy levels are well known to induce tissue regenerative effects. Transthoracic application of shock waves (SW) could be shown to augment myocardial vascularization in a porcine model of myocardial infarction. SW even improve myocardial perfusion and cause relief of angina symptoms in human patients with severe coronary artery disease. Nevertheless the underlying mechanism remains largely unknown.

**Materials and Methods:** Primary cell cultures of endothelial cells and fibroblasts were established from native rat hearts. Additionally H9C2-cardiomyocytes (American Type Culture Collection) were used. A thermostatically controlled water bath was designed to avoid distracting physical effects. Adherent cells in common cell culture flasks filled with culture medium were dunked into the water bath. Unfocused SW at an energy flux density of 0.15 mJ/mm<sup>2</sup> were applied with a frequency of 5 Hz to the cells. Non-treated cells were used as a control group. Number of cells and their vitality then were analysed over a period of 7 days. Several analysis of immunohistochemistry and molecular biology were performed.

**Results:** SW stimulate every cardiac cell type to a different

extent. Each cell type reacts at another timepoint after treatment. The distance between the applicator and the cells, as well as the energy flux density have a strong influence on the cells' behaviour. Duplication time of treated cells was significantly shorter compared to controls. Treated cells do also alter their cytoskeleton (Vimentin, Tubulin, beta-Actin), show significantly more proliferation (Ki-67) and changes in the expression of Connexin 43. No apoptosis was found in the treatment group.

**Conclusion:** SW activate proliferation of cardiac cells. Endothelial cells proliferate fastest, underlining the known effect of neovascularization *in-vivo*. Moreover cells alter the assembly of microfilaments, thus seem to ameliorate cell migration. Changes of the MMP and TIMP levels, as well as the expression of adhesion molecules seem to be strongly involved in the SW tissue regenerative effect on ischemic myocardium.

### Clipping or coiling – what should be the method of treatment in poor-grade subarachnoid hemorrhage?

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**Introduction and aim:** Both endovascular coil embolization and surgical clipping are now firmly established as treatment options for the management of cerebral aneurysms; however both regimens have inherent risks. The standard treatment for several decades has been surgical clipping of the neck of the aneurysm. In recent years, endovascular embolization has evolved worldwide. Moreover, controversy still surrounds especially the management of poor-grade subarachnoid hemorrhage (SAH) that historically fared poorly and often were excluded from aggressive treatment. The aim of our study was to assess the angiographic and clinical outcomes, and cerebral complications associated with both modalities of cerebral aneurysms treatment in patients with poor-grade SAH.

**Material and Methods:** We retrospectively reviewed the charts, operative and embolization reports and imaging of 328 patients with the diagnosis of SAH, who underwent aneurysm treatment at single center between January 2006 and December 2008. Clinical status of the patients was assessed using World Federation of Neurological Surgeons (WFNS) grade and Glasgow Coma Scale (GCS). 27 patients were selected as cases with IV and V grade WFNS SAH who underwent surgical clipping or endovascular embolization. These group consisted of 18 female between the ages 30 and 71 years (mean 52,7) and 9 male between ages 24 and 70 years (mean 52,5). Patients were divided into 2 groups: group A, patients who underwent clipping; group B, patients who underwent coil embolization. The following data were evaluated: age, sex, localization of aneurysm, morphological parameters of aneurysms found in CTA and DSA. Short-term outcome was measured with Glasgow Outcome Scale (GOS).

**Results:** 33 aneurysms were treated. The aneurysm location were middle cerebral artery (45,5%), anterior commu-

nicating artery (24,2%), internal carotid artery (12,1%), pericallosal artery (6,1%), basillar artery (6,1%), vertebral artery (3%) and posterior communicating artery (3%). The diameter of the aneurysms ranged from 2 to 47 mm with mean 8,8 mm. The mortality rate after 14 days in group A was 47,8% and 25% in group B. In group A 30,4% were GOS 2, 43,5% were GOS 3 and 26,1% were GOS 4 after the operation. In this group in 52,2% patients conditions had worsen during one to fourteen days. In group B 50% were GOS 3 and 50% were GOS 4. In this group in 25% patients conditions had worsen. The periprocedural technical complication rate was 17,4% in group A and 25% in group B. Coil embolizations were unsuccessful due to vasospasms and atherosclerosis.

**Conclusion:** The results of this study demonstrate that endovascular treatment is associated with better outcome in patients with ruptured intracranial aneurysms with WFNS grades IV and V. This is especially important in patients within these grades due to their potential for obtaining higher functional and physical recoveries.

### The role of Patent Blue® in the sentinel lymph node procedure in breast cancer

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**Introduction:** Since its introduction in the early 90's, a lot of controversy exists on the method to perform a sentinel lymph node procedure. Multiple studies showed that a combination of radioactive tracer and blue dye results in the best detection of the sentinel lymph node (SLN), although some groups obtain the same detection ratio by single use of radioactive tracer.

**Aim:** The aim of our study was to evaluate the value of Patent Blue® in the detection of sentinel lymph node (SLN) and the staging of the axilla. Subsequently the adverse effects of Patent Blue® during SLN procedure were studied.

**Methods:** 1004 patients who underwent a SLN procedure between 2003 and 2008 were enrolled in a retrospective study. SLNs were detected by a combination of peritumorally Tc-99m nanocolloid (40-80 Mbq) and periareolarly Patent Blue® (0.5-1ml). Preoperatively, a lymphoscintigraphy was performed and the SLNs were marked on the skin. In case of a negative lymphoscintigraphy an extra dose of radioactive tracer was injected. During the operation the SLNs were detected by a hand-held gammaprobe and the Patent Blue®. A positive SLN procedure was followed by an axillary lymph node dissection (ALND). When no hot, nor blue nodes were detected, the SLN procedure failed and an ALND was performed. Severe adverse reactions on Patent Blue® were registered. Statistical analysis was performed by one-sided chi-squared test.

**Results:** In 37 of 1004 patients no hot nodes were detected. Twenty-four of them were blue and SLN procedure was still successful. SLNB failed in 13 patients (1,3%). The patients without hot nodes -failure of radioactive tracer- were older (63 versus 57 years,  $p = 0.0072$ ) and the tumors were more frequently localized in the inner quadrants of the breast ( $p = 0,0239$ ), when compared to patients with

hot nodes. In 219 patients positive nodes were hot. By contrast, in 20 patients a cold node only was positive, of whom in 12 patients it was blue and in 8 patients it was white. Severe allergic reactions were notified in two patients (0.2%).

**Conclusion:** In 24 (2,4%) patients the SLN was detected thanks to Patent Blue®. In 12 patients (1%) the axilla was correctly staged by the use of Patent Blue®. Especially older patients with tumors located in the inner quadrants of the breast could benefit the most of the use of Patent Blue®. The benefits of the use of Patent Blue® in detection of the SLN and staging of the axilla exceed the risk of severe allergic reactions (0,2%). Therefore, Patent Blue® has a surplus value in SLN procedure and should remain a part of the SLN protocol.

### Using of ischemic preconditioning (IPC) in intestinal transplantation

ESC-ID 967  
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It is well known that decrease of graft damage range during intestinal transplantation (ITx) increases viability of the graft, otherwise resulting in serious complications. The aim of this study was to observe the impact of ischemic preconditioning (IPC) on graft damage during ITx. Sprague Dawley rats ( $n = 21$ ) were randomly divided into 3 groups: control group S ( $n = 7$ ) – graft harvest, preservation; experimental group SIPC ( $n = 7$ ) – short-term IPC (i.e. 4cycles of 4min ischemia by clamping of a. mesenterica sup. with 10min reperfusion between ischemic attacks) following by graft harvest and preservation; experimental group LIPC ( $n = 7$ ) – long-term IPC (i.e. 2cycles of 12min mesenteric ischemia with 10min reperfusion between ischemic attacks) following by graft harvest and preservation. For preservation HTK (histidinetryptophan-ketoglutarate) solution was used. The samples were taken during preservation in time 0, 3, 6, 9, 12, 18 and 24h. Following staining by Hematoxylin & Eosin method the histopathological injury index (HII) was calculated using Park/Chiu scoring system. Bleb cells (BC) and serotonin positive cells (SPC) were quantified with Alcian blue staining and anti-serotonin antibody, respectively. All results were assessed by statistical analysis. In all samples of both groups with IPC (SIPC, LIPC) were detected lower values of HII in comparison to control group S, comparing of both variant IPC has showed lower values of HII in group LIPC. Number of BC decreases with the range of damage. Higher amounts of BC were observed in groups with IPC (SIPC, LIPC), after comparing of groups with IPC was proved higher amount of BC in group LIPC. With increasing range of damage increases the SPC activity. SPC activity was lower in all samples of group SIPC as well as LIPC comparing to the control group S, mutual comparing of groups with IPC showed lower SPC activity again in group LIPC. Using of

IPC in ITx leads to significantly lower damage of the graft. In conclusion, long-term IPC (LIPC) shows significantly lower range of graft damage after intestinal transplantation in rat by comparison with short-term IPC (SIPC).

### Evaluation of Quality of Life of patient with obesity after surgical procedure

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*Background:* WHO, since 1998 classified obesity as chronic disease which is defined as a body mass index (BMI) 30 kg/m<sup>2</sup> or higher, in which excess body fat has accumulated to an extent that health may be negatively affected. Obesity is regarded as pandemic of the 21st century and its occurrence still grows up. WHO's latest projections indicate that globally in 2005 approximately 1.6 billion adults (age 15+) were overweight and at least 400 million adults were obese. We can distinguish some methods of treatment of obesity such as conservative treatment (diet, lifestyle, exercises), pharmacotherapy. However more often surgical intervention is offered for patients.

*Aim:* Evaluation of Quality of Life of patient with obesity after surgical procedure with the help of questionnaire Impact of Weight on Quality of Life – Lite (IWQOL-Lite). Review of literature.

*Material and methods:* We qualified 12 patients who were operated due to obesity between April 2005 – December 2007 with the help of sleeve gastrectomy and Roux-en-Y gastric bypass. The group included 75 % female and 25% male patients. 50% patients were <35 years old. Average age was 39.8 years. IWQOL-Lite Polish edition questionnaire was sent to every patient. IWQOL-Lite Polish edition questionnaire includes questions about five aspects of life such as: physical function, self-esteem, sexual life, public distress and work.

*Results:* We received eight filled questionnaires. Compliance reached 66,6%. Patients presented the highest level of satisfaction in the questionnaire in two aspects of life such as public distress and work. They were least satisfied with self-esteem aspect of life. Average weight before surgical procedure was 149.5 kg (100-156, SD 20.08 ), after operation was 98.4 kg (72-144, SD 21.68). Average time from surgical procedure to completion of the questionnaire was 20.25 months (6-37, SD 8.84). We measured blood glucose level before (8.24 mmol/l) and after (6.16 mmol/l) surgical procedure. Reduction in hypertension also occurred.

*Conclusion:* IWQOL-Lite Polish edition questionnaire provides useful, objective evaluation of quality of life patients after surgical treatment of obesity. Surgical treatment of obesity improves of quality of life and patients present the highest level of satisfaction in two aspects of life such as public distress and work.

### Femoral vein valves in the area of the saphenofemoral junction – functional anatomy

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*Background:* Venous drainage of the lower limb is controlled by a complex sapheno-femoral valves system. The most common patterns include 2 valves of great saphenous vein (GSV) and 2 or 3 valves of femoral vein (FV). In case of lack of saphenous valves (10% of people) suprasaphenic and infrasaphenic valves of FV play an important role, as reflux from the deep venous system could be considered as an initial point of venous incompetence. Despite the fact of clinical significance the literature on this subject is very poor.

*Aim:* The aim of this study was a detailed anatomical description with precise measurements and functional assessment of femoral vein valves near the saphenofemoral junction (SFJ).

*Material and method:* The study was performed on 20 (7 R, 13 L) femoral veins from cadavers of both sexes aged 15-75 years. The valves were examined with the use of rigid endoscope (4 mm diameter, 11 cm of length) with 0 and 30 degree lenses. The endoscope was introduced through femoral vein against the direction of blood flow with constant water flow. Detailed observations and measurements were performed on entirely dissected veins. We estimated the number of leaflets, distance from SFJ, the function of valves and diameter of GSV. All data were analyzed using Statistica StatSoft 8.0 software.

*Results:* Suprasaphenic valve of FV was observed in 15/20 cases (75%). Its mean distance from SFJ was 46.7 mm above the junction (range 19.4-74.0 mm). First infrasaphenic valve was present in 19/20 veins (95%) with mean distance of 23 mm below the SFJ (range 0-46 mm). Second infrasaphenic valve was found in 8/20 cases (40%). It was observed most commonly in men (6/8, 75%) Its mean distance from the SFJ was 59.7 mm (range 38.6-80.8 mm). Sex and age do not influence on the distance between valves and SFJ. Valves were composed of 2 leaflets in 40/42 cases (95%). Incompetence was observed in 17/42 (41%) valves. Statistical analysis showed the competence of valve does not correlate with the circumference of the FV at valve's level.

*Conclusions:* 1. Valves of FV consist mostly of 2 leaflets. 2. Their competence is not related to the circumference of the FV at valve's level. 3. The most constantly observed valve is the first infrasaphenic valve. 3. Second post-saphenous valve occurs most commonly in men.

## Radiation induced rectovaginal fistulas - risk factors, development and healing

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*Introduction:* Rectovaginal fistula is an abnormal, epithelium-lined tract between the rectum and vagina. These are rare conditions, accounting for about 5% of all perianal fistulas. Radiation induced rectovaginal fistulas are defined by Radiation Therapy Oncology Group scale as grade IV, late complication of radiation therapy, developing at least 90 days after radiotherapy.

*Aims:* 1. To evaluate the effect of radiotherapy on location, time of fistula's development and natural course of the disease. 2. To estimate the prognostic factors of healing of the fistula 3. To evaluate the quality of life of patients with rectovaginal fistulas.

*Materials and methods:* 40 patients with diagnosis of rectovaginal fistula hospitalized in the Department of General and Colorectal Surgery between 2003-2008 and treated in the Gynecological Radiotherapy Unit of UM in Lodz were investigated concerning the primary disease, previous surgical and oncological history, adjuvant therapy, current treatment and post-operative follow up. Data was gathered from the medical documentation and original questionnaire with AIS (Acceptance of Illness Scale) and SWLS (Satisfaction With Life Scale) answered by the patient on the control visit. All patients underwent careful physical examination at different points of follow up.

*Results:* Median age of analyzed group was 60 y.o. (from 37 to 84 y.o.) Follow up ranged from 1,2 to 12 years (mean 5,3; median 4,8 years). At the end of the study 23 patients were alive. The primary disease in most cases was cervical cancer (63%) endometrial cancer in 12%. About half of the cancers were classified by FIGO as II stage. Treatment included surgery and radiotherapy (53%) or radiotherapy alone (42%). Chemotherapy was administered additionally in 20% of patients. All patients received standard dose of radiation during both brachy- and teletherapy. The correlation between the risk of fistula development and radiotherapy scheme, brachytherapy followed by teletherapy was the leading factor of fistulation ( $p < 0,014$ ). Gynecologic surgery together with radiotherapy increases the risk of fistula development but has no influence on the formation of medium and high fistulas ( $p < 0,04$ ). Early complications of radiotherapy affected 42% of patients, late complications other than fistula appeared in 15%. Only in 7 patients the fistulas were healed (20%), patients age in this group vary from 47 to 82. Patients were staged by FIGO as I or II. 4 patients doesn't receive full scheme of radiotherapy. Fistula persisted in 33 patients. In one case stoma closure was performed. Mean time of fistula's healing was 2 years and 7 months. In AIS test patients shows moderate acceptance of illness and high consciousness of morbidity. In SWLS test satisfaction with life was assessed as moderate or high.

*Conclusions:* 1. Applying brachy- and teletherapy at the same time in terms of therapeutic dose doesn't increase the risk of development of rectovaginal fistulas, which tends to appear rather after long-time scheme of radiotherapy. 2. Fecal diversion alone gives hardly a chance for closure of the postradiotherapy rectovaginal fistulas. Patients age tends to have no influence on fistulas devel-

opment. In fistulas healing important factors are histological type of neoplasms and low staging by FIGO. 3. The results of AIS and SWLS test shows that despite poor treatment outcomes and high conscious of morbidity, patients satisfaction of life was assessed as moderate or high.

## Urology

### External Validation of an Artificial Neural Network for Prostate Cancer Detection with Population Dependent Variability

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*Purpose:* Multivariate models are commonly used to increase specificity of prostate-specific antigen (PSA) and percent free PSA (%fPSA). An external validation of the retrospectively trained artificial neural network (ANN) program "ProstataClass" was performed with prospective data using the Elecsys total PSA (tPSA) and free PSA (fPSA) assays (Roche Diagnostics).

*Materials and Methods:* Data from 393 men with tPSA, %fPSA, age, prostate volume and digital rectal examination (DRE) status were applied to established ANNs. The outcome of the ANN "ProstataClass", the ANN with external validation data (ANNev) and the ANN with data from both groups (ANNall) and other multivariate models were compared.

*Results:* Within a tPSA range 1-22.8  $\mu\text{g/l}$ , 229 men (58.3%) had prostate cancer (PCa). tPSA, %fPSA and the number of positive DRE differed significantly from the cohort of 787 patients of the ANN "ProstataClass" whereas age and prostate volume were comparable. Areas under the ROC curves (AUC) for tPSA, %fPSA and the ANN "ProstataClass" were 0.53, 0.73 and 0.75 ( $p = 0.085$  between %fPSA and ANN). The AUCs of the ANNev (0.75) and ANNall (0.77) were significantly better compared with %fPSA ( $p = 0.021$  and  $0.0008$ ) but all 3 ANNs had no differences between each other.

*Conclusions:* Differences in the cohort composition with less positive DRE status (-26.8%) and higher %fPSA values (+3.6%) in the external validation cohort are most likely responsible for the decreased AUCs for all ANN models in the external validation cohort and different predicted and observed PCa probabilities.

### Correlation between Gleason score and surgical margins and seminal vesicles involvement in clinically localized prostate cancer

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*Aim:* Our goal was to find correlation between GS and state of surgical margins (bladder neck and urethra) and

seminal vesicles in clinically localized prostate cancer and determine possible impact of GS on course and progression of the disease.

**Material and methods:** In this retrospective study 67 patients (pts) (aged 57 - 75), with clinically localized prostate carcinoma (T1, T2 stage), underwent radical prostatectomy (RP), during 2007. and 2008. Patients were divided into 3 groups depending on the GS level: I group (GS <6), II group (GS = 7) and III group (GS >8). Other than GS, we also monitored surgical margins' and seminal vesicles' involvement.

**Results:** Margins and/or seminal vesicle involvement were positive in 5%, 18,5% and 60% of the pts, according to group (I, II, III), respectively. Significantly positive margins/seminal vesicles were seen in III group (12/20) (60%), ( $p < 0,001$ ). Significantly negative margins/seminal vesicles were present in I and II group: 19/20 (95%) and 22/27 (81,5%), ( $p < 0,001$ ).

**Conclusion:** The results show a significant correlation between GS and positive surgical margins and seminal vesicles involvement in group of pts with  $GS \geq 8$ . This confirms the predictive value of these parameters to disease progression and supports the fact that GS can be a factor of prediction of disease progression and of influence in further therapy.

### Evaluation of the association between serum level of alkaline phosphatase and white blood cells with tumor grade in Transitional Cell Carcinoma (TCC) of the ureter

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**Introduction:** Upper urinary tract transitional cell carcinoma (TCC) is a rare disease, accounting for about 5% of all urothelial tumors. In comparison with other pelvic and renal malignancies, TCC has poor prognosis with more recurrences and metastasis. Therefore, it is of great value to find prognostic factors in order to improve their prognosis. This study was aimed to evaluate the association between serum level of alkaline phosphatase and white blood cells with tumor grade in TCC of the ureter and to determine the diagnostic values of these factors.

**Materials and Methods:** This analytical cross-sectional study was performed on 33 patients with TCC of ureter who were referred to Hasheminejad Hospital in Tehran from 1997 to 2007. Diagnosis of TCC was confirmed pathologically for all the patients. Rather than demographic data, main variables including tumour grade, number of white blood cells (WBC) and serum level of alkaline phosphatase (Alk-P) were recorded. Grades I and II were considered as low grade tumour; while the grades III and VI were considered high. All data were collected and analyzed using SPSS v.15 software and Kruskal-Wallis, Mann-Whitney U test, correlation and receiver operative curve (ROC) analysis were performed.

**Results:** Out of 33 patients, 28 (84.8%) were male and 5 (15.2%) were female with the mean age of 61.70 (SD =

10.10). The highest number of WBC was significantly seen in patients with grade VI with the mean of 11306 (SD = 3591.4) /mm<sup>3</sup> ( $P = 0.002$ ). Results of ROC analysis showed that the number of WBC (AUC = 0.825,  $P = 0.001$ ) had a greater area under curve (AUC) compared with serum level of Alk-P (AUC = 0.770,  $P = 0.008$ ). The cut-point value of 7800 /mm<sup>3</sup> for WBC had the sensitivity of 81.3%, specificity of 76.5%, positive predictive value (PPV) of 76.5% and negative predictive value (NPV) of 81.2%. Moreover, the cut-point value of 178 IU/L for Alk-P had the sensitivity of 93.8%, specificity of 52.9%, PPV of 65.2% and NPV of 90% to detect high versus low grade patients.

**Conclusions:** It was shown that the number of WBC and serum level of Alk-P is considerably increased while the tumour grade increase. Furthermore, the number of WBC has more prognostic value than serum level of Alk-P to predict high grade tumours. Our results may lead to detect the patients with poorer prognosis which are in need of more effective approaches.

**Key words:** Transitional Cell Carcinoma (TCC), Grade, Alkaline phosphatase, White blood cells

### Use and Misuse of Nomograms for Prostate Cancer Detection Without Consideration of Analytical Differences Between PSA Assays

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**Purpose:** PSA inter-assay variability is a common problem in daily clinical practice, which has not been considered in nomograms for prostate cancer (PCa) detection.

**Patients and Methods:** Total and free PSA were measured with five different assays (Abbott, Bayer, Beckman, DPC and Roche) in 780 biopsy-referred men. Together with age, prostate volume and digital rectal examination (DRE) status these data were applied to five established online available nomograms.

**Results:** PCa was found in 455 (58.3%) men and 325 had no evidence of malignancy (NEM). Median total PSA concentrations ranged from 5.50 (Bayer) to 7.04  $\mu\text{g/l}$  (DPC) while the median percent free PSA ranged from 10.63% (DPC) to 16.42% (Bayer). Areas under the ROC curve (AUC) were around 0.80 for the Sextant 1 model. All other nomograms with %fPSA like the Sextant 2, Initial Extended, and Kawakami 1 and 2 nomograms showed comparable AUCs around 0.85. However, the outputs of the five nomograms differed significantly when changing the PSA assay. Here, the Kawakami 1 nomogram calculated a median output for the Abbott assay with 0.59 but already 0.76 when using the DPC assay. Considerable differences between the five assays were also seen when analysing the sensitivities and specificities at various cutoffs. **Conclusions:** ROC curve analysis suggests unimpaired nomogram prediction in spite of changing PSA assays. In contrast, the considerably different outputs as well as the different sensitivities and specificities indicate a substantial effect of PSA assay variability on nomogram performance.



## Fatty-acid binding proteins in cell lines of urological tumors

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*Aim:* Fatty acid-binding proteins (FABPs) are a family of carrier proteins for fatty acids and other lipophilic substances. They are members of the superfamily of lipid-binding proteins (LBP). So far 9 different FABPs have been identified: L (liver), I (intestinal), H (heart), A (adipocyte), E (epidermal), Il (ileal), B (brain), M (myelin) and T (testis). The primary roles of all the FABP family members are regulation of fatty acid uptake and intracellular transport. Recent trials show significant differences in the expression of FABPs between healthy tissue and carcinoma tissues. Therefore we did a systematic characterisation of standard permanent urological cell lines on the level of mRNA and protein regarding the expression of FABPs. We want to explore if the cell lines show the same pattern of FABP (expression) like the corresponding tumor tissues. With these results a better selection of cell lines for further in vitro experiments should be possible.

*Methods:* We used 9 human renal carcinoma cell lines (Caki-1, Caki-2, ACHN, SW 839, 786-0, A498, SN-12, A 704, HK-2), 4 human prostate carcinoma cell lines (LNCaP, PC-3, Du-145, BPH-1, 22RV-1) 7 human urinary bladder carcinoma cell lines (J 82, HCV 29, RT-4, RT-112, SCaBER, UM-UC-3, HT-1376) and also couples of healthy human tissue and carcinoma tissue. To detect FABPs on mRNA level we used RT-PCR (LightCycler) and for protein detection we used Laemmli SDS-PAGE and Semidry Western-Blotting. Currently antibodies are available for 7 FABPs.

*Results:* The pattern of FABP mRNA expression are quite heterogeneous concerning the type of FABP and the quantity. E-FABP and IL-FABP appear in most of the tested cell lines. Only LNCaP does not express E-FABP. L-FABP, H-FABP and I-FABP could not be detected in any cell line. RT-4 and RT-112 are the only two cell lines that contain A-FABP. Only a small number of cell lines express B-FABP. This mRNA was found in one prostate carcinoma cell line (22RV-1) and in two renal carcinoma cell lines (Caki-2, A704). The protein detection of the various FABPs shows similar results. The renal healthy and carcinoma tissue was positive for B-FABP and IL-FABP mRNA but the expression level was different.

*Conclusion:* B-FABP, IL-FABP and E-FABP are expressed in cell lines of prostate, renal and urinary bladder carcinoma. A-FABP is highly expressed in human urinary bladder carcinoma cell lines (RT-4, RT-112) and might be specific for urinary bladder cancer. Moreover, an analogy concerning the expression of B-FABP and IL-FABP between selected cell lines and human renal healthy and carcinoma tissue could be detected. Further research is necessary to analyse the importance and role of certain FABPs in the above mentioned cell lines.

## A comparison of the perioperative data after open radical retropubic prostatectomy or robotic-assisted laparoscopic prostatectomy

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*Aim:* To compare the perioperative biochemical data, the postoperative need for help for hygiene and mobility, and duration of bladder catheterization, hospitalization and ICU stay of patients undergoing radical retropubic prostatectomy (RRP) versus robotic-assisted laparoscopic prostatectomy (RALP) performed by an experienced open yet naive laparoscopic surgical team, in a peripheral low-volume urological centre.

*Methods and materials:* Over a 4-year period (2004-2008), 22 men underwent radical prostatectomy without lymphadenectomy at the study institution. The mean age of the patients was 63.9 years and the mean PSA value at time of diagnosis was 9.2 ng/mL.

*Results:* Patients in the robotic-assisted laparoscopic prostatectomy group presented a significantly lower decrease in hemoglobin, hematocrit and total plasmatic protein and a significantly smaller need for help for hygiene and mobility and a shorter duration of bladder catheterization, hospitalization and ICU stay.

*Conclusion:* The results of this study have shown that robotic-assisted laparoscopic prostatectomy is associated with a lower perioperative morbidity and a shorter hospital stay than radical retropubic prostatectomy, even when only considering the first performed robotic-assisted laparoscopic prostatectomies by a yet unexperienced robotic team in a peripheral low-volume urological centre.

## MicroRNAs as prognostic and diagnostic biomarkers in renal clear cell carcinoma

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*Aims:* MicroRNAs (miRNAs) are small non-coding ssRNAs which regulate gene expression posttranscriptionally and are implied in oncogenic pathways. Changes in their expression were found in a variety of human cancers. Only few data are available on microRNAs in clear cell renal cell carcinoma (ccRCC). The aim was to identify differentially expressed miRNAs in renal cell carcinoma by comparing miRNA expression in tumor and adjacent normal tissue and to correlate miRNA expression with clinico-pathological data.

*Methods:* Previous microarray-based experiments identified 13 over-expressed and 20 down-regulated microRNAs in ccRCC samples. Based on these data up- and down-regulated microRNAs were quantified by real-time RT-PCR using a LightCycler 480 system. Matched malignant and benign tissue samples from 72 renal cell carcinomas were analyzed. Expression data were normalized to RNU6B. Receiver operation characteristics (ROC) analyses were

performed to estimate the potential of miRNAs to discriminate between normal and tumor tissue.

*Results:* The expression levels of hsa-miR-16, -452\*, -224, -155, -210, -122a were increased and that of hsa-miR-200b, -363, -429, -200c, -514, -141, -184 were decreased in ccRCC. A combined analysis of miR-141 and miR-155 resulted in a 97% overall correct classification of ccRCC and normal renal samples. None of the expression profiles of these differentially expressed miRNAs, however, were associated with tumor stage, tumor grade or tumor-specific survival.

*Conclusions:* Renal cell carcinoma is characterized by significant miRNA expression changes that clearly discriminate tumor and normal tissue. The study provides a solid basis for further functional studies of microRNAs in renal cell cancer.

## POSTER PRESENTATIONS

### Anaesthesiology

#### Safety and efficacy of intravascular and conventional cooling in patients after cardiac arrest

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*Background and Aim:* Mild therapeutic hypothermia is considered by the European Resuscitation Council as the optimal technique for preserving brain viability after sudden circulatory arrest. The aim of the study was to assess the safety and efficacy of conventional and intravascular cooling used in patients with a history of out-of-hospital cardiac arrest.

*Material and Methods:* 19 patients (pts) received conventional (group C, n = 7) or intravascular (group IV, n = 12) cooling (Alsius, Coolgard, USA). The goal was to decrease and maintain body temperature to 32 - 34°C for the consecutive 24 hours. Temperature readings obtained from the blood (Swan-Ganz catheter), oesophagus and urinary bladder were analyzed using ANOVA. Frequency and severity of cardiovascular, infectious and metabolic disturbances were recorded and subsequently compared between patients who reached the desired temperature and those patients in whom the desired temperature was not achieved.

*Results:* Desired temperature was reached in 4 patients in group C (57.1%) and in 10 patients in group IV (83.3%). Temperature profiles obtained from these two groups were significantly different ( $p = 0.02$ ). Stable temperature profile (24 hours) was reached in 6 pts in group IV and in none in group C ( $p = 0.04$ ). Patients in both groups who reached the desired temperature had a high incidence of transiently increased serum amylase (75%) and infection (64.3%). Bradycardia and coagulopathy requiring blood transfusions were noted only in 3 pts (21.4%) in this patient subset. All complications excluding infections resolved when body temperature was reestablished.

*Conclusions:* Intravascular hypothermia is more effective than conventional cooling in patients after cardiac arrest. Transient increase of serum amylase and infection may be expected in patients who reach a target temperature, regardless of the cooling method.

#### Prevalence of adrenal insufficiency and its relation with mortality in Iranian patients admitted to intensive care unit of Hazrat Rasool hospital in the year 2003

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*Introduction:* Adrenal insufficiency (AI) is common in critically ill patients and affects their prognosis. It can

cause many problems for these patients such as electrolyte imbalance, hormonal disorders, skeletal disorders, and even death. This study was conducted to determine the prevalence of AI in ICU of a hospital in Tehran.

*Materials and methods:* This analytic cross-sectional study was performed on 100 patients who were admitted to the ICU of "Hazrat Rasul" hospital in Tehran in the year 2003. The demographic data for these patients were recorded and also the clinical data between two serum cortisol determinations were collected. According to the other studies, random serum cortisol level less than 25 mcg/dl was considered as AI. All collected variables were analyzed by the statistical software, spss v16, using chi2 and independent T-test.

*Results:* From 100 patients who satisfied the inclusion criteria, 43 patients (43%) were men and the other 57 (57%) were women, with the mean age of 62.73 (SD = 19.03) years. The mean level of patients' serum cortisol was 24.58 (SD = 20.09) mcg/dl. Finally from all patients, 44 individuals (44%) were expired, 25 of them included our criteria as adrenal insufficiency and the other 19 didn't fit the criteria. The mean level of serum cortisol was not significantly different between discharged and expired patients ( $p > 0.05$ ).

*Conclusion:* Although the majority of acutely ill patients who remained in a critical condition had AI, but there was no statistically significant relation between this frequency and the mortality of the patients. One reason of this finding is more cortisol secretion in the patients admitted to ICU, because of the additional stress. On the other hand, the cutpoint of 25 mcg/dl for definition of AI doesn't seem to be suitable in these patients. This study could be a basic knowledge for further investigations

### Biochemistry

#### Melatonin as a modulator of microwave radiation effects on rat thymocyte proliferation, apoptosis and oxidative stress

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*Introduction:* Exposition to microwave radiation (MW), from mobile phones and other sources induce disturbances in thymocytes. The pineal secretory product, melatonin (Mel), exerts a variety of effects on the immune system. Melatonin inhibits oxidative stress, DNA fragmentation and apoptosis of T lymphocytes.

*Aim:* The aim of the study was to evaluate the intensity of proliferation, apoptosis and oxidative stress in rats' thymocytes under exposure of MW from mobile phones and potential protective effects of melatonin.

*Materials and methods:* Wistar rats (40 animals) were divided in 4 experimental groups: I (control-sham exposed), II (Mel group)-rats treated with Mel every day (2 mg/kg b.w., i.p.), III (MW group)-rats exposed to MW (4

h/day during 40 days), IV (MW+Mel)-rats treated with Mel every day (2mg/kg b.w., i.p) and exposed to MW radiation (4 h/day during 40 days). MW was produced by a mobile test phone (SAR = 0.043-0.135 W/kg).

**Results:** MW significantly decreased thymocytes' proliferation, induced by ConA ( $p < 0.05$ ) and increased apoptosis, detected using the Annexin V-FITC/PI detection kit ( $p < 0.01$ ). DNA fragmentation in exposed thymocytes is probably triggered by the increase activation of alkaline-DNase I (caspase 3-activated) and acid-DNase II ( $p < 0.05$ ). A significant increase in the thymus malondialdehyde (MDA) and carbonyl group concentration ( $p < 0.001$ ), and decreased activity of catalase ( $p < 0.01$ ) was registered during exposure. Melatonin was found to be effective on MW induced injury of thymocyte: (1) increase thymocyte proliferation ( $p < 0.05$ ), (2) decreased apoptotic rate of thymocytes ( $p < 0.01$ ), (3) decreased DNase I and DNase II activity ( $p < 0.05$ ), (4) decreased MDA and carbonyl group levels ( $p < 0.01$ ), (5) increased activity of catalase ( $p < 0.05$ ).

**Conclusion:** Melatonin exerts protective effects on rat thymocyte by reducing proliferation, apoptosis and oxidative stress in rats' thymocytes under exposure of microwave radiation.

**Key words:** melatonin, microwave radiation, thymocytes, apoptosis, oxidative stress

### Antioxidant effect of water soluble vitamin E in blood plasma of rats with Diurnal Rhythm Disturbance

ESC-ID 457  
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**Background:** Long lasting OS due to Diurnal rhythm disturbance (DRD) may help to initiate large variety of pathological conditions and OS-related diseases. Trolox is a soluble form of Vitamin E, which shows very good antioxidant effect. The effect of Trolox on the OS due to DRD is not yet investigated. The OS evaluation in the blood plasma permits to in vivo monitoring during clinical manipulations, even surgery. In this research, the effect of Trolox on the OS level in the blood plasma of rat exposed to DRD was assessed in vivo. The MDA formation was used as a marker of the OS damage, while MTT transformation to formazan indicated the free-radicals formation (FRF) in the blood plasma.

**Materials and Methods:** 20 male Wistar Albino rats ( $110 \pm 10$  g each) were separated in four groups. The control groups (C, CT) lived at normal light/dark cycle (12/12 hrs), while two groups (DRD, DRDT) were exposed to a constant light, for a period of three weeks. The groups CT and DRDT received everyday 200mg/kg Trolox (p.o.). All animals were provided with fresh food and water ad libitum, and kept at room temperature ( $22 \pm 1$ )°C. At the end of the third week they were decapitated under anesthesia (ether). The blood plasma was separated and stored at -81°C. The OS was monitored by measuring the levels of two markers – Malondialdehyde (MDA), and conversion of MTT to MTT-formazan due to FRF in presence of blood plasma. The amounts of proteins in the blood plasma were determined. Then, the amounts of MDA and MTT-formazan

formed in presence of blood plasma for 5 min., were determined from the Absorbances at 245 and 576 nm for MDA and MTT-formazan, respectively.

**Results:** Our results showed that DRD (group DRD) caused a significant increase of both FRF (2.17 times) and MDA (1.44 times) in the blood plasma compared with the corresponding levels of the control animals (group C). Similar but weaker effects were observed among animals treated with Trolox (1.22 and 1.11 times more MDA and FRF, respectively for group DRDT compare with group CT). For the rats living at normal light/dark cycle, the administration of Trolox did not affect the MDA level of the blood plasma, but the free-radicals formation was diminished at about 20%. Comparison between groups DRD and DRDT revealed a decrease of the MDA level by 10%, while the free-radicals decreased by 65%. These observations were in agreement with the well known radical scavenging effect of Trolox.

**Conclusions:** Our results suggest that diurnal rhythm disturbance may increase both free-radicals formation and oxidative damage in the blood plasma of rat. Application of Trolox (200 mg/kg, p.o.) substantially reduces the free-radicals formation and by this way, the resulting Malondialdehyde synthesis. Therefore, Trolox exhibits a significant in vivo antioxidant effect in the blood plasma of rat exposed to DRD. **Keywords:** Diurnal rhythm disturbance, Oxidative stress, Blood plasma, Malondialdehyde, Free-radicals formation, Trolox. **Acknowledgement:** This work was possible with the financial support of Grant 19/2008, scientific Board of the Medical University – Sofia, Sofia, Bulgaria

### Virgin coconut oil is non-mutagenic and anti-mutagenic: Results of Ames test and Comet assay

ESC-ID 359  
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Virgin coconut oil (VCO) is currently one of the most popular and widely used natural oils here in the Philippines. Considered by Dr. Dayrit as a "Drugstore in a Bottle", it may be used as an energy supplement, antimicrobial agent, HIV treatment, etc. However, VCO safety assessment appears to be limited providing ground for the group to investigate its mutagenicity. Anti-mutagenic potential was also studied, due to claims that VCO shows anti-carcinogenic activity. This study determined the mutagenic and anti-mutagenic potential of VCO using randomized-controlled trials of Ames Salmonella mutagenicity assay using Salmonella typhimurium TA98 and S. typhimurium TA100 and alkaline single-cell gel electrophoresis (comet) assay using 60 Swiss Webster ICR mice lymphocyte DNA.

Results showed that VCO was non-mutagenic in vitro and in vivo, regardless of dose. Moreover, VCO appeared to be anti-mutagenic at high dose in both Ames test (TA98 and TA100) and comet assay. Low dose VCO exhibited anti-mutagenicity in TA98 and comet assay, but not in TA100. Dose dependence was observed in vivo using comet assay.

Moreover, Ames test using TA100 also shows higher (but not statistically significant) anti-mutagenic capability of a higher dose of VCO. No dose dependent relationship was however found from the Ames test using *S. typhimurium* TA98. In summary, Ames test and comet assay show that VCO is non-mutagenic and anti-mutagenic. The results of this study could lead to further studies involving the therapeutic use of VCO as an anti-cancer agent.

### A Biochemical Analysis of Endogenous Hydrogen Sulphide Generating Enzymes

ESC-ID **85**  
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*Aim:* Hydrogen sulphide (H<sub>2</sub>S), a well known toxic gas, has recently been defined as a physiologically relevant gasotransmitter. H<sub>2</sub>S has been found to open KATP channels, stimulate NMDA-receptors and act as a cytoprotective signalling at an endogenous concentration of 1-300 μM. The actual whole tissue H<sub>2</sub>S concentration is under debate. Three enzymes endogenously generate H<sub>2</sub>S in mammalian tissues via the desulphydration of cysteine to pyruvate; cystathionine γ-lyase (CGL), cystathionine β-synthase (CBS) and 3-mercaptopyruvate sulphurtransferase (MCST). The present study focused on a biochemical analysis of CGL, which is thought to synthesise H<sub>2</sub>S in the liver, kidney and vasculature.

*Methods and Materials:* Rat liver cytosol was utilised as a source of CGL. Enzyme activity was recorded using an adapted form of the spectrophotometric methylene blue method of H<sub>2</sub>S determination with 100 μM sodium sulphide used for calibration.

*Results:* L-cysteine was confirmed as the preferred substrate for CGL, with a Km of 2mM in this assay system. DL-propargylglycine (PPGG), a suicide inhibitor of CGL, was found to be effective at 10 μM, but also to inhibit CBS in rat kidney cytosol at higher concentrations. Amino oxaloacetic acid (AOAA), previously considered to be a specific CBS inhibitor, was also found to inhibit CGL (IC<sub>50</sub> = 13 μM). ATP (IC<sub>50</sub> = 3.6mM) and ADP (IC<sub>50</sub> = 6.3mM) were identified as novel CGL inhibitors, while AMP stimulates CGL activity. Synthetic H<sub>2</sub>S enzyme activity was in the order liver >kidney >brain in the rat. 10 μM PPGG (CGL specific at this concentration) and 100 μM were used to calculate CGL and CBS activity in selected rat tissues. H<sub>2</sub>S generation in the rat; (1) was predominately catalysed by CGL in the liver, (2) predominately catalysed by CBS in the brain, (3) was catalysed by both CGL and CBS in the kidney.

*Conclusion:* The investigations into CGL inhibitors allow a hypothesis to be made on the rate H<sub>2</sub>S generation in vivo. CGL activity could transiently increase by removal of tonic ATP inhibition in conditions such as hypoxia. This would result in ATP generation from pyruvate and a net increase in H<sub>2</sub>S levels. This could generate a concentration great enough for H<sub>2</sub>S to become physiologically relevant and trigger the appropriate mechanical effects on vascular beds and cytoprotective properties, hence defining cysteine desulphydration as a cellular survival mechanism.

### Association of methionine synthase A2756G SNP and methionine synthase reductase A66G SNP with male infertility in a Romanian population group

ESC-ID **239**  
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Folate metabolism is essential for proper cellular function and plays a crucial role in spermatogenesis. Methionine is an essential amino acid required for synthesis of S-Adenosyl methionine, the principal methyl donor group involved in protein, lipid and nucleic acid transmethylation. Methionine synthesis is catalyzed by the enzyme methionine synthase (MTR), which eventually becomes inactive due to the oxidation of its cobalamin cofactor. The protein encoded by 5-methyltetrahydrofolate-homocysteine methyltransferase reductase (MTRR) gene regenerates a functional methionine synthase via reductive methylation. We investigated for the first time in the Romanian population if two polymorphisms in the MTR gene A2756G SNP and MTRR gene A66G are associated with idiopathic male infertility in a group of Romanian patients. We studied the genotype and allelic distribution of these two SNPs in two groups, a control group of 65 Romanian healthy men and a case group of 63 patients suffering from idiopathic azoospermia or severe oligozoospermia. We performed genetic analysis by means of molecular genetics techniques, respectively PCR-RFLP (Polymerase Chain Reaction – Restriction Fragment Length Polymorphism). The genotype and allelic distribution of these two polymorphisms are the following: MTR genotype distribution: 3% G/G, 35% G/A, 62% A/A case group, respectively 1.5% G/G, 37% G/A, 61.5% A/A in the control group, allelic distribution: 20.6% G allele, 79.4% A allele case group vs. 20% G allele, 80% A allele control group and for the MTRR gene: genotype distribution 9.5% G/G, 69.4% G/A, 20.6% A/A case group vs. 9.2% G/G, 64.6% G/A, 26.2% A/A control group, while allelic distribution 44.4% G allele, 55.6% A allele case group vs. 41.5% G allele, 58.5% A allele control group. Our results show that the genotype and allelic distribution of MTR A2756G and MTRR A66G are similar in our two study groups; hence these two studied polymorphisms in the folate enzyme encoding genes are not genetic risk factors for male infertility in our Romanian population group. It may be necessary to enlarge the study groups in order to obtain more significant conclusions and to evaluate other polymorphisms in genes that code for key enzymes in the folate and homocysteine metabolism, for being able to interpret the eventual complex gene-gene interactions with possible implications in the studied pathology. Key words: gene, folic acid, homocysteine, polymorphism, azoospermia

### Effect of PACAP on retinal degeneratoin in neo-natal rats (in-vivo study)

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Pituitary adenylate cyclase activating polypeptide (PACAP) and its receptors are present in the retina and exert several distinct functions. PACAP has well-known neuroprotective effects in neuronal cultures in vitro and against different insults in vivo. We have shown that PACAP is neuroprotective against monosodium glutamate (MSG)-induced retinal degeneration. In the present study we investigated the possible signal transduction pathways involved in the protective effect of intravitreal PACAP administration against apoptotic retinal degeneration induced by neonatal MSG treatment and we further more pushed this experiment to study the effect of PACAP1-38 on a more specific excitotoxic model system using Ornithine, which represent an accepted rodent model for Stargardt's disease. In the MSG experiment we used 100pmol PACAP dissolved in 5  $\mu$ l physiological saline injected intra-vitreally. Immediately after each PACAP treatment, rat pups were treated with 2mg/g bodyweight MSG s.c. In the Ornithine experiment we used: 100pmol PACAP injected intra-vitreally and 0.25 mol/l of ornithine in 0.01 ml bilaterally into the vitreous cavity as well. MSG induced activation of proapoptotic signaling proteins and reduced the levels of antiapoptotic molecules in neonatal retinas. Co-treatment with PACAP attenuated the MSG-induced activation of caspase-3 and JNK, inhibited the MSG-induced cytosolic translocation of apoptosis inducing factor (AIF) and cytochrome c, and increased the level of phospho-Bad. The Ornithine results are not yet completed, but we expect positive signs because Vitamine E is known to slow down the progression of the disease so we think Stargardt disease causes degeneration by Oxidative stress pathway and PACAP acts on this specific pathway. In summary, our results show that PACAP treatment attenuated the MSG-induced changes in apoptotic signaling molecules in vivo and suggest that also endogenously present PACAP has neuroprotective effects. These results may have further clinical implications in reducing glutamate-induced excitotoxicity in several ophthalmic diseases. The ornithine induced degeneration may provide new insights into the pathomechanisms of ornithine-induced retinal degeneration, as well as new molecular targets for the clinical management of Stargardt's disease.

### Dual Inhibition of Cyclooxygenase and Lipoxygenase pathways by Lipoproteins in Human Platelets

ESC-ID **688**  
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Products of Arachidonic acid (AA) metabolism i.e. prostaglandins (PGs), prostacyclin (PGI<sub>2</sub>), thromboxane-A<sub>2</sub>

(TXA<sub>2</sub>) and lipoxygenase metabolites server important roles in the pathogenesis of ischemic heart disease and thrombosis. In the present study, we investigated the effects of lipoproteins on the products of AA metabolism produced by bovine seminal vesicles (BSV) and human blood platelets. Lipoproteins were separated by density gradient zonal ultracentrifugation and their effects on the synthesis of various AA metabolites were measured using radioimmunoassays. The results show that human lipoproteins VLDL, LDL and HDL inhibited AA metabolism to varying extents. VLDL, LDL and HDL inhibited the biosynthesis of prostaglandin E<sub>2</sub> and 6-keto-prostaglandin F<sub>1</sub> $\alpha$  (a stable metabolite of prostacyclin) in a concentration related manner. All three lipoproteins also exerted an inhibitory influence on the biosynthesis of prostaglandin F<sub>2</sub> $\alpha$  (PG F<sub>2</sub> $\alpha$ ) although not in a concentration-related manner. The effect of the lipoproteins on the production of AA metabolites by human platelets i.e. TXA<sub>2</sub> and 12-hydroxy-eicosatetraenoic acid (12-HETE) were also studied using radiometric thin layer chromatography coupled with automated data integrator system. In human platelets, HDL had a concentration-dependent inhibitory effect on the production of 12-HETE and TXA<sub>2</sub>, whereas LDL had a strong inhibitory effect on TXA<sub>2</sub> production but weakly inhibited the production of 12-HETE. In contrast VLDL did not exhibit any effect on AA metabolism by platelets. These results demonstrate a hitherto unrecognized property of human lipoproteins and point to a new facet of lipoprotein action.

### Evaluation of paraoxonase and arylesterase activity of serum PON1 in patients with acute heart disease

ESC-ID **856**  
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Serum paraoxonase-1 (PON1) is an esterase associated with apolipoprotein (apo) A-I in HDL that is believed to protect against cardiovascular disease (CVD) by inhibiting oxidation of LDL. This enzyme is active toward multiple exogenous and endogenous substrates and it is found to hydrolyze organic phosphate, aromatic carboxylic acid esters, estrogen 3-esters, polyunsaturated fatty acid oxidative products, a variety of aromatic and aliphatic lactones and some other biologically active compounds, such as PAF (platelet-activating factor). The latter four enzyme activities are considered physiological and may be responsible for the protective effect of PON1 against the development of atherosclerosis and CVD. Although reduced serum PON1 activities and protein level have been reported for patients with different heart disease by several research groups, still there are quite many other controversial results. The aim of the current pilot study was to set up the methods for assessment of paraoxonase (PON) and arylesterase (ArEs) PON1 activities in serum and to compare them between patients with acute heart disease and healthy controls.

*Materials and methods:* The two enzyme activities were assessed by applying adapted kinetics methods based on

the continuous spectrophotometrically recording of products generated after hydrolysis of substrates: paraoxon for paraoxonase and phenylacetate for arylesterase activity, respectively. The enzyme activities were evaluated in the sera of 16 patients with acute heart disease and in 28 controls individuals.

**Results:** The two enzyme activities of PON1 were very heterogeneous both in the group of controls and in that of patients. Although not significantly, the level of arylesterase activity was lower in patients (mean of 52.31 kU/l) compared to that of control group (67.57 kU/l,  $p = 0.199$ , Mann-Whitney U test), whereas the levels of paraoxonase activity of patients and controls were commensurable (118 U/l vs. 113 U/l,  $p = 0.887$  Mann-Whitney U test). The double substrate method showed a trimodal repartition in control group: there were individuals with a) high ArEs/low PON activities, b) with low ArEs/low PON, and c) low ArEs/high PON activities. In the patient group there were mainly individuals with two phenotypes: a) low ArEs/low PON, and b) low ArEs/high PON activities. The differences of the phenotypes may be due to the polymorphisms in the gene encoding PON1, which determined enzymes with distinguished affinity and activity to the used substrate.

## Cardiology

### Drug-eluting stents: A far-reaching evaluation

ESC-ID **439**  
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**Introduction:** The way to interventional cardiovascular medicine was opened by Werner Forsman, a German physician whom in 1929 performed the first human cardiac catheterization on himself. Then in 1977 Gruntzig performed the first balloon angioplasty, procedure that would subsequently become of reference in treatment of diseased blood vessels. Though a great breakthrough, the method posed a series of complications such as abrupt vessel closure, myocardial infarction and death. Meeting these problems, later Schatz modified The Palmaz stent, thus giving birth to the first commercially successful stent, the Palmaz-Schatz stent. The first human stent implantation was done in 1986 by Puel and Sigwart almost 20 years after Dotter and Judkins came with the concept. The arrival of the bare metal stent(BMS) partially improved the outcome of balloon angioplasty but still maintained a high incidence in restenosis and stent thrombosis. This led to the development of the drug-eluting stent(DES) that still carries on a risk factor but a more reduced one than the BMS.

**Objectives:** The study aims at bringing forward a comprehensive overview on the wide array of DES in use today all over the world, underlining through it the main indication in each type of stent.

**Methods and Materials:** We based our evaluation on experience gained over one year of practice in University Hospital of Bucharest (2008) in using 292 DES. The survey shows the use of DES in patients with risk factors,

recording clinical, demographical data stent types: 154 Xience, 94 Endeavour, 26 Cyph, 16 Taxus as well as the number of stents used( by gender: 49 on females-243 on males, smoking status: 245 on smoking persons- 47 on non-smokers, elevated blood pressure:206 on patients with high blood pressure; diabetes mellitus:56 on patients with DM,MI:103 on patients with previous myocardial infarction). The evaluation correlates the conclusions with the pre existing studies.

**Conclusions:** After having reviewed the history of stents, current indications and our experience, two major problems remain: In-Stent Restenosis and Stent Thrombosis. For the future hopes are to reduce these complications with the further development of substances coating DES and of the immunological and biodegradable stents.

### Blood velocities measured in the sinus coronarius by 3T- MRI in patients operated for coarctation of the aorta

ESC-ID **701**  
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**Background:** Coarctation of the aorta (CoA) counts for approximately 5% of the congenital heart defects. Most patients are operated during infancy. Late complications are rest- or re-coarctation of the aorta, abnormal blood pressure response to exercise and hypertension at rest. Life-long follow up is therefore recommended. A number of papers report an increased risk of coronary heart disease in patients with congenital heart defects. Many factors may accelerate the arteriosclerotic process, but little is known about CoA as a risk factor. In addition to hypertension, an altered blood stream profile and flow reserve in the coronary arteries might be of importance.

**Objective:** We want to uncover changes in the coronary circulation in patients with CoA by studying the blood velocities and -reserve in sinus coronarius.

**Design and method:** This study is an open prospective study of 10 controls and 10 patients operated for CoA (15-25 years of age). The patients are recruited from our outpatient clinic. Patients with a bicuspid aortic valve or a blood pressure difference of 20 mmHg or more between the right arm and leg, were excluded from the study. Velocities were measured by the phase shift velocity mapping technique (3.0T GE Signa Excite scanner) in the sinus coronarius before and after infusion of adenosine (0.14 mg/kg/min). A special designed workstation based on java makes it possible to select the region of interest and analyse the velocities.

**Results:** Preliminary data from five patients and five controls indicate a small difference in the average velocity and flow between the CoA-group (16 cm/s and 8,34 ml/s) and controls (11 cm/s and 4,4 ml/s) at rest. A larger increase in average velocity and flow in the CoA-group (31,7 cm/s and 13,8 ml/s ) than in controls (11,4 cm/s and 5,7 ml/s) was noted after infusion with adenosine. There are differences in the flow pattern between individuals within each cardiac cycle in both groups, more so in the CoA-group.

**Conclusion:** At rest there is just a small difference of the blood velocity and blood flow in controls and patients operated for CoA. However individuals in the CoA-group have more heterogeneous flow patterns. The CoA-patients have also a larger increase in average blood velocity and blood flow after infusion with adenosine. The changes within the cyclic flow pattern must be explored further.

### **Venous drainage of the sinoatrial and atrioventricular nodes**

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**Background:** Until present no data are available in the international literature on the venous drainage of the sinoatrial and atrioventricular nodes (SAN, AVN). Moreover, there have been also only a few reports on the veins of the right atrial wall. If particular veins of SAN and AVN exist, the occlusion of these veins could cause sinoatrial and atrioventricular dysfunctions by venous stasis and decreased perfusion of the nodes.

**Aims:** The arterial blood supply of the SAN, and AVN was widely investigated, and arteries of them are known. In the present study we focused on the investigation of nodal veins.

**Aims:** 1.) demonstration and analysis of the venous system draining the SAN, AVN, and the atria using corrosion cast preparations; 2.) exact anatomical description of these veins.

**Material and Methods:** We applied a special multi-component resin material for the corrosion cast preparation, described by M. Kiss (2006). After the cannulation of the ascending aorta, the inferior and superior vena cava, we injected the corrosion material into the right atrium, and into the aorta (casting of coronary arteries), with positive pressure (venous system: 30-40 Hgmm, arterial system 80-90 Hgmm). After the polymerization of the resin, the heart tissue was corroded by hydrochloric acid.

**Results:** 1.) The vein originating in the region of the SAN, after a short, straight course opens directly into the right atrium, at the crossing of the crista terminalis and the inlet of superior vena; 2.) we have found two variations of the atrioventricular nodal veins: a.) The vein takes its origin in the atrioventricular septum, below the non-coronary aortic sinus (region of the AVN) and after a short run (15-25 mm) towards the coronary sulcus it enters the right atrium, in the middle part of the interatrial septum; b.) The vein originates in the atrioventricular septum, below the non-coronary aortic sinus (region of the AVN) and runs perpendicular to the coronary sulcus and opens into the middle cardiac vein, approximately 0,5 cm before it joins the coronary sinus; 3.) The veins collecting from the right atrial wall, after a short (1-2 mm) run (perpendicular to the atrial lumen) open directly to the right atrium.

**Conclusion:** We first demonstrated the veins which drain the SAN, AVN. The damage or occlusion of these veins caused by catheter ablation therapy or some heart surgical interventions may lead to venous stasis and perfusion disturbances of the SAN, AVN and the atrial wall resulting in SA, AV, and atrial dysfunctions.

### **Blood Pressure and Cognitive Performance in Iranian Elderly Subjects**

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**Aim:** Dementia is one of the most disabling neurological disorders of aging populations. The progressive and irreversible nature of the disease sheds light on the importance of risk factors identification and primary prevention. Arteriosclerotic angiopathy might contribute to the development and progression of dementia. The association between blood pressure and cognitive function is not well identified. The purpose of the present study was to investigate the cross-sectional association between blood pressure and cognitive performance among the elderly population.

**Methods and Materials:** A total of 154 subjects (55% female) aged 65-101 years were enrolled in the present study. Cognitive performance was assessed by means of MMSE and the results were interpreted as being normal or impaired according to the age- and education-specific norms. Hypertension was defined as SBP >140, DBP >90, or positive history of hypertension being on anti-hypertensive medications.

**Results:** Hypertensive subjects showed no significant difference in cognitive performance from those with lower blood pressures. Blood pressure had no significant effect on cognitive performance between subjects of  $\geq 75$  years and <75 years. Following adjustment for age, sex, literacy and history of stroke, no association was observed between cognitive performance and systolic, diastolic, and mean blood pressures. To exclude the interfering impacts of other cardiovascular risk factors, analyses were also adjusted for final diagnosis of DM, dyslipidemia, history of smoking, and BMI. Once more, no association was observed.

**Conclusion:** In the present study, we failed to demonstrate any cross-sectional association between blood pressure and cognitive performance among Iranian elderly population. Previous cross-sectional studies have reported inconsistent results. In order to formulate a hypothesis, large-scale longitudinal studies are warranted.

### **Influence of contrast induced nephropathy on inhospital prognosis in patients with acute myocardial infarction treated with percutaneous coronary intervention**

ESC-ID **697**  
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**Introduction:** Percutaneous coronary intervention (PCI) is connected with regimen of contrast agents which are main factor influencing the incidence of contrast induced nephropathy (CIN). There are some coverages that CIN in



acute myocardial infarction (AMI) may influence prognosis of patients treated with PCI.

**Aim:** The aim of our study was to compare results of treatment and prognosis according to the incidence of CIN in patients with AMI treated with PCI.

**Material and method:** We examined consecutive patients with AMI treated with PCI, admitted to III Chair and Department of Cardiology, Silesian Medical University, Silesian Center for Heart Diseases in Zabrze, Poland between 2007 and 2008. CIN was defined as an increase of >25% or 0,5 mg/dl in the baseline creatinine concentration within 48-72 hours after PCI. Analysed group consisted of 531 patients. They were divided into two groups according to the incidence of CIN: the first group included patients with CIN and the second group patients without CIN. For the purpose of this study selected parameters were compared during in-hospital observation.

**Results:** There were 79 patients with CIN and 452 patients without CIN in analysed population. Patients with CIN were older, more often female, less often smokers and more often had diabetes. CIN was connected with higher percentage of anterior wall MI, cardiogenic shock (CS) (20,51% vs 7,52%;  $p = 0,0003$ ), reocclusions (7,69% vs 4,52%;  $p = 0,028$ ) and with higher level of CK-MB (266,53 vs 180,17 U/l;  $p < 0,0001$ ). In patients with CIN, according to patients without CIN, lower left ventricular ejection fraction (LVEF) (38,11% vs 42,98%;  $p < 0,0001$ ) and higher percentage of gastrointestinal haemorrhages (6,41% vs 0,88%;  $p = 0,0005$ ) was observed. The incidence of CIN was significantly related with higher in-hospital mortality in investigated group (16,46% vs 5,09%;  $p = 0,0002$ ). In multivariate analysis the independent prognostic factors of in-hospital mortality was CS, diabetes, age, time of pain duration and LVEF. Independent factors influencing the incidence of CIN in patients with AMI treated with PCI was CS [OR = 5,28 (1,28-21,8);  $p = 0,022$ ], anterior wall MI [OR = 2,44 (1,06-5,64);  $p = 0,37$ ], baseline glomerular filtration rate [OR = 1,02 (1,01-33,27);  $p = 0,007$ ], age [OR = 1,06 (1,01-18,41);  $p = 0,021$ ] and final TIMI 3 flow [OR = 0,18 (0,05-0,18);  $p = 0,009$ ].

**Conclusion:** The incidence of CIN in AMI treated with PCI is associated with higher mortality and higher percentage of complications during in-hospital observation. In this analysis factors predisposing to CIN were selected in this group of patients.

### Does serum sodium level on admission influence short- term and one-year mortality in patients treated with percutaneous coronary intervention for ST elevated myocardial infarction?

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**Introduction:** The level of electrolytes is an important predictor in acute myocardial infarction (AMI). It is interesting whether the baseline sodium level influences prog-

nosis in patients with AMI treated with percutaneous coronary intervention (PCI).

**Material and method:** The aim of this study was to compare the results of treatment and prognosis according to the baseline sodium level in AMI treated with PCI. We examined 2556 consecutive patients with AMI treated with PCI, admitted to III Chair and Department of Cardiology, Silesian Medical University, Silesian Center for Heart Diseases in Zabrze, Poland between 1998 and 2006. Patients were divided into three groups according to the baseline serum sodium level: I group-patients with sodium levels hyponatraemia was connected with the highest one-year mortality (15,38% vs 9,16% vs 10,59%;  $p = 0,0091$ ). In multivariate analysis independent factors influencing one-year mortality were: cardiogenic shock [HR = 4,99 (3,76-6,63);  $p = 0,041$ ]. There was no statistical significance for hipo- and hipernatraemia in this analysis.

**Conclusion:** Improper serum sodium level on admission is connected with higher in-hospital and one-year mortality in AMI treated with PCI. In multivariate analysis hyponatraemia and hipernatraemia are not independent factors influencing one-year mortality in MI.

### Presence of 4a allele of 4a/4b eNOS polymorphism may contribute to the risk of complications in post-acute period of myocardial infarction (MI)

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**Background:** Endothelium-derived nitric oxide (eNO) is a crucial regulator of cardiovascular functions. It is involved in smooth muscle relaxation, inhibition of leukocyte adhesion to vascular endothelium, platelet aggregation, apoptosis of myocardiocytes. Thus, eNO may be involved in development of MI. It is produced by endothelial NO-synthase (eNOS) in endothelium. Endothelial NO-synthase gene (eNOS) is located on chromosome 7q35-36 and spans 21 kb of genomic DNA. eNOS was fully screened for polymorphisms. Much attention was focused on 4a/4b polymorphism. 4a/4b polymorphism is a diallelic minisatellite in intron 4. Alleles 4a and 4b contain four and five 27-bp tandem repeats, respectively. 4a allele was associated with high risk of MI in some populations. Results of further studies are inconsistent, therefore the polymorphism has yet to be completely characterized.

**Aim:** To investigate genotype distribution and allele frequencies of 4a/4b eNOS polymorphism in male patients of different age with acute myocardial infarction (AMI) and those who had survived MI being at the same age a few years ago.

**Material and methods:** 13 male patients with AMI under the age of 45 (group I), 57 male patients with AMI older 60 (group II), 275 men who had survived MI being under 45 (group III), and 100 men who had survived MI being older 60 (group IV) a few years ago. 4a/4b eNOS polymorphism was genotyped by PCR.

**Results:** Frequency of 4a allele was significantly higher in men from the group I compared to the group III (31% and

15% respectively,  $p = 0.047$ ,  $OR = 2.5$ ). Percentage of 4a allele carriers (men with 4a/4a and 4a/4b genotypes) was twice as much in the group I compared to the group III (53,8% and 26,9% respectively,  $OR = 2.0$  95 % CI 1.17-3.4). There was no difference in the allele frequencies and genotype distribution in men from groups II - IV.

**Conclusion:** Our results allow suggesting that presence of 4a allele of 4a/4b eNOS polymorphism may contribute to the risk of complications in post-acute period of MI in young male patients.

### The tight junction protein CAR regulates cardiac conduction in AV-and Sinusnode

ESC-ID **940**

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The Coxsackievirus-adenovirus receptor (CAR) mediates both virus uptake and cell-cell attachment. It is closely associated with the tight junction in epithelial cells and it is a component of the intercalated disk in cardiomyocytes. CAR is highly expressed in the developing heart and deletion of CAR results in embryonic lethality. The healthy adult myocardium shows lower expression of CAR, but in myocardial infarction or cardiomyopathy, CAR is up-regulated. We have generated a heart specific and a heart specific inducible CAR knockout (KO) to investigate the role of CAR in the embryonic and adult mouse heart. Echocardiography of heart specific KO embryos showed an increased PR interval and lethality at age E12.5. Analysis of the cardiac function in CAR deficient adult mice revealed the development of an atrioventricular block and sinus node dysfunction that paralleled the loss of CAR. We documented the expression and specific distribution of CAR in the AV-node as compare to the surrounding myocardium. Functional analysis of the gap junction by dye coupling experiments revealed an altered communication between cardiomyocytes that might involve ZO-1, an adaptor protein shared between CAR and connexin 45 (Cx45). CAR deficiency results in a selective effect on a subset of connexins, with downregulation of both ZO-1 and Cx45 in the CAR-KO. Our novel mechanistic insights into the interaction of tight and gap-junction proteins could help explain the association of viral disease and arrhythmia.

### Diagonal ear lobe crease predict the severity of coronary artery disease

ESC-ID **874**

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**Background:** Although the association between Diagonal Ear lobe Crease (ELC) and Coronary Artery Disease (CAD) has been proposed and ELC is suggested as a simple mark-

er of CAD, the relationship with severity of CAD has not been investigated.

**Objective:** The aim of this study was to evaluate the association between ELC and the presence and severity of CAD.

**Method:** In a prospective double blind cross sectional study 100 patients with CAD documented by coronary angiography (case group) and 60 patients with normal coronary angiography (control group) were examined for the presence of ELC. Also the associations of ELC with severity of CAD (number of coronary arteries with significant stenosis) and with conventional risk factors of CAD were evaluated.

**Result:** A significant association between ELC and CAD was seen ( $P < 0.0002$ ). The observed sensitivity, specificity, positive predictive value and negative predictive value of ELC for CAD were the followings respectively (65%; 78%; 83.6%; 55.6%). Also the presence of ELC was associated with severity of CAD ( $P < 0.013$ ). Although there was direct correlation between frequency of ELC and increasing age, association of ELC and CAD was independent of age and sex. patient's Statistical analysis revealed that ELC was not associated with conventional CAD risk factors.

**Conclusion:** The study indicates a positive association between ELC and the presence and severity of CAD.

**Keywords:** Coronary Artery Disease (CAD), Diagonal Ear lobe crease (ELC)

### Deletion of the catalytic subunit $\text{i}\beta\text{1}$ of immunoproteasomes is linked to less severe enterovirus myocarditis in mice

ESC-ID **1014**

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Coxsackievirus B3 (CVB3) is a frequent cause of acute myocarditis. One of the late sequela of acute CVB3-myocarditis is dilated cardiomyopathy (DCM), which is associated with adverse prognosis. The ubiquitin-proteasome system (UPS) is a key regulator of protein balance and also crucially involved in the generation of viral epitopes. In systemic inflammation, immunoproteasomes (ip) are formed, which are characterized by an enhanced substrate turnover. Here, we studied the impact of immunoproteasomal subunit LMP2 in a murine model of CVB3-induced myocarditis. LMP2<sup>-/-</sup> mice were infected with  $1 \times 10^5$  PFU CVB3 Nancy strain and cardiac function was determined at the acute stage of myocarditis (8 days post infection) by conductance catheter measurements. Whereas the cardiac output was reduced in wt littermates from  $13011.5 \mu\text{l}/\text{min} \pm 504.1 \mu\text{l}/\text{min}$  to  $9200.8 \mu\text{l}/\text{min} \pm 652.6 \mu\text{l}/\text{min}$ ,  $p < 0.05$ , there was no change in the global cardiac output in LMP2<sup>-/-</sup> mice in CVB3-myocarditis (wt  $12514.4 \mu\text{l}/\text{min} \pm 651.8 \mu\text{l}/\text{min}$ ; LMP2<sup>-/-</sup>  $12920.7 \mu\text{l}/\text{min} \pm 683.2 \mu\text{l}/\text{min}$ ). Likewise, parameters of systolic and diastolic function pointed to preserved LV function in CVB3-myocarditis in LMP2<sup>-/-</sup> mice in comparison to their wt littermates ( $p < 0.05$ ). These findings are in perfect agreement with the less severe myocarditis that was

observed in LMP2<sup>-/-</sup> mice at day 8 p.i. (wt mice:  $1.95 \pm 0.20$  vs. LMP2<sup>-/-</sup> mice:  $0.71 \pm 0.06$  (scale 0-4),  $p < 0.001$ ). Interestingly, CVB3 replication was tremendously diminished in LMP2<sup>-/-</sup> mice, which could be verified by plaque assay, TaqMan and in situ hybridization. In conclusion, LMP2<sup>-/-</sup> mice display a decreased susceptibility to acute CVB3-myocarditis, which is likewise linked to tremendously reduced impairment of left ventricular function CVB3-myocarditis.

### Use of electrocardiographic Butler-Leggett criteria to assess right ventricular volume overload in adults with ostium secundum atrial septal defects

ESC-ID **485**  
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**Background:** The electrocardiographic Butler-Leggett (BL) criterion have been shown to estimate the amount of right ventricular hypertrophy (RVH) in patients with right ventricular (RV) pressure overload, but have not been examined in conditions of RV volume overload. This study was performed to test the hypothesis that there exists a correlation between the BL criterion on the electrocardiogram (ECG) and the Qp/Qs ratio determined by cardiac catheterization in adults with secundum atrial septal defects.

**Methods:** Demographic, cardiac catheterization, atrial septal defect (ASD) closure and ECG data were acquired on 70 consecutive adults with ostium secundum ASDs closed percutaneously. A modified version of the original BL criterion was used. Simple linear regression and logistic regression models were created to test the hypothesis.

**Results:** The mean age was 51 years; 67% were female. The mean Qp/Qs ratio was  $1.61 \pm 0.46$ . ECG data showed a mean BL criterion value of  $0.11 \pm 0.41$  mV. The BL criterion values correlated with shunt ratios ( $r^2 = 0.11$  and  $p = 0.004$ ). A BL criterion value of  $> 0$  mV predicted a significant shunt ratio (Qp/Qs  $> 1.5$ ) (OR 4.8; 95% CI 1.3, 18.1;  $p = 0$  mV may be useful in predicting a significant shunt ratio in adult individuals with known secundum ASDs.

## Dentistry

### The chronology of eruption of the first permanent molares and incisors of the first-graders

ESC-ID **878**  
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**Introduction:** The eruption of teeth includes both a series of complex movements of the dental foundation from the place of formation to the setting into the dental line and

its movements within the function it performs. Permanent dentition consists of 32 teeth (dentes permanentes s. adulti) whose eruption begins with the 6th year of life, and ends after the 18th. Dentition goes through three phases: the preruptive, prefunctional and functional.

**Aim:** The aim of study was to examine the chronology of eruption of the first permanent molars in relation to the permanent central and lateral incisors on the sample of the first graders of primary schools in Nis.

**Methods and materials:** The study included 552 first-graders of six primary schools in Nis, which represents 25.7% of the examinees mentioned. Based on the month and year of birth, all students of the first grade are divided into three groups: the 1st children who were born in the first half of 2001, the 2nd children who were born in the second half of 2001, the 3rd children who were born in the first half of 2002. Each subject undergone a systematic test of teeth as well as the order of appearance of teeth: 6, 1 and 2.

**Results:** The results obtained in this study show that there was a significant relationship between eruption chronology and the location of teeth- the first tooth most often happened to be the dentate six, the second central incisor and the third lateral incisor. The research shows that there was no statistically significant difference between the sexes in the springing chronology of the afore mentioned teeth.

**Conclusion:** On average, in all three examined groups of the first grade students of primary schools in Nis, dentates six appear first, then the central incisors and lateral incisors teeth appear as the third dentates.

### The application of Er:YAG laser in scaling and root planning

ESC-ID **106**  
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**Introduction:** The primary aim of periodontal treatment is controlling the bacterial deposits by removing the colonies inside the dental calculus, plaque or even the infected bulk of root surface cementum and periodontal tissues. Lasers with different wavelength can be used for removing calculus as an effective device. Er:YAG (erbium: yttrium-aluminum-garnet) laser of 2940 nm wavelength with high absorption in water and hydroxyapatite is a suitable device for scaling and root planning.

**Materials and Methods:** The Er:YAG laser was used for removing caries. The output energies used for this procedure varies from 50 to 250 mJ. Irradiation was done about 1-2mm distance of root surface. The scaling was continued under water irrigation until the calculus removed thoroughly.

**Results:** Reduction of pocket depth was seen after Er:YAG scaling. Also, after 6 months, higher reduction of bleeding on probing and improvement in attachment level were found comparing to conventional methods.

**Conclusion:** Using Er:YAG laser for initial periodontal treatment is minimally invasive and safe which results in instrumentation of deep and narrow pockets without causing trauma to surrounding tissues.

**Key words:** Er:YAG:laser, Scaling and root planning, Calculus, Attachment level

## Study upon dental students' attitude toward obese patients

ESC-ID **942**  
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*Introduction:* Obesity is a medical condition obviously related to the reduction of life expectancy. We are now witnesses of the alarming worldwide extension of this disease. The serious associated pathology has assured the medical community of the necessity of implementing prophylactic programmes, aimed to reduce the prevalence of obesity. Precocious support and treatment are desired for obese patients and must be initiated by any doctor, regardless of his speciality. However, obesity generates a heterogenous echo throughout medical society, as it is bounded to both organic and habitual aetiological factors. Therefore, students in medicine must be given the complete package of information about the exact meaning of obesity and how it can be treated in order to enable them to give medical support on a scientific basis.

*Aim:* This study was conducted with the aim of evaluating the perception on obese patients' medical and social condition and of identifying the attitude towards them (empathy or on the contrary, unprofessional judgemental attitude) among dental students, considering their medical defined point of view.

*Materials and methods:* We have surveyed a representative group of dental students from the University of Medicine and Pharmacy „Carol Davila”- Faculty of Dentistry, in the 3rd, 4th, 5th and 6th year of study. At this point, they have accomplished the Nutrition and Food Hygiene course, Internal Medicine and General Surgery courses and clerkships at „Sf. Ioan” hospital in Bucharest ( the most prestigious clinic of bariatric surgery in Romania). The survey took place between the 5th and 21st of May. It consisted of 15 single choice questions and 3 „fill-in” tasks. Respondents were asked, on condition of anonymity, to mention personal data such as age, sex, weight, height, year of study. The valid answers have been interpreted on a statistical basis, which led to the construction of graphs revealing the most popular answers among the students.

*Results:* 386 questionnaires were filled by students, most of them – 150 – in the 3rd year of study. When it comes to the main cause in generating obesity, 58% of the students incriminated the loss of nutritional control and a sedentary life, fact emphasized by the high percentage -46%- of the students who believe that a balanced, well-conducted lifestyle would not lead to this severe pathology. The main benefit that should motivate an obese person to lose weight is considered to be life quality improvement– 78%. Although the surgical option for obesity treatment is likely to be efficient (54%), students seem confused in choosing the optimal surgical technique, probably because of their lack of information about each technique's indications. From a professional, social and emotional point of view students show sympathy, as they do not manifest any discrimination feelings towards obese persons. 74 % students expect themselves to be well trained at the moment of graduation so that they easily recognize clinical signs of general affections, obesity in

this case. Moreover, most of them are willing to redirect an obese patient to a specialist (64%), considering this an appropriate reaction, for he is a doctor in the first place – 58%. The specialist that most students recommend is the specialist in nutrition and diabetes– 59%. When it comes to systemic complications generated by obesity, that could interfere with the treatment in the oro-maxillo-facial region, 44% of the students, as future dentists, consider precaution means necessary, and are willing to analyse before the beginning of treatment, para-clinical investigation results .On the contrary,39% would skip this step.

*Conclusions:* Dental students should be well trained and prepared to recognize severe diseases and associated oral lesions in order to prevent both systemic and oro-maxillo-facial complications of obesity. In addition, they should be able to advise, conceal and direct obese patients to qualified specialists in order to stop the evolution of obesity and even start the remission of this affection. Future dentists are the most required specialists by most of the people, considering the fact that when it comes to eating or fonation disfunctions, severe pain in the oro-maxillo-facial territory or the alteration of the aesthetic principles, patients don't hesitate appealing to the dentist.

*Key words:* obesity, dental students, empathy, judgemental attitude, survey

## A comparison between Liquid-based cytology versus conventional cytology for detection of precancerous lesions in oral cavity

ESC-ID **925**  
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*Introduction:* Oral Squamous Cell Carcinoma (OSCC) is the most common cancer of head and neck globally. Despite the progresses achieved in past decades, OSCC has remained to have a low 5 years survival rate. The most important factor in the lack of improvement in prognosis is significant portion of OSCCs are not diagnosed until they have reached to an advanced stage. So, early detection of cancerous and precancerous lesions seems to be the most important way to reduce this mortality and morbidity. The aim of the current study is a comparison between liquid based cytology (LBC) –which has been used for diagnosis of such lesions in other parts of the body- as a new way of screening precancerous lesions in oral cavity with conventional cytology.

*Material and methods:* Samples were collected by cyto-brush device from 36 patients with precancerous lesions (17 leukoplakia, 12 erythroplakia and 6 erythroleukoplakia) to be checked with both methods of LBC and conventional cytology. A conventional smear was first prepared by stroking the brush along the glass slide which was later fixed in 95% ethanol. In LBC method, the brush containing the residual material was immersed in a preservative fluid and processed according to the manufacturer's formula. Slides of both techniques were stained by Papanicolaou method. Fisher's exact test was used for data analysis and  $p < 0.05$  considered as statistical significance. *Results:* Both techniques agreed on cytologic diagnosis in every case they yielded an adequate specimen. In 4 cases (2 leukoplakia, 1 erythroplakia and 1 erythroleuko-

plakia) conventional smear resulted hypocellularity and therefore inadequate for analysis. LBC indicated a significant overall improvement in smear thickness, cell distribution, reduction in cell overlapping and blood presence on specimen analysis. On the other hand, cell morphology was better visualized in LBC preparations.

*Discussion:* Although LBC showed an overall improvement on sample presentation specimen adequacy and visualization, both methods are diagnostically reliable and can be used routinely. It should be highlighted that further studies are still needed to improve the issue of late diagnosis.

### Statistical analysis of 1035 odontogenic cysts: A retrospective study

ESC-ID **861**  
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 Institution **Tehran University of Medical Sciences**

*Background and Aim:* Odontogenic cysts are true cysts arising from odontogenic epithelium and constitute a major part of oral lesions. Information regarding the distribution pattern of these cysts is different worldwide and can be helpful in diagnosis. The aim of this study was to determine this information in an Iranian sample population and compare it with other investigations.

*Material and Methods:* In this retrospective study, we reviewed all files in the oral pathology department of the faculty of dentistry, Tehran university of medical sciences, from 1993 to 2007. Cases of odontogenic cysts were retrieved and histological slides were re-evaluated for diagnosis confirmation. Age and sex of patients as well as type and site of lesions were recorded. Statistical analysis was performed by SPSS software.

*Results:* Among 6483 files and after diagnosis confirmation, 1035 cases (15.9%) were accepted as odontogenic cysts. In 58 cases (5%) the diagnoses changed, most of which involved keratocysts. 57.6% of cases were men and 42.4% were women (M:F = 1.35:1). Radicular cyst was the most common diagnosed type (35.9%) followed by dentigerous cyst (23.1%) and keratocyst (18.3%). Radicular cyst was more frequently reported in the anterior region (43.7%) whilst, dentigerous cyst and keratocyst were more often seen in molars and posterior region of the jaws (33.9% and 36% respectively). 24.3% of patients were younger than 16 and dentigerous cyst was the most common lesion in this age group.

*Conclusion:* Our results being similar to other studies, differ them in some respects which should be considered in further studies based on such information.

*Key words:* Cyst-Odontogenic-Radicular-Dentigerous-Keratocyst

### Evaluation of PH rate of saliva in patient with chronic renal failure

ESC-ID **455**  
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*Aim:* The patients with chronic renal failure present different oral manifestations and changes in salivary proper-

ties due to their specific systemic conditions. These manifestations include change in taste, enamel hypoplasia, dry mouth, gingival bleeding and calculus destruction. Salivary properties and flow rate also change in the end stage renal failure for their systemic conditions resulting in the change in the saliva functions such as buffering, teeth development, antimicrobial activity, lubrication, and taste and digestion. The present study was done to assess the salivary pH in the patients undergoing dialysis referred to Imam Khomeini and Shahid Mostafa Khomeini Hospitals in Tehran during 2008.

*Methods and Materials:* In this clinical trial, 100 patients with end stage renal failure were selected by sequential referring method and their salivary pH were determined in three time intervals of before, during and after dialysis. The patients underwent dialysis for 4 hours in average. Unstimulated saliva samples of the patients were collected by direct method of spitting in 5 till 10 minutes. The pH of the salivary samples was determined by pH ion technique using electrometric device. The data were subjected to analysis of variance test and central tendency indices.

*Results:* Twenty seven percent of the patients were females and 73% of them were males. 67% of them were received dialysis in Shahid Mostafa Khomeini hospital and 33% in Imam Khomeini hospital. The mean age of the patients was 38.01 years with the standard deviation of 14.81 years. The patients received dialysis treatment for 3.45 years in average. Salivary pH of the patients was  $7.83 \pm 0.23$  before dialysis,  $7.82 \pm 0.23$  during dialysis and  $7.81 \pm 0.24$  after the dialysis. No significant differences were found between the three time intervals regarding patients salivary pH ( $p = 0.85$ ).

*Conclusion:* Under the study limitation, the salivary pH of the patients undergoing dialysis was remained fixed almost during three time interval of before, during and after the dialysis. Comparing the salivary pH of the healthy specimens as published in the studies, higher salivary pH was noted in the patients with renal failure.

## Dermatology

### Epidemiology and evaluation of 1073 burn patients in the southeast of Iran

ESC-ID **902**  
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*Background:* Burn injury is one of the major causes of morbidity and mortality worldwide. In addition to the financial burden it inflicts on the health care system, it can cause psychological, social and physical problems to the patient and family. The aim of this study is to determine the epidemiological parameters in hospitalized burn patient.

*Methods:* In a cross-sectional study we evaluated data of 1073 hospitalized burn patients in Zahedan Khatam Al Anbia hospital from 2005-2008. All data extracted from medical record and analyzed by SPSS software. A P value less than 0.05 was considered significant.

**Results:** Among 1073 patient with acute burn, 55.4% were male and 44.6% were female. The mean ages was 20.94 year old and mean burn size was  $49.11 \pm 29.65$  total body surface area (TBSA). Total body surface area burned was significantly higher in those with self burn ( $p = 0.0001$ ). In children younger than 15 year-old scalds (hot liquid) was the most frequent cause of burn (44%). There was a higher incidence of self burning in women ( $p = 0.0001$ ) and the mean length of hospital stay was  $6.32 \pm 5.27$  days. The mortality rate in self burn patient was 87.34 % and in total cases was 41.47 %.

**Conclusion:** In the present study, most of the burns were registered in 16 to 20 years age group, and more in male than in female subjects. Burn was more frequent in children and adulthood as a high risk groups with higher mortality and morbidity. This shows a need for burn prevention programs.

**Key words:** burn, epidemiology, hospitalized patients

## Endocrinology

### Does synthetic somatostatin analogues differentially affects lipid metabolism in isolated rat adipocytes?

ESC-ID **1045**  
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Several clinical observations indicate a significant weight loss in patients during somatostatin (SST) synthetic analogs therapy (e.g. octreotide). In order to determine a possible mechanism which underplaying these phenomena, we analyzed expression of somatostatin receptors isoforms (SSTR) and potential pro-lipolytic effects of SST and their analogs (octreotide, SSTR2 selective Compound 2) in isolated rat adipocytes.

**Materials and methods:** • Expressions studies SSTR isoforms expression profiling was performed by Real Time PCR. • Lipolysis Cells were incubated with KRB buffer supplemented with SST, octreotide and Cpd. 2 (1nM, 10nM and 100 nM) in absence (basal) or presence of epinephrine. Lipolysis rate was assayed by measuring released glycerol concentrations. • Lipogenesis Incubation of adipocytes with KBR buffer in presence of different doses of SST, octreotide and Cpd. 2 without (basal) or with insulin (10 nM) and in the additional presence of [U-14C] glucose was performed. Lipogenesis was determined as the incorporation of [U-14C]glucose into lipids. • Glucose uptake Cells were preincubated in KRB buffer with different doses of SST, octreotide and Cpd. 2 in absence (basal glucose uptake) or presence of insulin (insulin stimulated glucose uptake) for 30 minutes. Glucose transport was initiated by the addition of 2-deoxy-D-[1-3H]glucose and after 3 min terminated by addition of cytocholasin B solution. Then the radioactivity of samples was measured.

**Results:** Real Time PCR results showed predominant SSTR2 isoform expression in adipose tissue. SST and

their synthetic analogues (octreotide and Cpd. 2) significantly elevated basal and stimulated lipolysis. In addition, we observed decreased TG accumulation and glucose uptake. However, those effects was prompted by the highest concentration of tested agents seems to be much more discreet.

**Conclusion:** Our preliminary data suggest that direct regulation of lipid and carbohydrate metabolism in adipose tissue might be responsible for well-documented body weight lowering during octreotide therapy. In summary, distinguished somatostatin receptor expression profile and most potent action of Compound 2 suggest substantial role of SSTR2 activation.

### Disturbances of PTH-vitamin D axis in noncholestatic chronic liver diseases

ESC-ID **172**  
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**Background and Aims:** The liver has an important role in metabolism of vitamin D. Our aim was to determine the prevalence and type of vitamin D-parathyroid hormone (PTH) disturbance in patients with noncholestatic chronic liver disease (CLD) and its relationship with disease severity and liver function.

**Method and Materials:** We studied 90 consecutive patients (50 men, 40 women; mean age,  $42.3 \pm 12.7$  [SD] y) with noncholestatic CLD caused by hepatitis C (n = 28), hepatitis B (n = 26), autoimmune hepatitis (n = 19), and cryptogenic CLD (n = 17); 51 patients had cirrhosis. Serum concentrations of 25-hydroxyvitamin D (25[OH]D), PTH, calcium, phosphate, magnesium, creatinine, and liver function tests were determined.

**Results:** Serum 25(OH)D levels were inadequate in 61 patients: vitamin D deficiency (6.8 pmol/L) was present in 6 patients. The prevalence of vitamin D deficiency was significantly higher in cirrhotic vs noncirrhotic patients (76.5% vs 17.9%;  $P = .0001$ ). In Child-Pugh class B and C patients, 25(OH)D levels were significantly lower than in class A patients (P topenia).

**Conclusion:** Vitamin D inadequacy is common in noncholestatic CLD and correlates with disease severity, but secondary hyperparathyroidism is relatively infrequent. Management of CLD should include assessment of vitamin D status and replacement when necessary.

### Downregulation of hepatic glucose 6-phosphatase-alpha in patients with nonalcoholic fatty liver

ESC-ID **772**  
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**Background:** Microsomal glucose-6-phosphatase-alpha (G6Pase-alpha) and glucose-6-phosphate transporter (G6PT) perform the terminal step in both glycogenolysis and gluconeogenesis. Deficiency of G6Pase-alpha leads

to glycogen storage disease type 1a, whereas deficiency of G6PT leads to glycogen storage disease type 1b. Partial inhibition of G6Pase in rats results in increased hepatic triglyceride contents and de novo lipogenesis leading to hepatic steatosis. Hepatic steatosis (nonalcoholic fatty liver), affecting 34% of the U.S. population, frequently represents a hepatic manifestation of the metabolic syndrome. The molecular mechanisms underlying the relationship between fatty liver and G6Pase-alpha have not been evaluated in humans.

**Methods:** 27 patients (11 men, 16 women) underwent liver biopsy. Histological diagnosis identified normal liver in 8 patients and nonalcoholic fatty liver in 19 patients. We quantified G6Pase-alpha and G6PT mRNA expression by real-time PCR with 18S as housekeeping gene. In addition, anthropometric measurements and analysis of plasma lipids and liver enzymes were performed.

**Results:** The groups of normal and fatty liver showed no significant differences in age, HOMA-IR, BMI, liver enzymes or waist-to-hip ratio, but total plasma cholesterol levels were higher in the fatty liver group ( $p < 0.05$ ). G6Pase-alpha and G6PT mRNA expressions were down-regulated significantly in fatty liver compared to normal liver ( $p < 0.05$ ). G6Pase-alpha and G6PT mRNA expressions correlated positively ( $R^2 = 0.4581$ ,  $p < 0.001$ ). Both expressions did not correlate with age, BMI, liver enzymes, triglycerides or glucose levels.

**Discussion:** G6Pase-alpha activity is largely controlled by transcriptional levels. Our data suggest that the expression of hepatic G6Pase-alpha and G6PT are closely interlinked. The observed downregulation of G6Pase-alpha in fatty liver seems to play a major role in hepatic fat accumulation and pathogenesis of fatty liver.

### Perceptions towards glycaemic control strategies among diabetes patients in sub-Saharan Africa: A case study of Dar es Salaam, Tanzania

ESC-ID **97**  
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**Aim:** To assess perceptions towards glycaemic control strategies among adults with type 2 diabetes mellitus in a metropolitan area of Africa.

**Materials and Methods:** A cross-sectional survey was done in July – Sept 2007 involving adult patients with type 2 diabetes mellitus attending diabetes clinics in the city of Dar es Salaam. Data were collected using a semi-structured questionnaire with 10 likert type items designed specifically to assess the pattern of exercise, eating habits and oral hypoglycaemics. Data were analysed using Epi-Info version 3.3.2. Statistical significance tests included the usage of X2 test to check for the association between dependent variables and P-values related to the level education of the respondent ( $P = 0.0001$ ).

**Conclusion:** Cost of oral hypoglycaemics was perceived to be very high in this study population. The usage of traditional medicaments for blood sugar control was so high among the study population.

### The effects of recombinant human insulin-like growth factor I on glycaemic control, body composition and physical fitness in healthy amateur athletes

ESC-ID **336**  
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**Introduction:** There is anecdotal evidence that recombinant human insulin-like growth factor I (rhIGF-I) is being abused by elite athletes to gain a competitive advantage. There is, however, limited published clinical trial data to confirm that rhIGF-I has beneficial effects on physical performance.

**Objectives:** Primary hypothesis: individuals administering rhIGF-I will show reduced insulin resistance. Secondary hypothesis: individuals administering rhIGF-I will show a significant increase in lean muscle mass and reduced levels of adipose tissue resulting in improved physical performance.

**Methods:** 18 volunteers (17 male and 1 female) were recruited. The following baseline assessments were performed: fasting glycated haemoglobin, lipids and non esterified fatty acids, glucose and insulin before and during a standard 75g oral glucose tolerance test (OGTT). The following body composition measurements were also taken: body mass index, waist, hip and arm circumference measurements, Dual Energy X-ray Absorptiometry, bio-electrical impedance and skinfold thickness. Physical fitness was determined by a maximal treadmill test following the Bruce protocol. The volunteers were then randomised to administer either low or high dose rhIGF-I/rhIGFBP-3 complex or placebo for 28 days. All physiological measurements were then repeated on the day after the last injection.

**Results:** Mean glucose ( $p = 0.047$ ), insulin ( $p = 0.035$ ) and insulin resistance ( $p = 0.025$ ) decreased significantly in individuals who administered rhIGF-I for twenty eight days, supporting my primary hypothesis. No significant change in body composition or physical fitness was shown in participants that administered rhIGF-I.

**Conclusion:** Insulin resistance is reduced in healthy adults after administering twenty eight days of the recombinant protein complex consisting of rhIGF-I and rhIGFBP-3. The effect of rhIGF-I on body composition and physical fitness remains unclear.

### Otorhinolaryngoscopy

#### Corrosive stomatoesophagitis by children - surgical or therapeutical problem

ESC-ID **872**  
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Chemical burns of the esophagus by the corrosive substances are a serious medical and social problem. Most of the patients in Bulgaria are children and young people, which determines largely the social significance of this serious debilitating disease. In this study, we made a deep

analysis of the age characteristics of the patients, the type of the corrosive agent, the severity of the damage, the chosen method for dilatation of the oesophagus and the final outcome of the treatment. We used the database of the children up to 15 years of age, hospitalized with a diagnosis corrosive Stomatooesophagitis in the Department of ENT diseases in the University Hospital in Stara Zagora, Bulgaria. Of all 45 examined cases, the number of boys is nearly 2 times bigger than girls- 29/16. After a subsequent endoscopic examination and therapeutic treatment of children in 38 cases the conventional bouginage gave excellent results. Two children died and by 4 others the normal passage of the oesophagus was not recovered. This brought the necessity of using retrograde bouginage, as well as extirpation and surgical replacement of the esophagus with a large intestine by a 11-year-old boy. In conclusion, there are advantages and disadvantages of the conservative and surgical treatment of the corrosive stomatooesophagitis, and the indications for choosing the method should be well considered.

**Key words:** oesophagus, corrosive stomatooesophagitis, chemical burns, bouginage, dilatation, surgical treatment.

### The experience of treatment of seasonal allergic rhinitis with intranasalis glucocorticosteroids

ESC-ID **682**

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The seasonal allergic rhinitis (SAR) is widespread disease almost in all countries. In the literary data we can find the trend to the growth of its incidence. So, the medicamentous treatment is still actual. The only group, having pathogenetic invoice to the all phases of allergic process are glucocorticosteroids.

**Aim:** To investigate the invoice of topical glucocorticosteroid Benarin (0,05% Budesonide) on clinical expressions of SAR.

**Materials and methods:** We observed 42 persons with manifested form of SAR, of them 27 women and 15 men, age 18 to 53. The average age was  $28,6 \pm 3,6$ . The plurality of them was in the most able-bodied age (30 persons – 71,4%). The lasting of the disease was in average  $3,4 \pm 0,6$ , from 7 to 3, and amount of exacerbations 1-3 per season of pallination, dependent of the blossom calendar. 3 patients (7,1%) passed ultrasonic disintegration of nasal conchae with temporary effect for winter time only. All patients received the treatment with topical GCS 0,05% Budesonide, in drops during 2 weeks. All patients evaluated objective and subjective clinical signs, common condition, unwanted side-effects. All the symptoms were evaluated in 3 grades – 0- absence, 1- some, 2-moderately and 3 – strongly expressed symptoms. Also we investigated the data of rhinocytograms – eosinophilia, fungal mycelium, index of epithelium destruction. 80% of patients presented on the second examination, 60% - on the 3rd and 4th.

**Discussion:** On the self-evaluation lists patients noted the effect on the 2nd-3rd day, and achieved maximum at 7th-10th. On the 7th day rhinorrhea and nasal obstruction were less 5 times, nasal itch 10 times, sneezing was absolutely absent. Objective examination shown fast reduction of edema and stagnant color, stop of rhinorrhea

and eosinophilia. Fungal mycelium in rhinocytograms was found it in 4,4% of patients.

**Conclusions:** 0,05% Budesonide is a modern topical GCS, fastly efficient on main SAR's symptoms with small amount of side-effects.

## Epidemiology

### Estimation of MRSA prevalence in the hospital environment of Gomel, Belarus

ESC-ID **883**

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**Aim:** To estimate MRSA prevalence among S.aureus strains isolated from environment objects in Gomel hospitals.

**Methods:** Isolation and identification of S.aureus strains were carried out with routine microbiological methods. Primary inoculation was done on mannitol-salt agar, plates was incubated for 48 h 370C. Identification was carried out according its catalase activity, mannitol and glucose aerobic and anaerobic fermentation and plasmocoagulation reaction. Sensitivity to vancomycine was estimated by disc-diffusion method on Mueller-Hintone agar with addition of 4% NaCl and 6 mg/l of oxacilline. For internal quality control S.aureus ATCC 38591 (MRSA) and S.aureus ATCC 29213 (MSSA) strains were used.

**Results:** A total 18798 wipe-samples from hospital environmental objects, in 86 samples (0.46%) S.aureus was found. More frequently S.aureus strains were isolated in infectious diseases unit (1.49%), out-patients departments (0.88%), obstetrics unit (0.55%); the least – in surgical departments (0.06%) and gynecological departments (0.16%). Additionally examined 284 hospital's members to reveal carrying of S.aureus. Positive results obtained from 25 (8.8%) of examined. Resistance to methicilline revealed in 7 strains of S.aureus, isolated from environment objects (8.14%). All MRSA were isolated from 5 Gomel hospitals (3- from surgical units, 2 – from neonatal unit, 1 – from obstetrics and intensive care unit). No MRSA were isolated from out-hospital departments and from carriers. All strains of S.aureus preserved sensitivity to vancomycine.

**Conclusions:** 17.5% of S.aureus strains isolated from environment objects in Gomel hospitals were MRSA. Neonatal and surgical units are of high risk, where effective infection control measures should be introduced.

### Epidemiological situation of Echinococcosis among the population in Karaganda (Kazakhstan)

ESC-ID **836**

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Epidemiological situation of Echinococcosis among the population in Karaganda (Kazakhstan) Introduction



Echinococcosis is a serious parasitic disease of humans and animals, inflicting a big social-economic damage. During the last 12 years, the incidence of Echinococcosis increased at least 3 times in Kazakhstan. It was noted that people of middle age are affected more often (70%). Death-rate varies from 2 -7%, disablement from 4-9%, while relapse of the disease occurs in 6-16% and necessitates recurring operations. The economic losses are estimated at several millions of tenge (KZT) annually. The most alarming is the increasing number of children having Echinococcosis, reported to vary from 6 - 39% in the age group from 10 months to 15 years old. Particularly the children (boys) from rural areas, who take care of animals, are more often affected.

**Aims:** Study the manifestation of the epidemic process of echinococcosis, among the population of the Karaganda region since 1997 to 2008 y.

**Tasks of research:** 1. Retrospective epidemiological analysis of the long-term dynamics of echinococcosis diseases in Karaganda region (Kazakhstan) within 1997-2008 yrs. 2. Retrospective epidemiological analysis of the long-term dynamics of echinococcosis diseases in Karaganda region (Kazakhstan) among children and adult population since 1997 to 2008 yrs. 3. The comparative analysis of children and adults sickness rate in Karaganda region within 1997-2008 yrs. 4. Retrospective epidemiological analysis of the long-term dynamics of echinococcosis diseases in Karaganda region (Kazakhstan) amongst urban and rural population. 5. The comparative analysis of echinococcosis among urban and rural population in Karaganda region since 1997 to 2008 yrs.

**Materials and Methods:** The research was conducted at Karaganda State Medical Academy with the help of Karaganda Region Department of State Sanitary Control. The following materials were used: The Journal of Infectious Diseases Registrations, the Information Lists of Infectious Diseases with Epidemiological Examination, the information lists about Helminthes carriers (Helminthes Card) and Form No.058. Retrospective epidemiological analysis is a method of calculation of spreading of infectious disease for more than 5 years. The basic rate change of retrospective analysis is: Epidemical tendency, Periodicity, Reduction and Increasing of disease. In calculation retrospective epidemiological analysis we used the method of LEAST SQUARES. LEAST SQUARE METHOD  $Sick\ Rate = (cases * 100000) / population$   $Jactual = (Jabsolute * 100000) / population$   $Jmid = \sum Jactual / N$   $Jtheoretical = Jmid + (B * X)$   $B = \sum (Jactual * X) / \sum X^2$   $T (reduction - increasing) = (B / Jmid) * 100$  (in %) (For Prognosis)  $Jtheoretical\ for\ prognosis = Jmid + (B * X)$   $\Delta = Jactual - Jtheoretical$   $\sum \Delta + \sum \Delta -$   $\Delta + mid = \sum \Delta + / n$   $\Delta - mid = \sum \Delta - / n$   $Jactual - actual\ level\ of\ spreading\ disease\ every\ year.$   $Jabsolute - cases\ of\ the\ disease\ every\ year.$   $Jtheoretical - theoretical\ level\ of\ spreading\ disease\ every\ year.$   $Jmid - middle\ arithmetical\ level\ of\ spreading\ disease\ studied\ period.$   $B - Coefficient\ which\ shows\ the\ difference\ between\ theoretical\ levels\ of\ spreading\ disease\ for\ adjacent\ years.$   $\Delta + \Delta - = Quantity\ of\ deviation\ of\ results\ below\ (\Delta + )\ and\ above\ (\Delta - ).$   $Jprognosis + = below\ limit\ of\ prognosis$   $Jprognosis - = above\ limit\ of\ prognosis$   $T (reduction\ \&\ increasing) - average\ rate\ of\ the\ reduction\ and\ Increasing\ of\ the\ year.$   $X - Number\ below\ and\ above\ mid\ line$   $N = numbers\ of\ years.$   $n = number\ of\ \Delta +\ or\ \Delta -\ respectively$   $\Sigma - Sum$  The estimation of the tendency of spreading disease is done by V.D.Belyakovs gradation: - if T is from 0 till + - 1.1 = tendency is stable (no change) - if T is from 1.1 till + - 1 = tendency is moderate (reduc-

tion and increasing) - if T is more than + - 5.1 = tendency is Worse (reduction and increasing).

**Results:** Echinococcosis in Karaganda 1997-2008: the highest incidences were from 2000-2008, ranging up to 2.5-3.9 (per 100,000). The average rate change during the epidemiological process is 4.3%. Among children: the highest incidences were in 1999-2008 and made up to 4.8-5.3 (per 100,000) and their average rate change was 2.9%. Among adults, the highest incidences were in 2000-2008 and made up to 2.1-3.7 (per 100,000) with an average rate change of 5.2%. Among the rural population the incidences were in 2000-2008 and made up to 9.3-12.4 (per 100,000), with an average rate change of 6.2%. Among urban population, the highest incidences were in 2003-2008 and ranged 1.5-2.9 (per 100,000). The average rate change during the epidemiological process is 6.4%.

**Conclusion:** 1. The analysis of spreading Echinococcosis : • Among population in Karaganda region is constantly increasing i.e. from 1997 – 2000 it is increased 3 times, 2001 – 2002 it is decreased 0.7 times and 2003 – 2008 it is increased 2 times. • Among children's, 1997 – 1999 it is increased 3 times, 1999 – 2000 it is decreased 0.7 times, 2001 – 2002 it is increased 2 times, and 2003 – 2008 it is increased 1.5 times. • Among adults, 1997 – 1998 it is increased to 2.2 times, 1998 – 1999 it is decreased 5 times, 1999 – 2000 it is increased 3.5 times, 2000- 2002 it is decreased 0.1 times; 2002 – 2008 it is increased 9.2 times. • Among rural population, 1997 – 2000 it is increased 6.2 times, 2001 – 2002 it is decreased 0.7 times, 2002 – 2008 it is increased 3.6 times. • Among urban population, 1997 – 1999 it is decreased 0.8 times, 1999 – 2001 it is increased 2.6 times, 2001 – 2002 it is decreased 1.1 times, 2002 – 2004 it is increased 1.6, 2004 – 2005 it is decreased 0.7 times, and 2005 – 2008 it is increased 2.2 times. 2. The Echinococcosis disease in Karaganda region tends to growing. Prognosis of diseases on 2009 – 3.6 (per 100,000). 3. The Echinococcosis among the children population exceeds the adult one. 4. The diseases among the rural population exceed the urban one. In social-professional structure of the echinococcosis diseases of the population, the epidemiological significant group is cattle-breeders. **Key words:** Mortality, Disability Epidemical tendency, Periodicity, Reduction & Increasing of disease, LEAST SQUARES, V.D.Belyakovs gradation , Helminthes Card , Form 058 .

## Characteristics of family homicide in Serbia

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**Aim:** This paper explores differences in characteristics of the various types of family homicides, either between intimates, children, parents, siblings or other family members, its motives and mechanisms, and highlights the needs for specific prevention.

**Materials and methods:** Data used in this research was collected from autopsy files of cases of family homicide from The Forensic Institute in Belgrade for the period of 6 years (2002-2007). Information for this research were collected from autopsy reports, death certificates, toxicological findings and crime-scene police reports.

**Results:** Out of 313 examined cases of homicide, it was determined for 60 to be family-related, that is 19.2%, which makes 10 family homicides in average per year. Victims were aged 0-85, in average 43.5 years. Demographic analysis of the victims revealed 38.3% to be males and 61.7% females. Males dominate among the offenders with 81.7%. Positive family history of either homicide, suicide, mental disorder, alcohol abuse or combined was present in 22 cases (36.7%). Predominantly, the locality of the event (90%) was a family dwelling. In terms of the type of weapons used, most commonly it happened to be firearms. Suicide followed by a case of either simple or multiple homicide was noted in 20, attempted in 4 cases, exclusively committed by males. Our study also emphasizes 3 cases of familicide.

**Conclusion:** A significant similarity was noted between filicide and intimate partner homicide cases, as well as, between parricide and siblicide in terms of the weapons used and suicide-homicide rates. Most commonly present variables noted as risk factors were: living with a mentally ill member of the family, presence of some kind of family violence, physical, verbal, psychological or sexual, alcohol abuse, low financial standard of the family. Since the underlying motives of such violent family behavior remain hidden even to the closest environment, preventability of family homicides is being questioned but we stress that broader availability of the family violence resources, as well as increasing female independence are good foundations for prevention.

### High prevalence of multidrug resistance of *Vibrio cholerae* strains in a cholera outbreak in Karaj, Iran during June-September 2008

ESC-ID 927  
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**Introduction:** Cholera, a fatal epidemic diarrheal disease is still endemic in Africa and Asia. Despite improved sanitary aquatic conditions, outbreaks of cholera still happen in Tehran. In case a patient is affected by *Vibrio cholerae* (*V. cholerae*), an antibiotic to which the bacteria is susceptible can be used to reduce the duration and volume of diarrhea. Following the occurrence of suspected cases in Karaj (situated in the west of Tehran province) between June and September of 2008, we undertook this study to determine whether the disease was caused by cholera and to describe the prevalence of serotypes, route of transmission and antimicrobial resistance profile.

**Material and methods:** In this descriptive study 6505 Rectal swabs were collected from patients with acute gastroenteritis referred to hospitals in Karaj. Serotypes and biotypes of the isolates were determined by standard procedures. A checklist was developed to gather demographic information such as water supply properties and food or drinks consumed by the patient within 5 days before the onset of symptoms. Antimicrobial susceptibility of 45 Inaba and 30 non-agglutinating (NAG) strains was determined. All data were analyzed by SPSS 16.0.

**Results:** From 6505 specimens, 110(1.69%) were defined as *V. cholerae*, including 70(63.3%) *V. cholerae* O1 biotype El-Tor and serotype Inaba and 40(36.4%) NAG vibrios. Only 1 death was reported (case fatality rate = 0.9%). The majority of cases had access to safe potable water,

while 74.5% of patients had used uncooked vegetable during 5 days before the onset of symptoms. Antibiogram demonstrated that Inaba strains were 100% resistant to Nalidixic acid and Amoxicillin, 95.7% to Trimethoprim-sulphamethoxazole and 91.3% to Furazolidone while the highest resistance frequency in NAG vibrios was 77.4% to Erythromycin. The lowest resistance rate belonged to Ciprofloxacin to which just one NAG strain was resistant.

**Conclusions:** Despite similar studies that reported contaminated water as the main cause of cholera outbreaks, the majority of our study's patients benefited from safe potable water supply. According to the fact that most of the subjects had consumed uncooked vegetables and the report that vegetable farms of Tehran's suburbs are watered with drainage, consumption of uncooked vegetable might be the main route of cholera transmission in this outbreak. Comparison with the results of the latest epidemic in 2005, suggests a worrying increase in the resistance of *V. Cholerae* to several antibiotics. It also shows that Ciprofloxacin can still be used as the first line of Cholera treatment in this region.

## Gastroenterology

### The influence of aging and ethanol on oxidative stress parameters in rat liver and gastric mucosa

ESC-ID 323  
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**Aim:** The aim of our study was to investigate the influence of aging and ethanol on lipid peroxidation and antioxidative mechanisms in rat liver and gastric mucosa.

**Methods and Materials:** Adult (3 months) and old (18 months) male Wistar rats (n = 28) are divided into following subgroups: 1. control, 2. ethanol-treated groups. Ethanol was administered in 5 doses of 2 g/kg at 12-hour time intervals by orogastric tube. After last treatment liver and gastric mucosa samples were collected for determination of oxidative stress parameters.

**Results:** Ethanol induced a significant increase in malondialdehyde (MDA) level in gastric mucosa and liver in both adult and old animals in comparison with adult control group (pced in the gastric mucosa than in liver. The increased susceptibility of the gastric mucosa to lipid peroxidation is caused, at least partly, by greater reduction in GSH level in gastric mucosa. In aging an additional contributing mechanism to this susceptibility is greater SOD activity reduction in gastric mucosa.

### Hydatid disease of the liver-clinical presentation and WHO classification

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**Introduction:** Hydatid disease remains an important health problem in endemic areas, while due to the increased

mobility of a modern man it is becoming a worldwide concern. Hydatid disease the most commonly affects the liver. Hepatic cystic echinococcosis may be classified into five types according to the widely accepted imaging classification of Gharbi et al., based on ultrasonography patterns. Different types of cysts have different natural history and therapeutic implication. World health organization (WHO), gave a new classification with the intention to follow the natural history and imaging patterns of the hydatid cyst in 2003. WHO encourage physicians to compare their data on the occurrence of hydatid cysts in different parts of the world.

*Aim:* The aim of this descriptive study is to present our one year experience in examination of the patients with liver echinococcus cysts.

*Methods:* The study includes 52 patients treated for liver echinococcus cyst in the Institute for Digestive diseases, Clinical Center of Serbia, Belgrade (Serbia). We analyzed patients' data, symptoms (abdominal pain, nausea, vomiting, fever), and presence of extra hepatic cysts. All liver cysts were explored by ultrasonography, and data on their size, segmental localization, type and number were recorded.

*Results:* The study includes 16 male and 36 female patient, mean age 53.15±17.51 years (minimum 17; maximum 78). Abdominal pain was reported by 69.2%, nausea by 23.1%, and vomiting by 15.4% of patients. Fever was present in 3.8% of patients. No symptoms were reported by 38.5% patients. Spreading of the disease, and extra hepatic localization of echinococcus cysts were present in 11.5% of cases. Solitary echinococcus cyst has been found in 69.2% patients. In 19.2% patients we identified Gharbi type I cyst, in 29.8% Gharbi II, while Gharbi III type of cyst were determined in 33.7%, Gharbi IV type in 15.4% and Gharbi V in 1.9%. In vast majority of the cases cysts were localized in liver segment IV, VII, and III (34.6%, 23.1%, and 11.5% respectively).

*Conclusion:* This descriptive study based on application of ultrasonography, and standardized classification contributes to improvement of the knowledge on characteristics of hydatid disease and features of echinococcus cysts in Balkans. Also, this is a useful review of diagnostic standards. Key words Echinococcus granulosus, hydatid cyst, echosonography

### Differentiation of human stem cells using co-culture systems with hepatic cell lines

ESC-ID **618**  
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*Aim:* Co-culture systems of human embryonic and adult haematopoietic stem cells with murine hepatocytes were used to study the conditions and potentiality to differentiate them into hepatocyte-like cells in vitro.

*Methods:* For differentiation studies were the embryonic stem cell line SA002 and CD34+cord blood-derived stem cells used. CD34+cells were separated by density gradient centrifugation and magnetic cell separation. The differentiation potential was investigated by using direct co-culture with the murine hepatocyte cell line AML12 and conditioned media. Conditioned media was produced by culti-

vating AML12 cells with serum-free medium for diverse periods of time. The expansion and differentiation of both stem cell types were investigated. CD34+ and SA002 cells were cultivated for 1 or 2 weeks, respectively. Both cell types were cultivated in co-culture with AML12 cells or conditioned media. For ultrastructural analysis electron microscopy was used to determine morphological changes, closed cell-cell contacts and junction formation. Connexin (gap junction protein) 32 and 43 expression of both stem cell types were analysed by immunocytochemistry. After different time points of co-culture or cultivation with conditioned media a selected hepatic, endodermal expression pattern of both stem cell types were determined by Real-Time RT-PCR. Further investigation of cell-cell-interactions between CD34+ and AML12 cells were performed by calceinAM dye transfer.

*Results:* CD34+cells showed a low expression of Cx43 and a higher expression of Cx32. In contrast, SA002 cells possess a unique Cx43 expression and Cx32 is only expressed in areas of stem cell colonies. Conditioned media of AML12 cells induced a very low endodermal and hepatic differentiation of CD34+cells determined by Real-Time RT-PCR. There are no major morphological changes in the cell structure, except a higher production of phago(ly)some-like structures. Cultivation of SA002 cells with conditioned AML12 media induced distinct changes in cellular and ultrastructure morphology, indicating a differentiation towards an endodermal and hepatic lineage. On gene expression level changes in albumin expression were noticed. Direct co-culture of murine AML12 hepatocytes with stem cells, showed unincisive cellulare and morphological parameters of hepatic differentiation of CD34+ cells. In contrast, co-cultivation of SA002 with AML12 cells exerted several hepatic differentiation features by showing morphological similarities to early hepatic differentiated cells. High gene expression levels of early endodermal and hepatic markers like AFP or SOX17 in the SA002 cells were detected by Real-Time RT-PCR. CalceinAM dye transfer demonstrated dye exchange between AML12 and CD34+cells. Modulators of gap junction formation influenced this interactions, whereby retinol acid increased and 3-heptanol reduced the dye transfer.

*Conclusion:* In contrast to the restricted transdifferentiation potential of CD34+cells, the established co-culture systems proved a distinct differentiation potential of SA002-cells in vitro.

### The importance of determining the quotient of the number of platelets and diameter of spleen as well as the determination of diameter of portal vein in the assessment of esophageal varices

ESC-ID **162**  
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*Introduction:* Portal hypertension is a condition of increased pressure in the portal system, which is followed by increased flow in the portal vein and increased portal resistance. Bleeding from esophagus varices developed as a result of portal hypertension is serious complication in liver cirrhosis.

**Purpose:** Aim was to prove that quotient of the number of platelets and diameter of spleen greater than 909 has a 100% negative predictive value for the presence of varices esophagus. Also, to prove that the diameter of portal veins less than 15 mm has a negative predictive value for the presence of portal hypertension.

**Material and methods:** This retrospective study included 143 patients who were treated hospitaly in the KCS. In all patients complete clinical examination and laboratory was run: complete blood picture, hepatogram, coagulation factors, INR, viral markers, immunological analysis, lipidogram, ultrasound view with color Doppler-based, esophagogastroduodenoscopy

**Results:** quotient of the number of platelets and diameter of spleen in the group of patients with varices esophagus was 471, which had 100% positive predictive value for the presence of varices, while the quotient of the number of platelets and diameter of spleen in the group of patients without varices esophagus was 976, which had 100% negative predictive value for the presence of varices esophagus. In patients with esophagus varices average diameter of portal veins was 15.8 +2.11, and in the group without varices was 12.33 +2.04 (p <0.0002) and in this regard found statistically significant differences between these groups.

**Conclusion:** Based on the results of our study we can conclude that there is significant correlation between diameter of portal veins and varices esophagus. Also, we can conclude that the quotient of the number of platelets and diameter of spleen reater than 909 has a 100% negative predictive value for the presence of varices esophagus.

**Key words:** portal hypertension, esophagus varices, platelets

### **One week Furazolidone in combination with two weeks Amoxicillin, Bismuth subcitrate and Omeprazole for Helicobacter pylori eradication**

ESC-ID **212**  
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**Background:** Resistance to Metronidazole is one of the most common reasons for H-pylori treatment failure with classic triple therapy. The clarithromycin based regimen is not cost effective for developing countries . Furazolidone is a good substitution, but has many side-effects. Decrease Furazolidon treatment period to 1 week may be decrease the drug side effects.

**Methods:** 177 patients with duodenal ulcer , were randomized and received the following medications Group I : (First week: Omeprazole 2 \_20mg + Amoxicillin2\_1g+ Bismuth saubcitrate 4\_120mg + Furazolidon 2\_200mg) and ( second week: . the same regimen except Furazolidon) GroupeII: (First week: Omeprazole 2 \_20mg + Amoxicillin2\_1g+ Bismuth subcitrate 4\_120mg + Furazolidon 2\_200mg) and(second week: the same regimen except that Furazolidon followed by metronidazole) Control endoscopy was performed after 6 weeks.3 biopsies specimens from the antrum and 3from the corpus were taken for a urease test and histology. Eradication was concluded if all tests were negative for H-pylori .

**Results:** 157 patients completed the study . 2 patients from GroupeI and 3 from GroupeII didn't tolerate the regimen and were excluded. No serious complication was detected . Eradication rate by per-protocol Analysis (PPA) and intention -to-treat (ITT) were 89% and 79.3% in groupI and 86.6% and 74.4% in group II.

**Conclusion:** one week Furazolidone in combination with 2 weeks Amoxicillin, Omeprazole and Bismuth subcitrate is safe and, cost effective regimen for eradication of H-pylori. Adding Metronidazole to above regimen, didn't increase eradication rate.

## *Genetics*

### **Prenatal tests - the role and meaning of genetic amniocentesis**

ESC-ID **734**  
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Amniocentesis is one of the procedures carried out on a pregnant woman to obtain a prenatal examination, evaluate health condition of the fetus or to treat the high risk pregnancy. Because of many different complications which can occur during or after the intervention, we have to consider concrete indications depending from the age (in hbd) of pregnancy, the fetus and mothers condition. In this work we have analysed general and detailed indications for amniocentesis performed on 325 patients in year 2008 in The 2nd Gynaecological, Obstetrical and Neonatological Clinic in Wroclaw Medical University.

The aim of the work was to research the prevalence of each: non and relative indications and their recurrence in groups (general population, early/late amniocentesis, primiparas/multiparas). We have statistically analysed the results of biochemical noninvasive tests as implications to make a genetic amniocentesis. We have also analysed results of the genetic amniocentesis and obtained the prevalence of the chromosome abnormalities in general population.

**Results:** genetic amniocentesis was the most frequent (72%) among all amniocentesis (diagnostic and therapeutical amniocentesis were 28%). In general population the most frequent indication was the age above 35 years (49,6%) and the positive results of noninvasive tests (24,4%). Mostly used to coexist age of mother with abnormalities in ultrasonography or age with positive noninvasive biochemical tests. Serological incompatibility occurred in 20% Rh minus patients.

**Genetic amniocentesis:** the examination was made most often in 16th hbd, youngest pregnancy age-12 hbd, oldest one-23 hbd. Genetic amniocentesis was most frequently carried out in 2nd gestation, 2nd delivery. The number of fetus with chromosomal aberrations was 35 (10,7%). Most common were Down and Edwards syndrome.

**Findings:** 1. The role of genetic USG in detecting chromosomal fetus abnormalities has increased rapidly during last years. 2. Genetic amniocentesis is an ultimate prenatal examination which confirms the chromosomal abnormalities, but doesn't always show the final fetus condition. 3.

False positive biochemical tests results were a significantly common indication to genetic amniocentesis.

**Key words:** genetic amniocentesis, genetic USG, noninvasive genetical tests, chromosomal aberrations

## Gynaecology

### Spontaneous abortion: Chlamydia trachomatis in as one of possible etiological factors

ESC-ID **1038**  
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**Background:** Timely detection and treatment of Chlamydia trachomatis infection during pregnancy is an important public health issue. It has been suggested that there is association between Chlamydia infection and spontaneous abortion, post partum endometritis, preterm labor and delivery, premature rupture of the membranes and low birth weight babies.

**Aim:** The study was undertaken to determine the association between Chlamydia trachomatis infection and symptoms of spontaneous abortion.

**Methods:** The chosen sample underwent testing by a direct immunofluorescence method of determining the Chlamydia trachomatis antigens in endocervical smear in patients with symptoms of spontaneous abortion (contraction of the uterus, moderate effacement of the cervix, bleeding for more than the 7 days).

**Results:** The investigation comprised 37 patients with symptoms of spontaneous abortion and 20 women's with normal course of pregnancy. In the investigated group the positive finding of Chlamydia trachomatis was recorded in 8 (21.6%) patients in the cervix and in 2 (10%) patients from control group. Our study has documented the efficacy of Erythromycin treatment in eradicating Chlamydia infection in pregnant women and preventing vertical transmission of Chlamydia to neonates.

**Conclusion:** The results of investigations point to the necessity of diagnostics and treatment of Chlamydia trachomatis in prevention of spontaneous abortion particularly in the women with previous unsuccessful pregnancies.

**Key words:** spontaneous abortion, Chlamydia trachomatis

## Haematology/Oncology

### “Human placental extracts in the prevention of radiation-induced oral mucositis”

ESC-ID **230**  
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**Introduction:** Pain and mucositis reduces the quality of life of oral and oropharyngeal cancer patients undergoing radiation therapy<sup>1</sup>. The reactions start from the second week and worsen as the treatment continues. Several topical and systemic agents are directed to the decrease and the acceptance of this acute toxicity, but few have shown

a significant effect<sup>2,3,4</sup>. Human placental extract has been used as biological dressing in various conditions such as ulcers, oral submucous fibrosis and burns<sup>5,6</sup>.

**Aim:** To evaluate the effects of injections of human placental extracts in the prevention of oral mucositis in patients of oral and oropharyngeal cancers undergoing radiation therapy.

**Methods and Materials:** The study accrued 42 patients who underwent radiotherapy on telecobalt with 60 Gy in 30 fractions over a period of 6 weeks. The patients were randomized to test where patients received injections of human placental extracts 2ml deep intramuscular along with radiation (5 days a week for 15 days, starting from the second week of radiation) and control arm who received symptomatic treatment with anesthetic antacid gels. Patients were assessed at baseline and once a week till treatment completion with mucositis, swallowing status and pain.

**Results:** It was observed that the onset of mucositis and pain was delayed and of lesser grade in the test group as compared to the control group. They also had better swallowing and salivary status.

**Conclusion:** Human placental extracts when administered intramuscularly reduces the radiation induced mucositis and pain in patients undergoing radiotherapy. These injections are safe to use, does not produce any local reaction and improves the quality of life.

### Comparing preoperative and postoperative diagnosis in 490 patients with non-neoplastic thyroid diseases who had a thyroidectomy

ESC-ID **187**  
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**Aim:** There are always discrepancies between preoperative and postoperative diagnosis of thyroid diseases. This discrepancy has been proven in studies comparing preoperative fine needle aspiration (FNA) results with postoperative pathologic diagnosis. We aimed to compare preoperative diagnosis of non-neoplastic thyroid diseases with their pathological diagnosis.

**Methods and Materials:** We conducted a retrospective study in Imam Khomeini and Amir Alam hospitals, Tehran, Iran, in which 490 patients with pathologically confirmed non-neoplastic thyroid diseases who received surgery from September 1996 to June 2006, were recruited. Preoperative diagnosis, clinical and paraclinical figures were gathered. Postoperative surgical diagnoses were compared with preoperative diagnoses and clinical figures.

**Results:** According to pathology results 83.7% of cases were goiter (nodular, diffuse and adenomatous), 7.1% were hyperplasia (Grave's disease), 9.2% thyroiditis (Hashimoto's, lymphocytic, chronic, granulomatous thyroiditis and Riedel's). 18.8% cases were male and 81.2% were female (female: male ratio of 4:1) with average age of 38.7 years (range 12-82). More frequent preoperative diagnosis were nodular goiter in 34.7% cold nodule of thyroid in 31.4% and 8.6% of patients operated with preoperative diagnosis of thyroid malignancy, other preop-

erative diagnosis were less frequent. Preoperative diagnosis of thyroid malignancy was significantly higher in thyroiditis in comparison with goiters (nodular, diffuse and adenomatous) and hyperplasia (p-value = 0.009 and p-value = 0.005 respectively). Also lymphadenopathy was significantly higher in thyroiditis cases. Compression symptoms were significantly lower in cases of thyroid hyperplasia and ophthalmic signs were higher in this group. No significant difference among these 3 groups was found for pain, tenderness and fixation of thyroid mass.

**Conclusion:** Our study showed that among postoperative diagnosis of non-neoplastic thyroid diseases, thyroiditis has the most possibility of preoperative misdiagnosis as thyroid cancer. Maybe more evaluations for thyroiditis (checking antithyroid peroxidase antibody) in addition to other signs and symptoms and FNA can help to confirm benign nature of disease before surgery. **Keywords:** thyroidectomy, preoperative diagnosis, non-neoplastic thyroid disease

### The role of beta1-integrin for the formation of medulloblastoma

ESC-ID 746

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Besides leukemia medulloblastoma is one of the most common malignant tumors in childhood. Despite enormous advances in diagnostics and therapies, the 5-year overall survival rate is still only between 50 and 70% and surviving patients suffer from irreversible neurological and cognitive problems caused by treatment. Therefore, it is important to develop more targeted therapies. Previous studies in mice showed that the function of the adhesion cell receptor beta1 integrin plays a critical role for induction and maintenance of breast cancer. Moreover, beta1 integrin expression is correlated with a bad survival of human breast cancer patients. The role of beta1 integrin in tumorigenesis of medulloblastoma remains unclear, although it has crucial roles in the proliferation of cerebellar granule neurons precursors (GNPs), which are believed to represent the cell of origin for medulloblastoma. To determine the clinical relevance of beta1 integrin in medulloblastoma, we have analyzed mRNA levels of beta1 integrin in human medulloblastoma tissue from 21 patients using quantitative real-time PCR. We detected that beta1 integrin is highly overexpressed in all subtypes of medulloblastoma in comparison with normal cerebellar tissue. Survival of medulloblastoma patients, however, seems not to be associated with the expression of beta1 integrin in our series. In order to investigate the role of beta1 integrin during medulloblastoma formation and maintenance, we used a conditional Cre/loxP-based mouse model with an attempt to activate the sonic hedgehog signalling pathway and consequently generate medulloblastoma from GNPs and, at the same time in the same cellular compartment, to delete beta1 integrin. Preliminary analyses of 3-days-old mice revealed significantly smaller tumors in mice with homozygous deletion of beta1 integrin as compared to heterozygous mice (p less

than 0,05). Surprisingly, adult mice showed no differences in survival or morphology of the tumors, and expression of beta1 integrin was similar in all tumors. Thorough analysis of the tumor mRNA proved incomplete recombination of the beta1 integrin allele and a strong subsequent selection for tumor cells with wildtype beta1 integrin. These results suggest a critical role for beta1 integrin during the formation of medulloblastoma and further studies are needed to evaluate whether beta1 integrin may serve as a target of more specific medulloblastoma treatments.

### In vitro / in vivo differentiation potential of human embryonic H1 and SA002 stem cells

ESC-ID **254**

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**Aim:** The aim of these study was the comparison of human embryonal stem cells H1 and SA002 concerning to their spontaneous differentiation potential in vitro, teratoma formation capacity and germ layer formation in immunodeficient NOD/SCID mice.

**Method:** Undifferentiated H1 cells were cultivated on mouse embryonic feeder cells (MEF), whereas undifferentiated SA002 stem cells grown on human foreskin fibroblast (HFF) in presence of 4ng/ml basic fibroblast growth factor (bFGF) for 7 days. For spontaneous differentiation the embryonal H1 and SA002 cells were cultured on matrigel coated dishes without feeder cells in absence of bFGF for 14 days. Differentiation potential was evaluated by real time-PCR using hepatocyte specific primers, immunohistochemistry with markers for the undifferentiation state and by histopathology. For real time-PCR studies RNA was isolated with a Qiagen-Micro- or Minikit. Undifferentiated H1 and SA002 stem cells were sub cutaneously (s.c.) transplanted into NOD/SCID mice for evaluation of in vivo differentiation potential (teratoma formation capacity). Mice were sacrificed after 49 and 106 days, teratomas were shock-frozen for RNA-isolation and paraffin slices of teratomas were prepared and stained with hematoxylin/ eosin (H/E) for histopathology.

**Results:** Both embryonic cell lines express the pluripotent markers Oct-4, SSEA, TRA-1 and alkaline phosphatase. In addition, these cells were characterized by low SOX17, CXCR4, AFP, HNF3b, C/EBP $\alpha$  and nestin transcripts, as revealed by real time-PCR, but albumin was not detectable. In vitro differentiation of the cells in the absence of bFGF and cultivation on matrigel without feeder cells revealed spontaneous differentiation of both cell lines and increased the albumin, AFP, CK19, nestin and C/EBP $\alpha$  expression. In vivo differentiation of embryonic stem cells was induced by s.c. transplantation of undifferentiated stem cells in NOD/SCID mice. The results demonstrate comparable low teratoma growth of both cell lines during the time period up to 56 days. Both cell lines showed predominantly differentiation into cells of mesodermal and endodermal cell types. After s.c. application no cell dissemination into other mouse organs was found

by human PCR. With respect of the in vivo differentiation potential, both embryonic cell lines showed comparable germ layer formation capacity in NOD/SCID mice.

**Conclusion:** The results suggest, that both H1 and SA002 stem cells showed in vitro and in vivo an endodermal differentiation potential and were suitable for application of directed hepatic differentiation.

### Improved nonviral gene transfer and transgene expression by using the minimalistic MIDGE-vector system

ESC-ID **329**  
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**Aim:** Plasmid-based vector systems belong to the most widespread vectors used in clinical gene therapy trials. However, their transfer efficiencies are comparatively low and minor safety concerns still remain due to bacterial backbone sequences. We have previously shown in a phase I gene transfer clinical trial that transfer of plasmid-DNA by intratumoral jet-injection is safe and results in transgene expression. However, the plasmid-vector design, size and limited expression efficiency demands further improvements. Due to these facts, great efforts are made for nonviral vectors to reduce size and proportion of needless sequences to improve transfer- and expression efficiencies.

**Methods and Materials:** The minimalistic immunologically defined gene expression (MIDGE, Mologen AG, Berlin, Germany) vector system is a nonviral linear DNA molecule that is depleted of any bacterial origin or replication backbone sequence, resistance genes or additional CpG islands, which significantly reduces vector size. This makes MIDGE of high value with respect to potential clinical use. We analyzed transfer and reporter gene expression efficiency of the MIDGE-vector compared to plasmid-vectors in different human colon carcinoma and melanoma cell lines (HCT116, SW480, A375, SK-MEL-28, MeWo) in vitro by luciferase reporter assay and quantitative RT-PCR after lipofection and electroporation. Additionally, we tested a human tumor necrosis factor alpha (hTNF $\alpha$ ) expressing MIDGE-vector (MIDGE-CMVhTNF) for in vivo application. Vector distribution (blood and organs) and tumor vector load were analyzed by quantitative PCR and transgene expression was analyzed by ELISA following gene transfer by intratumoral jet-injection in nmri nu/nu mice with pre-established A375 melanomas.

**Results:** These analyses revealed that MIDGE-mediated luciferase reporter expression is significantly higher in the tested cell lines compared to plasmid-mediated luciferase expression at mRNA and protein level. By using the more efficient MIDGE system for TNF- $\alpha$  expression, again this vector led to an increase in transgene expression compared to the respective plasmid vector. In vivo the MIDGE vector led to efficient transgene expression. This expression was highest 1 to 3 days after jet-injection and was strictly DNA-dose dependent. Measurement of blood samples using real-time PCR revealed the low leakiness and rapid clearance of the MIDGE-vector DNA in all animals.

**Conclusion:** These in vitro and in vivo studies indicate that the MIDGE-vector system leads to improved transgene expression and has great potential for clinical use in local cancer gene therapy.

### Tumor-specific suicide gene therapy using the Clostridium perfringens enterotoxin (CPE)

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**Introduction:** Clostridium perfringens enterotoxin (CPE) is a toxic protein, which is produced by the bacterial Clostridium strain type A. The Claudins 3 and 4 have been identified as the specific cellular receptors for CPE. Claudins are transmembrane proteins found in tight junctions of epithelial cells and are involved in the regulation of the cell permeability. It has been shown, that CPE binds to claudin 4 with higher affinity than to claudin 3. Binding of CPE to these receptors triggers formation of membrane pore complexes leading to rapid cell death. Interestingly, claudin 3 and 4 were found highly overexpressed in numerous human epithelial tumors such as breast, ovarian, colon and pancreatic cancer. This tumor-specific overexpression can be exploited for the selective anti-tumor action of CPE. The anti-cancer activity of external application of recombinant bacterial CPE has already been reported by others. The aim of this study was to achieve tumor cell killing by CPE gene transfer. For this approach, we generated expression vectors bearing "wild-type" CPE gene or modified CPE gene, which has been codon-optimized for eucaryotic expression. The CPE-expressing vectors were tested for in vitro and in vivo gene therapy of claudin 3 and claudin 4 overexpressing cancer cell lines.

**Methods:** The "wild-type" CPE gene and the optimized variant of the CPE gene were cloned into the eucaryotic expression vector pCpG-mcs-G2. To both CPE gene variants was added the Kozak sequence. For the in vitro gene transfer experiments the human tumor cell lines- HCT116 (colon cancer), MCF-7 (breast cancer), Panc-1 (pancreatic cancer) and SK-MEL-5 (melanoma) were tested for claudin 3 and claudin 4 expression at the mRNA and protein level by quantitative real-time PCR and Western-blotting, respectively. These cell lines were transfected with the generated CPE expressing constructs. CPE expression was analyzed by quantitative real-time PCR and by Western-blotting. The cytotoxicity of CPE was analyzed 24, 48 and 72 hours after transfection by cell counting. The effect of gene transfer of the optimized CPE in vivo was tested in MCF-7 tumor-bearing nude mice.

**Results:** The CPE expression analysis at the mRNA and protein level revealed, that the optimized CPE is significantly higher expressed compared to the "wild-type" CPE. Furthermore, the expression of optimized CPE showed improved cytotoxic activity, compared to the "wild-type" CPE. The highest cytotoxic effect (up to 70 % dead cells) was observed 48h after gene transfer. The cytotoxicity of

CPE is clearly dependent on presence and level of claudin 4 expression. MCF-7 cells with the highest claudin 4 expression showed the highest sensitivity to CPE. The in vivo gene transfer of optimized CPE led to reduced tumor growth in MCF-7 tumor-bearing mice compared to the untreated control group.

**Conclusion:** This result provides first evidence, that the optimized CPE can be used for a novel suicide gene therapy of claudin 3 and 4 overexpressing tumors, leading to a rapid and efficient cell killing in vitro and in vivo.

### **In vivo imaging of colon cancer metastasis in mice: Impact of the newly identified gene MACC1**

ESC-ID **956**  
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The prognosis of survival in case of colorectal cancer strongly depends on the occurrence of metastases. Currently used markers turned out to be insufficient to predict development of distant metastasis already for early stages. Our workgroup identified the previously undescribed gene MACC1 (metastasis-associated in colon cancer-1). MACC1 is an important prognostic indicator for metastasis formation in colon cancer. It induces migration, invasion and proliferation in vitro as well as metastases in several mouse models. Moreover, MACC1 transcriptionally controls the gene encoding the receptor tyrosine kinase Met, thereby activating the hepatocyte growth factor (HGF)/Met signalling pathway. In this study, we aim at in vivo imaging of metastasis formation in colon cancer xenograft mouse models. Therefore, we generated constructs which contain the MACC1 gene and the reporter gene firefly luciferase in an IRES-vector. In order to evaluate the importance of the SH3 domain (Src homology 3 domain) occurring in wild type MACC1, we also generated IRES-constructs that harbour mutant MACC1 (with a deletion of the SH3-domain) together with the reporter gene. Constructs harbouring the reporter gene exclusively served as controls. Successful cloning was confirmed by using specific restriction endonucleases as well as by sequencing. Constructs were then transfected into colon cancer cells that intrinsically express MACC1 at very low levels. We generated stably expressing clones for wild type MACC1 and for mutant MACC1, respectively. Clones are characterized by determining luciferase activity via bioluminescence measurements and by MACC1 expression using quantitative real time RT-PCR. The functional impact is evaluated by performing migration experiments and the most effective clones are selected for in vivo imaging. Mice are examined three times a week by measuring the bioluminescence with a high sensitive in vivo imager. These techniques allow the monitoring of the development of metastases, a more detailed understanding of the metastasis organ tropism, and of metastasis kinetics. Thereby, the foundation for intervention strategies with the aim of metastasis prevention will be created.

### **Enhanced in vitro uptake of noncytotoxic magnetic nanoparticles in the presence of external magnetic field**

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Following the fast development of magnetic nanotechnology and its implication to the medicine, magnetic nanoparticles' strict physical and chemical properties, low toxicity and increased cell internalization nonetheless, are required. According to these concerns silica coated maghemite nanoparticles were prepared and dispersed in a physiological medium before testing for cytotoxicity in different cell lines in vitro and its cellular internalization and trafficking pathways determined in the absence and presence of external magnetic field. Silica (SiO<sub>2</sub>) coated maghemite ( $\gamma$ -Fe<sub>2</sub>O<sub>3</sub>) nanoparticles were synthesized in situ by the precipitation in microemulsion system water/SDS,1-butanol/cyclohexane. The material was dispersed in 0.9% sodium chloride solution before it was autoclaved and pH measured. The size and morphology of the nanoparticles were evaluated using transmission electron microscopy (TEM) and X-ray diffractometry (XRD). Mean maghemite crystallite size was calculated according to the broadening of the X-ray diffraction pattern using the Debye-Scherrer formula. By magneto-susceptometer the specific magnetization of the prepared samples was measured and hysteresis loop was obtained. The potential cytotoxicity of nanoparticles to human mesothelial (Met-SA), mouse fibroblast (L929), human melanoma (SK-MEL28) and human hepatocellular carcinoma (HepG2) cells was assessed by MTS assay. Two groups of L929 cells were given pulse of magnetic nanoparticles when exposed to NdFeB permanent magnets for 1 h while one group was followed by a 3 h chase. The cellular uptake and trafficking pathways of magnetic nanoparticles were semi-quantitatively evaluated in the absence and presence of external magnetic field by TEM using a pulse-chase approach. The results of TEM analysis demonstrated monodispersity of core-shell type nanoparticles without changes in the properties of the autoclaved material. Measured pH was 6.9 directly after the preparation of dispersion and was not significantly changed after a period of 5 months when the pH value was 7.1. According to X-ray diffraction characteristic peak of the spinel structure of maghemite, the mean crystallite size was 7.8 nm. Hysteresis loop obtained by magneto-susceptometer demonstrated superparamagnetic behaviour of the nanoparticles with the specific magnetization of 37.9 Am<sup>2</sup>/kg. No cytotoxicity to Met-SA, L929, SK-MEL28 and HepG2 cells exposed to different concentrations of nanoparticles dispersion was observed, however the highest concentration minimally decreased the survival of cells in all cell lines. Cellular uptake in L929 cells of magnetic nanoparticles was enhanced when exposed to the external magnetic field resulting in increased growth in number and size of endocytotic vesicles, however no difference in internalization between the pulse and pulse-chase group of L929 cells was observed. This study demonstrates in a physiological medium stable silica coated maghemite nanoparticles, synthesized by the precipitation in microemulsion system water/SDS,1-



butanol/cyclohexane, which possess principal properties required for application to biological materials, being noncytotoxic and having enhanced cellular internalization in the presence of external magnetic field.

### Identification, morphological and functional analysis of a panel of novel DCIS markers

ESC-ID **850**  
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The ductal carcinoma in situ (DCIS) of the mammary gland represents an early, pre-invasive stage in the development of invasive breast carcinoma. Since the introduction of high quality mammography screening diagnosis of DCIS has increased, but only DCIS with microcalcification ( and causes the development of DCIS around month 3 after lactation. Later DCIS progresses into invasive adenocarcinoma. In order to analyze the expression profile at transcriptional level from different stages within this complex process, Affymetrix GeneChip® analysis of mammary glands of different time points after whelping was performed. First, genes differentially expressed between invasive tumors and controls were extracted from the data set. In order to look for genes that already indicate the initiation of DCIS, mice from month 1 to 3 after lactation were compared to controls as well. Genes significantly expressed in both comparisons represent potential candidate genes for early DCIS detection. Genes with constant upregulation during DCIS-development and low variance within the groups were chosen as final candidate genes. Expression of the seven most interesting genes was validated by quantitative RT-PCR. As a next step these genes were analyzed in human DCIS samples. This analysis revealed a strong upregulation of the candidate genes in human DCIS leading to the conclusion that they work as early detection markers in human DCIS, as well. Hierarchical clustering using our seven candidate genes showed that DCIS and invasive carcinomas cluster together apart from healthy samples. Expression of our candidate genes in human tissues was validated by quantitative RT-PCR and semiquantitatively by immunohistochemistry. Our newly detected set of tumor markers, which effectively distinguish between healthy and DCIS serve as a further step in improving prognosis and therapy of DCIS.

### Sensitivity and Specificity of Photodynamic Diagnostics in Detection of Malignant Pharyngeal Tumours

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*Background:* The aim of this work was to evaluate sensitivity and specificity of photodynamic diagnostics in detection of malignant pharyngeal tumours.

*Material and methods:* 63 patients with 83 verified malignant pharyngeal tumours were examined and treated in Vilnius University Institute of Oncology (VUOI) – they underwent sensitized tumour therapy (STT) and photodynamic diagnostics of pharyngeal tumours: after 24 hours of intravenous photosensitizer infusion their oral and pharyngeal mucosa was irradiated by a diode, emitting white and violet (405 nm length) light. With consent, photodynamic diagnostics of pharyngeal and oral cavities was also applied to 30 patients who underwent sensitized tumour therapy in VUOI for treatment of tumours of other locations in order to evaluate the frequency of fluorescence artefacts. These patients who exhibited artefacts were asked to clean their teeth; afterwards photodynamic diagnostics was repeated for them.

*Results:* When inspecting normal mucosa, only ordinary violet light was seen. However malignant tumours exhibited intense red fluorescence. The margins of the fluorescence were conterminous with the tumour margins, determined by morphologic tests. All malignant pharyngeal tumours except melanoma (6 patients, 7 tumours) exhibited specific red fluorescence. The sensitivity of photodynamic diagnostics of pharyngeal tumours is 90,5%. 8 out of 63 patients with pharyngeal tumours exhibited fluorescence artefacts – additional radiation of lilac shade in healthy mucosa. Artefacts of the same kind were also observed in 17 out of 30 patients who underwent STT for treatment of tumours in other locations (not pharynx). Five of them exhibited only weak fluorescence. The specificity of photodynamic diagnostics of pharyngeal tumours is 77,27%. Fluorescence artefacts were observed mostly in the gums (22 patients) and in the radix of the tongue (7). The colour of the false fluorescence was different from the colour, typical for malignant tumours – its shade was more lilac than red. According to literature, such additional fluorescence could be caused by oral microorganisms, however, it remained after teeth brushing.

*Conclusions:* Photodynamic diagnostics is an effective method of detection of pharyngeal tumours of all histologic types, except melanoma. Specificity of photodynamic diagnostics is less in tumours, located in gums and in the radix of the tongue because fluorescence artefacts were common in these sites. The colour of fluorescence artefacts differs from the colour, typical for malignant tumours – it is of lilac, but not of red shade.

## Immunology

### Genetic susceptibility to Graves' ophthalmopathy: the role of polymorphisms in pro-inflammatory cytokine genes

ESC-ID **248**  
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*Purpose:* In order to investigate the underlying genetic mechanisms of Graves' Ophthalmopathy (GO), this study examined the association of single nucleotide polymor-

phisms in the five important pro-inflammatory cytokines, namely IL-12, TNF- $\alpha$ , IFN- $\gamma$ , IL-2 and IL-6 with GO in a sample of Iranian adults.

**Methods:** A total of 57 patients of Graves' disease without GO, 50 patients with GO and 140 healthy controls were enrolled. Patients were recruited consecutively from the outpatient endocrine clinic of a large university general hospital. Genotype and allele frequencies of the following pro-inflammatory cytokines were compared between the groups: IL-12(-1188A/C), TNF- $\alpha$  (-308A/G, -238A/G), INF- $\gamma$  (UTR 5644A/T), IL-2 (-330T/G, 166G/T), and IL-6 (-174C/G, nt565A/G). A corrected (for multiple testing) P value (Pc) less than 0.05 was considered statistically significant.

**Results:** The IL-12 -1188C allele (OR = 2.65, Pc:0.00) and CC genotype (OR = 7.58, Pc:0.00) were significantly more frequent in patients with GO than patients without GO. The TNF- $\alpha$  -238A allele was more frequent in patients with GO than those without GO (OR = 2.99, Pc:0.00). The frequency of the IFN- $\gamma$  UTR 5644T allele (OR = 2.67, Pc:0.045), AT (OR = 13.33, Pc:0.04) and TT (OR = 18.46, Pc: 0.00 respectively) genotypes were significantly higher among patients with GO than those without GO. No significant association was found for other polymorphisms.

**Conclusions:** We demonstrated that specific polymorphisms in IL-12, IFN- $\gamma$  and TNF- $\alpha$  genes are associated with susceptibility to GO in the Iranian population. Our results open a new perspective for future studies on the genetic associates of Graves' ophthalmopathy.

### Construct of GM-CSF-BRAFV600E fusion protein

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**Introduction:** Malignant melanoma is the most lethal and metastatic form of skin cancer. In approximately 70% of human melanomas, position 600 of BRAF protein is mutated from Valine to Glutamic Acid (BRAFFV600E). Because BRAFFV600E is only expressed in human tumors, it provides a potential immunotherapeutic target. Since BRAFFV600E is expressed intracellularly, it is a target for T cells. In order to induce cellular immunity, we are working on the development of vaccines with high probability of inducing T cell immunity. One of our vaccine approaches is the production of GM-CSF- BRAFFV600E or GM-CSF-BRAFFWT fusion proteins. GM-CSF is an immune modulator that amplifies the immune response, acting as an immune adjuvant. Published research had shown that GM-CSF fused to PAP (prostatic acid phosphatase) induces tumor protective immunity in cancer patients, despite the presence of this protein in normal pancreatic tissue.

**Methods and Materials:** GM-CSF cDNA was obtained by isolating RNA from stimulated spleen cells followed by GM-CSF specific RT-PCR. We produced two variants of GM-CSF gene: long and short [with and without (Gly4Ser)<sub>3</sub> flexible linker]. This flexible linker was included, to evaluate if it is necessary for the biological activity of GM-CSF (e.g. protein folding). BRAFFV600E, BRAFFWT and GM-CSF cDNAs were cloned into intermedi-

ate vectors, then subcloned into the final vector pFastBac.

**Results:** The BRAF kinase domain was amplified by PCR from full length cDNA clones. The isolated PCR products were cloned into pCR-BluntII-TOPO vector. Digestion with Eco RI confirmed the presence of the cloned inserts. These fusion proteins will be expressed in insect cells, purified by affinity chromatography, followed by their functional analysis. Finally mice will be immunized with different vaccines, including these fusion proteins, and the immune response will be measured to determine the optimal vaccine.

### The evaluation of osteocalcin and Osteoprotegerin, as osteogenic bone remodeling markers in rheumatoid arthritis

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**Introduction:** Osteoprotegerin (OPG) is a 60 kDa glycoprotein, which belongs to the family of TNF's receptors (TNF-R) having strong functions such as bone protection. It is a protector factor of the bone, being considered a 'decoy receptor' and a strong inhibitor of RANKL binding RANK receptor. Osteocalcin (OC) is a protein that contains three residues of the amino acid gamma-carboxyglutamic acid (Gla), which, in the presence of calcium, promotes binding to hydroxyapatite and subsequent accumulation in bone matrix.

**Aim:** The aim of our study is to evaluate some osteogenic markers involved in bone remodeling, such as the estimation of osteocalcin (OC) and osteoprotegerin (OPG/RANK) concentrations in the synovial fluid as well as in the serum of rheumatoid arthritis (RA) patients.

**Material and Methods:** This retrospective study held on a period of 2 years, included a group of patients diagnosed with RA (n = 76) and a group of healthy subjects (n = 70). The evaluation of OPG and OC were performed in the initial stage of the disease, without using any anti-inflammatory non-steroidal therapy for at least 1 month before our study began, using the immunoenzymatic technique ELISA. As for data analysing, we used t-Student test.

**Results:** The concentration of OPG in the synovial fluid represented  $14.25 \pm 0.75$  pmol/l while in the serum the concentration was  $1.69 \pm 0.80$  pmol/l, which was significantly increased compared to the values determined in the serum of healthy controls ( $1.19 \pm 0.30$  pmol/l). The increase of OC concentration evolves in the same manner in both sides of our measurements, the values being of  $6.43 \pm 4.80$  ng/ml and  $19.9 \pm 4.8$  ng/ml respectively, compared to the values determined in healthy controls, in the synovial liquid ( $0.5$  ng/ml) and in the serum ( $3.1 \pm 0.1$  ng/ml).

**Conclusion:** High levels of OPG found in the synovial liquid of patients with RA, leads us to conclude that it is a process localized only at articular level. High levels of OC demonstrates that in RA, it is strongly stimulated the mineralization of bone matrix. The conclusion of our study demonstrates that OPG and OC are markers of bone metabolism, with strong correlations concerning bone remodeling, by stimulating the osteoblasts (bone synthesis).

## The stem cell niche

ESC-ID 75  
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In spite of the advances in the knowledge of adult stem cells (ASCs) during the past few years, their natural activities *in vivo* are still poorly understood. Mesenchymal stem cells (MSCs), one of the most promising types of ASCs for cell-based therapies, are defined mainly by functional assays using cultured cells. Defining MSCs *in vitro* adds complexity to their study because the artificial conditions may introduce experimental artifacts. Inserting these results in the context of the organism is difficult because the exact location and functions of MSCs *in vivo* remain elusive; the identification of the MSC niche is necessary to validate results obtained *in vitro* and to further the knowledge of the physiological functions of this ASC. Here we show an analysis of the evidence suggesting a perivascular location for MSCs, correlating these cells with pericytes, and present a model in which the perivascular zone is the MSC niche *in vivo*, where local cues coordinate the transition to progenitor and mature cell phenotypes. This model proposes that MSCs stabilize blood vessels and contribute to tissue and immune system homeostasis under physiological conditions and assume a more active role in the repair of focal tissue injury. The establishment of the perivascular compartment as the MSC niche provides a basis for the rational design of additional *in vivo* therapeutic approaches. This view connects the MSC to the immune and vascular systems, emphasizing its role as a physiological integrator and its importance in tissue repair/regeneration.

## Infiltration of regulatory T cells (Tregs) in the brain after traumatic injury

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Regulatory T cells (Treg) play a major role in controlling immune responses under physiological conditions as well as in various systemic and central nervous system inflammatory diseases. However, little is known about the mechanisms of the induction of Tregs after traumatic brain injury. So far, investigations from several animal models such as entorhinal cortex lesion (ECL) revealed that although autoreactive T cells are present in traumatic CNS injury, white matter damage such as found in disseminated autoimmune encephalomyelitis, does not occur, even when highly susceptible mouse strains were employed. Thus, the initial expansion of myelin-specific T cells and subsequent inflammation in regions of (traumatic) axonal degeneration seems to be transient and eventually is kept under tight control, implying active maintenance of immune tolerance. Aim of this study was to investigate the temporary infiltration and localization of Tregs after ECL. ECL was performed in FoxP3EGFP<sup>tg</sup> mice, and T cell and Treg infiltration was analyzed by flow cytometry on day 7, 14, and 30 after ECL. Tregs were identified by the

expression of CD4 and Foxp3. We could find at each day a significant increase of T cells as well as Tregs in the ipsilateral hemisphere with a maximum on day 14 compared with infiltrates of sham operated mice. Additionally, an increase of Tregs in the CD4<sup>+</sup>/Tregs ratio in the ipsilateral hemisphere at day 14 compared to day 7 occurred. Interestingly, CD25 was downregulated in the Tregs subset on day 7 and 14, which raises the question whether proliferation or neogenesis of Tregs takes place in the brain. In order to localize Foxp3<sup>+</sup> T cells in the brain, cryosections were stained with antibodies directed against CD3 and EGFP on day 14 after ECL. CD3<sup>+</sup>FoxP3<sup>+</sup> cells were detected directly within the lesion and in the parenchyma adjacent to the lesion. Further investigations are necessary to differentiate between infiltrations versus neogenesis and to determine the regulatory potential of the Tregs after ECL. Elucidating the trafficking and function of Tregs in the context of ECL may lead to a better understanding of general mechanisms involved in adaptive immune responses after CNS trauma.

## Effect of calcium-rich diet on calmodulin containing spleen macrophages

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*Background:* Calmodulin (CM) - a calcium ion (Ca<sup>2+</sup>)-sensor protein, is ubiquitous distributed in eukaryotes. CM - an intracellular calcium receptor, which can bind up to four Ca<sup>2+</sup>, change its conformation and regulate cellular functions. CM modulates biological activities of many cellular proteins, enzymes and ion channels. Changes in intracellular free Ca<sup>2+</sup> are involved in the transmembrane signalling of different cells, including spleen macrophages. Activated macrophages are play important roles in the induction and maintenance of innate immune defence.

*Aim:* The aim of this study was the investigation of spleen macrophages structure, which contain calmodulin; quantitative analysis and optical density measurement of calmodulin immunoreactivity on their membrane in norm and after calcium rich diet.

*Methods:* All experimental animals (32 male rats) were divided in two groups: first group - control animals (n = 16) drank normal water without addition of microelements; animals from second group (n = 16) received water with high level of Ca<sup>2+</sup> (CaCl<sub>2</sub>, concentration 200 mg/l) during two months. Spleens were removed from deep anesthetized animals and fixed in formaldehyde. Cryosections were immunostained with anti-calmodulin antibody.

*Results:* Microscopic analysis of non-treated animal's group revealed three populations of calmodulin immunoreactive spleen macrophages: big (141–291 micron<sup>2</sup>), middle (51,5–140 micron<sup>2</sup>) and small (6,4–51,4 micron<sup>2</sup>). They were predominantly distributed in red pulp and along the border zone of sinuses. In white pulp there were detected only single CM-positive cells. Morphologically calmodulin containing spleen macrophages have round shape with irregular body sides and exhibited relatively homogenic CM-immunoreactivity. Two months calcium-rich diet lead to increasing of spleen follicles size, some follicles joint each other, the volume of CM

containing macrophages increased. The number of middle and big cells increased on 16,3% and 5,9% respectively. But the quantity of small macrophages significantly decreased on 23%. Quantitative analysis in 5 fields of view demonstrated the dramatically decreasing of common number of calmodulin containing spleen macrophages: 353 cells in control vs. 178 cells in calcium treated group. The optical density of membrane protein – calmodulin – in control group is 0,55 CU. After calcium-rich diet this density slightly increased till 0,56 CU.

**Conclusions:** Our study demonstrated compensatory reaction of spleen macrophages after two months calcium-rich diet. Decreasing of macrophages number and at the same time increasing of their size and volume can indicate the stimulation of phagocytic activity of spleen calmodulin containing macrophages and have favourable effect on immune status of organism.

## Infectiology

### Clinic and microbiological characteristics of patient with sepsis

ESC-ID **114**  
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**Introduction:** Sepsis is a multisystemic inflammatory response syndrome to microorganisms. The most frequent causes of sepsis are Gram - positive and Gram – negative bacteria. Origins might be infections of any organ and organic systems.

**Material and Methods:** This study represents the patients treated at Clinical Pharmacotherapy Department of the Institute of Infectious and Tropical diseases of the Clinical Centre of Serbia, during the period from November 2004 to December 2005.

**Aim:** The aim of this study is to represent the age and gender characteristics of patients, and also their clinical symptoms; then to define the existence of a statistically significant difference of diagnostic parameters, therapy results, the most frequent causes and sensitivity of causative microorganisms to tested antibiotics.

**Results:** The sample includes 66 patients – 70% of males and 30% of females. Most of them were in the age range from 41 to 60 years. One third of the patients was febrile less than 10 days before admission. All of them had high temperature., but also we reported exhaustion (41%), headache (38%), myalgia and arthralgia (38%), shivering and fewer (36%). Chronic diseases as risk factors were present in 35% of the patients. All patients had accelerated sedimentation rate on admission, fibrinogen was increased in 91% of the patients, 85% of the patients had increased C-reactive protein (CRP). Aspartat – transferaze was increased in 32%, alanin transferaze in 30%, lactate – dehydrogenaze increase in 17% and creatin – phosphokinase in 14%. On dismissal SE was accelerated in 54%, and fibrinogen was increased in 54%. CRP on dismissal was measured in 54%, and in 31% was increased. Leukocytosis, with leukocyte count higher than 10 was present in 45% of the patients, and leucopenia with leukocyte count lower than 4 in 4.5%. hemoculture was positive in 27.3% of the patients, major of isolates consisted

of E.coli (22%), Staphilococcus aureus (19.5%) and Enterococcus spp. (44.6%). The most frequently used initial therapy was the combination of cephalosporines and amino glycosides (68.4%).

**Conclusions:** Age and sender structure of the patients were directly determined by the population that the Institute treats. The most secure indicator was high temperature. Chronic diseases came out as the most important risk factors. The indicators of inflammation (SE, fibrinogen, CRP) and leukocyte count were shown as excellent evaluation parameters of patients' recovery.

### Neuroborreliosis and Antiphospholipid Syndrome - Dissociated or Linked?

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**Aim:** The investigation of the cause of cerebellar microvascular degeneration and refractory hypertension in a patient with neuroborreliosis.

**Methods and Materials:** We present the case of a 59-year old woman with binocular diplopia, vestibular syndrome and new-onset hypertension one year after a tick bite. Low anti-Borrelia IgM titers prompted the reevaluation of the patient using the Mikrogen recomBlot for Borrelia IgM and IgG. MRI showed multi-focal demyelinating lesions along the corpus callosum and ventricles and injection of a contrast medium revealed microvascular degenerative changes in the cerebellum. In order to treat these changes, the patient was started on Sulodexide, which had the added benefit of lowering her BP. This forced us to seek a new explanation for her condition, which (at this stage of the diagnostic process) could not be completely attributed to neuroborreliosis. We therefore further investigated the patient with fundoscopy, a lupus anticoagulant test and serial dosage of several autoantibody titers.

**Results:** The Western Blot revealed a non-complete IgM response with hyperreactivity to Borrelia garinii. The patient had a persistently high titer of anticardiolipin antibodies and a positive lupus anticoagulant test, but was negative for antinuclear antibodies, thus discounting SLE as a possible cause. The fast regression of her symptoms upon treatment with Sulodexide also suggests that the primary mechanisms behind her symptoms are microvascular thrombosis and endothelial alterations. Thus, the diagnosis of antiphospholipid syndrome (APLS) was made, but the fact that her past medical history was negative for both thrombotic events and spontaneous abortions prompted the question of whether the advent of APLS was brought on by the infectious process. A review of the literature indicates that secondary APLS has so far been linked to various infections, thus supporting our hypothesis.

**Conclusion:** We propose a link between borreliosis and antiphospholipid syndrome and suggest that severe metabolic changes can be included in the as-yet incompletely explained post-borreliosis syndrome.

## Microbiology

### Community acquired methicillin resistant *Staphylococcus aureus*: carriage in medical students

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Methicillin resistant *S.aureus* (MRSA) is the world leading cause of nosocomial infection. The more serious problem is emerging of community acquired MRSA (CA-MRSA). Numerous cases of necrotizing pneumonia and sepsis caused by CA-MRSA were described. Spread of multidrug-resistant strains in hospitals potentially can be concerned with asymptomatic MRSA carriage of medical students during clinical classes.

*Aim:* To estimate prevalence of CA-MRSA among medical students.

*Materials and methods:* Screening microbiological testing of 335 second-year medical students was performed. All observed students had no signs of upper respiratory airway disease, was not undergoing in-hospital classes, and was not treated in-hospital during past month. 335 throat swabs were study material. Isolation and identification of *S.aureus* strains were carried out with routine microbiological tests. Primary inoculation were done on mannitol-salt agar, inoculated plates were incubated for 48 h 37°C, assessment of bacterial growth was semiquantitative. Identification was carried out according its catalase activity, mannitol and glucose aerobic and anaerobic fermentation and plasmocoagulation reaction. Sensitivity to clindamycin (Cd), gentamycin (G), ciprofloxacin (Cf), erythromycin (E) and vancomycin (Va) was estimated by disc-diffusion method on Mueller-Hinton agar with addition of 4% NaCl and 6 mg/l of oxacycline. For internal quality control *S.aureus* ATCC 38591 (MRSA) and *S.aureus* ATCC 29213 (MSSA) strains were used. Extraction of bacterial DNA was carried out using thermal lysis in TE-buffer. Amplification of *mecA* gene was done with PCR using primers suggested by Mehrotra M., Wang G., and Johnson W.M. (2000).

*Results:* *S.aureus* strains were isolated from 148 (44.2%) students. In 36 (24.3%) from each has poor growth (+), 49 (33.1%) – moderate growth (++) , 42(28.4%) – valuable growth and in 21 (14.2%) – massive growth (++++) of *S.aureus*. Results of antimicrobial resistance testing (% of resistant strains): Cd - 3.4%, G - 1.4%, Cf - 2.0, E - 2.0%, Va - 0.0%. Most of isolate were sensitive or has resistance to 1-2 antibiotics. In screening phenotypical tests in 21 strains (14.2%) were revealed resistance to methicillin. In 20 of each *mecA* gene were revealed with PCR.

*Conclusions:* High prevalence of *S.aureus* carrying (44.2%) among medical students. 14.2% of studied strains had resistance to methicillin. Methicillin-resistant isolates has no associated resistance with non- $\beta$ -lactam antibiotics, indicating on its community origin. There in necessity in further epidemiological studies to ascertain probable role of medical students as infection source in hospitals.

### Antimicrobial effect of coatings containing silver nanoparticles against multiresistant microorganisms

ESC-ID **672**  
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Spread of antimicrobial resistance among bacteria responsible for serious nosocomial infections is a potential healthcare threat, demanding development of new effective antimicrobial therapy strategies. An antimicrobial activity of some metals was shown, indicating its potential usefulness as therapeutic alternative.

*Aim:* to estimate antimicrobial activity of silver nanoparticles on multiantibiotic-resistant and sensitive referent strains.

*Materials and methods:* Gauze with thin-layer silver nanoparticles coating (diameter of particles 10-14 nm) were used as study samples. Thin coat was applied on a gauze substrate in active gaseous phase generated by electron beam dispersion of AgNO<sub>3</sub> powder under vacuum. Coatings morphology and nanoparticles size were estimated by atomic force microscopy and infrared spectrophotometry. For susceptibility testing series of test-tubes with 5 ml of trypticase-soy broth and 24h-cultures of microorganisms with concentrations about 10<sup>5</sup>, 10<sup>6</sup>, 10<sup>7</sup>, 10<sup>8</sup> and 10<sup>9</sup> microbial cells/ml. Microbial concentration controlled with densitometry by MacFarland optical density units. Cultures of *Staphylococcus aureus* ATCC 25923 (susceptible), *S.aureus* ATCC 35591 (methicillin-resistant, MRSA), *E.coli* ATCC 25922 (susceptible), *E.coli* ESBL CTX M-3 (producing extended spectrum beta-lactamase CTX-M), *Klebsiella pneumoniae* ATCC 13883 (susceptible), *K.pneumoniae* ATCC 700603 (producing extended spectrum beta-lactamase SHV), *Pseudomonas aeruginosa* ATCC 27853 (susceptible), *P.aeruginosa* 257 MBL VIM (multiantibiotic-resistant, methallo-beta-lactamase producing), *Salmonella Typhimurium* ATCC 13311, *Shigella sonnei* ATCC 29930, *Candida albicans* ATCC 10231, *C.kruzei* ATCC 6258. Gauze containing silver nanoparticles samples were inserted in test-tubes with microorganisms (25 mg on 1 test-tube). Tubes with were shaken for 30 min. Concentration of silver nanoparticles in nutritive media was estimated by masspectrofotometry and were about 75-80 mg/l. Inoculations were incubated in 37°C for 24 h. Bacterial grows were assessed visually and with densitometry.

*Results:* Marked bactericidal effect of studying coating on all tested cultures was revealed. Growth of *E.coli* ESBL CTX M-3, *P.aeruginosa* ATCC 27853, *P.aeruginosa* 257 MBL VIM and *S.sonnei* ATCC 29930 was absent in tube with initial microbial concentration about 10<sup>5</sup> to 10<sup>8</sup> cells/ml. For *S.aureus* ATCC 25923 and *E.coli* ATCC 25922 bactericidal effect were pointed in tubes with initial microbial concentration 10<sup>5</sup> to 10<sup>7</sup> cells/ml. For the rest referent strains bactericidal effect were pointed on initial microbial concentration about 10<sup>5</sup> to 10<sup>6</sup> cells/ml, on initial microbial concentration 10<sup>7</sup> cells/ml bactericidal effect was absent, but optical density of bacterial suspension wasn't risen after 24 h of incubation (bacteriostatic effect).

*Conclusion:* Developed technology of forming of silver nanoparticles containing coatings has a marked bacteri-

dal activity against all studied bacteria and fungi referent strains. Estimated bactericidal effect is universal and does not depend on antibiotic susceptibility-resistance of tested strains. Potential usefulness of using of developed coatings for local antimicrobial treating of infections caused with multiantibiotic-resistant strains was shown.

## Nephrology

### Post renal transplantation erythrocytosis: A report of 67 cases

ESC-ID **666**  
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*Introduction and aims:* Posttransplantation erythrocytosis is defined by most as a persistently elevated hematocrit to a level greater than 51% in renal transplant recipients. Our aim was to evaluate the prevalence, natural course and the probable risk factors of PTE.

*Methods:* In this retrospective study we reviewed 596 renal transplant recipients of whom 67 (11.2%) had erythrocytosis.

*Results:* The male sex was strongly associated with the development of PTE ( $P = 0.003$ ). The onset time was 1 to 103.6 months (mean value: 18.1 22.7) after transplantation with the mean duration time being 16.2 17.6 months. Most of them had received cyclosporine (95.5% vs. 4.4%). The majority of the patients did not have acute rejection (77.61% vs. 22.38%), delayed graft function (98.5% vs. 1.4%), autosomal dominant polycystic kidney disease (95.5% vs. 4.4%) or diabetes mellitus (88% vs. 11.9%). No thromboembolic disease attributable to erythrocytosis was observed among the patients.

*Conclusions:* Posttransplantation erythrocytosis is a frequent problem in renal transplant recipients. It is a self limited complication that can rarely lead to thromboembolic disease. keywords: postrenal transplantation erythrocytosis, renal transplantation, thromboembolic diseases

### Cyclosporin compared to Tacrolimus is an independent risk factor for the rate of renal function decline after lung transplantation

ESC-ID **951**  
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*Introduction:* Renal failure is an important source of comorbidity after lung transplantation (1), with calcineurin-inhibitor (CNI) toxicity as a major contributor. (2) The purpose of this study was to analyze whether differences in renal toxicity existed between different CNI-based

treatment-regimens used after lung transplantation, in the context of other possible risk factors.

*Patients and Methods:* All 340 primary adult lung and heart-lung recipients between 1990 and 2008 were evaluated. Patients received 3 different consecutive CNI-based regimens: between 1990-2001 cyclosporine (CsA,  $n = 156$ ), between 2001-2004 tacrolimus (Tac,  $n = 54$ ) and between 2004-2008 tacrolimus with lower postoperative target levels (Tac-low,  $n = 130$ ). The efficacy of these regimens on long-term patient and graft outcome was at least equivalent. As primary endpoint for renal function decline 'time to doubling of serum creatinine' was assessed for all patients. Survival analysis and Cox proportional hazard models were used to investigate differences in the risk of time to doubling of serum creatinine between therapy-groups, censoring for death and CNI-switch.

*Results:* The mean serum creatinine at baseline was  $79 \pm 18 \mu\text{mol/L}$ . The cumulative incidence of patients that reached doubling of serum creatinine was 35% after one year and 65% after five years. In a multivariate analysis Tac-low compared to cyclosporin was independently associated with a lower risk of doubling of serum creatinine ( $RR = 0.50 (0.33-0.77) P = 0.002$ ), taking into consideration the baseline differences between the therapy-groups (age, hypertension and BMI) and the significant confounders found in univariate analysis (pulmonary indication, type of transplantation and baseline serum creatinine). The same trend towards protection of renal function was seen for the Tac group, but this difference was not significant ( $RR = 0.65 (0.39-1.08) P = 0.09$ ). A lower serum creatinine and hypertension at baseline were also independently associated with the endpoint.

*Conclusion:* Based on these results we conclude that tacrolimus with low post-operative target levels independently spares renal function compared to cyclosporin, in the presence of equivalent efficacy (survival, BOS).

### Coronary artery calcification in renal transplant patients

ESC-ID **536**  
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*Introduction:* In end stage renal disease (ESRD) patients, the prevalence of coronary artery calcification (CAC) is very high and recently some studies disclosed clear relationship between CAC, chronic inflammation (CI) and low serum levels of calcification inhibitors. Cardiovascular disease (CVD) accounts for 30-50% of all cause of mortality after renal transplantation (Tx), which is two-fold higher CV risk than in general population. Developing of CVD after Tx depends of atherosclerosis degree before transplantation and of the numerous factors of risks (traditional and specific) associated with this process after operation.

*Aim:* The aims of the present study were to evaluate the prevalence of coronary artery calcification in renal transplant patients (Tx pts) and to evaluate the association between CAC and renal function (estimated by GFR), markers of chronic inflammation [high sensitive C reactive protein (hs-CRP), serum amyloid-A (SAA) and interleukin -6 (IL-6)], lipids, both divalent ions, homocysteine, and calcification inhibitors (fetuin A).

*Patients and methods:* The study involved 49 Tx patients.

There were 31 males and 18 females, aged  $41.98 \pm 10.58$  years and previous chronic kidney disease (CKD) duration was  $144.27 \pm 69.40$  months. CAC score was evaluated by cardiac multi-slice computed tomographic (CT), and than they were separated in group with or without calcification.

**Results:** The prevalence of Tx patients with CAC (score >100) was 44%. Pts with CAC were significantly older ( $48,23 \pm 9,46$  vs  $36,85 \pm 18,8$  years), had longer CKD duration ( $169,86 \pm 63,42$  vs  $123,19 \pm 69,44$  months), higher interleucin 6, C reactive protein, but lower fetium A, and GFR serum levels compared to pts without CAC. Significant positive correlation was found between CAC score and age, and also between CAC score and males. However, multivariate regression analysis revealed that only age ( $\alpha = 0.537$ ,  $P = 0.000$ ) was a significant determinant of elevated total CAC score in our renal transplant recipients.

**Conclusion:** Coronary artery calcifications are essential predictors of cardiovascular disease (CVD) in patients with renal transplantation. If we define CAC score and coronary artery lesions on time, in future we will reduce morbidity from CVD.

**Keywords:** end stage renal disease, renal transplant patients, coronary artery calcification, fetium A, C reactive protein, interleukin -6

### **G894T polymorphism of the endothelial nitric oxide synthase gene in patients on the haemodialysis**

ESC-ID **549**  
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**Introduction:** Nitric oxide (NO) is product of L-arginine by nitric oxide synthase (NOS). The gene polymorphism G894T in egzone 7, of the endothelial nitric oxide synthase (eNOS), leads to reduced enzyme activity. This polymorphism can be genetic risk factor associated with chronic renal failure.

**Aim:** The aim of the study was to analyze the frequencies of alleles and genotypes for G894T gene polymorphism in hemodialysis patients, and to investigate the possible differences between frequencies of alleles and genotypes in group of patients and a healthy control.

**Material and methods:** The research included 97 patients on hemodialysis and 84 healthy individuals. The detection of G894T polymorphism has been performed by polymerase chain reaction restriction fragments lenght polymorphism (PCR-RFLPS) method.  $\chi^2$  test was used to estimate the difference in genotypes frequency (GG, GT and TT) and allele frequency (G and T) in group of hemodialysis patients and control patients.

**Results:** In our study the frequency of genotypes in group of hemodialysis patients and in control group were: 54 GG (56%), 34 GT (35%), 9 TT (9%) and 42 GG (56%), 32 GT (38%), 10 TT (12%), respectively. No significant difference in distribution of the TT genotype between group of patients on hemodialysis and healthy control has been observed. T allele frequency in our patients group (0.27) is lower then the T allele frequency in the control group (0, 31).

**Conclusion:** Our study did not confirm association of the G894T polymorphism of eNOS gene with chronic renal

failure in patients who have this polymorphism and are included in the chronic hemodialysis program.

**Key words:** endothelial nitric oxide synthase (eNOS), G894T gene polymorphism, chronic renal failure

### **Evaluation of the factors affecting the outcome and prognosis in neonates with acute renal failure**

ESC-ID **939**  
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**Introduction:** Acute renal failure (ARF) is a rapid loss of renal function over days to weeks resulting in an inability to excrete nitrogenous wastes and creatinine. It is still a common condition in neonatal intensive care units (NICU) by affecting up to 8-24% of newborns. In addition, ARF has a considerable economic pressure on health care system besides the possibility of losing the kidney. Unfortunately, despite several advanced therapeutic techniques, ARF still has poor prognosis in children. Therefore, it would be more effective to utilize prognostic indicators at the beginning of disease, in order to identify those patients who need early aggressive treatment for better prognosis. The aim of this study is to evaluate the factors affecting the outcome and prognosis in neonates with acute renal failure.

**Methods:** In this retrospective study, the etiology and prognostic parameters of acute renal failure in 56 neonates referring to Ali-Asghar Hospital NICU center in Tehran, Iran from 1989 to 2003 were assessed. ARF was defined as a serum creatinine level of more than 1.5 mg/dL for at least 24-48 h, while maternal renal function was normal in term infants. Risk factors of mortality such as sex, sepsis, respiratory distress, need for mechanical ventilation and ... were assessed in all participants. All the variables were analyzed and compared in survivors and non survivors by multiple logistic regression analysis. In addition, Chi square and student's T-test were used for qualitative and quantitative variables, respectively.

**Results:** A total of 3925 neonates were treated in our NICU during the 8 years study period, and 56(1.42%) of these babies had ARF. Premature newborns were 29 (51.8%) of the cases. Sepsis was the most frequent contributing condition ( $n = 35$ , 62.5%) followed by feeding problems ( $n = 19$ , 34.5%) and congenital disorder ( $n = 2$ , 3.5%). Multiple logistic regression models ( $p < 0.001$ ,  $R^2 = 0.693$ ) revealed that among factors which showed significant difference between survivors and non-survivors in all neonates (term and preterm), the need of mechanical ventilation ( $p = 0.002$ ,  $B = 4.180$ ), anemia ( $p = 0.015$ ,  $B = -0.668$ ), gastrointestinal bleeding (GI bleeding) ( $p = 0.008$ ,  $B = 3.756$ ) and low blood urea nitrogen (BUN) ( $p = 0.043$ ,  $B = -0.024$ ) could be regarded as independent predictors of prognosis of ARF in neonates.

**Conclusion:** The present study showed acute renal failure in neonates is frequently associated with preventable con-

ditions, specifically sepsis and feeding problems. Supportive therapy is effective in most cases of neonatal acute renal failure. Early recognition of risk factors and rapid effective treatment of contributing conditions will reduce mortality in neonatal acute renal failure.

**Key words:** Acute renal failure, Neonate, mortality, prognosis

### **Autosomal Dominant Polycystic Kidney Disease Is Not Associated with Increased Risk of Post-Transplant Diabetes Mellitus**

ESC-ID 726

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Autosomal dominant polycystic kidney disease (ADPKD) is a common cause of renal failure which accounts for nearly 10% of end stage renal disease (ESRD) cases. There is controversy in the literature about the association of ADPKD and development of post-transplant diabetes mellitus (PTDM), a complication which impacts on both the patient and graft survival in renal transplant recipients. In this study we aimed to compare the prevalence of post transplant diabetes mellitus in patients with and without ADPKD.

**Materials and Methods:** In a cross-sectional study we analyzed data from 596 renal transplant patients who had been followed for at least one year. 5.3% (n = 32) of patients had ADPKD as the cause of renal failure.

**Results:** Of 596 renal transplant patients 58.6 were male and 40.3 female. Patients were  $37.7 \pm 13.4$  years old. The mean duration of follow-up was  $44.2 \pm 32$  months. Age was strongly associated with the development of PTDM ( $P = 0.003$ ) but not sex ( $P = 0.24$ ). The prevalence of PTDM in patients with and without ADPKD were 12.5% vs. 14.7%, respectively, with difference between the two prevalence being not statistically significant ( $p = 0.48$ ).

**Conclusion:** Our findings show that ADPKD as the cause of ESRD in renal transplant recipients is not associated with the development of PTDM. **Keywords:** Autosomal dominant polycystic kidney disease, post-transplant diabetes mellitus, renal transplantation

### **Regulation of Annexin A1 in the macula densa: association with neuronal nitric oxide synthase and cyclooxygenase 2**

ESC-ID 293

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**Aim:** Aim of the present study was to determine the effects of salt transport on the expression of Annexin A1 (ANXA1) in the macula densa (MD) and its association with cyclooxygenase-2 (COX-2) and neuronal nitric oxide synthase (NOS1).

**Methods:** The MD associated expression of ANXA1, NOS1 and COX-2 were determined by immunohistochemistry and cell counting on kidney sections of furosemide treated rats. Association of ANXA1 with NOS1 and COX-2 was studied by double labelling immunofluorescence.

**Results:** Furosemide treatment caused a strong increase in the number of ANXA1 expressing macula densa cells ( $51 \pm 16$  vs.  $126 \pm 19$  cells/100 glomeruli;  $p$  is less than 0.05). Double labelling immunofluorescence showed a frequent (90-100%) co-expression of ANXA1 with NOS1 and COX-2 in control- and furosemide treated animals. In summary we have shown that ANXA1 expression in the MD of rats is stimulated by furosemide treatment and colocalizes with the MD enzymes NOS1 and COX-2. ANXA1 may therefore be critically involved in the regulation of MD signalling.

### **Correlation between gum overgrowth and atherosclerosis in renal transplant recipients**

ESC-ID 660

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**Introduction and aims:** Gingival overgrowth (GO) is the main oral manifestation in transplant recipients (TR). The risk factors for GO occurrence are calcineurin inhibitors, bacterial dental plaque, gingival inflammation index, calcium channel blockers, transforming growth factor-beta, HLA-A24, IL-1A polymorphisms and down-regulation of matrix metalloproteinase levels in gingival fibroblast. Interestingly some risk factors for atherosclerosis such as bacterial dental plaque and gingival inflammation are similar with risk factors of GO. So we suggest that there may be a correlation between GO and atherosclerosis.

**Methods:** In this cross sectional case control study we enrolled 343 renal TRs which received their allograft between 1997-2004. Carotid intimal medial thickness (CIMT) as a marker of atherosclerosis was measured by ultrasonography in TRs by one radiologist and the CIMT 7.5 mm considered positive. All TRs were examined by one dentist and GO scoring determined based on Mc Gaw scoring system. Other demographic and clinical data obtained from medical records and the TRs and entered in the questionnaire. data was analyzed using chi-square, T test and logistic regression.

**Results:** Among 343 TRs 57.7% were male, mean age was 40.54 13.08 years. CIMT 7.5 mm was found in 33.8% of TRs. GO was found in 37.6% of TRs. Mean of follow up from transplantation was 74.88 67.92 months. Mean duration of dialysis was 17.39 16.74 months. Mean body mass index (BMI) was 25.13 4.74. 74.9% of TRs have hypertension, 31.3% dyslipidemia, 8.7% history of smoking, 3.5% family history of ischemic heart disease, and 51% were diabetic. We found a strong correlation between CIMT and GO (chi-square test,  $P = 0.0001$ ). And also there were correlations between age ( $P = 0.0001$ ), dyslipidemia ( $P = 0.0001$ ), diabetes ( $P = 0.002$ ), BMI ( $P = 0.006$ ), and dialysis duration ( $P = 0.02$ ) with CIMT. There were correlation between age ( $P = 0.007$ ), using calcium channel blocker ( $P = 0.03$ ) and GO. By logistic regression analysis GO ( $P = 0.0001$ , OR = 0.19, 95% CI: 0.1-0.35), age ( $P = 0.0001$ ,



OR = 9.28, 95% CI: 3.97-22.68), sex (P = 0.019, OR = 0.47, 95%CI: 0.25-0.88), dialysis duration (P = 0.02, OR = 0.97, 95%CI: 0.96-0.99), dyslipidemia (P = 0.007, OR = 0.34, 95%CI: 0.23-0.79), diabetes (p = 0.01, OR = 0.27, 95%CI: 0.1-0.73) remained correlated with CIMT.

**Conclusions:** In this study we found a correlation between CIMT and GO. To our knowledge this is the first study. We suggest further studies to clarify this correlation which may use in clinical practice as a risk factor of atherosclerosis.

### Effect of zinc supplementation on red blood cell osmotic fragility in hemodialysis

ESC-ID 724  
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**Introduction:** Zinc deficiency may aggravate the effect of oxidative stress on RBC (Red Blood Cell) of chronic uremia. In this study, there is an attempt to show the relationship between the plasma zinc level and RBC osmotic fragility in hemodialysis patients.

**Patients and Methods:** Thirty five patients with low level of serum zinc (serum zinc levelistically significant (pay 0 (59+/- 3.5) were significantly (P than those at day 43(38+/- 2.9), (at NaCl 40%). No significant changes were noted in the level of Hb.

**Discussion:** In this trial, oral zinc supplementation caused a significant rises in the plasma zinc level after 6 weeks. Although Hb concentration was not changed during the trial the level of osmotic fragility in red cells reduced significantly.

**Key words:** osmotic fragility, hemodialysis, zinc

### Establishment of a primary glomerular parietal epithelial cell line

ESC-ID 263  
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Recently, we have shown that parietal cells (PECs) are important for the regeneration of podocytes. In addition, we demonstrated that PECs are the main constituents in cellular glomerular lesions in crescentic glomerulonephritis and collapsing FSGS. Despite the crucial role of PECs in glomerular regeneration and pathology, the tools to perform mechanistic studies on PECs in vitro are limited. In this study, we used the triple-transgenic PEC-rtTA mouse to obtain pure PECs. This mouse was recently generated by us and allows specific and irreversible labelling of PECs upon administration of doxycycline. Glomeruli from doxycycline treated PEC-rtTA mice were isolated using an optimized protocol for the isolation of capsuled glomeruli. The glomeruli were cultured and several clonal cell lines were obtained by subcloning of the glomerular outgrowths. The PEC origin of the cells was proven by testing their beta gal expression (i.e. the

genetic tag) using a Xgal staining and a chemiluminescence assay. Several stable beta gal positive clones were obtained, of which three representative clones were further characterized by immunostaining and western blot analysis. The clones were negative for an endothelial marker (von Willebrand Factor), a myofibroblast marker (alpha smooth muscle actin), and for the podocyte markers nestin, synaptopodin and podocin. In contrast, the cells were positive for markers normally expressed by PECs, caveolin1, claudin1 and Pax2. Limiting dilution experiments revealed a high cloning efficiency of the cells. Furthermore, the clones were surprisingly stable as the clones possessed the same growth and phenotypic characteristics after up to 60 passages. In this study we have established for the first time a primary mouse PEC line with proven cellular origin, which exhibit specific characteristics of PEC in vivo.

## Neurology

### Marcel Proust was neuroscientist?

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**Aim:** Marcel Proust has been a famous writer and well known in the History of Literature as the creator of the masterpiece "A la recherche du temps perdu". In his seven-volume work he tries to turn his memories into the only reality he acknowledges. The aim of the abstract is to follow the writer in his deepest thoughts and at the same time distinguish his remarkable empirical observations about the way our memory works.

**Methods and material:** Studying the work of Marcel Proust has been really requiring due to the complicated style of the writer. However, it is a great experience to discover that Proust has been a pioneer by describing brand new scientific neurological mechanisms and conclusions, such as enorasis and the pathways of memory. His observations and descriptions are verified by the most up to date bibliographies on the subject.

**Results:** It is quite interesting and encouraging to find out that actual scientific facts and evidence have been detected in the past by the intelligentsia of centuries ago. Marcel Proust was one of the most prominent artistic scientists.

**Conclusions:** Science and Art are tightly bonded. Science needs Art in order to find an expression and Art needs Science in order to find an aetiological explanation. Marcel Proust has been a great representative of this bond

### Anatomy of emissary foramina and veins in fetuses and children

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**Introduction:** Emissary veins (EVs) connect the intracranial and extracranial venous system. In pathologies with

increased intracranial pressure, they play a role in its regulation. Their significant role was proved in craniosynostoses, in which the width of the EVs and foramina is increased. Injuries of these vessels during operative cranioplasty, may lead to severe haemorrhage. The EVs can be a route for the inflammatory processes, especially from the face and the oral cavity to the cavernous sinus. The presence of the EVs should be considered during operations, especially the mastoid emissary vein (MEV) in cochlear implantation. Despite the significance in some clinical situations, the literature about anatomy of these veins and the emissary foramina in fetuses and in children is very poor.

*Aim:* The aim of our study was to evaluate the anatomy of the EVs and emissary foramina in fetuses and children.

*Material and methods:* Our study was performed on fetuses from the collection of Department of Anatomy. 15 fetuses from 10 to 34 Hbd fixed in 4% formaline were examined with microsurgical technique and 6 fetuses from 16 to 23 Hbd were prepared with corosive method. In the second part of the study we evaluated CT of 30 healthy children aged from 1 to 17 years old with mild brain concussion.

*Results:* The parietal foramina and veins were not found in fetuses in any case, whereas in children CT scans the foramen was present in 2 cases (6.67%). The occipital emissary foramen and vein were present in 3 fetuses (20%) and in 5 children (16.7%). The vein originated from the confluens sinuum and had an oblique course directed upward and posteriorly. The condylar emissary veins or the external orifices of their canal were present in all fetuses and almost all children, on one or both sides. They appeared as single vessels in 36.4 % or plexiform in remaining cases. The MEVs were present in almost all fetuses and children on both sides in a number of one. Double MEV was found in 3 children (10%), and in 3 fetuses (20%). In children it always ran posteriorly and to the lateral side, whereas in fetuses it usually ran in transverse plane. The foramen of Vesalius was found only in 5 children (16.7%), bilaterally.

*Conclusions:* In fetuses and children, the MEVs appear most constantly and bilaterally. Their course changes during development. The least frequently present veins are the occipital and parietal EVs. CT is a good method of examining the presence and course of the EVs in children.

### ExpressExpression of 8-oxoguanine DNA glycosylase 1 and p53 protein in peripheral lymphocytes of patients with Alzheimer's disease

ESC-ID **276**  
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Reactive oxygen species (ROS) generate various modified DNA bases. Among them 8-oxo-2'-deoxyguanosine (8-oxo2dG) is the most abundant and seems to play a major role in mutagenesis. 8-Oxo2dG is removed from DNA by the specific 8-oxoguanine DNA glycosylase 1 (OGG1). OGG1 mutation may be associated with the GC-to-AT transversion type point mutations. This type of mutation

is commonly observed in the tumor suppressor p53 gene. The aimed of the study was to determine the extent of oxidative DNA damage (8-oxo2dG) and expression of three isoforms OGG1-1a, 1b, 1c, and OGG1 and p53 proteins in peripheral lymphocytes of Alzheimer's disease (AD) patients and in controls. The studies were conducted on 41 patients with AD, including 25 women and 16 men aging 34-84 years. The control groups included 51 individuals, 20 women and 31 men aging 22-83 years. The level of 8-oxo2dG was determined by HPLC/EC/UV technique, and expression of OGG1-1a, 1b, 1c by RQ-PCR, and the level of OGG1 and p53 proteins by Western Blot method. We were observed increase of the level of 8-oxo2dG after 60 years of age (insignificant) and in AD patients ( $p < 0.05$ ) as compared to the controls. Simultaneously, the level of OGG1 protein was decreased in individuals after 60 years of age ( $p < 0.001$ ) and in AD patients ( $p < 0.01$ ) as compared to the controls when the level of p53 protein was increased in individuals after 60 years of age ( $p < 0.05$ ) as well as in AD as compared to controls. The OGG1-1c isoform seems to be associated with early stage of AD, while increase in expression of OGG1-1b isoform and levels of OGG1 protein appear to be similar related to the progression of AD. Moreover, in patient with mild dementia (in MMSE scale) were observed the lowest level of 8-oxo2-dG and OGG1 and the highest level of p53 protein, when in the patients with severe dementia (in MMSE scale) higher level of 8-oxo2dG and the lowest level of OGG1 and p53 proteins. It appears that OGG1 and p53 proteins are involved in pathogenesis of AD by repair of oxidative DNA damage.

### Handicap caused by spinal column injuries

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Spinal column injuries are consequences of direct or indirect impact of force as a result of automobile or sports injuries, or by falls from considerable heights. They can cause spinal cord damage and constant or transmittend motor system disfunction. Besides, they are most common cause of handicap and limitation in professional and social life. The aim of the study was analysis of handicap caused by spinal column injuries in patients hospitalized in Orthopaedics and Rehabilitation Department in Lublin in 2002-2008 years.

*Material and Methods:* Retrospective analysis of medical documentation of 32 patients hospitalized after spinal injury.

*Results:* In the group of 19 men (59%) and 13 women (41%) in average age 37,5 year (19-66 year) the most common type of accident was car crash (43%), fall from a window (12,5%), motor accidents (6,25%) and jump to water (6,25%). 9,3% of accidents happened during work and 3,3% happened in hospital. 21% were uncounscious after accident. 16% were under the influence of alcohol. 47% of injuries were localized in lumbar, 43% in thoracic and 18% in cervical region of the spine. 76,7% had spinal cord damaged. 56% were operated and rest received conservative therapy. 37,5% used wheelchair for locomotion.

*Conclusion:* The most common fracture of vertebrae in

compression mechanism during car crash. Most injuries were located in lumbar and low thoracic region and in high percentage caused spinal cord damage. More than one third of patients had high movement restrictions and used wheelchair.

### The Frequency of Different Ictal Patterns Recorded by Scalp EEG in Patients with Mesial Temporal Epilepsy

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*Introduction:* We analyzed ictal electroencephalographic (EEG) data of patients with mesial temporal epilepsy. This analysis was performed in order to show which kind of EEG changes was the most frequent as the ictal onset pattern.

*Material and methods:* Retrospectively, we analyzed 106 ictal EEG data of 17 patients. All the seizures were recorded with the five-day long, continuous video EEG telemetry. Analyzing the EEG data, we considered as ictal beginning every sudden change of background activity if it was in correlation with the recorded seizure semiology. For the classification of ictal onset patterns we used previously defined terminology: a) rhythmical 2-4 Hz discharges (R24); b) rhythmical 5-10 Hz discharges (R51); c) spike repetition (RPS); d) recruiting rhythm (REG); e) sudden low-voltage activity (NVA).

*Results:* As the scalp EEG ictal onset pattern we found rhythmical 2-4 Hz discharges (R24) in 45,28% seizures, rhythmical 5-10 Hz discharges (R51) in 22,64%, spike repetition (RPS) in 4,72% seizures, recruiting rhythm (REG) in 13,21%, while the sudden low-voltage activity (NVA) was found in 14,15% seizures.

*Conclusion:* Our results showed that among patients with mesial temporal lobe epilepsy the most frequent ictal onset pattern recorded by scalp EEG was rhythmical activity 2-4 Hz. This finding is in correlation with previous experiences that epileptogenic zones localized in deep brain structures are more likely to cause rhythmic slow ictal activity recorded with scalp EEG. However, rhythmic slow discharges can be considered as characteristic ictal pattern for mesial temporal epilepsy. Keywords: Ictal – EEG – pattern – 2-4 Hz – temporal lobe

### Miss-splicing of GPHN transcripts in the hippocampus of temporal lobe epilepsy patients produces dominant negative gephyrins

ESC-ID **795**  
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Roughly 50 million patients world-wide are afflicted with epilepsy, making it one of the most common neurological disorders. About 2.4 million new cases per year are report-

ed globally, and in at least 50% of cases epilepsy begins in childhood or adolescence. Deficits in GABAergic function have often been related to epilepsies. For example, in temporal lobe epilepsy (TLE) patients with temperature-sensitive mutations in the GABRG2 gene decreased (20%) amplitude and decay time constant of inhibitory synaptic potentials already is sufficient for ictogenesis. Gephyrin is involved in postsynaptic anchoring of GABAA receptors (GABAAR), and GPHN knockout mice display reduced amplitude of inhibitory synaptic potentials. Furthermore, alternative splicing is known to produce gephyrins with dominant negative functions. Because gephyrin orchestrates the control of postsynaptic GABAA receptor numbers it is not surprising that decreased gephyrin immunoreactivity in the cornu ammonis of epileptic hippocampus parallels reduced immunofluorescent signals of GABAA receptors. However, we still do not know why gephyrin immunoreactivity drops in the epileptic hippocampus. We have screened hippocampotomies of pharmacoresistant TLE patients with heterogeneous course of disease and various degrees of hippocampal sclerosis for gephyrin expression and alternative splicing. Four miss-spliced gephyrins with dominant negative functions were isolated, among them two with premature STOP codons due to exon skipping.

### Neuropathology of experimental toxocariasis in Syrian golden hamsters

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*Introduction:* Toxocariasis is a disease caused by a larval stage of *Toxocara* sp., and predominantly involves *Toxocara canis* and *Toxocara cati*. *Toxocara* larvae tend to accumulate in brain tissue and can remain alive and motile for years, producing a different neuropathological damage. Very little information is available on toxocariasis in the hamster.

*Aim:* The aim of this study was to investigate a neuropathologic characteristic of experimental toxocariasis in hamsters.

*Material and methods:* The research included upon 15 Syrian golden hamsters (10 experimental and 5 control). The animal from experimental group was given orally with about 10000 eggs of *T. canis*. The experimental animals were sacrificed in moribund state after two (3 animal), three (3 animal), and five weeks (4 animal) of inoculation. Brains in experimental and control animal removed from skull and histologically analysed using hematoxylin & eosin, Walton stain, and May Grunwald Giemsa.

*Results:* Histopathologically, the cerebellum was the most affected area, but we can also see the migrating larvae in every other part of brain. Loss of Purkinje cells, glial nerve fibers and nerve sheaths were characteristic and common findings. We found granulomatous lesions with migrating larvae in the center, but larvae and lesions also existed independently, suggesting that some of the degenerative changes might be the result of indirect effects of the larvae. Also we found haemorrhagic lesions and exudative lesions with or without migrating larvae. Leucocytes around the vessel walls suggest vasculitis, consisting of eosinophils and lymphocyte infiltration.

We also found area of gliosis and haemosiderosis. All animals had intensive meningitis.

**Conclusion:** Because of specific neuropathological evidences, which we also see in human (especially in children), we suggested that the experimental infection of hamsters may be a useful model of toxocariasis in humans.

### The pattern of drainage of solutes from the brain in the presence of Cerebral Amyloid Angiopathy. Significance in Alzheimer's Disease

ESC-ID **164**

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**Introduction:** In Alzheimer's disease and normal ageing, amyloid- $\beta$  (A $\beta$ ) accumulates in the walls of blood vessels as Cerebral Amyloid Angiopathy (CAA). Experimental studies show that solutes similar to A $\beta$  drain out of the brain along the basement membranes of capillaries and arteries. In this project, using a transgenic mouse model (Tg2576) of AD, I am testing the hypothesis that the perivascular drainage of solutes is disrupted in the presence of extracellular deposits of A $\beta$ .

**Objectives:** 1) study the pattern of distribution of fluorescent tracer injected intracerebrally; 2) quantify the number of blood vessels labelled with tracer.

**Method:** 3kDa soluble fluorescent dextran was injected into the hippocampus of 3, 7 and 22 months old Tg2576 (n = 9) and age matched naïve control mice (n = 9) by my supervisor. Brains were fixed, frozen and sectioned using a cryostat. A $\beta$  and laminin were identified by immunofluorescence and visualised using a Leica confocal microscope. The number of vessels labelled with dextran was counted using a Zeiss Analysis system.

**Results:** The 22 month old Tg2576 had more A $\beta$  positive plaques and CAA. Blood vessels were identified by the laminin positive staining. Overall, the 22 month old Tg2576 mice had fewer blood vessels labelled with dextran compared to the 3 and 7 month old ones. The 22 month old Tg2576 mice had fewer capillaries labelled with dextran compared to controls and to 3 and 7 month old Tg2576 mice.

**Conclusions:** The presence of CAA and insoluble A $\beta$  along the blood vessels appears to disrupt the drainage of solutes along the perivascular pathways. This may lead to further accumulation of A $\beta$  and other compounds within amyloid plaques. Further studies are required to clarify the factors that influence the dynamics of perivascular drainage in the brain and their failure in ageing. This will help in devising therapeutic methods that enhance the drainage of solutes through the perivascular pathways and prevent accumulation of A $\beta$  along these pathways in old age.

### Knowledge of risk factors and symptoms of stroke in Estonia

ESC-ID **656**

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**Background:** Due to high mortality and chronic disability, the global burden of stroke is greater than that of heart disease. The outcome of stroke can be improved by effective therapies, including intravenous (iv) thrombolysis within 4.5 hours of onset of ischemic stroke. One of the most important reasons that patients are not treated with iv thrombolysis is that they do not attend hospital within the treatment time window. Late arrival to the hospital could be the result of poor appreciation of stroke warning signs by the patient and relatives.

**Aim:** The current study was undertaken to assess the knowledge of stroke risk factors and symptoms among the Estonian population and to compare our results with the results of studies carried out elsewhere.

**Methods:** The study was conducted from October 2008 to February 2009 as face-to-face interview in streets, shopping malls, at schools or other public places in Estonia. A simple closed-ended questionnaire was used. The items of the multiple-choice questionnaire were: What kind of disease is stroke?, What are the warning signs of stroke?, What are the stroke risk factors?, Which action needs to be taken at onset of stroke symptoms?

**Results:** Three hundred and fifty five persons filled in the questionnaire, 31% of them were men and 69% were women. The mean age of respondents was 39.6 (SD 21.2) years for men and 40.6 (SD 19.7) years for women. The study was conducted in two big cities (Tallinn, n = 122 and Tartu, n = 228, missing place of residence n = 5). 85% percent of respondents answered correctly that stroke is an acute disease. However, 42% of respondents thought that dyspnea is a stroke symptom and 45% that drinking coffee is a stroke risk factor. Most of the respondents (99%) knew that one should call the ambulance at onset of stroke, although 33% would also call to a friend or a relative. The knowledge about stroke was without differences between the sexes. Higher level of education was related to higher and younger age with lower level of stroke awareness. The best results were obtained by persons around 40-60 years. In Estonia overall awareness is a bit higher than elsewhere; however, similar questions were answered incorrectly and stroke was similarly mistaken for heart attack. According to our study the results of the respondents in the age group 60-90 were a lot better than found in other studies.

**Conclusion:** Knowledge about stroke symptoms and risk factors was better among middle-aged persons and for those with higher education. Although most of the respondents were aware that ambulance must be called immediately at onset of stroke, several questions about stroke were answered incorrectly both by the younger and older respondents. Therefore, stroke awareness campaigns are still needed to address this knowledge gap.

## Tau protein as a possible biological marker in amyotrophic lateral sclerosis

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**Background:** Biological markers are actively searched in amyotrophic lateral sclerosis (ALS). Cerebrospinal fluid (CSF) concentrations tau, have been measured in ALS with levels found increased in some studies and unchanged in others. Traditionally ALS has been viewed as disease of the motor system, but today ALS is recognized as a multi-system disorder. The cognitive impairment revealed in some non-demented ALS patients is characterised mostly by executive dysfunction.

**Aims:** To examine the diagnostic potential of CSF tau (total tau and phosphorylated tau) in patients with ALS. To investigate the correlation between level of CSF biomarkers and cognitive impairment in non-demented patients with ALS; to correlate functional status of ALS patients with level of CSF biomarkers.

**Material and methods:** Using sandwich ELISA, we investigated the levels of CSF total tau (T-tau), and CSF phosphorylated tau (P-tau), in 12 ALS patients and in 13 age-matched controls. 52 non demented ALS patients and 27 healthy controls underwent comprehensive neuropsychological assessment. The battery of neuropsychological tests included: Mini Mental State test; Test Raven Progressive Matrix; Letter Fluency Test; Category Fluency Test (Animal Naming Test); Boston Naming Test; Cambridge Neuropsychological Test Automated Battery (CANTAB) and measures of affective status that included Scale of Apathy and Hamilton Rating Scale For Depression. The Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) was an instrument for evaluation the functional status of patients with ALS.

**Results:** Mean levels of CSF T-tau and P-tau were not significantly different between groups. Behavioral changes, in the form of apathy, were revealed. The most striking impairment was found on tests of verbal fluency, indicating executive dysfunction and on Boston Naming Test.

**Conclusion:** There were no significant changes in the level of CSF biomarkers in patients with ALS. The cognitive performances, in the form of executive dysfunction do not correlate with the levels of CSF tau. Functional statuses of the patients with ALS do not correlate with the levels of CSF biomarkers. CSF tau is not a biological marker for ALS. Key words: ALS; tau protein; executive dysfunction.

## Animal verbal fluency in patients with mild cognitive impairment

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**Background:** Mild cognitive impairment (MCI) is characterized by memory complaints and deficits in the absence

of dementia, global cognitive decline, or significant problems with activities of daily living. Numerous epidemiological studies have documented an accelerated rate of progression to Alzheimer's disease (AD) in MCI patients. Traditionally, emphasis has been placed on episodic memory deficits, but recent research indicates that some MCI patients show subtle, non-amnestic cognitive difficulties. Changes in aspects of executive function, such as verbal fluency, have been observed in people years before the clinical diagnosis of AD. Performance on verbal fluency tasks has often been included in predictive conversion models, and older adults who eventually develop AD may show baseline decline in verbal fluency 3–5 years before diagnosis (Raoux et al, 2008). Verbal fluency is typically defined as the ability to produce words under specific constraints (semantic category or specific letter), and within a fixed time interval. Recently animal naming was found to be the most sensitive among other verbal fluency tasks to distinguish normal aging from dementia. Troyer et al. (1997) proposed two main processes underlying both semantic and phonemic verbal fluency tasks: clustering, a semantic process of word generation within subcategories, and switching, an executive process of strategic search and shifting between subcategories. Recent research hypothesizes that clustering and switching variables could be differentially affected in pre-clinical phase of AD with greater impairment of shifting abilities rather than cluster size in MCI patients. (Gomes et al, 2006; Murphy et al, 2006).

**Aim:** To examine the utility of both quantitative and qualitative aspects of animal verbal fluency in distinguishing between MCI patients and healthy subjects.

**Methods and Material:** Subjects were 26 MCI patients and 11 healthy age and education matched controls. They were asked to generate as much as possible animals for the period of 1 minute. Three indices were calculated according to the original method of Troyer et al. (1997) – number of clusters, number of switches, mean cluster size, in addition to traditional measure total number of words generated.

**Results:** Both groups were with comparable general cognitive ability assessed by Mini Mental State Examination scale. All studied fluency measures were reduced in MCI group relative to cognitively intact older adults. MCI patients generated significantly small total number of correct words (without repetitions) than control group ( $p = .03$ ) as well as they generated significantly less subcategories (clusters) ( $p = .009$ ). We also observed group differences in two other verbal fluency measures, mean cluster size and number of switches, but they did not reach significant level. One possible reason is small number of subjects. The examined quantitative and qualitative aspects of animal verbal fluency revealed differences in clustering and switching parameters indicating subtle changes in the quality of the semantic store and retrieval strategies in MCI patients.

**Conclusions:** Our findings highlight the importance of including verbal fluency tests and detailed neuropsychological analysis in assessment procedures targeting pre-clinical dementia populations.

## Changes in cortical inhibition after fatiguing exercise in stroke patients

ESC-ID 311  
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*Introduction:* 40-70 % of all stroke patients experience problems with fatigue during the acute and subacute phase of recovery. This may be due to changes in function of intracortical GABA-dependent inhibitory circuits. We want to study changes in cortical excitability after fatiguing exercise in hemiparetic stroke patients within three months after stroke.

*Methods:* Twelve stroke patients are to be compared with age and gender matched healthy subjects. Cortical excitability and indirectly the intracortical GABA-level will be assessed by transcranial magnetic stimulation (TMS), including paired-pulse stimulation, before and up to 30 minutes after a single fatiguing exercise, in which, the patients must develop and keep a force equivalent to 50 % of their maximal voluntary contraction (MVC) for as long as possible. Motor evoked potentials will be recorded from the biceps brachii muscle. Lack in the voluntary activation of the paretic muscle will be assessed with the twitch interpolation technique.

*Results:* Data will be presented.

## Compliance of acute stroke patients with autoCPAP treatment for OSAS

ESC-ID 941  
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*Objective:* Aim of this study is to investigate the compliance of automated Continuous Positive Airway Pressure (autoCPAP) treatment in acute stroke patients.

*Background:* Many authors suggest a correlation between obstructive sleep apnoea (OSA) and cerebrovascular disease(1). Recent literature demonstrates that OSA is an independent risk factor for acute stroke(2). Moreover, while there is a high prevalence of OSA in patients with acute stroke, this combination is also associated with a worse clinical outcome(3). Especially in the acute phase of stroke, OSA can have detrimental effects on critically ischemic, but viable brain tissue (the so called penumbra)(4). This is due to the physiological consequences of recurrent obstructive apnoeas, such as hemodynamic fluctuations, periods of hypoxia and an increased thrombotic risk. Currently, small studies are testing the hypothesis whether application of CPAP in acute stroke can reduce secondary damage to the penumbra by reducing the OSAS component(5;6). However, important questions about acceptance need to be answered. In order to identify consecutive stroke patients that will accept this kind of treatment in the acute setting, we set up this pilot study.

*Methods:* Two sleep studies with SOMNOsmart2 (Weinmann, Hamburg, Germany) will be performed within 48 hours after stroke onset. During the first diagnostic night pressures are set at 4 cmH<sub>2</sub>O for both upper and lower limit. In the second therapeutic night, the automatic mode

will be used with a lower and upper pressure limit of 4 and 10 cmH<sub>2</sub>O, respectively. We will record patients' characteristics, reasons for non-compliance, duration of therapy, and respiratory disturbance index(RDI)/average pressure. We will compare the parameters of both nights  
*Results:* Preliminary results will be presented focussing on compliance of acute stroke patients with autoCPAP treatment. Data of both prevalence of OSA and compliance with auto CPAP will be presented for the first 48 hours.

*Keywords:* obstructive sleep apnoe, acute stroke, autoCPAP, compliance

## The Role Of Microglia and Neuropeptides in Regulating Hippocampal Neurogenesis

ESC-ID 387  
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Adult mammalian neurogenesis is evident in the hippocampal dentate gyrus where it plays a role in learning and memory and is implicated in the pathophysiology of several brain disorders. Microglia, the innate immune cells of the brain, have recently emerged as an important component of the neurogenic niche, however their role in the regulation of neurogenesis under physiological and pathophysiological conditions is a matter of debate. The aim of this study is to investigate the effect of microglia on hippocampal neurogenesis and to look at how vasoactive intestinal peptide (VIP), a potent immunomodulatory neuropeptide found in dentate gyrus interneurons, modulates the effects microglia have on neurogenesis. In this study, we have investigated the effect of microglial depletion (using MAC-SAP), microglial co-culture and addition of microglia-conditioned-medium on primary hippocampal cell cultures derived from post-natal rats. We have also looked at how pre-treatment of microglia with VIP alters their effect on hippocampal cultures. Bromodeoxyuridine was used as a marker of cell proliferation. Quantification of cell death was achieved using the nuclear stain 4',6-diamidino-2-phenylindole and Propidium Iodide. Immunohistochemistry was used to phenotype cells for nestin, GFAP and Tuj1. We have shown that microglial depletion results in a reduction in the numbers of nestin, GFAP and Tuj1 expressing cells. This reduction has been shown to be attributable to a decrease in cell survival and proliferation. Conversely, co-culture of microglia with hippocampal neurons or addition of their conditioned medium results in increased cell survival and proliferation. Pre-treatment of microglia with VIP was shown to increase both their proliferative and trophic effect on hippocampal cultures. In conclusion, this study demonstrates that microglia induce proliferative and trophic effects on neural stem cells and immature neurons through the release of soluble factors. Furthermore, we provide evidence that VIP regulates the release of these soluble factors, thus identifying a novel neuro-immuno-neurogenic link.

## Gender-dependent involvement of the endothelial and neuronal nitric oxide synthetase in the angiotensin-(1-7)-mediated enhancement of LTP in the lateral amygdala

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The amygdala is discussed in terms of its role in receiving afferent sensory input and in processing information related to hydromineral balance. Angiotensin acts on and through the amygdala to stimulate thirst and sodium appetite. In addition, different angiotensins seem to play a role in cognition and learning mechanisms by acting on and through the amygdala. Long-term potentiation (LTP) is the predominant experimental model used for studying the mechanisms of learning and memory. Besides inhibitory actions of angiotensin II on mechanisms of learning and memory, angiotensin-(1-7) also affects LTP. Angiotensin converting enzyme 2 metabolizes angiotensin II to the vasodilatory peptide angiotensin-(1-7), while neprilysin generates angiotensin(1-7) from angiotensin I. Recently, we have shown that angiotensin-(1-7) enhanced the magnitude of LTP in the lateral nucleus of the amygdala (LA) by acting on the Mas receptor. Here we show that L-NAME, an unspecific inhibitor of nitric oxide synthetase (NOS), is able to block this angiotensin-(1-7)-specific effect on the magnitude of LTP. The present study also supports the involvement of nitric oxide (NO) in the mediation of LA-LTP by the decrease in LTP amplitude in animals lacking either the endothelial (eNOS<sup>-/-</sup>) or the neuronal NOS (nNOS<sup>-/-</sup>). To analyze the mechanisms of angiotensin-(1-7) in modulating the magnitude of LA-LTP in detail, we induced LA-LTP in horizontal brain slices derived from 9-months-old wild type mice (C57BL/6J) or mice homozygous for disruption of the eNOS or nNOS. Interestingly, in male eNOS<sup>-/-</sup> mice angiotensin-(1-7) enhanced LA-LTP, whereas in female eNOS<sup>-/-</sup> mice the enhancing effect of angiotensin-(1-7) was missing. Therefore, the LTP enhancing effect of angiotensin-(1-7) is mediated by eNOS in females. In contrast, in female nNOS<sup>-/-</sup>, Ang-(1-7) caused a strong enhancement of LTP, whereas the effect of Ang-(1-7) was missing in male nNOS<sup>-/-</sup>. Thus, in males angiotensin-(1-7) induced an increase in the magnitude of LTP by the involvement of nNOS. Our data support not only the hypothesis that NO contributes to plasticity changes in the lateral amygdala, but also a gender-dependent involvement of NO which is deriving from different sources in the mediation of angiotensin-(1-7)-induced effects on synaptic plasticity.

## Ophthalmology

### Prevalence of Different Types of Ptosis between 2002-2008 at labafinejad hospital Tehran Iran

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*Introduction:* (Blepharo-) ptosis can affect one or both eyes. Ptosis is generally classified as congenital or acquired and as myogenic, aponeurotic, neurogenic, mechanical and traumatic. The aims of this study to describe the relative incidence and the outcome of surgery in a large cohort of patients.

*Materials and Methods:* A descriptive review of 233 ptosis patients managed in a large referral ophthalmologic centre, between 2002-2008, documenting the etiology, age, sex, laterality, family history, amblyopia if present, past medical history and postoperative complications.

*Results:* Of the 233 patients [female 50.2%, mean age 18.7 ± 12.8 years] with ptosis, 84.9% of them had unilateral ptosis. Positive family history was seen in 3.4% of patients. We found that 19.2% of patients had amblyopia. Past ocular surgery has been done in 85% of cases and 6.9% had undergone strabismus and ptosis surgery. 78.9% of cases had congenital ptosis and the others had acquired ptosis. Myogenic type (68.7%) was the most common cause of ptosis in congenital ptosis other types prevalence were neurogenic (10.9%), traumatic (12.2%), aponeurotic (5.7%), mechanical (2.6%). We achieved 63.7% success in first surgery. Under-correction (19.%) was the most common complication and overcorrection (4.9%) was the second complication in patients after surgery.

*Conclusion:* In this series, ptosis was mostly congenital and the number of patients with amblyopia was significant. So early, early detection and refer of children with ptosis is necessary to prevent amblyopia. Traumatic ptosis especially surgical traumatic was the second common cause of ptosis. All congenital mechanical ptosis were due to eyelid tumors, that emphasizes, thorough evaluation of ptosis patients before surgery.

### Comparison of interlaminar decompression and decompressive laminectomy in lumbar spinal stenosis – A retrospective analysis

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*Background:* There is an ongoing debate about the adequate surgical intervention for lumbar spinal stenosis. Classically, decompressive laminectomy was the intervention of choice but added unwanted instability due to removal of the laminae, spinous process and other osteoligamentous structures. Interlaminar decompression is a minimally invasive technique which involves introducing fenestrations into adjacent laminae, thereby retaining

the vital structures necessary for spinal stability.

**Methods:** 99 patient files were retrospectively reviewed and information was gathered according to a preformed questionnaire of patients who had undergone interlaminar decompression or decompressive laminectomy. All patients were operated on by the same surgeon. Some of the outcome variables that were analyzed included pre-operative symptoms, estimated blood loss, length of procedure, length of hospital stay and post-operative resolution of symptoms.

**Results:** 55 patients (31 males; 24 females) underwent interlaminar decompression while 44 patients (30 males; 14 females) underwent decompressive laminectomy. 80.8 % of patients presented with low back pain with leg pain, 8.1 % with only leg pain, and 10.1 % with only back pain. The mean estimated blood loss for interlaminar decompression and decompressive laminectomy was 211.6 ml and 256.3 ml respectively. No significant difference in hospital stay was found. Post-operative resolution of symptoms was found to be higher in patients who underwent interlaminar decompression.

**Conclusion:** Interlaminar decompression was more effective in relieving pain. However, the results highlight the need for further prospective studies and randomized controlled trials for lumbar spinal stenosis.

## Orthopaedics

### A pilot study to evaluate functional ultrasound elastography (FUSE) in Achilles tendon rupture: developing the rules governing elasticity image interpretation

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**Purpose:** Functional ultrasound Elastography (FUSE) of Tendo Achilles is an ultrasound elasticity technique utilising controlled, measurable movement of the foot to non-invasively evaluate its elastic and load-deformation properties. The study purpose is to assess Achilles tendons, paratenon and bursae in healthy volunteers and to compare the findings with acute and healing TA rupture patients.

**Methods:** Trial patients with Achilles tendon rupture confirmed by dynamic USS and 12 tendons in healthy volunteers underwent FUSE scan (Z.one, Zonare Medical System Inc., CA, USA, 8.5 MHz) and compared it to findings in gray scale sonography (8.5 MHz). Tendon insertion, mid-portion and musculotendinous junction were examined during lateral movement and axial compression/decompression modes. Sagittal scans were acquired at rupture area. B mode and elasticity images were derived from the raw ultrasound radio frequency data. The anatomical structures were evaluated by a semi-quantitative score of different colours representing stiff tissue (blue) to more soft tissue (green, yellow, red).

**Results:** Tendons in healthy volunteers showed all blue colouring in elasticity images, consistent with stiff normal tendon tissue and normal findings at gray scale B mode images. Patients showed tendon and paratenon

thickening and a visible rupture gap. By tracking the lateral movement of the tendon, significant higher detection of intratendinous colour alteration detected by FUSE (green, yellow, red) at the ruptured tendon substance in comparison to axial compression/decompression technique and B mode. In all patients the softer areas increased with dorsal flexion and decreased with planter flexion. The healing TA Elastography scan revealed the change to blue as the scaring tissues harden.

**Conclusion:** Our findings show that FUSE seems to be a sensitive method for assessment of acute and healing TA rupture. In interpreting images, elastic properties of normal tissue must be understood, the B mode and elasticity images must be viewed simultaneously.

### Functional and morphologic analysis of the osseous precursor cells differentiated from the mesenchymal stem cells extracted from bone marrow aspirate.

#### Implications in Orthopedic Surgery

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**Aim:** The treatment of pseudarthrosis, osteonecrosis, bone defects after surgical treatment of tumors appeals more often to cell cultures. Osteoblasts are specialized cells in producing extra cellular matrix and are involved in bone remodeling. We proposed ourselves to perform a morphologic analysis of the osteoblasts differentiated from mesenchym stem cells extracted from bone marrow aspirate; furthermore, we studied osteoblast adhesion to biomaterials used in orthopedic surgery.

**Methods and Materials:** We harvested bone marrow during orthopedic surgical interventions. We isolated the stem cells using ficol separation method at the National Institute for Biochemistry Bucharest; later, the cells were characterized by flow cytometry and were differentiated in osteogenic medium; we studied the osteoblasts with specific markers: alkaline phosphates, collagen I, BSP, osteocalcine, osteonectine, CD29 and the formation of mineralization centers with Alizarin Red staining. We made tests for viability, morphology and adhesion (electronic and fluorescence microscopy) of osseous precursor cells after their attachment to diverse biomaterials: bio glass films deposited on titanium by pulse laser deposition (PLD) hidroxyapatite films (HA) deposited on cuart by radio-frequency magnetron sputtering (MS).

**Results:** Osteoblasts differentiated in vitro express osteocalcine, a protein from the extra cellular matrix that bounds calcium and participates to the formation of the mineralized matrix. In the same time, they express osteonectine and bone sialoprotein especially inside cytoplasm. In case of HA deposited by MS, the cells elaborate a large amount of matrix that transmit signals to integrins and kinases from the cell periphery; HA deposited by PLD induces a cell adhesion based on the interaction between vinculin and actine with the formation of cytoskeleton. Signals from integrins are weaker, probably because of the lack of integrin-integrin interaction usually induced by the bounding to the extra cellular



matrix.

**Conclusions:** The study of the osteoblasts differentiated from stem cells is important because a large population of active osteoblasts is needed for bioengineering and the understanding of osteoblast differentiation process presents great interest for the revealing of genes implicated in diverse bone diseases. The adhesion mechanism is not influenced by the material chemical structure but by the nanostructure of the film that can stimulate integrins that connect actine and influence cellular adhesion, migration, proliferation and differentiation. Key words: osteoblast, mesenchym stem cell, actine, vinculin, integrin, cellular adhesion

### Characterization of human skeletal muscle satellite cells

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**Background:** In vivo, human skeletal muscle has the potential to repair and regenerate until old age. The regenerative potential is thought to be mediated by satellite cells, muscle specific stem cells located in close proximity to the outer surface of the muscle fiber. However, in vitro the proliferative and regenerative potential of satellite cells is rapidly lost. Satellite cells would be the favourite carriers of gene therapy approaches in so far untreatable devastating muscular dystrophies. Problem: Whereas murine satellite cells have been characterized to quite some extent, knowledge about human satellite cells is still in its beginning. We wanted to characterize these cells.

**Material and methods:** All procedures were IRB approved. Fresh human skeletal muscle (n = 27, age 5-87 years, m:f 9:19) was obtained during surgery. Immediately after excision single muscle fibers were isolated under a dissecting microscope using fine needles. The fibers were either placed into defined skeletal muscle growth medium and incubated at 37C, 10% CO<sub>2</sub>, or immediately stained for Pax7 and distinct surface antigens by immunofluorescence. Other samples were digested by collagenase and cell suspensions were immobilized by cytospin and stained as above. Cell suspensions were also characterized by FACS analysis. Serial Western blot analysis was performed on proliferating myoblasts.

**Results:** Pax7 positive cells consisted of 3 ± 2% of all fiber nuclei. The number of Pax7 positive cells was age dependent and decreased with age. Other satellite cell markers that have been previously characterized in the mouse did not colocalize with Pax7 positive cells (c-met, CXCR4, Syndecan 4, alpha 7 integrin, CD 34). Myf5 and MyoD became detectable during proliferation and differentiation, but not in the quiescent state. Preplating of tissue culture dish with Delta-factor DLL1, an antagonist of Wnt signaling, resulted in reduced cell numbers.

**Conclusions:** We developed the technical tools to study freshly isolated human satellite cells. Further characterization of relevant surface markers and signaling pathways is important.

### Determination of the optimal method and device of osteosynthesis in the fracture of the anatomical neck of the humeral bone (Experimental and clinical studies in disertation work)

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**Introduction and Aim:** Analysis of both achieves and follow-up material of various methods of osteosynthesis in the fracture of the anatomical neck of the humeral bone was conducted. The data showed that after 21 days, control X-rays reveals various degrees of displacement of the proximal fragment along the line of fracture especially after passive motion has been initiated in the shoulder joint. This necessitated our experiment to determine the resistance of the different device of osteosynthesis used in the management of the fracture of the anatomical neck of the humerus bone.

**Method:** The experiment was divided into five series, conducted with fresh bones of cadavers not older than 24hrs. osteotomy of the anatomical neck of the humeral head was performed to imitate fracture of the region. First series • osteosynthesis with Kirschner wire, second series • osteosynthesis with cannulated screws, third series • osteosynthesis with T shape "AO" compression plate, fourth series • osteosynthesis with tension band wiring and the fifth series • osteosynthesis with blocking screw/pins patent of Ukraine # 20791 from 2007. The experiment samples were place in a universal testing machine (ZD- 10 Germany). The specimens were subjected to different degrees of forces and the changes along the line of fracture were recorded. To calculate the variation of forces acting on the specimens, determine the direction of these forces and stability of the osteosynthesis the forces were grouped into their various components. Calculation was conducted using finite element method and standard (ANSYS) and geometry was constructed with auto CAD 2006 system.

**Conclusion and result:** The analysis of the results showed, resistance of the method of osteosynthesis in direct relationship to the contact between the various devices and substances of the bone. In blocking osteosynthesis the resistance increases with increase in forces acting on the specimens while it was reverse in every other series.

### Evaluation of patients with coxarthrosis using the Harris Hip Questionnaire

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**Introduction:** Coxarthrosis is a degenerative condition of the coxo-femoral joint which involves impairment in the patients' everyday lives. It has a progressive and irre-

versible evolution which leads to serious limitations of the movements of the limb and invalidity.

**Aim:** The aim of our study is the pre and postoperative assessment of 115 patients hospitalized for total hip arthroplasty in the Orthopaedics Department of the Clinical Emergency Hospital Bucharest. We evaluate the evolution status of the patients and the functional outcome of the surgical treatment.

**Methods and Materials:** The study involves 115 patients, 73 men (63,47%) and 42 females (36,52%) with ages between 67 and 84, 38 (33,04 %) from rural environment and 77 (66,95 %) from urban environment, hospitalized for the surgery during June 2007 – February 2009. We used the Harris Hip Questionnaire, a set of questions divided into 4 categories (pain, function, functional activities and physical exam) with a total of 100 points. For each patient the scores were calculated preoperatory and at the periodic 2 weeks, 6 weeks, respectively 3 months postoperative evaluations. The results are grouped into 4 categories of score: poor (under 70 points), fair (between 70-79), good (between 80-89) and excellent (between 80-89). Observations Scores under 70 points were obtained by patients at the preoperative assessment, indicating a major damage of the hip joint. High scores, over 85 points were obtained at the postoperative assessments indicating a good functionality of the limb. We notice an important increase of the functional Harris Hip Score post arthroplasty, demonstrating the improvement of the patients' status and a good functional outcome of the surgery.

**Conclusion:** The Harris Hip Questionnaire represents a useful and objective method which helps the surgeon evaluate the patients status before and after total hip arthroplasty.

### **The modified Latarjet procedure – A series of cases**

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Arthroscopic treatment of recurrent post-traumatic anterior instability has been proven successful in all patients except those with significant bone deficiency of more than 25% of the inferior glenoid diameter or engaging Hill-Sachs lesion. In our report we present the cases of 21 young professional athletes, with a history of post-traumatic anterior-inferior glenohumeral instability in their dominant shoulders. Glenoid bone deficiency was documented radiographically with antero-posterior, true antero-posterior, West Point and Bernageau views, and 3D computed tomographic scans. We performed modified Latarjet coracoid transfers to replace glenoid bone loss and augment the concavity of the glenoid articular arc. An open deltopectoral approach was used to harvest the coracoid graft, with the attached conjoint tendon (vascularized graft). The exposure of the anterior shoulder was achieved by splitting of the subscapularis tendon, while the articular capsule was incised vertically and reflected laterally. The graft was provisionally fixed with guide pins for optimal alignment with the glenoid arc, then permanently secured with 2 bicortical screws. A sling was worn for 4 weeks, gentle external rotation stretching was

begun at 6 weeks, graft consolidation was documented at 9 weeks, and return to sports was allowed at 6 months. At present (up to 36 months postoperative), patients have fully returned to their pre-injury level of activity. We consider that the modified Latarjet procedure effectively diminishes the shoulder's tendency for dislocation during off-axial loads. Major advantages of the technique include the augmentation of the glenoid bone loss and conjoint tendon transfer serving as a constraint to antero-inferior translation.

### **Comparative analysis of mini-invasive endoscopic treatment versus open treatment of carpal tunnel syndrome**

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**Introduction:** Carpal tunnel syndrome is one of the most common peripheral neuropathies. It affects mainly middle aged women. The classic symptoms of CTS include nocturnal pain associated with tingling and numbness in the distribution of median nerve in the hand. In the majority of patients the exact cause and pathogenesis of CTS is unclear. Our study compares the results of open release of carpal tunnel syndrome with an endoscopic release, which uses a minimal access approach.

**Methods:** A retrospective study was conducted on two groups of patients operated on by the same surgical team between January 2006 and April 2009, in the Orthopedic department in "St. Anna" University hospital. All cases presented with numbness of six months duration or more, and a positive Phalen's test. Open carpal tunnel release (CTR) was done in the first group of 24 consecutive patients before the minimal access surgery introduced in January 2006. The new approach used in a second consecutive group of 62 patients. In two patient the minimal access surgery was abandoned because the median nerve could not be safely separated from the transverse carpal ligament.

**Results:** The two groups were shown to be comparable with respect to clinical presentation and nerve conduction studies. The main advantage of the minimal access surgery came from the shorten period of observation, less postoperative pain and risk of wound fibrosis, which often return the carpal syndrome with more severe complains. The numbness and patient satisfaction, followed in 6 months after procedure was better. Patients must take neurotrophic medicaments, and go to physiotherapy.

**Conclusion:** There is an evidence supporting the need for replacement of standard open carpal tunnel release by existing alternative surgical procedures as minimal access surgery or the treatment of carpal tunnel syndrome. Compared with open CTR, endoscopic CTR has the advantage of reduced pain of scar and shortened time of hospitalization and recovery. It is a safe and effective method for treating idiopathic CTS. The decision to apply endoscopic carpal tunnel release instead of open carpal tunnel release seems to be guided by the surgeon's and patient's preferences. **Keywords:** Carpal tunnel release, minimal access surgery, nerve conduction

## Harris Hip Score in the Evaluation of Postoperative Results in Trochanteric Fractures

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During the last 50 years, the treatment of trochanteric femur fractures has evolved with improved understanding of both fracture biology and biomechanics. Previously, nonsurgical treatment of these fractures was associated not only with significant shortening and malrotation but also with the morbidity and mortality of prolonged immobilization.

**Objective:** The aim of the present paper is to compare the results obtained using two osteosynthesis methods developed for the surgical treatment of fractures of the trochanteric region of the femur: the Gamma nail and the Dynamic Hip Screw (DHS) using the Harris Hip Score.

**Material and method:** A retrospective study is presented of 73 cases of patients sustaining different types of trochanteric fractures treated surgically in the second Orthopedic Department at "Floreasca" Emergency Hospital, Bucharest, Romania between March 2007 and September 2008. There were 48 females with an average age of 68 (46 – 93 years old) and 25 men with an average age of 63 (40 – 89 years old). Thirty-one trochanteric fractures of the femur were treated using the Gamma nail and forty-two were treated with the Dynamic Hip Screw (DHS) in our service with the prerequisite of a minimum follow-up of three months. Main Outcome Measurements: Radiographs were analyzed for fracture classification, evaluation of fracture reduction, implant positioning, later loss of reduction, and other complications. Postoperative functional status of the patients was recorded, with a minimum of three months follow-up using the Harris Hip Score.

**Results:** Nine percent of the patients in the DHS group and four percent in the Gamma group suffered significant secondary redisplacement during the three months follow-up, leading to a varus malunion, lag screw cutout, or excessive lag screw sliding with medialization of the distal fracture fragment. One patient (2.0 percent) in the DHS group suffered an implant-related femoral fracture below the plate and needed surgical reintervention, and one had a deep infection in the DHS group. All but one fracture in the Gamma group and two fractures in the DHS group healed within three months. After six weeks and then later at three months we evaluated the patients both radiologically and clinically using the Harris Hip Score Questionnaire. On the six weeks follow-up we obtained an 86 (66-97) medium Harris Hip Score for the Gamma nail group and a 77 (63-98) for the DHS group. On the three months follow-up we noticed that the medium Harris Hip Score for the Gamma nail group increased up to 91 (75-99), while for the DHS group was 89 (69-98).

**Conclusion:** The functional outcome of the surgical treatment in trochanteric fractures using the Gamma nail at six weeks evaluation seems to be above the results obtained using DHS, due to less exposure of the fracture, limited blood loss and early mobilization, however at the three months evaluation the Harris Hip Score leveled for the two groups, offering similar functional results.

## Clodronate for prevention of glucocorticoid-induced osteoporosis

ESC-ID 427  
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**Aim:** Osteoporosis is a common complication of long term glucocorticoid therapy for which there is no well proved preventive or restorative remedy. This double blind, placebo-controlled study evaluated safety and efficacy of clodronate intermittent cyclical therapy in prevention of corticosteroid induced bone loss.

**Method:** We randomly assigned 160 patients, 18 to 90 years of age commencing or already taking glucocorticoid and had bone mineral density more than 2.5 SD below mean young normal values at lumbar spine or femoral neck [patients did not have other diseases or take drugs known to influence bone metabolism] to receive intermittent cyclical Clodronate (400 mg daily for 2 weeks) plus Calcium (500 mg daily for 11 weeks) or intermittent cyclical placebo with Calcium. The primary outcome measure was the amount of change in bone mineral density of lumbar spine from baseline to week 97.

**Results:** At 97 weeks bone mineral density was maintained of the lumbar spine (+1.4%) in the intermittent cyclical clodronate group, while significant bone loss occurred in the placebo group (-1.6%,  $p = 0.03$ ) At the femoral neck there was a non significant bone loss in the cyclical regimen group (-1.0 %) while in the placebo group bone mass decreased significantly (-3.6 %,  $p < 0.001$ ). The difference between placebo and cyclical clodronate groups was significant at the lumbar spine ( $p = 0.009$ ) but did not reach statistical significance at the femoral neck. Treatment withdrawal led to bone loss in the cyclical clodronate group that was significant at lumbar spine. A similar number of patients experienced adverse events (including upper GI events) across treatment groups and clodronate was generally well tolerated. **Conclusion:** The results of this study confirm the protective influence of clodronate on glucocorticoid-induced bone mass loss (at lumbar spine) [as documented in mean values in BMD reported at the end of treatment compared with baseline values].

## Comparative histological analysis of standard and defect healing in a rat femoral osteotomy model

ESC-ID 648  
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**Introduction:** Bone healing is an efficient process, but delayed and non-unions are challenging problems that may occur following fracture or osteotomy. Bone healing is a highly complex process, so it is not surprising that current therapies can often be ineffective in treating delayed or non-unions. Current therapies may not ade-

quately address the (patho-) physiological healing situation. Thus, for the development of effective therapies to treat patients with these complications, it is essential to obtain a more in-depth understanding of the pathophysiology of healing leading to delayed/non-union. The aim of this study was to establish a rat femoral segmental defect model that would result in a non-union and to compare the biological processes in this model to those of a standard healing model.

**Methods:** A mid-shaft transverse osteotomy of the left femur (1 mm and 4 mm gap) was created in female Sprague Dawley rats (n = 128) and stabilised with an external fixator. Animals were sacrificed at 2, 3, 4 and 6 weeks post-operatively, according to characteristic time points of fracture healing in rats. Examinations of decalcified paraffin-embedded and undecalcified PMMA-embedded (polymethylmetacrylate) bone samples were performed by histology and immunohistochemistry.

**Results:** Preliminary results of the histological examination of sections through the osteotomy region demonstrate (1) complete bony bridging of the 1 mm osteotomy gap by week 6 postoperatively in the standard healing group, (2) no bony bridging of the 4 mm defect by week 6 postoperatively in the defect healing group with resorption of cortical bone ends, gap filling with fibrous tissue, and sealing of the medullary cavity by bony tissue.

**Conclusion:** The development of a reproducible small animal non-union model based on a 4 mm segmental femoral bony defect provides a valuable platform for evaluation of biological changes that occur during defect healing that can be used to establish novel approaches in tissue engineering.

## Paediatrics

### Venous extension of Wilm's tumor in Iranian children

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**Introduction:** Wilm's tumor is the most common renal malignancy and the second common solid tumor in children.

**Aim:** The aim of this study is to define incidence of wilm's tumor venous extension, clinical and pathological manifestations of this neoplasm in a pediatric oncology referral center in Tehran.

**Methods and materials:** In this cross sectional study all patients with pathologically approved Wilm's tumor at oncology department of Aliasghar pediatric hospital in time period of April 1998 to June 2008 were studied retrospectively. History of illness, imaging reports, surgical and pathological reports were reviewed to collect following data: gender, age, presenting symptoms, site of primary involvement, IVC extension, Renal vein extension, extension to portal vein and right atrium, site of distant metastasis, pathologic grade and stage of neoplasm.

**Results:** Of 100 patients, who were studied, 53% male 43% female with age mean of 40 months, primary site of tumor was 47% at right kidney, 46% at left and 5% bilat-

eral. Venous extension was detected in 6% of patients, in 5% of patients IVC, 4% renal vein, 1% portal vein and 1% right atrium were involved. 66.6% of venous extensions were detected before surgery. The most common symptom was abdominal mass and the most common site of distant metastasis was the lung. There were no statistically significant difference between belonging to specific gender or age group and having venous extension, but there was statistically significant difference between favorable and unfavorable pathologic grade in having venous extension (p = 0.03).

**Conclusion:** prevalence of venous thrombosis was evaluated 6% in this study which is compatible with the report of "National Wilm's Tumor Study 4", but in contrast with their result that all these extensions were diagnosed pre-operatively we had diagnosed 66.6% of all and the rest was detected during surgery, that could be due to lack of complete diagnostic imaging processes.

### A rare case of hepatosplenomegaly, cytopenia and fever in a new-born

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**Background:** Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening disease that usually affects infants and very young children, a condition that affects only one in 1,2 million people. The disease, often misdiagnosed, has a 60% mortality rate.

**Aim:** We report an unusual case of hepatosplenomegaly, cytopenia and fever in a pediatric patient, diagnosed with an extreme rare disease and its management. This case illustrates the various interpretations of these clinical symptoms and the difficulty to find the right diagnosis.

**Methods and Material:** A 3-months-old little girl with fever, pallor, hepatosplenomegaly, anemia and thrombocytopenia was referred to our pediatrics department. She was born at term, with a weight of 4210g and a length of 54cm, with APGAR 9 (10). In the patient's family history it is important to mention the death of another 6 months brother in 2004 with the same clinical presentation. The presumptive diagnosis at that time was neonatal hepatitis (the clinical findings were: hepatomegaly, pallor, jaundice; and paraclinical: pancytopenia). Starting with April the fourth 2009, the little girl presents lost of appetite and agitation. During the medical consultation we consider the differential diagnosis between various infectious diseases, a malignant process, autoimmune lymphoproliferative syndrome (ALPS) and hemophagocytic lymphohistiocytosis (HLH).

**Results:** Using HLH 2004 guidelines, we were able to confirm the positive diagnosis of HLH, fulfilling six out of eight possible criteria (minimum necessary being five): fever, splenomegaly, cytopenia (affecting >2 or 3 lineages in the peripheral blood: hemoglobin (53 g/L) and platelets (56 x 10<sup>9</sup>/L) hypertriglyceridemia (369 mg/dl) and/or hypofibrinogenemia (160 mg/dl), hemophagocytosis in bone marrow, ferritin (10.600 µg/L). A peripheral blood sample of patient and her 3 y old brother was sent for molecular analysis in a highly specialised laboratory abroad. The patient started the treatment according HLH-

2004 protocol. After the first week the child responded very well. The spleen decreased from +7 cm below costal margin to the normal size, liver decreased from +5 to +2; also the platelets raised from  $56 \times 10^6/L$  to  $240 \times 10^6/L$ . *Conclusions:* It should be detained that HLH is a under diagnosed disease in our country and it also should be taken into consideration as a possible diagnosis in a young children.

**Analysis of clinical, laboratory, radiological, pathohistological and cytogenetical characteristics of high risk neuroblastoma and response to the therapy - A single institution experience**

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*Introduction:* Neuroblastoma is a tumor derived from pluripotent sympathetic cells and it is the most common extracranial solid tumor in infancy. Poor prognosis of patients with neuroblastoma is associated with risk factors such as: age, advanced stage with metastases, unfavorable histopathology and presence of genetic alterations - n-myc amplification, 1p deletion, 2q gain, 17p deletion, 17q gain and DNA ploidy.

*Aim:* Analysis of clinical, laboratory, radiological, pathohistological and cytogenetical characteristics of high risk neuroblastoma and response to the therapy in patients with this malignancy.

*Material and Methods:* Our study was carried out on 16 children (11 male, 5 female), aged 6 days - 7,25 years who had been diagnosed with high risk neuroblastoma, based on clinical manifestations, laboratory results, radiological findings of primary and metastatic tumors, histopathological and/or cytogenetical analysis of tumor and bone marrow aspiration and biopsy. 13 out of 16 children were treated in accordance with the HR-NBL-1/ESIOP protocol. 3 patients died prior to treatment initiation.

*Results:* Primary tumors were operable and fully extirpated in 4 (25%) out of 16 children before the administration of induction chemotherapy. 13 out of 16 children (81,25%) received induction chemotherapy according to COJEC protocol. Complete chemotherapy consisting of 8 cycles was administered to 10 patients, 1 patient received 5 cycles, and in 1 patient chemotherapy is in progress. After they received chemotherapy, the tumor became operable in 6 more patients, and was totally resected in 3 patients, and partially resected in 3 patients. The most common side effect of induction chemotherapy was febrile neutropenia, that had occurred in 5 children (38,46%), and as a result 1 of them developed febrile convulsions. Ileus developed in 2 patients (15,38%) who had previously underwent an abdominal tumor operation; in 1 patient the tumor was fully extirpated before induction chemotherapy and ileus developed in the 31st week of treatment. The other patient's tumor was partially resected after induction chemotherapy, and ileus developed during radiotherapy. In both children the treatment for ileus was conservatory. Treatment in accordance with HR-NBL-1/ESIOP protocol was completed in 11 patients, and it is still in progress in

2 patients. 8 out of 11 patients died (72, 72%) - 1 due to the toxic effects of treatment, other 7 patients due to progression of primary disease. 3 patients (27,27%) have been disease free for 72, 49 and 48 months since the beginning of treatment.

*Conclusion:* Our results correspond to those of other institutions that use the same protocol for high risk neuroblastoma treatment. However, for a final conclusion it is necessary to conduct a multicentric cooperative study with a higher number of patients.

**The spectrum of manifestation of Tuberculosis among pediatric patients admitted at Muhimbili National Hospital (MNH) Dar es Salaam, May 2007 – April 2009**

ESC-ID 920  
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*Introduction:* About one third of the world's population is infected with Mycobacterium tuberculosis and each year, about 9 million people develop TB, of whom about 2 million die. Of the 9 million annual TB cases, about 1 million (11%) occur in children (under 15 years of age). Of these childhood cases, 75% occur annually in 22 high-burden countries that together account for 80% of the world's estimated incident cases (WHO, 2006). Tanzania ranks 14th on the list of 22 high-burden TB countries in the world (USAID, 2009).

*Aim:* The study aimed at describing the spectrum of manifestation of TB among pediatric patients admitted at MNH Dar es Salaam, May 2007 to April 2009.

*Methods and Materials:* A retrospective, descriptive study was done. Data was retrieved from medical files of pediatric patients who were diagnosed and treated for tuberculosis at MNH between May 2007 and April 2009.

*Results:* A total of 96 children were diagnosed and treated for TB in the 2 year period. Their ages ranged from 2- 84 months. Of these, 60.4% were males while 39.6% were females. 34% had a history of contact with a source case. 74% were diagnosed with pulmonary TB (PTB), 21% with extra-pulmonary TB (EPTB) and 5% with both PTB and EPTB. The most common site of EPTB was the lymph node (40%). 68% of the children were tested for HIV, 28% of these tested positive while 40% tested negative. Child's HIV Sero-status was not significantly associated with PTB or EPTB, perhaps because a large number (32%) were not tested for HIV. 76% were discharged home to continue treatment while 24% died in the ward.

*Conclusion:* The lymph node is the most common site of extra-pulmonary occurrence of TB among pediatric patients at MNH. HIV testing of children diagnosed and treated for TB is sometimes not done at MNH in spite of Tanzania being a high HIV prevalence setting.

### The body mass index influence on exertion tolerance and upper airway infectious diseases frequency in pre-school children

ESC-ID **653**  
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*Aim:* The estimation of the exertion tolerance in pre-school children has a fundamental importance due to its great weight for morbidity on cardiac diseases in the future. The aim of the study was to evaluate the influence of the pre-school children's Body Mass Index (BMI) on exertion tolerance and upper airway infectious diseases frequency.

*Methods:* The study group was consisted of 252 children (girls = 144, boys = 108) at age of 3- 6 years attending Warsaw's nurse schools. The questionnaires concerning a child's labour and later physical development were distributed to their parents. Body and vital parameters including: height, body mass and heart rate at rest as well as after exertion were measured. BMI was counted for each child. Exertion consisting of 20 knee bends but no longer than 30 seconds was performed in the same pattern in each patient. The results were given in percentiles according to the percentile charts for Polish children. The study group was divided into 5 groups concerning the BMI- percentile value. Group 1: = 90pc (overweight children)

*Results:* Group 1 (27 children): Mean heart rate at rest (HR1) = 90/min, mean heart rate after an effort (HR2) = 113/min. Mean heart rate after an effort (HR) increased by 23/min, BP = 103/71. Group 2 (54): HR1 = 91/min, HR2 = 116/min, HR = 25/min, BP = 103/70. Group 3 (66): HR1 = 88/min, HR2 = 114/min, HR = 26/min, BP = 104/71. Group 4 (58): HR1 = 91/min, HR2 = 120/min, HR = 29/min, BP = 108/71. Group 5 (49): HR1 = 96/min, HR2 = 121/min, HR = 25/min, BP = 110/74. 100% patients from the groups 1,2,3, 4 (no overweight children) and 62% children from the 5. group did an exertion test in a accurate time span. Among patients in the groups 1, 2, 3 the infection's frequency was 0,65/child/2 months, while in the group of overweight children- 1,24/child/2 months (p 90pc withstand worse the physical effort in comparison with their peers with proper BMI (25- 75pc). 2.The value of blood pressure is statistically significant higher in overweight children than in children with proper BMI. 3.Children with high value of BMI (> 75pc) reluctantly execute the physical effort and in consequence they do not achieve expected results in increase of heart rate. 4.The frequency of upper airway infectious diseases is higher in overweight children than in patients with correct weight.

### Pharmacology

#### Underlying mechanisms for the medicinal use of Morus alba (Shahtut) in diarrhea, constipation and asthma

ESC-ID **396**  
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*Aim:* The study was aimed to investigate the antispasmodic, spasmogenic and bronchodilatory effects of the crude extract of Morus alba (Ma.Cr) commonly called The White Mulberry. Introduction: Recent literature cites Morus alba as an anti-oxidative and anti-melanin agent that also demonstrates anti-leukaemic and anti-inflammatory activity. It regulates the levels of lipids by lowering cholesterol levels and also acts as a hypouricemic agent. Ethyl acetate extract from the leaves induces endothelium-independent relaxation and contraction of rat aorta. It also has ameliorative effects on alcohol-induced fatty liver and inhibitory effects on intestinal disaccharidase activity that justify its use in the treatment of diabetes. Its constituents include adenine, albufuran-c, arachidonic acid, arsenic, ascorbic acid, benzaldehyde, biotin, caffeic acid, campesterol, beta-carotene, choline, chysanthemin, citric acid, m-cresol, o-cresol, p-cresol, cyclomulberrin, cyclomulberochromene, beta-ecdysterone, eugenol, folic acid, folinic acid, glutathionine, cis-5-hydroxy-l-pipecolic acid, trans-5-hydroxy-l-pipecolic acid, linoleic acid, methyl-salicylate, moracenin-d, moracetin, morachalcone-a, morin, morusin, mulberranol, mulberrin, mulberrochromene, mulberrofuran-b, mulberrofuran-m, mulberrofuran-n, niacin, pelargonidin-3-glucoside, phytate-phosphorus, pipecolic acid, riboflavin, sarcosine, scopoletin, scopolin, beta-sitosterol, tannin, thiamin, valeric acid and xanthophylls.

*Methods and Materials:* Prepared rabbit jejunum and guinea-pig ileum ex-vivo assays were used. Ma.Cr as well as its various fractions, separated by fractional distillation after dissolution in various organic solvents, were tested using isolated tissue bath assemblies attached to polygraph or Powerlab to record the results. The results were tabulated and analysed using Graph Pad Prism.

*Results:* Ma.Cr caused a dose-dependent (0.03-3 mg/mL) relaxation in jejunum, similar to verapamil or dicyclomine, while atropine exhibited partial relaxation. Ma.Cr relaxed carbachol and K<sup>+</sup> (80 mM)-induced contractions similar to dicyclomine, but different from verapamil and atropine. Ma.Cr caused a rightward shift of the Ca<sup>2+</sup> dose-response curves (DRCs) to the right, similar to dicyclomine or verapamil. In the presence of atropine (0.1 μM), the spasmolytic effect of Ma.Cr was potentiated in jejunal preparations. In guinea-pig ileum, both Ma.Cr and its aqueous fraction (Ma.Aq) exhibited atropine-sensitive contraction. Ma.Cr caused a rightward parallel shift of the acetylcholine DRCs at lower dose (0.03 mg/mL) and suppression of maximum response at next higher dose (0.1 mg/mL) like dicyclomine. In rabbit trachea, Ma.Cr caused full relaxation of CCh (0.03-5 mg/mL) and partial relaxation on K<sup>+</sup> (80 mM)-induced contractions. Bio-guided fractionation revealed the presence of anti-muscarinic like constituents in the petroleum fraction (Ma.Pet),

whereas the chloroform fraction (Ma.CHCl<sub>3</sub>) was enriched with Ca<sup>2+</sup>-channel antagonizing components.

**Conclusion:** These results suggest that the antispasmodic and bronchodilatory effects of *Morus alba* are mediated through a dual blockade of muscarinic receptors and Ca<sup>2+</sup> channels, while the laxative effect is due to the activation of muscarinic receptors, which may rationalize its medicinal use in diarrhea, constipation and asthma.

**Key words:** *Morus alba*; Antispasmodic; Spasmogenic; Bronchodilator

### Studies regarding the implication of nitric oxide in peripheral and spinal nociceptive sensibility

ESC-ID 761

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**Aim:** The implication of nitric oxide in the algescic process is already known, the purpose of this study was to specify the two components- peripheral and spinal, of the nociceptive sensibility, and the modification induced by the L-arginine/NO synthase/nitric oxide.

**Method and Materials:** There were used lots of male Wistar rats, in accordance with the international guidelines regarding ethics. To estimate, at peripheral and spinal levels, the role of NO in nociceptive processing, there were examined on different lots the effects of intraperitoneal (i.p.) and intrathecal (i.t.) of: 1) L-arginine, precursor of nitric oxide (i.p. 50-100mg/kg, i.t. 1mg/rat), 2) NG-nitro-L-arginine-methyl-ester (L-NAME), an nitric oxide synthase inhibitor (i.p. 20-75mg/kg, i.t. 0,1mg/ rat), 3) the association of L-NAME and L-arginine. The administration of the substances was made by injecting percutaneous for the intraperitoneal administration and by introducing a catheter for the intrathecal administration. To test pain sensibility there were used mechano-algescic and thermo-algescic (tail flick and plantar test) tests.

**Results:** The results were compared with the ones obtained on the witness lots for every method. Statistical analysis of the results was performed using t test and ANOVA one-way method. The results obtained using the tail flick test weren't statistically significant. The mechano-algescic and the plantar test registered an increase of response latency to nociceptive stimuli. This result was evident after the administration of NO synthase inhibitor (L-NAME).

**Conclusion:** The data obtained show new proof in supporting the role of L-arginine/NO synthase/nitric oxide system in the nociceptive process.

### The effect of tocopherol on iron content in serum and thoracic aorta tissue in experimental atherosclerosis

ESC-ID 599

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**Aim:** This paper deals with the effect of tocopherol on iron content in serum and thoracic aorta tissue in experi-

mental atherosclerosis. Having in mind the importance of iron as a catalyser of some oxidants production, we examined the iron content in serum and thoracic aorta tissue of rabbits with atherosclerosis induced by hypercholesterolemic diet (4% solution of crystalline cholesterol in edible oil).

**Methods and Materials:** For this study six groups of seven rabbits each were used: C - control group on the usual diet for this species, O - control group on an oil-containing diet, Ch - experimental group fed on a hypercholesterolemic diet, T - experimental group received tocopherol intramuscularly, ChT - experimental group treated with cholesterol and tocopherol, and OT - experimental group received oil and tocopherol. Iron content was quantified by atomic absorption spectrophotometry. Experimental atherosclerosis was pathohistologically confirmed.

**Results:** After two-months of treatment highly significant decrease of iron content was registered in serum of T and OT group (p <0.01) compared to both control groups. In comparison with Ch group serum iron content was highly significantly decreased in T, OT and ChT group (p <0.01). Aorta tissue iron content was highly significantly decreased in O group (p <0.01) compared to C, Ch and ChT group. Our findings indicate that tocopherol exerts influence on iron content in serum and thoracic aorta tissue in rabbits with experimental atherosclerosis.

**Key words:** iron, experimental atherosclerosis, hypercholesterolemic diet, iron, rabbits, tocopherol

### Isolation and identification of polyphenolic compounds from defatted seeds of *Oenothera paradoxa*

ESC-ID 474

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**Introduction:** *Oenothera paradoxa* Hudziok is known as the main source of seeds that are used in pharmaceutical industry to obtain cold press oil. Evening primrose oil (EPO) is widely used in medicine to treat many affections. EPO contains relatively high amounts of  $\alpha$ -linolenic acid that is precursor of prostaglandin E<sub>1</sub> which shows anti-inflammatory activity. After EPO extraction pharmaceutical companies get big amounts (about 50 tons per year) of defatted seeds that must be utilized.

**Aim:** The aim of study was to isolate and identify some phenolic compounds from defatted seeds of *Oenothera paradoxa*. Examining phytochemical profile of defatted seeds may open new possibilities of using them as potential source of bioactive compounds that would partially solve problem of expensive utilization.

**Material and methods:** Aqueous extract of defatted seeds was obtained from Agropharm S.A. (Tuszyn, Poland). First purification was carried using extraction with ethyl acetate. Isolation of unknown compound using open-column chromatography (CC) and flash chromatography applying different sorbents such as polyamide, silica gel, toyopearl and RP-18. Thin layer chromatography using polar and non-polar eluents. Identification was performed using HPLC-DAD, MS and NMR spectroscopy.

**Results:** Preliminary research using TLC and HPLC showed that, apart from known compounds such as catechin, gallic acid, elagic acid, caffeic acid and penta-

galloyl glucose aqueous extract also contains several unknown substances. Using column liquid chromatography we managed to isolate three compounds which we suspect to be catechin's and gallic acid's derivatives. We confirmed using MS spectrum and HPLC that one of them is ethyl gallate which has not been found before in *Oenothera paradoxa*. Apart from phenolic compounds we confirmed that aqueous extract contains considerable amount of glucose and linolenic acid.

**Conclusion:** Aqueous extract from defatted seeds of *Oenothera paradoxa* contains wide variety of polyphenolic compounds which shows possibility of its usage in medicine. To determinate exact structure and bioactivity of undefined compounds further research must be carried.

### Nitric oxide's metabolic pathway and cascade of arachidonic acid in pathogenesis of primary hypertension and endothelial dysfunction in young men

ESC-ID **590**  
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**Background:** Endothelium plays a crucial role in regulation of vascular tone and endothelial dysfunction (ED) is an important risk factor of cardiovascular diseases. The mechanisms involved in decreased vasodilative activity of endothelial cells include decreased bioavailability of nitric oxide, oxidative stress and disorders in metabolism of prostanoids, which could thus play important role in the pathogenesis of essential hypertension. The aim of this study was to explore the role of nitric oxide's metabolic pathway and of prostanoids in pathogenesis of ED in young men with and without essential hypertension (HTN).

**Material and methods:** N = 70 men aged 18-40y (N = 23 with essential hypertension and N = 47 normotensive) were investigated. Initial concentrations of: 1) metabolites of nitric oxide's pathway (ADMA, L-Arg, SDMA), 2) biochemical markers of increased cardiovascular risk 3) markers of oxidative stress (MD/HNN, GSH/GSSG) and 4) prostanoids (TxB2, 6-keto-PGF1alpha) were measured. Ultrasound assessment of endothelial function - flow mediated vasodilation (FMD) of brachial artery was studied before and after intravenous infusion of 16g L-Arginine. All measurements were repeated after oral administration of non-selective inhibitor of cyclooxygenase, indomethacin - 75mg/24h for 2 days.

**Results:** The prevalence of endothelial dysfunction was similar in hypertensive and in normotensive group. There were no significant differences in ADMA level and L-Arg/ADMA ratio in HTN(+) vs. HTN(-) subjects at baseline and after treatment with indomethacin as well. Higher baseline plasma level of TxB2 and lower of 6-keto-PGF1alpha in group with hypertension were observed. A different response on indomethacin depending on the presence of hypertension as assessed by prostanoids' level as well as by FMD was observed.

**Conclusions:** 1. For the first time we have shown that ED in young men without hypertension is mainly dependent on nitric oxide deficiency, but in young men with coexis-

tent hypertension a significant role of prostanoids' pathway is observed as well. 2. Oxidative stress plays an important role in pathogenesis of ED in both groups. 3. A novel observation from our study is that HTN and ED are partially independent of each other and are characterized by different regulation patterns of endothelium dependent vascular tone.

### The investigation of the oxidative stress parameters in hyperhomocysteinemia induced in rats

ESC-ID **534**  
 Author **Jigaranu A**  
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 Country **Romania**  
 Institution **University of Medicine and Pharmacy "Gr.T.Popa" Iasi**

**Introduction:** Hyperhomocysteinemia is a pathological condition characterized by high blood levels of homocysteine. It is known the fact that increased plasmatic levels of homocysteine affect the epithelial function and are associated with cardiovascular diseases. The mechanism through which homocysteine produces these effects is not discovered yet but it is believed that in this process are involved reactive species generators of free radicals.

**Aim:** In this paper we studied the influence of hyperhomocysteinemia experimentally induced on systems involved in antioxidant defence at lab animals (rats).

**Method and Materials:** There were determined the plasmatic concentrations of homocysteine and the intracellular activity of superoxide dismutase (SOD), glutathione peroxidase (GPx) and the total serum antioxidant capacity (CAT).

**Results:** The results show an increase of the SOD activity which means an increase of reactive species. At the same time there is a decrease in the activity of both GPx and CAT as result of the reduced detoxification capacity of reactive species.

**Conclusion:** We can say that high plasmatic levels of homocysteine are associated with a diminution of the antioxidant capacity.

### Study of protective Effect of Enalapril in paraquate induced fibrosis of lungs in Rats

ESC-ID **550**  
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**Introduction:** Interstitial lung disease is a serious problem, which led to lung fibrosis and this process seem to be progressive and irreversible. There are many drugs used to decelerate this process. In this article we study protective effect of Enalapril in lung fibrosis as some pervious study support it and it is a routine one in drugstore.

**Material and Methods:** We had two groups of rats. The first one is 20 rats which get enalapril through their water and control group 20 rats which is fed by just pure water without additive. The rats had been induced for lung fibrosis with paraquate, Time and dosage spent for this process was based on previous pilot study.



**Results:** After staging fibrosis of lungs with trichrome staining, lungs in rats which get enalapril are less fibrotic than the other one statistically meaningful.

**Conclusion:** We get to this idea that enalapril and maybe other ACE inhibitors can have protective effect in lung fibrosis even in human and other organs. Anti oxidative effect of Enalapril can explain it to some how.

**Key words:** Enalapril, Lung fibrosis, Rat, Anti oxidative

### The investigation of the oxidative stress parameters in hyperhomocysteinemia induced in rats

ESC-ID **680**  
 Author **Murariu O**  
 Coauthor **Jigaranu Anca-Olivia, Onofrei Alexandra, Burlui Alexandra, Florescu Victor**  
 Country **Romania**  
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**Introduction:** Hyperhomocysteinemia is a pathological condition characterized by high blood levels of homocysteine. It is known the fact that increased plasmatic levels of homocysteine affect the epithelial function and are associated with cardiovascular diseases. The mechanism through which homocysteine produces these effects is not discovered yet but it is believed that in this process are involved reactive species generators of free radicals.

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**Method and Materials:** There were determined the plasmatic concentrations of homocysteine and the intracellular activity of superoxide dismutase (SOD), glutathione peroxidase (GPx) and the total serum antioxidant capacity (CAT).

**Results:** The results show an increase of the SOD activity which means an increase of reactive species. At the same time there is a decrease in the activity of both GPx and CAT as result of the reduced detoxification capacity of reactive species.

**Conclusion:** We can say that high plasmatic levels of homocysteine are associated with a diminution of the antioxidant capacity.

### Cellular uptake and delivery of a liposomal carrier across an epithelial monolayer model depends on the membrane properties

ESC-ID **802**  
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 Coauthor **Zeisig, Reiner; Koklic, Tilen; Sentjurc, Marjeta; Wiesner, Burkhard; Lemm, Margit and Fichtner, Iduna**  
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**Background:** Treatment of brain tumors and metastasis is a serious challenge of oncology because of the limited transport of drugs into the brain. Chemotherapy with systemically used hydrophilic cytostatics often failed, because of the physiological barriers, mainly the blood-brain barrier. The transport of compounds via the BBB can

be improved using liposomes, because of their high lipophilic properties. An encapsulation of drugs into liposomes could improve in this way the drug transport to the brain.

**Aim:** Investigation of the effect of liposomal membrane composition on the interaction with an epithelial cell monolayer.

**Methods:** Liposomal formulations differ in the content of lipids, membrane fluidity and charge were prepared by lipid film hydration. The fluorescent marker calcein was encapsulated. Size, stability, fluidity and amount of entrapped calcein were characterized. Uptake and transcytosis of liposomes by Madin-Darby canine kidney cells, employed to mimic a tight epithelial barrier model, was quantified by fluorescence measurements of liposomal calcein. Tightness of the cell monolayer was controlled by transepithelial electrical resistance measurement. Cells were incubated with liposomes for 24 hours. The marker concentration was determined in the cells after cell lysis and in the basal media of a transwell chamber. Therapeutic studies were performed in a mouse xenograft brain tumor model after an intracerebral injection of MT-3 cells in nude mice. Animals were treated with free or liposomal encapsulated Mitoxantron.

**Results:** Prepared vesicles had a diameter between 100 and 200nm and encapsulated about 90µg calcein/µmol total lipid. Liposomes with a positive charge and formulations which contained the helper lipid DOPE showed the most efficient uptake with values of 190-257nmol calcein/well. Confocal laser scanning microscopy demonstrated that liposomes were clearly endocytosed by the MDCK cells, but were also found bound to the cell membrane. Transcytosis experiments showed the best results with liposomes containing a combination of the helper lipids DOPE and OPP with 591pmol calcein/cm<sup>2</sup> found in the basal medium. Electron paramagnetic resonance measurements revealed a clear increase in transcytosis if membrane fluidity was enhanced. In vivo studies present a significantly better anti-tumor effect of MTO-liposomes compared to the free drug based on the tumor volume reduction. Furthermore the liposomal treatment evidenced lower side effects.

### Prostanoids and nitric oxide's metabolic pathway in pathogenesis of essential hypertension in young men

ESC-ID **595**  
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 Country **Poland**  
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**Background:** Essential hypertension (HTN) is one of the most common diseases in the worldwide population. Although per se is an asymptomatic disorder, in the long term outcome it is characterized by serious cardiovascular complications as well as shortening of expected life time. The aim of this study was to explore the role of nitric oxide metabolic pathway, prostanoids and oxidative stress in pathogenesis of HTN in young men. The modulating influence of i.v. L-Arg infusion and treatment with cyclooxygenase inhibitor on vascular reactivity was investigated.

**Material and methods:** N = 70 men aged 18-40y, with and without HTN (N = 23 and 47, respectively) were studied. Initial concentrations of: 1) metabolites of nitric oxide's pathway (ADMA, L-Arg, SDMA), 2) prostanoids (TXB2, 6-ketoPGF1 $\alpha$ ) and 3) markers of oxidative stress (MDA, thiol index) were measured. Ultrasound assessment of endothelial function - flow mediated vasodilation (FMD) of brachial artery was studied before and after infusion of 16g L-Arginine. All measurements were repeated after oral administration of non-selective inhibitor of cyclooxygenase, indomethacine - 75mg/24h for 2 days.

**Results:** Significant improvement of FMD values in response on infusion of L-Arg as well as on treatment with indomethacine was observed in both groups. Moreover, assessment of both drugs resulted in improvement of endothelial function but only in hypertensive subjects. Group with HTN was characterized by higher initial concentration of TxB2 and lower of 6-ketoPGF1 $\alpha$ . A different response on indomethacine in hypertensive vs. normotensive group was observed. MDA and thiol index have decreased after treatment with indomethacine in both groups.

**Conclusions:** 1. Both, nitric oxide's and prostanoids' metabolic pathways are involved in the pathogenesis of essential hypertension in young men. 2. Treatment with indomethacine and L-Arg has synergistic effect on improvement of endothelial function in young hypertensive men. 3. Hypertensive men are characterized by different reactivity on inhibitor of cyclooxygenase as compared to those normotensive. Key words: essential hypertension, nitric oxide, prostanoids, endothelial function

### The implications of polyphenols in metabolic pregnancy effects of experimental diabetes

ESC-ID 713  
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**Introduction:** Pregestational diabetes mellitus, a condition easy to diagnose before pregnancy, is an important risk factor for the normal faetal development. This is due to a number of mechanisms, and especially through the effects of reactive oxygen species, which stimulate the lipid peroxidation of polyunsaturated fatty acids. Thus it becomes mandatory to apply a treatment during the diabetic pregnancy, which will maintain the blood glucose level as close as possible to the normal value.

**Aim:** In this study we investigated the effects of some natural polyphenols extracted from the Sambucus nigra fruits on both glucose and lipid metabolism and on pregnancy evolution in streptozotocin induced diabetes in rats.

**Material and Methods:** In the experimental model, diabetes mellitus was induced by the intraperitoneal administration of streptozotocin (STZ), 60 mg/kbw, in a single dose. The animals were divided into 4 groups, each group consisting in 10 female adult Wistar rats with an average weight of 250 – 280 grams: a witness group (M); a (DZ) group injected with streptozotocin; a (P) group which received an extract of vegetal polyphenols, in dose of 0.034 g/kbw and a (DZ+P) group, that received the polyphenols extract during 16 weeks after induced diabetes. We followed glucose levels and lipidic profile:

total cholesterol (Ch-T), high-density lipoproteins (HDL), triglyceride (TG) and low-density lipoprotein (LDL). We also followed the pregnancy evolution in female rats.

**Results:** According to the experimental studies, polyphenols extracted from the Sambucus nigra fruits increase the antioxidant activity of serum (in vivo), having an antiradical activity for the reactive oxygen species, which are excessively produced in oxidative stress conditions. The hyperglycemia in rats from the (DZ+P) group was reduced compared to those in the DZ group, after 10 weeks. The serum concentrations of total cholesterol (Ch-T) in the (DZ+P) group were lower than the values in the M group. In DZ group high-density lipoproteins (HDL) had lower values in comparison with both M and (DZ+P) groups. The polyphenols protection of diabetic rats brought the triglyceride (TG) values back to normal limits. Low-density lipoprotein (LDL) determinations showed a significant increase in DZ group and a decrease in (DZ+P) group. The female rats which received polyphenolic extract after streptozotocin induced diabetes, (DZ+P) group, had normal pregnancies, in terms, and they gave birth to living newborn rats. The female rats from DZ group had spontaneous abortions or they gave birth to dead newborn rats.

**Conclusions:** Hyperlipemia, characteristic for the lipid metabolism even in normal pregnancy, will be indirectly influenced and controlled by the use of natural polyphenols. Through their positive influence on experimental diabetes, polyphenols improve hyperglycemia, secondary dyslipidemia and oxidative stress leading to the normal faetal development. Keywords: diabetes mellitus, natural polyphenols, lipidic profile

### The investigation of the oxidative stress parameters in hyperhomocysteinemia induced in rats

ESC-ID 691  
 Author **Jigaranu A-O**  
 Coauthor **Murariu Oana, Onofrei Alexandra, Burlui Alexandra, Florescu Victor.**  
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**Introduction:** Hyperhomocysteinemia is a pathological condition characterized by high blood levels of homocysteine. It is known the fact that increased plasmatic levels of homocysteine affect the epithelial function and are associated with cardiovascular diseases. The mechanism through which homocysteine produces these effects is not discovered yet but it is believed that in this process are involved reactive species generators of free radicals.

**Aim:** In this paper we studied the influence of hyperhomocysteinemia experimentally induced on systems involved in antioxidant defence at lab animals (rats).

**Method and Materials:** There were determined the plasmatic concentrations of homocysteine and the intracellular activity of superoxide dismutase (SOD), glutathione peroxidase (GPx) and the total serum antioxidant capacity (CAT).

**Results:** The results show an increase of the SOD activity which means an increase of reactive species. At the same time there is a decrease in the activity of both GPx and CAT as result of the reduced detoxification capacity of reactive species.

**Conclusion:** We can say that high plasmatic levels of homocysteine are associated with a diminution of the antioxidant capacity.

### **GdCl<sub>3</sub>, a kupffer cell inhibitor, attenuates hepatic injury in a rat model of chronic cholestasis**

ESC-ID **454**

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**Introduction:** In response to tissue insults, kupffer cells release different kinds of toxic cytokines and profibrogenic mediators (e.g. transforming growth factor B1, TGF $\beta$ 1) causing hepatocellular injury and fibrosis (1, 2). It has been demonstrated that gadolinium chloride (GdCl<sub>3</sub>, a selective kupffer cell inhibitor) ameliorates alcohol-induced hepatic injury and also attenuates carbon tetrachloride and thioacetamide-induced hepatic fibrosis in rats (2-4). However the effect of GdCl<sub>3</sub> on hepatic injury induced by chronic cholestasis in bile duct ligated (BDL) rats has not been reported yet and is the aim of this study.

**Materials and methods:** Either bile duct ligation (BDL) or sham operation was performed on male Sprague-Dawley rats (230-260 gr). Rats were divided into four groups. In the control-saline and BDL-saline groups, rats received sterile saline solution (1 ml/kg/day i.p.), but in the control-GdCl<sub>3</sub> and BDL-GdCl<sub>3</sub> groups they were treated with GdCl<sub>3</sub> (20 mg/kg/day i.p.) for 28 days following BDL or sham operations. All the animals were sacrificed and serum samples were obtained for measuring biochemical markers of liver injury. Liver samples were collected rapidly and snap frozen in liquid nitrogen for assessment of lipid peroxidation (by measuring malondialdehyde concentration, MDA) and liver antioxidant enzyme activities like glutathione peroxidase (GPx) and catalase (CAT) as we described previously (5). Formalin fixed paraffin embedded sections of liver were obtained for measuring fibrosis, ductular proliferation and portal inflammation due to previously described scoring systems (6,7).

**Results:** From biochemical markers, GdCl<sub>3</sub> administration partially prevented the elevation in alkaline phosphatase, alanine aminotransferase and gamma-glutamyl transpeptidase, but these markers did not reach normal levels. In BDL-GdCl<sub>3</sub> group MDA concentration was more than control groups and less than BDL-saline group. The hepatic CAT and GPx activity significantly decreased following BDL, but improved by GdCl<sub>3</sub> administration. Biliary obstruction for 28 days resulted in marked morphological changes in liver tissue including formation of extensive fibrosis, portal tracts enlargement with inflammation and extensive ductular proliferation. Degree of fibrosis and inflammation were reduced by GdCl<sub>3</sub> treatment, however ductular proliferation remained prominent. **Discussion:** Kupffer cells by engulfing apoptotic bodies, are triggered to increase cytokine and death ligands expression which contributes to more apoptosis and fur-

ther tissue injury in a feed-forward loop (8). Our results suggest that GdCl<sub>3</sub> by disrupting this loop decreases hepatic inflammation and injury following BDL. As mentioned earlier, activated kupffer cells release profibrogenic mediators like TGF $\beta$ 1, which can be suppressed by GdCl<sub>3</sub> administration (2). It is also demonstrated that GdCl<sub>3</sub> stimulates kupffer cells to release matrix metalloproteinase 13 (an enzyme responsible for degrading fibrotic fibers) in different fibrosis induced models (9, 10). Our results showing less fibrosis score following GdCl<sub>3</sub> is consistent with aforementioned findings. Similar ductular proliferation score in both BDL groups suggests that kupffer cells may not be essential for bile duct proliferation. In conclusion, GdCl<sub>3</sub> diminishes cholestasis-induced hepatic injury. Further research along this line may pave a new path for therapeutic interventions in cholestatic disorders.

## *Physiology*

### **Protective effect of polyphenols in transient cerebral ischemia**

ESC-ID **260**

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**Background:** Polyphenolic compounds possess neuroprotective activities (anti-oxidant, anti-inflammatory, increase in Na-K ATPase activity) that may be beneficial for treating cerebral ischemia. Our objective was to determine whether treatment with grape seed polyphenol extract reduces the effects of cerebral ischemia concerning spatial memory and exploring capacity, protects against oxidative stress and enhances recovery.

**Methods and materials:** 24 Female Wistar rats (Weight = 200g, Age = 3 months) divided in 2 groups: Group 1 were given orally grape seed polyphenol extract (50mg/kg dissolved in 0.5ml carboxymethylcellulose) for 12 days and Group 2 were given carboxymethylcellulose for the same period of time. Then, they were all submitted to water maze testing for 4 days. The following day, they were subjected to transient common carotid artery occlusion for 30 seconds followed by reperfusion. After 72h they were retested for another 4 days. After the testing period, they were sacrificed in order to obtain blood, brain, liver and heart samples for oxidative stress analysis and brain tissue for histopathological analysis.

**Results:** In what regards spatial memory, we noticed an increase in the time spent in quadrant D on the 4th day of testing (20,98  $\pm$  4,7 s) and the decrease in the number of circles (3,25  $\pm$  1,42) in Group 1, which was given polyphenols, before the ischemia. Thus, the rats proved to have memorized the quadrant in which the platform was priorly placed. After the ischemia, the latency time in water maze training decreased in both groups. On the 4th day of testing there was no noticeable difference in the learning process, compared to the pre-ischemic testing. The results for oxidative stress analysis proved that malondialdehyde (marker of lipid-peroxidation) decreases in brain tissue after polyphenol treatment. Histology analysis showed less brain damage in the group given polyphenols.

**Conclusion:** Therefore, in the ischemic injury reactive oxygen species are involved. Polyphenols have a antioxidant effect, improve spatial memory and provide protection against neurodegenerative changes.

### Acute hemodynamic overload during ischemia - cardioprotective effects of ARAs

ESC-ID **418**  
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**Introduction:** Acute hemodynamic overload frequently occurs in myocardial ischemia, leading to a decline in myocardial contractility and an increase in myocardial stiffness. Considering that locally produced angiotensin II (AngII) influences cardiac function in this setting, our objective is to evaluate the systolic and diastolic responses to an acute hemodynamic overload during an ischemic insult in the presence of AngII receptor antagonists.

**Methods:** Rabbit papillary muscles (modified Krebs solution, 0.2Hz, 1.8mM Ca<sup>2+</sup>, 30°C) were mechanically overloaded from 92% L<sub>max</sub> to 100% L<sub>max</sub> (length at which maximal force is developed), during an ischemic insult (Isch, stretch during ischemia, followed by reperfusion; n = 7); during an ischemic insult in the presence of ZD-7155, a selective AT<sub>1</sub>-receptor antagonist (Isch-AT<sub>1</sub>: 10-6M; n = 7) and during an ischemic insult in the presence of ZD-7155 (10-6M) and PD-123,319 (10-6M), a selective AT<sub>2</sub>-receptor antagonist (Isch-AT<sub>1</sub>+AT<sub>2</sub>; n = 8). Immediate and delayed responses to muscle stretch were evaluated.

Results as mean ± standard error (ptolic counterpart of this effect is partially dependent on AT<sub>2</sub> receptor activation. Our results highlight the importance of using ARBs early, during the acute phase of myocardial ischemia. This can probably avoid further hemodynamic decompensation. They also support the use of AT<sub>2</sub> agonists as a potentially new therapeutic target in myocardial ischemia.

### Neuromodulatory effects of norepinephrine on stimulus-induced sharp wave-ripple complexes in the adult rat hippocampus in vitro

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Sharp waves-ripple complexes (SPW-Rs), which have been described in the rat hippocampus in vivo, are thought to be substantially involved in learning and memory by mediating the transfer of declarative information from the hippocampus into the cortical mantle in a process of memory consolidation. As previously reported, in hippocampal slices the induction of SPW-Rs is associated to the induction of long-term potentiation

(LTP) induced by repeated high frequency stimulation (HFS). Here, we employed the in vitro paradigm of hippocampal SPW-Rs to investigate the effects of norepinephrine (NE, 50-100 μM) on both the induction and expression of such network oscillations. We found that NE facilitated the LTP-associated stimulus induction of SPW-Rs in area CA3 and also reversibly blocked their expression in both areas CA3 and CA1 when the drug was applied on established SPW-R activity. Following wash out, we observed a significant increase in both the incidence and amplitude of SPW-Rs. In more detail, we found that the unspecific beta adrenoceptor agonist isoproterenol (2 μM) as well as the beta1 agonist dobutamine (100 μM) significantly enhanced SPW-R activity. In contrast, SPW-Rs were significantly reduced by both alpha1 and alpha2 adrenoceptor agonists methoxamine (100 μM) phenylephrine (100 μM) and clonidine (100 μM), respectively. Moreover, when the unspecific alpha adrenoceptor antagonist phentolamine (100 μM) was administered 30 min before NE was then co-applied, we observed that both the SPW-R incidence and amplitude were increased. In addition, the co-application of the more specific alpha1 antagonist prazosine (100 μM) resulted in a prevention of the previously observed suppression of SPW-Rs altogether suggesting a beta adrenoceptor-mediated effect. Intracellular recordings obtained from both CA3 or CA1 pyramidal cells simultaneously recorded during SPW-R network activity revealed that the blocking effect of NE was accompanied by a pronounced hyperpolarization of about 5-10 mV in the majority of recorded neurons in both regions. Together, our data show that hippocampal SPW-R activity is substantially modulated by NE, and that these effects are mediated by beta1 adrenoceptors. Based on our results that the SPW-R induction was facilitated in presence of unspecific beta agonist and beta 1 agonist (dobutamine), we suggest that the threshold for the induction of hippocampal LTP might be modulated by the activation of beta1 adrenoceptors.

### Strain correlation between preproorexin and interleukin-1 genes expression in cells of rat hypothalamus after injection of subseptic dose of LPS

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Meanwhile participation of orexin-containing neurons of hypothalamus in the process of brain response to antigen challenge is under investigation, cerebral interleukin-1 is well known as a marker of response to antigen challenge. Recently the increase of preproorexin gene expression in cells of rat hypothalamus was shown in 2 hours after i.v. injection of lipopolysaccharide (LPS) in subseptic dose 500 mkg/kg. The aim of this research was to investigate preproorexin and interleukin-1 genes expression levels in hypothalamic cells of male Wistar rats during several hours after i.p. injection of LPS (500 mkg/kg). An intensity of both genes expression was evaluated by RT-PCR in 1 and 2 hours after antigen challenge. 6-fold increase of both genes expression was observed only in 2 hours. Strain correlations (p < 0,01) were observed between pre-

proorexin and interleukin-1 both in 1 and 2 hours after LPS injection. Such correlations were not observed in control groups of animals (i.p. saline injection). This data allow suggesting the possibility of participation of orexinergic neurons in the mechanisms of brain reaction to antigen challenge, and possible involvement of interleukin-1 in these mechanisms.

### Renal excretory function and systolic arterial blood pressure during chronic neuronal nitric oxide synthase inhibition in spontaneously hypertensive rats

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*Aim:* The aim of the study was to examine the participation of neuronal nitric oxide synthase (nNOS) in the regulation of water, calcium (ECa), sodium (ENa), chloride (ECl) excretions and systolic arterial pressure (SAP) in spontaneously hypertensive rats (SHR).

*Methods and Materials:* The experiments were carried out on 32 conscious male rats separated in 4 groups. All rats were housed in metabolic cages with free access to drinking fluids and rat food. First and second group were composed of normotensive Wistar rats (WR, n = 8) and hypertensive rats (SHR, n = 8) drinking tap water. Third and fourth group consisted of WR (n = 8) and SHR (n = 8), which were treated with 7-nitroindazole (7-NI) in dose 10 mg/kg per day dissolved in drinking water for 6 days. The systolic arterial blood pressure was measured daily through tail-cuff method (UgoBasile 5500). The effect of nNOS inhibition was observed for 6 days by collecting urine for 6 hours daily. The urine flow rate (UFR) concentrations of sodium, (Corning 410C) chloride, (Corning 925) and calcium (spectrophotometrically) were determined and the excretions of the investigated parameters were calculated.

*Results:* The SAP in SHR ( $181.04 \pm 0.8$  mmHg) was higher as compared to WR ( $123.8 \pm 1.1$  mmHg), ( $p < 0.001$ ). The inhibition of nNOS causes an increase in the SAP only in WR on the 1st day to  $132.3 \pm 0.3$  mmHg and on the 6th day to  $147.4 \pm 1.4$  mmHg, ( $p < 0.01$ ). In control condition UFR, ENa, ECl, ECa did not differ between WR and SHR. The 7-NI increased UFR in WR from  $1.36 \pm 0.09$  to  $1.84 \pm 0.18 \mu\text{l}/\text{min}/100$  g b.w., ( $p < 0.05$ ) on 5th day and to  $2.75 \pm 0.28 \mu\text{l}/\text{min}/100$  g b.w., ( $p < 0.05$ ) on 6th day after the nNOS inhibition. The UFR also increased in SHR even on the 1st day from  $1.12 \pm 0.08$  to  $1.59 \pm 0.02 \mu\text{l}/\text{min}/100$  g b.w., ( $p < 0.05$ ) and this effect is prolonged to 6th day:  $1.56 \pm 0.28 \mu\text{l}/\text{min}/100$  g b.w., ( $p < 0.05$ ). In normotensive Wistar rats ENa and ECl increased on 4th day after 7-NI application by 136.1% and by 122.3%, ( $p < 0.05$ ) respectively, but ECa did not change. In SHR ENa and ECl also increased after nNOS inhibition, however on the 1st day by 89.6% and 56.4%, ( $p < 0.05$ ) respectively. As a contrast to WR in SHR ECa increased on 2nd day from  $6.99 \pm 0.73$  to  $11.57 \pm 2.73$  nmol/min/100 g b.w., ( $p < 0.05$ ) and remained steady to day 6 of the experiment ( $13.11 \pm 2.73$  nmol/min/100 g b.w., ( $p < 0.05$ )).

*Conclusion:* In SHR the nitric oxide, generated by nNOS is not involved in the regulation of SAP, but more significantly participates in the regulation of renal excretory function compared to Wistar rats. The nitric oxide, gener-

ated by nNOS, contributes to the long term regulation of calcium excretion in SHR.

### iPLA2: Expression in the kidney and its regulatory mechanisms concerning NaCl reuptake in the thick ascending limb of the loop of Henle

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The activation of the thick ascending limb type 2 Na-K-2 Cl<sup>-</sup> -cotransporter (NKCC2) by circulating vasopressin (AVP) is essential for the function of the renal concentration mechanism. AVP activates NKCC2 by promoting phosphorylation of threonine residues in the n-terminal part of the molecule. The actions of AVP are counteracted by locally produced inhibitory agents such as the eicosanoids 20-HETE and PGE<sub>2</sub>. Little is known about the production and regulation of these factors and the potential interactions between AVP signaling and the inhibitory systems. One of the rate limiting steps of eicosanoid synthesis is the release of arachidonic acid from membrane phospholipids by a phospholipase isoenzyme and recent pharmacological studies suggest that a calcium-insensitive isoform of phospholipase A<sub>2</sub> (iPLA) is involved in the regulation of NKCC2 activity. However, the renal localization of iPLA and the effects of AVP on its expression are unknown. Aim of the present study was to demonstrate iPLA expression in the TAL and to characterize its effects on NKCC2 phosphorylation. We further determined the effects of AVP on iPLA expression. PCR, Western blot, and double labeling immunohistochemistry were used to localize iPLA in kidneys of control rats and in immortalized rat medullary TAL cells (mTAL). The role of iPLA as a regulator of TAL transport was studied by Western blot and immunostaining on mTAL cells treated with the iPLA inhibitor bromoenol lactone (BEL). The effects of AVP on iPLA expression were evaluated on kidneys of male adult Brattleboro (BB) rats that were treated with the selective vasopressin 2 receptor analogue desmopressin (dDAVP) via osmotic minipump (5ng/h; 3d). Immunohistochemistry showed a strong expression of iPLA in the basolateral membrane and in the cytosol of the TAL and a weaker signal in the remaining nephron segments. Expression of iPLA in mTAL cells was confirmed by PCR and Western blot. Treatment of mTAL cells with 5 μM BEL over 1 hour markedly increased the amount of phosphorylated NKCC2 (1.25 fold of control;  $p < 0.05$ ) while the amount of total NKCC2 remained unchanged. Treatment of BB rats with dDAVP caused a marked increase in phospho NKCC2 signal (2.25 fold of control;  $p < 0.05$ ) while iPLA expression was decreased (0.52 fold of control;  $p < 0.05$ ). In summary we have shown that iPLA is expressed in the TAL where it exerts an inhibitory effect on NKCC2 phosphorylation. In BB rats chronically treated with dDAVP an stimulation of NKCC2 is associated with decreased levels of iPLA. These results suggest that iPLA is an important target and mediator of vasopressin action in the TAL.

## Towards the molecular mechanisms regulating circadian olfactory sensitivity

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*Introduction:* Biological rhythms are found in a wide variety of living organisms. Daily rhythms, which persist in constant conditions and exhibit an endogenous period of about 24 hours are termed circadian, from Latin “circa” (about) and “dies” (day). These rhythms are generated by one or several specialized tissues, which contain circadian pacemakers that synchronize their oscillations with external zeitgebers, for example, the light-dark cycle. In mammals, a hypothalamic structure, the suprachiasmatic nuclei (SCN), were identified to be the master circadian pacemaker. The SCN control a vast variety of endogenous circadian processes, e.g. the sleep/wake cycle, metabolic processes, body temperature, and blood pressure. A large number of other tissues express circadian rhythms as well; however, most of these peripheral clocks appear to be driven by the SCN. In contrast to that, the olfactory bulbs (OB) have been demonstrated to express SCN-independent circadian rhythms in vivo and in vitro. In addition our own data show that also the olfactory epithelium (OE), displays rhythmic clock gene expression, indicating the presence of a functional clock in the olfactory system. This is supported by the fact that olfactory sensitivity in insects and mammals is modulated in a circadian manner. Rationale and aim Recently, G protein-coupled receptor kinase 2 has been identified as a mediator of rhythmic olfactory responses in *Drosophila* by means of linking circadian clock output to rhythmic olfactory receptor accumulation in the antennae. Are similar mechanisms realized in the mammalian system? Does the olfactory clock drive rhythmic olfactory sensitivity in mice? The proposed study will address this question and aims to identify the molecular basis for rhythms in olfactory sensitivity in mammals.

*Material and Methods:* Global transcriptome analysis of mouse olfactory epithelia will be performed to identify rhythmically expressed genes. Candidates for the regulation of circadian olfactory sensitivity, e.g. genes involved in olfactory sensation, will be evaluated and validated for rhythmic expression by qPCR, western blot, in situ hybridization and immunohistochemistry. Furthermore, a primary olfactory epithelial cell line and an immortalized olfactory receptor neuronal line will be established and tested for cell-autonomous or inducible molecular rhythms. Thereby, molecular mechanisms underlying the circadian control of olfactory regulation will be identified employing overexpression and knock-down studies of candidate genes. Combining these techniques with the transduction of a luciferase reporter gene, I will be able to measure the effects of molecular manipulations on circadian rhythmicity in real-time using single cell bioluminescence imaging.

*Results and conclusions:* The first stage of my project – the transcriptome analysis of OE tissue – will be performed in June and, hence, preliminary results will be presented at the conference.

## Preconditioning is lost in hyperlipidemia: role of nitrosative stress

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*Introduction:* Peroxynitrite (ONOO-) – induced nitrosative stress has been shown to trigger ischemic stress adaptation of the rat myocardium. The aim of this study was to investigate the role of nitrosative stress in the mechanism of preconditioning in experimental hyperlipidemia. Therefore, rats were fed with 2% cholesterol-enriched diet for 12 weeks.

*Materials and methods:* Hearts were then subjected to a preconditioning protocol (3 intermittent periods of global ischemia/reperfusion or exogenous peroxynitrite infusion (final 3  $\mu$ M) of 5 min duration each) followed by a 30-min test ischemia and 120 min of reperfusion).

*Results:* In the normocholesterolemic group preceding preconditioning induced either by global ischemia or ONOO infusion decreased infarct size from  $45 \pm 3.8\%$  to  $5.7 \pm 2.0\%$  and  $13.1 \pm 4.1\%$ , respectively. ONOO infusion did not induce preconditioning in the cholesterol-fed group (infarct size:  $33.3 \pm 3.8\%$ ). To investigate the role of endogenously formed ONOO- we measured the ONOO-marker nitrotyrosine content during preconditioning and observed that the 1st period of ischemia/reperfusion increased nitrotyrosine formation, which was attenuated after the 3rd period of ischemia/reperfusion. This increased formation of nitrotyrosine was not observed in hyperlipidemic rats.

*Conclusions:* Nitrosative stress plays an important role in the triggering mechanism of preconditioning. This mechanism is deteriorated in cholesterol-diet induced hyperlipidemia.

## Resistin influence on hormones secretion from pancreas islets and INS-1 and InR1-G9 cell lines

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*Aim:* The aim of this study was to determine possible effect of resistin on insulin and glucagon secretion.

*Methods and Materials:* Experiments were performed with fresh isolated pancreatic islets and cell lines (INS-1 and InR1-G9). Incubations with resistin were carried out for 90 minutes. Applied concentrations of resistin in each cases were 1, 10 and 100 nM and control without that peptide. Additionally, we carried on that experiments in different concentrations of glucose: 1 and 6 mM for glucagon secretion and 6 and 24 mM for insulin secretion. Incubation of islets and cells occurred in 1 ml of Krebs buffer and RPMI 1640 medium respectively. The experiment was performed according to rules accepted by the Local Ethical Commission for Investigation on Animals. *Results:* Our results suggest that resistin can affect devel-

opment of insulin resistance in Wistar rats. Experiments on cell lines have been showed distinct and opposing influence on insulin and glucagon secretion by 6 mM glucose and on glucagon secretion within the glucose concentration limits (1 and 6 mM). Insulin secretion was decreased in both glucose concentrations. Glucagon secretion was decreased at 1 mM glucose concentration and increased at 6 mM glucose concentration. In addition to cell lines experiment we observed similar trend in resistin effects on hormone secretion from pancreatic islets but without statistical differences.

**Conclusion:** Resistin seems to be tightly connected with insulin resistance particularly in rodents. Functioning of pancreas islets is one of several areas involved in insulin resistance and finally diabetes mellitus type II. Presented results suggests that resistin can contribute to decrease insulin secretion and additionally increase glucagon secretion which leads to rise glucose concentration in blood. Major reasons of insulin resistance are rather connected to target tissues and postreceptor cascade but contribution of islets can not be ignored.

### Effect of ethanol on bursting activity of the leech Retzius cells induced by nickel

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**Introduction:** Since ethanol can act as both proconvulsive and anticonvulsive substance, we have tested the effect of ethanol on bursting activity of the Retzius cells of the leech. The nervous system of the leech is a useful model system for investigating the cellular basis for epileptiform activity. Rhythmical epileptiform bursting activity on this model can be induced by several exogenous agents like inorganic Ca<sup>2+</sup>- channel blockers, such as Ni<sup>2+</sup>.

**Aim:** To study the effect of ethanol on Ni<sup>2+</sup> induced Na<sup>+</sup> dependent bursting activity of the leech Retzius cells.

**Material and Methods:** The experiments were performed on Retzius nerve cells of leech *Haemopsis sanguisuga*. Retzius neurons were treated with Ni-Ringer (containing 3 mmol/l NiCl<sub>2</sub>), 1% and 2% ethanol solutions. For registration of resting membrane potentials and action potentials, standard technique of intracellular registration with microelectrodes was used.

**Results:** Superfusion with Ni-Ringer solution induced minor depolarization of the membrane potential followed by spontaneous bursting activity characterized by rapid depolarizations to a plateau level during which bursts of action potentials occurred. Replacement of Ni-Ringer with 1% ethanol and nickel solution (1%EtOH-Ni-Ringer) led to a significant decrease in frequency of bursting activity and amplitude, but it had no effect on duration of plateaus and number of spikes per plateau. Application of 2% ethanol solution (2% EtOH-Ni-Ringer) led to highly significant decrease of all characteristics of bursting activity (frequency, plateau duration, amplitude and number of spikes per plateau).

**Conclusion:** Results of this study indicate inhibitory dose-dependent effect of ethanol on bursting activity of Retzius cells induced by Ni<sup>2+</sup>. This effect is probably mediated by Na<sup>+</sup> and Ca<sup>2+</sup> dependant K<sup>+</sup> channels. Key

words: ethanol, bursting, leech Retzius neuron, Ni<sup>2+</sup>, Ca<sup>2+</sup> activated K<sup>+</sup> channels

### Quality of adaptive reactions under intermittent hypoxia exposure (experimental research)

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**Aim:** A number of studies have investigated the performance of adaptive reactions in response to specific and nonspecific stress factors. Intermittent hypoxia exposure has been studied extensively as a nonspecific stress trigger with a purpose of experimental and clinical medicine. Recent studies have demonstrated that short-term daily intermittent altitude exposure was equally as effective as continuous altitude residence in inducing physiological adaptations. Intermittent hypoxic training (IHT) optimizes physiological body functions, integrates homeostatic system and physical working capacity. Although the positive benefits of IHT have been clearly defined, the potential negative consequences of IHT on health, specifically the immune system, remain undefined. Due to this, the main purpose of our research was to determine the types of adaptive reactions, based on the peripheral blood and haematological indices evaluation under intermittent hypoxia exposure.

**Materials and methods:** The experiments were carried out on the intact male rats (weight 180-220 g, control and experimental group). Experimental group was exposed to 10-days IHT. Then, the haematological parameters were analyzed such as red blood cells counts, haematocrit, haemoglobin content, leukocyte counts, number of monocytes and lymphocyte, polymorphonuclear neutrophils and eosinophil counts. Adaptation index was calculated as well.

**Results:** The effects of 10-days exposure to hypoxia in experimental rats is considered to be related to an increased production of red blood cells (6,3%), increase in haemoglobin content (6%), resulting in an increased oxygen carrying capacity of the blood and oxygen utilization. The leucocyte response at altitude is characterized by the decrease in leucocyte counts followed by increase in monocytes and lymphocytes counts, and at the same time decrease in polymorphonuclear neutrophils as well as eosinophil counts. Adaptation index was increased to 32,4% compare to control group. According to the well-known criterias, our data most probably correlate with development of general nonspecific adaptive response, evaluated as reaction of increased activation. Such type of response is generally characterized by most effective mobilization of protective and regulatory mechanisms of the main functional systems.

**Conclusions:** Our findings suggest that intermittent hypoxic training in experimental rats improve the efficiency of adaptive reactions and nonspecific body resistance. Obtained data correlate with the recent literature sources, according to which reaction of increased activation is associated with the mobilization of protective stress-limiting mechanisms.

## Monosodium glutamate changes Bcl-2/Bax protein ratio in rat thymocytes

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Apoptosis is a major biological process which is controlled through a class of different proteins. Some proteins keep cell living (anti-apoptosis protein, e.g. Bcl-2) while other proteins initiate the apoptosis (pro-apoptosis proteins, e.g. Bax). Monosodium glutamate (MSG) is often used as a flavor enhancer in modern nutrition. Recent studies show presence of glutamate receptors in thymic stroma, thymocytes, and lymphocytes.

*Aim:* The aim of this study was to examine the effects of MSG on thymocytes apoptosis and expression of Bcl-2 and Bax proteins.

*Materials and Methods:* We used rat thymocytes and exposed them to various concentrations of MSG (1-100mM) for 24 hours. Apoptosis was determined using the Annexin V-FITC/PI apoptosis detection kit. Protein expression of Bcl-2 and Bax were analyzed using flow cytometry with respective antibodies.

*Results:* Annexin V-FITC/PI staining shows that the treatment with MSG increases apoptosis ratio in rat thymocytes. MSG also caused decrease of Bcl-2 proteins, while expression of Bax proteins did not change significantly, during examination period.

*Conclusion:* Our studies show that the change in Bcl-2 and Bax expression after MSG treatment may be an important event in thymocyte apoptosis.

## Obestatin influence on brown adipocytes by GPR39-1a receptor – preliminary results

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*Aim:* The aim of this study was to find putative obestatin receptors GPR39-1a, GPR39-1b, GLP-1R and their changes during differentiation of preadipocytes from brown adipose tissue after obestatin treatment.

*Material and methods:* Brown adipocytes tissue (BAT) was taken from the neck brown fat pads of male Wistar rats (b.w. 100-120g). Animals were sacrificed and the brown neck fat tissue was cut off. After that tissue was digested with 2 mg/ml collagenase type II solution in Krebs-Ringer buffer. After 60 minutes of digestion in the 37°C water bath cell suspension were centrifuged and the pellet containing brown adipocyte-precursor cells was filtered through a nylon mesh of 150 and 30 µm-pore size and counted on a Bürker-Türk chamber with a trypan blue vital stain. Filtered cells in the concentration of 0.5 x 10<sup>6</sup> cells/ml were suspended in a DMEM/F12 culture medium with 10% FBS and penicillin-streptomycin solution and seeded on cell culture multi-well dishes. Preadipocytes were cultured in a 37 °C incubator with 5% CO<sub>2</sub>. Obestatin

was administered in the doses of 1, 10 or 100 nM for 1, 3, 5 and 7 days of exposition time. Total RNA and protein was isolated from preadipocytes using TriPure reagent (Roche). Reverse transcription was undertaken using ImProm-II Reverse Transcription System (Promega). The cDNA was amplified by multiplex PCR using primers and probes (Roche) for GPR39-1a, GPR39-1b, GLP-1R and internal standard GAPDH. Analysis was made using LightCycler Software Version 4.5 and was based on the relative quantification method with efficiency correction.

*Results:* We found expression of GPR39-1a and GPR39-1b in preadipocytes from brown adipose tissue. We also observed that GPR39-1a expression increased after obestatin stimulation whilst GPR39-1b expression did not change. Moreover, we demonstrated that there is no GLP-1R expression in BAT.

*Conclusions:* Our research indicates that both isoforms of GPR39 are expressed in brown preadipocytes. Moreover, in our study we found that obestatin may act by GPR39-1a receptor on differentiation of brown preadipocytes. On the other hand, we did not find expression of GLP-1R in these cells.

## Pneumology

### Bronchiectasis exacerbation score in preliminary outcomes prediction

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*Aim:* in order to emphasize the main indices combination to which the physician has to pay attention to and according to it was created bronchiectasis exacerbation score. This score aids to rapidly predict the intensiveness of needed empirical treatment. One of bronchiectasis treatment strategies is to slowdown the disease progression, hence, it is significant to decrease exacerbations' treatment duration and to use treatment as quickly as it possible.

*Methods and materials:* 25 adult patients with bronchiectasis exacerbation were retrospectively studied in 1045 beds university hospital from January, 2007 until January, 2009. According to the obtained data from our previous study and "PubMed" base the main bronchiectasis exacerbation indices combination was determined, including predisposition factors as the part I (the sum of age >65, bronchus obstruction states (COPD, chronic bronchitis, bronchial asthma), congenital (e.g. cystic fibrosis), systemic connective tissue (rheumatoid arthritis), decreased respiratory surface area (e.g.lobectomy, pulmectomy), immunosuppressive states, congenital anatomical defects (e.g. hypoplasia, tracheobronchomegaly). It was given 1 point for each predisposition factor. Into the II part the sum of dyspnoea according to the tolerance to physical activity (0 – not noticed; 1 – intensive; 2 – minimal physical activity; 3 - at rest), temperature (0–no; 1–subfebrile; 2 - >38.50C) and other (clubbing fingers, cyanosis, halitosis – 1 point for each feature) were included. In the III part the sum of CRP (CRP- C reactive protein) (0 – 50mg/l), blood gases values (paO<sub>2</sub> <60 mmHg; paCO<sub>2</sub> >40 mmHg –1 point), fibrobron-



choscopy (inflammation of mucosa; pus; deformation and atrophy of bronchi – for each feature - 1 point) and spirometry (FEV1/FVC>70% - 0; FEV1/FVC <70% and FEV1>70%-1 ; 50%tions. These ranges were correlated with the outcomes at discharge time: the symptoms (dyspnoea, temperature, sputum expectoration intensiveness) and auscultation sounds (productive rales) dynamics (0-totally absent; 1- decreased; 2-persisted).

**Results:** 25 bronchiectasis exacerbation cases were analyzed, Mean age of the patients was  $58.2 \pm 18.17$  (56% (N = 14) women and 44% (N = 11) men). There were 17.65% (N = 3) light; 29.41% (N = 5) - moderate and 52.94% (N = 9) - severe diseases' cases (overall 17 patients) based on bronchiectasis exacerbation score. The most significant correlation was established between outcomes ( $r = 0.491$ ,  $p = 0.045$ ). The significant correlations have been found between outcomes and CRP value ( $r = 0.520$ ,  $p = 0.011$ ) and with spirometry ( $r = 0.485$ ,  $p = 0.026$ ). Correlation between outcomes and sputum microbiological culture was statistically insignificant ( $r = 0.366$ ,  $p = 0.11$ ).

**Conclusion:** created bronchiectasis exacerbation score based on statistical estimation could be used to assist preliminary prediction of outcomes. The most significant correlation with outcomes was established with systemic inflammatory and lung function test values.

### Investigation of the role of null polymorphisms of glutathione-S-transferase genes (GST) for development of COPD and bronchial asthma

ESC-ID 858

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The glutathione-S-transferase isoenzymes play an important role both in cellular protection against oxidative stress and in the defense cellular response to air pollutants and to cytotoxic agents, present in cigarette smoke. For two of the isoenzymes, GSTM1 and GSTT1, null variant alleles have been found. Several studies have suggested that individuals homozygous for the null GSTM1 or GSTT1 alleles are with higher risk of developing COPD and Bronchial asthma; however the results are still quite controversial. The aim of the current study was to explore the possible role of GSTM1 and GSTT1 null polymorphisms as risk factors for COPD and Bronchial asthma.

**Methods:** In the current study we enrolled 73 patients with COPD, 26 adult patients with Bronchial asthma and 81 control persons. The GSTM1 and GSTT1 genotyping was performed by applying a modified multiplex PCR-based method.

**Results:** The presence of null GSTM1 genotype was associated with an increased risk for COPD (OR 2.24, 95% CI 1.18-4.27,  $p = 0.014$ ), but not for Bronchial asthma ( $p = 0.200$ ). GSTT1 null genotype did not affect independently the risk for COPD or asthma, however it increased the susceptibility to COPD in individuals with null GSTM1 genotype: subjects possessing double null genotype (GSTM1 null/GSTT1 null) had a 3.04-fold (95% CI, 1.05-8.73,  $p = 0.04$ ) increased risk of COPD when compared to

those with non-null genotype of GSTM1, GSTT1 or both.  
**Conclusions:** Our current results suggest that null GSTM1 polymorphism may contribute to the development of COPD, but not to Bronchial asthma, whereas the null GSTT1 polymorphism may only modulate the risk for COPD in individuals with GSTM1 null genotype. Key words: COPD, Bronchial asthma, GSTM1, GSTT1, null polymorphisms, health risk.

### Comparison of pulmonary manifestations in rheumatoid arthritis and systemic lupus erythematosus patients: 35 Case Series

ESC-ID **226**

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**Aim:** Pleuropulmonary manifestations of the Rheumatic Diseases (RDs) are a significant cause of morbidity and mortality worldwide. Rheumatoid Arthritis (RA) and Systemic Lupus Erythematosus (SLE) are significantly more common among these diseases. Respiratory involvement in RA and SLE occurs frequently but the diagnosis may be difficult because of heterogeneity of the anatomical and clinical presentations. However, there have been only a relatively few reports on comparison of pulmonary manifestations between RA and SLE patients.  
**Method and materials:** In a simple statistical study, we evaluate 20 RA patients and 15 SLE patients who admitted to Masih Daneshvari Hospital with pulmonary manifestations during 1996-2006 by records. RA and SLE were established previously in these patients by standard diagnostic criteria. Our evaluations include the patients' personal profile, symptoms, signs, and their main pulmonary diagnosis.

**Results:** The mean age of patients that we have studied in RA and SLE group were 53.8 and 36.6, respectively. The majority of patients in RA group was male (75%) but in SLE group was female (86%). In evaluation of respiratory symptoms, cough was more common in RA group (85% vs. 73%); Sputum was similar in both groups (60%) and Exertional dyspnea was a little common in RA group (90% vs. 87%). Hemoptysis (15%) and orthopnea (45%) were seen in RA group but weren't seen in SLE group. In evaluation of constitutional symptoms, fever was more common in SLE group (47% vs. 35%); but weight loss (40% vs. 33%) and arthralgia (80% vs. 73%) were more common in RA group. In evaluation of respiratory signs, both rale (65% vs. 47%) and wheezing (25% vs. 7%) were more common in RA group. In evaluation of other signs, arthritis was more common in RA group (75% vs. 53%); But, skin rash (73%), heart murmur (20%), and LAP (13%) were seen only in SLE group. In their main pulmonary diagnosis, ILD was significantly more common in RA group (55% vs. 7%); but pleural effusion (27% vs. 10%) and pneumonia (27% vs. 15%) were more common in SLE group. The other main pulmonary diagnosis wasn't similar. At last, all of our SLE patients were cured but 2 of our RA patients were died.

**Conclusion:** RA and SLE had broad spectrum Pulmonary manifestations which were relatively similar together. We

can be hopeful that if pulmonary manifestations of RA and SLE patients are noticed earlier the prognosis will be more satisfactory.

### Evaluation of effects of cetirizine on bleomycin induced pulmonary fibrosis in rats

ESC-ID **211**  
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*Aim:* To evaluate the protective effect of cetirizine against bleomycin induced pulmonary fibrosis in rats.

*Materials and Methods:* Male Sprague-Dawley rats (n = 30), received an intratracheal (IT) injection of bleomycin (7.5 IU/kg) in saline solution for induction of pulmonary fibrosis. The animals were divided into the following groups (n = 6): Group 1 received only a single-dose bleomycin (7.5IU/kg, IT) as "positive control". The rats in group 2 received vehicle (normal saline, IT) as "negative control". Group 3 or "Treatment-C1 group" received daily cetirizine 5mg/kg/day intraperitoneally (IP), 7 days before and 4 weeks after administering a single-dose bleomycin (7.5IU/ kg, IT). Group 4 or "Treatment-C2 group" received daily cetirizine 20mg/kg/day (IP), 7 days before and four weeks after administering a single-dose bleomycin (7.5 IU/kg, IT). And group 5 Cetirizine 5mg/kg/day (IP) plus the vehicle (IT) for five weeks as "sham C". We also investigated the effects of the drugs and the vehicle (without bleomycin) as sham groups. All the ethical issues were considered throughout the experiment based on the Urmia Medical University Ethical Protocols (UMUEP) on animal experiments. The cytokines (IL-8, TNF- $\alpha$ , TGF- $\beta$ 1) through ELISA kits, the amount of collagen in the lungs (hydroxyproline content), and pharmacological activity of the lung strip tissues were determined.

*Results:* Histological investigation of lung tissue showed that rats of group 3 and 4 had less pathological changes in lung tissues as compared to group 1 (p <0.05). The severity of changes in lung tissues is less in group 4 as compared with group 3 (p <0.05). The studies on contractility of lung-tissue strips showed that rats of group 3 and 4 had fewer contractions of lung strip preparations as compared to group 1. The amount of contractions of lung strip preparations is less in group 4 as compared with group 3. The study of collagen and hydroxyproline content of lung tissue revealed that rats of group 3 (C1: cetirizine 5mg/kg/day) and group 4 (C2: cetirizine 20mg/kg/day) had less hydroxyproline content of lung as compared to group one. The amount of lung hydroxyproline content is less in group 4 as compared with group three. Studying the Cytokine Profile in the Studied groups suggested that rats of group three and 4 had less amounts of fibrogenic cytokines as compared to group one. The cytokine levels have been decreased in the treated groups by cetirizine 5 (p <0.05) and 20 (p <0.01) mg/kg/day, in comparison to positive control group.

*Conclusions:* Cetirizine may have a protective effect against bleomycin induced pulmonary fibrosis as evident by the reduction of the severity of lung tissue changes,

collagen amounts and cytokines levels caused by bleomycin in rats lungs tissues.

*Key words:* Pulmonary fibrosis, Bleomycin, Cetirizine, Cytokines.

### Microvessel density as a prognostic marker in operable lung adenocarcinomas

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*Background:* Lung adenocarcinoma is most common cell type in females (smokers or non- smokers) and in non smoking males. Its incidence has been increasing until very recent years. Recent studies have demonstrated that angiogenesis assessed by microvessel density (MVD) is closely related to postoperative relapse, especially distant metastasis, indicating that MVD within tumor is prognostic factor in lung adenocarcinomas.

*Aim:* In this study we aimed to emphasize the intensity of angiogenesis, in terms of microvessel density, along with tumor stage, lymph node metastasis and overall survival of patients with resectable adenocarcinomas.

*Material and Methods:* Tumor tissue were obtained from 107 patients with operable lung adenocarcinomas and complete follow up information for five years were included. No adjuvant therapy was given prior surgery. Immunohistochemical detection of tumor-associated microvessels was performed using anti CD31 antibody directed against endothelial cells. Microvessel density were evaluated of the most vascular area on a x200 field.

*Results:* Microvessel counts were higher in patients with mediastinal nodal metastasis when compared with non-metastasing cases but without statistical significance (p = 0,244). Similarly angiogenesis was more intense in patients with stage IIIA disease when compared with the other lower stages but there was not statistical significance (p = 0,583). Furthermore, log rank analysis showed significant association with poor survival by higher stages (p = 0,022). And more intense angiogenesis of the tumors was a significant prognostic factor for reduced survival (p = 0,0024).

*Conclusions:* The most important prognostic factor for lung carcinoma is pathological stage. MVD may help in predicting the outcome of this group of cancers.

### Analysis of adult patients with bronchiectasis exacerbations in Centre of Pulmonology and Allergology, VUH SK

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*Aim:* This study was undertaken to establish the main risk, predisposition factors, symptoms, laboratory, instrumental and functional examinations indices correlation with bronchiectasis exacerbation, aetiology and

treatment among the patients hospitalized from January, 2007 until January, 2009.

**Methods and materials:** 25 adult patients' cases of bronchiectasis exacerbation were studied retrospectively in 1045 beds university hospital. In accordance with articles from "PubMed" base the risk (age>65, current or former smoker, BMI >18.5), predisposition factors (bronchus obstruction, acquired anatomical defects, immunosuppression, congenital defects, systemic connective tissue diseases), laboratory (included CRP, blood gas, bronchoalveolar lavage fluid tests), fibrobronchoscopy and spirometry examinations indices were determined and they were correlated with bronchiectasis exacerbation etiology (microbiological cultures) and treatment. The data was divided into two groups. The first group included above mentioned clinical indices, risk and predisposition factors, whilst the second consisted of aetiology and sputum microbiology test. Values of the both groups were correlated by SPSS 17.0 Pearson's correlation coefficient and MS Excel 2007.

**Results:** 25 bronchiectasis exacerbation cases were analyzed. The mean patient's age was  $58.2 \pm 18.17$  years (56% (N = 14) women and 44% (N = 11) men). 1. The significant correlation has been found between the age and the treatment with glucocorticoids + bronchodilators ( $r = 0.415$ ,  $p = 0.039$ ), rehabilitation ( $r = -0.397$ ,  $p = 0.049$ ) and oxygen therapy ( $r = 0.419$ ,  $p = 0.037$ ). 2. The study showed a weak correlation between smoking, BMI and the second group values. Among the predisposition factors the significant correlation was found between glucocorticoids + bronchodilators with COPD ( $r = 0.611$ ,  $p = 0.001$ ) and with all predisposition factors ( $r = 0.428$ ,  $p = 0.033$ ). 3. Among the main clinical symptoms of bronchiectasis exacerbation important correlation was found between glucocorticoids and bronchodilators with dyspnoea ( $r = 0.580$ ,  $p = 0.002$ ) and temperature ( $r = 0.408$ ,  $p = 0.043$ ). 4. Antimicrobial treatment has shown correlation with dyspnoea ( $r = 0.327$ ,  $p = 0.110$ ) and temperature ( $r = 0.463$ ,  $p = 0.020$ ). However, other symptoms have demonstrated insignificant correlation with the second group values. 5. There was found an interesting CRP Pearson's correlation coefficient with bronchi sanitation therapy ( $r = 0.483$ ,  $p = 0.020$ ). 6. The blood gas test has shown good correlation with antimicrobial treatment ( $r = 0.452$ ,  $p = 0.023$ ). 7. Endobronchial inflammatory fibrobronchoscopy changes has revealed significant correlation with microbiological cultures ( $r = 0.512$ ,  $p = 0.043$ ): inflammation signs in mucosa ( $r = 0.447$ ,  $p = 0.082$ ) is more valuable than sputum expectoration amount ( $r = 0.277$ ,  $p = 0.298$ ). 8. The spirometry, which indices define bronchial obstruction, has demonstrated significant correlation with sputum microbiology index (WBC>25 per field) ( $r = 0.565$ ,  $p = 0.023$ ). 9. Correlation between temperature and hospitalization time ( $r = 0.447$ ,  $p = 0.025$ ) was also noticed.

**Conclusion:** if high leucocytes value (>25 per visual field) in the sputum microbiology examination is found then more severe respiratory insufficiency can be expected. Another conclusion can be made, if inflammatory changes are observed during the fibrobronchoscopy, it shows the higher incidence that the causative agent is more aggressive, e.g. *Pseudomonas aeruginosa* or *Staphylococcus aureus*. Moreover, according to results CRP quantity can predict that the higher number of bronchi sanations will be needed during treatment. If the patient suffers from more severe dyspnoea and higher temperature, more intensive antimicrobial treatment will be needed.

## Psychiatry

### Gender peculiarities of suicidal behavior

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The problem of suicide is very acute in Belarus. Statistical data show rate of 27 suicide per 100000 population in 2008 year. Belarus is among 5 countries with the highest prevalence of suicide in the world. In the western countries, males die much more often by means of suicide than females do, although females attempt suicide more often. This pattern has held for at least a century. Identifying variables that indicate great risk of suicide in different genders, as well as investigating whether risk factors associated with suicide differ by gender, are important tasks. This research is aimed to create a system for prediction and prevention of suicidal behavior for men and women based on gender peculiarities of suicidal behavior. **Objectives of the research:** 1. To reveal relative risk factors of suicide attempt associated with features of family and individual anamnesis, socio-economic status, mental disorders among male and female population. 2. To identify means and circumstances, aims and causes, degree of suicidal intention and threat to life of suicides committed by observed men and women. Object of study is men and women, who committed a suicide or demonstrated risk of suicide and were admitted to the Hospital of Emergency Care of Minsk city or the Republican Psychiatric Hospital (100 men and 100 women).

**Methods:** We used the European Parasuicide Study Interview Schedule (EPSIS), the WHO Well-Being Index, the Bille-Brahe Measurement of Social Support, the Psychiatric Disability Assessment Schedule, the Beck Depression Inventory (BDI), the Brief Psychiatric Rating Scale (BPRS), the Brown-Goodwin Aggression Scale, the Barratt Impulsivity Scale, the Beck Hopelessness Scale and the Global Assessment Scale (GAS). Suicidal ideation was assessed by using the Suicide Scale for Ideations (A.T. Beck) and severity of medical injury were recorded by using the Pierce Suicide Intent Scale and Lethality Rating Scale, respectively.

**Preliminary results:** The study is still being carried out. However, authors expected to be able to produce preliminary results for the conference. By this time it has been exposed that men commit more suicide than women in Belarus like in the majority of other countries. There is still unclear situation with suicide attempts - none were fixed before year 2007. The system of its registration is currently being introduced and implemented in our country, which explains the lack of exact data at the moment. **Expected results:** By the end of the study risk factors of suicidal behavior for male and female population will be exposed. Authors will develop recommendations for prevention of suicidal behavior among men and women.

**Conclusion:** There is no doubt that suicide is a multi-casual phenomenon, therapy and prevention should be complex and gender differences should be considered in developing of the efficient strategies to combat this social problem.

## Evaluation of vicarious PTSD among children of Sardasht chemical warfare survivors 20 years after Iran-Iraq war

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*Introduction:* Post-Traumatic Stress disorder was reported before in 90% of chemical warfare survivors. Traumatic experiences in one person could affect the life of other family members, too. Clinical experiences and frequent observations demonstrated more psychological problems in families with a member of chemical attack victims. The aim of this study is to evaluate the frequency of vicarious PTSD among children of Iraq's chemical attacks survivors. *Material and Methods:* In a descriptive, Cross-sectional study, we enrolled 286 ?15 years old single children of chemical attacks survivors as case, and also 242 ages and sex matched normal civilian from the same city, Sardasht as the control group. PTSD in both groups was assessed by applying Mississippi Questionnaire.

*Results:* Mississippi scale for PTSD among the children of the Sardasht chemical attack survivors was  $128.88 \pm 13.92$ , and  $108.34 \pm 22.7$  in the control group ( $P < 0.05$ ). There was no significant difference in the Mississippi scale among different sex and age groups.

*Discussion:* This study demonstrated higher rates of PTSD among children of Sardasht chemical attack survivors compared with control group, suggesting the need to following up and treatment of severe cases.

*Key words:* Post-traumatic Stress Disorder (PTSD), vicarious trauma, chemical victims

## Public Health

### The effects of habitual cigarette smoking on blood count in men

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Harmful effects of habitual tobacco smoking are well-known and widely reported. Smoking is a risk factor for numerous civilizative diseases. The present study was aimed to investigate the influence of habitual cigarette smoking on the blood count in men and estimate whether there exists a relationship between the intensity of the smoking habit (expressed as a smoking index) and the blood count. The study was performed on 24 smoking (20 and more cigarettes per day for above 10 years) and 14 non-smoking men (age 35-50 years) recruited, based on a special questionnaire, from healthy volunteers being inhabitants of Bialystok (Poland). Blood from the cubical vein was collected (using a vacuum closed system) from all smokers and non-smokers. Complete blood count

involving white blood cells (WBC; including neutrophiles, lymphocytes, monocytes, eosynophiles and basophiles), red blood cells (RBC), haemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and platelets (PLT) were carried out. The smoking index, being a marker of the intensity of the smoking habit, was calculated by multiplication of the average number of cigarettes smoked per day and years of the smoking habit duration. In the smoking men, an increase in the total number of WBC (by 43.6%) and the numbers of neutrophiles (by 47.4%), lymphocytes (by 41.1%), eosynophiles (by 59.1%) and basophiles (by 63%) was noted compared to the non-smoking ones. There were no differences in the other measured parameters between smokers and non-smokers. There was no statistically significant correlation between the changes in the white blood cells in the smoking men and the intensity of the smoking habit. The results of the present study show that habitual smoking of 20 and more cigarettes per day for above 10 years may cause dysfunction of the immune system and induce an inflammatory reaction in men, but have no influence on the other parameters of the blood count.

### Paying the price to be modern: analysis of nutritional status in young adults

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*Introduction:* By 2010 cardiovascular diseases (CVD) will be the world's leading cause of death, with 2.3 billion people classified as overweight and an estimated 366 million suffering from diabetes mellitus (DM). WHO report found that "the uptake of the Western high fat, high sugar diet is especially pervasive amongst the newly urbanized populations where these foods become associated with being 'modern'." To determine the impact of these changing eating habits the nutritional status of a representative group ( $n = 95$ ) of young South Africans, between the ages 18-22 years, were investigated.

*Materials and Methods:* A 24-hour recall combined with food frequency questionnaire was completed by the participants. The dietary information obtained was processed using FoodFinder3 to obtain the nutrient intake of the population and results were statistically analysed by SAS statistical to compare regular intake to the standard RDA recommendations.

*Results:* The total energy (kJ) intake in females ( $9551.94; 4937.41$ ) indicated no significant difference ( $p > 0.05$ ) when compared to RDA of 9205 for the same age group, whilst the males ( $17153.95; 10929.54$ ) showed significant higher energy intake ( $p < 0.05$ ) found in the % total protein energy intake and % fat intake consisting of SFA (saturated fatty acids). Total % fat energy intake was significant (powered a significant difference ( $p < 0.05$ ); 5.82) and males ( $2.61; 4.23$ ), ( $-1.62; 4.68$ ). CHO (carbohydrates) energy intake was significant lower (pFe) compared to the RDA, reported significant lower (p in females and no difference in males).

*Conclusion:* The move away from traditional diets towards a more westernised "fast-food" culture was evident in this

representative young South African group. The male population showed dramatic increased total energy(kJ) intake and fat intake of both men and women were significant higher than the RDA. Significant lower values than RDA were found in females for CHO, Ca, Fe, Biotin, Vitamin D and E, which can indicate an increased risk of osteoporosis and Fe-deficiency anaemia. Micronutrient intake seems to be adequate but an increased intake of fat and energy may result to increased risk for obesity, hypertension, CVD and DM. Malnutrition, sedentary lifestyles and increased energy intake together with poor quality diets set the stage for chronic diseases developing.

### Does 5 a day keep breast cancer away?

ESC-ID **650**  
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Breast cancer is a common public health problem amongst women both in the UK and around the world (1-2). There has been much research suggesting that increased fruit and vegetable consumption may protect against breast cancer incidence, or improve its prognosis (3-9). This may be via a variety of mechanisms, including antioxidant effects, increased activity of detoxifying enzymes, effects on cell differentiation, maintenance of normal DNA repair, altered oestrogen metabolism, increased apoptosis, and decreased cell proliferation, to name a few (10-18). In 2003 the Department of Health launched the 5 A DAY programme as a means of encouraging the nation to consume at least five portions of a variety of fruits and/or vegetables per day. This was in an attempt to reduce early deaths from a number of diseases, including breast cancer (19-20). This study aimed to investigate the effects of the programme on breast cancer incidence and prognosis. It was hypothesised that individuals who consumed five or more portions of fruits and/or vegetables per day would be protected from developing breast cancer, and that individuals who already suffered from breast cancer would have an increased survival time, compared with individuals who consumed fewer than five portions per day. Data was extracted from four studies published in the previous five years which considered the effects of fruit and vegetable consumption on either the risk of developing breast cancer, or the risk of mortality in breast cancer sufferers. It was found that women who consumed fewer than five portions of fruits and/or vegetables per day were significantly more likely to develop breast cancer (OR = 1.142, 95% CI = 1.023, 1.275) but not significantly more likely to die if they already suffered from breast cancer (RR = 1.159, 95% CI = 0.977, 1.375) than women who consumed five or more portions per day. This analysis suggests that the 5 A DAY programme may decrease the odds of developing breast cancer and, to a lesser extent, decrease the risk of mortality in breast cancer sufferers. However, further studies must be carried out to confirm these findings.

### Protective factors for students' health: A comparison between 3 universities in Serbia

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*Aim:* The aims of this study were to assess which candidate variables were independently positive associated with Self-Rated Health (SRH) in university students; to compare these variables by university, and to investigate which of the variables was most important as a protective factor for students' health.

*Methods:* The data is from the survey conducted in 2009th in universities in Nis, Belgrade and Novi Sad. In this survey there were 807 students involved. SRH was assessed with a single question using a five-point scale ranging from "excellent" to "poor". The study measured a wide range of variables including: physical and psychological health, studying conditions, socio-demographic status and protective factors such as physical activity, leisure time, etc. The study is a cross-sectional design in which multiple regression analyse was conducted to examine associations among many variables.

*Results:* There were few differences in the effects of variables associated with SRH by university (wellbeing: a measure of psychological health). The remaining variables showed homogenous effects for both genders and for all three universities. More favorable health states (ie, greater psychological well-being and less distress) were positively associated with leisure time, physical and social activity, health values and religiousness for all three universities.

*Conclusion:* Socioeconomic conditions and lifestyle factors have been found to be related to self-rated health, which is an established predictor of morbidity. The results suggest that SRH can be reasonably used to compare students' health across the country. SRH is affected by different physical, psychological and psychosomatic aspects of health. However, its strongest association is with psychosomatic complaints. Results demonstrated that multiple protective contribute to the psychological well-being and less distress of university students. Health promotion practitioners should adopt strategies that strengthen the personality characteristics and values associated with university students' psychological health. Keywords: health status, students, protective factors

### Surveillance for pneumococcal meningitis in children in Southern Pakistan

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 Country **Pakistan**  
 Institution **Aga Khan University**

*Background:* Information about the burden of invasive pneumococcal disease (IPD) among children in Sindh, a province of over 35 million people in southern Pakistan is limited.

*Methods:* Surveillance of bacterial meningitis among children aged 30 pediatric admissions weekly, (2) avail-

ability of skilled personnel to perform lumbar punctures, and (3) close proximity to an Aga Khan University Hospital laboratory collection point.

**Results:** A total of 2690 children were admitted to the hospital with suspected acute bacterial meningitis, and 2646 (98%) underwent lumbar puncture. Of the 2646 cerebrospinal fluid specimens obtained, 412 (16%) were purulent, and pathogens were detected by culture or latex agglutination testing in 83 (20.1%) of the purulent specimens. Of the 83 isolates detected, 48 (57.8%) were *Haemophilus influenzae* type b, 32 (38.5%) were *Streptococcus pneumoniae*, and 3 (3.6%) were *Neisseria meningitidis*. Overall, 81% of the pathogens detected were from children aged

### Effects of CPAP(continuous positive airway pressure) therapy and APAP(automatic positive airway pressure) therapy on quality of life in patients with obstructive sleep apnea: A randomized clinical trial

ESC-ID 257  
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**Introduction:** Sleep apnea occurs when the airway from the mouth to the lung collapses during sleep. The person with sleep apnea may have hundreds of these episodes throughout the night, disrupting their sleep and reducing oxygen supply to vital organs. Sleep apnea is a common condition affecting about 5% of adults. Medical research has shown that Sleep apnea increases the possibility of having health problems such as hypertension, stroke, ischemic heart disease, and mood disorders, so the quality of life decrease in these patients. Fortunately effective treatment is available and once treated the person with sleep apnea leads a normal healthy life. In this study we compared 2 kinds of these treatments, CPAP(continuous positive airway pressure) therapy and APAP(automatic positive airway pressure) therapy in their effects on quality of life.

**Methods and Materials:** A randomized clinical trial was done in 2008. 30 patients with Obstructive sleep apnea, that they came to the Bamdad Clinic in Isfahan and their disease diagnosed with Polysomnography, randomly selected and divided with random allocation in to 3 equal groups. In first group patients received CPAP therapy for one month, in second group patients received APAP therapy for one month and the control group with no treatment. Quality of life measured in 3 groups before intervention and after that with SF-36 questionnaire and compared each other. SPSS v.16 used for data analysis.

**Results:** Out of 30 patients 20(66.6%) were male and 10(33.3%) were female with the mean age  $46.7 \pm 12.34$ . The mean of physical dimension before and after intervention in CPAP group was: 50.9 and 64.8, in APAP group was: 47.4 and 72.9 and in control group was: 46.7 and 47.8. The mean of mental dimension before and after intervention in CPAP group was: 39.4 and 66.7, in APAP group was: 41.6 and 73.4 and in control was: 39.7 and 41.9. With one way ANOVA test the quality of life in 3 groups before intervention had not significantly differences ( $P = 0.68$ ) but after the intervention it has significant differences ( $p = .0001$ ) and also a significant differ-

ence was shown between CPAP and APAP group and APAP group with control group and CPAP group with control group with Independent T-Test ( $p = .0001$ ). Eventually, quality of life increase more in APAP group than CPAP group and in CPAP group than control group.

**Conclusion:** According to the results of this study, sleep apnea decrease the quality of life in patients but we can help these patients. With treatment the sleep apnea finding suggest that APAP therapy is more effectiveness than CPAP therapy.

**Key words:** Sleep apnea, APAP therapy, CPAP therapy, quality of life, randomized clinical trial

### Gender differences of Sprague-Dawley Rats fed a Cafeteria Diet

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Obesity influences a wide-ranging level of the population. Animal models are suitable to study mechanisms of obesity as they are particularly helpful to clarify the involvement of single neurotransmitters and hormones, as for example leptin (e.g. Zucker-, ZDF-rat, ob/ob-, db/db-mouse).

**Aim:** Our purpose was to generate an obesity model which is not based on a genetic defect but on the manipulation of the diet. Such a model mimics the evolution of human obesity in a more lifelike way because it takes into account the important role of the environment for the development of obesity. Metabolic as well as behavioral changes induced by a so called cafeteria diet were detected in juvenile male and female Sprague-Dawley rats.

**Method:** Accessorily to standard chow, a selection of human food items with high caloric content (e.g. noodles, chocolate, cookies, cake, cheese) was offered over a period of 4 weeks to 6-week-old male and female Sprague-Dawley rats. Control groups of the same age received only standard chow. Water and food consumption, energy intake and body weight gain were documented daily. After 4 weeks of observation animals were subjected to behavioral testing in the Elevated plus maze-test (EPM) and Open field-test (OF). Rats were decapitated and fat tissue was dissected at the end of the study.

**Results:** In comparison to the control groups body weights of male and female animals fed the cafeteria diet were not significantly higher after 4 weeks of observation although they showed an elevated caloric intake. However, this increased energy intake resulted in a significantly augmented visceral fat mass in the cafeteria groups of both genders plus a significantly enlarged subcutaneous fat mass in male rats. This accretion in fat tissue was mainly due to a higher average consumption of fat. Upon behavioral testing, female cafeteria fed rats were not different from controls in the EPM, but showed significantly more rearings and entries into the inner zone of the open field. Within males the cafeteria diet had no effect on behavioral testing. **Conclusion:** The results show that the cafeteria diet administered for 4 weeks produced no increase of body weight but an enlargement of fat tissue. Behavior was affected by the cafeteria diet only in female Sprague-Dawley rats as indicated by the results from the OF.

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## Radiology

### Determination of sensitivity and specificity of ultrasonography in detection of free abdominal fluid in patients with blunt abdominal trauma

ESC-ID **556**  
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*Introduction:* Nowadays, trauma is the fourth cause of mortality and morbidity in developed countries. The major cause of these mortalities was intra-abdominal hemorrhage due to blunt abdominal trauma. Therefore, every action should be taken for early diagnosis of intra-abdominal hemorrhage to save the patient's life.

*Patients and methods:* All patient with blunt abdominal traumas admitted to emergency department of our tertiary center hospitals from 2006 to 2007 were included. All underwent both abdominal Ultrasonography and CT scan for detection of free abdominal fluid. We used sensitivity, specificity, positive predictive value, negative predictive value and accuracy as statistical tools for comparison.

*Results:* From 100 patients surveyed, 20% were female and 80% male. The most common presenting symptoms in the both groups were abdominal pain followed by abdominal tenderness. Sensitivity, specificity, negative and positive predictive value of ultrasonography in diagnosis of abdominal free fluid respectively were 84% , 86%, 91% and 75% also in the diagnosis of Intra abdominal damage were 54%, 90%, 65% and 79%.

*Conclusion:* Ultrasonography has sensitivity, specificity and accuracy which are enough to detect free fluid in blunt abdominal trauma compare to CT-Scan in children and adults age group. By combining abdominal viscera parenchymal abnormalities, these parameters will increase sensitively in children's age group.

*Key word:* CT -Scan, Ultrasonography , blunt abdominal trauma , sensitivity , specificity

### Transthoracic ultrasound – first choice diagnostic procedure in pulmonary fibrosis? – preliminary study

ESC-ID **355**  
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*Aim:* Pulmonary fibrosis (PF) is the end-stage consequence of interstitial lung disease. This a heterogeneous

group of non-neoplastic and non-infectious pulmonary disorders with common clinical, imaging, physiologic, or pathologic manifestations. The early diagnosis is difficult because of the non specific findings. The gold standard in diagnostic algorithm of pulmonary fibrosis is the lung biopsy and High-Resolution Computer Tomography (HRCT). However nowadays transthoracic sonography (TS) becomes more and more popular in diagnosis pulmonary diseases. Therefore despite little data on sonographic image of PF we decided to assess usefulness of this method in adult patients. The aim of our preliminary study was to analyze the image of pulmonary fibrosis in the transthoracic ultrasound examination and compare the result with the radiographic findings in HRCT.

*Methods and materials:* We performed transthoracic ultrasonography in 10 patients (8 men and 2 women, average age 56,3 years) hospitalized in Pulmonology Department of Medical University of Gdansk with diagnosed pulmonary fibrosis (HRCT confirmed). Ultrasound examination was performed using GE Logiq 500 and GE Logiq P6 with linear probe 8.2-11.0MHz and convex probe 3.5-6MHz. The results of these examinations were compared with the radiographic findings in HRCT. As a control group we examined 43 healthy subjects (18 men and 25 women, average age 47,2 years). All examinations were performed in the consent in writing of patients.

*Results:* The sonographic findings was irregular, fragmented, thickened pleural line; horizontal reverberation artifacts – B-line; subpleural cysts; reduction of the physiological gliding sign and pleural effusion. In all cases the findings in transthoracic ultrasound examination corresponded with the diagnosis based on HRCT. None of examined healthy subject presented such chest sonographic image.

*Conclusion:* 1. Transthoracic ultrasound could be useful and valuable method especially in early diagnosis as a screening test in people with risk factors and contribute to earlier referral to further highly specialist diagnostic procedures and treatment. 2. Transthoracic ultrasound is easy-to-learn method and can be performed both bedside and in outpatient clinic. 3. There is need to further study to evaluated the diagnostic value of transthoracic ultrasound in diagnosis of pulmonary fibrosis.

### The role of x-rays in diagnosing foreign bodies in children at different levels in the aero-digestive tract

ESC-ID **319**  
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 Institution **The Aga Khan University**

*Aim:* The aim of the study was to find the role of radiographic findings in diagnosis of FB's (foreign bodies) in the different parts of the aero-digestive tract before bronchoscopy/endoscopy.

*Methods and Materials:* We retrospectively reviewed the clinical records of a total of 157 (mean age: 3 years and 6 months, 97 males) patients with age the bronchus positive radiographic findings of a FB were identified in 32 patients (40.5%) whereas 47 (59.5 %) of the patients showed no signs of a foreign body. Foreign bodies were visualized in 9 of the cases. Out of the 59 cases of FBs in

the esophagus a FB was visualized on x-ray in 53 patients (90 %) and 6 patients (10%) had negative radiographic findings. Out of the 5 cases reviewed of a FB in the larynx 3(60%) patients had positive findings and 2 were negative. In all the 14 patients (100 %) with a FB in the pharynx a FB was visualized on x-ray.

**Conclusion:** Our results show that the diagnostic value of x-ray before bronchoscopy/endoscopy is low as far as the airway tract (bronchus, larynx) is concerned but it is helpful in the diagnosis of cases with a foreign body in the esophagus and pharynx.

### **Prospective evaluation of fast (Focused Assessment with Sonography for Trauma) in blunt abdominal trauma: The impact of ultrasonography on the management of patients with blunt abdominal trauma**

ESC-ID **566**  
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**Background:** A fast and accurate method for the evaluation of patients with blunt abdominal trauma as a major preventable cause of death results in significant mortality reduction. In this study we attempt to compare the focused assessment with sonography for trauma (FAST) examination with computed tomography (CT) and diagnostic peritoneal lavage (DPL).

**Methods:** This prospective study 4,557 patients with blunt abdominal trauma admitted to our institution's emergency room during a 24-month ending in May 2007. We compared 204 patients with blunt abdominal trauma examined with either FAST and CT scans or DPL. Sensitivity, specificity, accuracy of FAST in comparison with CT and/or DPL was calculated.

**Results:** In 140 cases with positive FAST results, CT scan or DPL confirmed positive findings in 124 patients. CT scans or DPL also were negative in 58 of the 64 patients with negative FAST findings. Sensitivity, specificity and accuracy of FAST were calculated 95.4% (95% CI 91.7-97.7), 78.4% (95% CI 71.9-82.4), 89% respectively.

**Conclusions:** We suggest that FAST examination can play significant role as a standard screening test in management protocols for patients with blunt abdominal trauma in order to reduce decision making time and unnecessary investigation but it should not be the only diagnostic modality.

## *Surgery*

### **Non-invasive investigation of pancreatic function after resection**

ESC-ID **354**  
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 Institution **University of Southampton**

**Introduction:** Pancreatic enzymes are required to digest food so it can be absorbed in the gut. Pancreatic insufficiency (PI) leads to maldigestion and malabsorption, causing malnutrition and steatorrhoea. Pancreatoduodenectomy, or Whipple's procedure results in many changes to the gastrointestinal tract, including PI, so patients must be managed effectively after surgery to ensure adequate nutrition. Existing investigations to assess pancreatic function are unpleasant for subjects and investigators and are not used in routine clinical practice. It is also possible to assess PI using a breath test. This measures the recovery of labelled  $^{13}\text{C}$  on the breath following administration of labelled triglyceride ([ $^{13}\text{C}$ ]2-octanoyl,1,3-distearin;  $^{13}\text{CMTG}$ ), that requires digestion by pancreatic lipase. This simple and non-invasive method could assess gastrointestinal tract function to digest and absorb fat in patients after pancreatic resection. There is a need to improve understanding of  $^{13}\text{CMTG}$  digestion to refine this investigation for routine clinical use.

**Objectives:** • To standardise a non-invasive test for gastrointestinal function that may have future applications in clinical practice. • To find a range of values for in healthy individuals using the  $^{13}\text{CMTG}$  breath test. • To investigate how  $^{13}\text{C}$  recovery is altered after pancreatic resection.

**Method:** 9 healthy volunteers (median age 54yrs, range 42-75yrs) and 5 patients after Whipple's procedure (median age 47yrs, range 30-60years) were recruited. After an overnight fast, each subject consumed  $^{13}\text{CMTG}$  (10 mg/kg body wt) in an emulsion with 2 slices of buttered toast. Breath samples were taken beforehand and every 30 minutes for 6 hours after.  $^{13}\text{C}$  enrichment was determined by CF-IRMS and mass of carbon on breath from  $\text{VCO}_2$ , measured by indirect calorimetry.  $^{13}\text{C}$  recovery was expressed as cumulative percentage dose recovered over 6h (cPDR). Graphs of percentage dose recovery/minute over 6 hours were plotted.

**Results:** Normal subjects show a consistent time-course of  $^{13}\text{C}$  recovery with a rapid increase in  $^{13}\text{C}$  in the first 120 minutes. The median cPDR for 6 hours was 33.4%, with range 26.2-48.2%. Patients showed a variety of patterns of  $^{13}\text{C}$  recovery. In contrast to the normal subjects, there was mostly a slow rise in  $^{13}\text{C}$  excretion in the early stages of the study. One patient had a  $^{13}\text{C}$  recovery pattern similar to the normal subjects. The median cPDR in the Whipple's patients was 17.6% and range 12.8%-39.3%.

**Conclusions:** The  $^{13}\text{C}$  MTG breath test shows decreased intraluminal lipolysis in patients soon after the Whipple's procedure. Pattern of  $^{13}\text{C}$  recovery can show different aspects of impaired gastrointestinal function. Delayed gastric emptying immediately after surgery may cause the slow rise in  $^{13}\text{C}$  recovery. Low  $^{13}\text{C}$  recovery throughout the study could be due to impaired function of



the pancreatic remnant. This test could be shortened in duration for use in clinical practice, as the differences between patients and normal subjects appear in the early stages. It has potential to monitor patient recovery after surgery and enhance patient management by improving nutrition.

### **Intussusception of efferent intragastric loop after gastrojejunostomy – An exceptional cause of high occlusion and hematemesis**

ESC-ID **464**  
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 Coauthor **Fabian Klein**  
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*Introduction:* Jejunogastric intussusception (JGI) is an extremely rare complication of gastrojejunostomy (GJS) that may appear any time after surgical intervention. Less than 200 cases have been reported so far, on very small series.

*Case report:* Young female, 32, who 12 years ago was operated for a gastro-duodenal disease that she doesn't know many details about. She presented severe pain in the superior abdominal segment posteriorly irradiated, incoercible biliary nausea followed by hematemesis. The endoscopic, imagistic and biological explorations suggested a huge gastric tumora that occupied the whole stomach and was bleeding diffusely. The rapid acute evolution asked for the urgent laparotomy that emphasized: soft tumour mass, intragastrically mobile without any scar at the stomach or duodenum level; adherent to the posterior of the stomach we discovered a ball of jejunal loops that couldn't be undone. The anterior gastrotomy sets the diagnosis: JGI of the efferent loops of a GJS. We hardly managed to reduce the intussusception, without resection, the loop being absolutely viable. In order to prevent a relapse, and because the anastomosis wasn't justified it was taken down.

*Conclusions:* JGI in a patient presenting GJS must be taken into consideration in the presence of the epigastric pains that wouldn't cease, biliary nausea followed by hematemesis and the rapid deterioration of general health status.

### **Brainstem and Thalamic Cavernous Malformations. Surgical approaches and outcome**

ESC-ID **758**  
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*Introduction:* Cerebral cavernous malformations (CCM or cavernous angioma, cavernous hemangioma, and cavernomas,) are vascular anomalies with undefined etiology, variable clinical manifestation and management. The brainstem cavernomas account for 18–22% of all CCM and together with the thalamic CCM represent a group of pathological lesions that are of specific neurosurgical

interest because of their localization, the approach to it, and the nature of the lesion itself.

*Aim:* To describe and analyze the clinical manifestations, neurosurgical management, postoperative results of operated patients with brainstem and thalamic CCM and to compare our findings with the one in the literature.

*Materials and Methods:* During the period 2000–2007, 15 patients with clinically manifest brainstem and thalamic CCM were treated in the Clinic of Neurosurgery, University Hospital “St. Ivan Rilski”. Retrospective analysis, based on the available clinical and neuropathological documentation was done. Review of the associated literature was performed.

*Results:* The gender proportion in the investigated group was approximately 1:1,5, male to female. The age at diagnosis ranged from 9 to 70 years with highest incidence in the 5th and 6th decade. The localization thalamus (67%), pons (33%). The primary onset symptoms were: hemiparesis, (53%), ICP (53%), cranial nerve palsies (27%), cerebellar symptoms (20%). Computer tomography (CT) and magnetic resonance imaging (MRI) were the primary methods for diagnosis. All the 15 patients were operated on, as 92% of the lesions were “gross totally” removed and 8% partially. Four of the operations were aided by neuronavigation and endoscopy. Improvement compared to preoperative status was established in 73% of the patients. Complication, such as cranial nerve palsies, ataxia and hemiparesis were observed in 27% of the cases. *Conclusion:* All the patients of this group follow the indications for an operation for CCM (hemorrhage and neurological deficit) and the postoperative results are similar to the worldwide literature about the experience with this peculiar type of pathology. Neuronavigation and endoscopic assistance improved the approach and the outcome of the surgical management.

*Key words:* Cerebral cavernous malformations, symptomatic epilepsy, intracerebral hemorrhage, surgical treatment, results

### **Electrolyte changes in mechanical ileus**

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*Aims:* The aim of this research was to analyse changes in the water and electrolyte balance occurring during mechanical ileus.

*Materials and Methods:* Retrospective analysis of medical histories and laboratory test results of 77 patients was used as the method of this research. The patients were operated due to mechanical ileus in the Surgical Ward of the Franciszek Raszeja's Hospital in Poznan during the period 2006–2008. The group consisted of 53 women and 24 men. 40 patients were operated because of high mechanical obstruction and 37 due to low obturation ileus. The border between high and low localization of the obstruction was determined by the ileocaecal valve. Adhesions, neoplastic tumours, inflammatory changes, stercoliths and hernias were the causes of mechanical ileus in the investigated group. The patients were consistently treated according to their electrolyte abnormalities. The electrolyte levels had been measured each day until they reached normal values.

**Results:** There were statistically significant changes in the levels of sodium and potassium in the form of hypokaliemia and hyponatremia in the investigated group. Inflammatory changes were the most common causes of water and electrolyte imbalance. It was statistically proven that the period of normalization of potassium in comparison to sodium levels was twice as long.

**Conclusion:** Mechanical ileus is an important factor causing water and electrolyte imbalance. Hypokaliemia is the most common outcome of this disease. The electrolyte abnormalities have a tendency not to respond well to treatment. Hypokaliemia usually requires longer therapy.

### Reevaluation of surgical treatment by external approach for Zenker's Diverticulum

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**Objective:** Surgical options for treating symptomatic Zenker's diverticulum include both traditional external procedures as well as endoscopic and minimally invasive techniques. The authors make a retrospective evaluation of the treatment for Zenker diverticulum by classic external approach.

**Methods:** The study included 7 cases (mean age 69 years) with different sizes Zenker's diverticulum, treated by external surgery in our department between 2003-2008. The presenting symptoms were dysphagia, halitosis, regurgitation, aspiration, hoarseness, weight loss. In 5 cases, Zenker diverticulum was associated with gastroesophageal reflux. Paraclinical assessment included barium esophagography, esophageal manometry, flexible endoscopy, CT scan. In the selected cases, different surgical procedures by external approach were performed. One of these cases is reported: a 61-year-old patient with large Zenker's diverticulum who underwent cricopharyngeal myotomy and diverticulectomy by laterocervical approach.

**Results:** Cricopharyngeal myotomy associated with diverticulectomy or diverticulopexy was the preferred treatment in the selected cases with large Zenker diverticulum and allowed a complete excision, without recurrences. Medical treatment for gastroesophageal reflux was associated. No major complications occurred and more than 90% of patients reported complete resolution of symptoms.

**Conclusions:** The surgical treatment should be performed according to the clinical and paraclinical characteristics of diverticulum and the patient. In certain selected cases, the external surgery remains a good option with favorable long term functional results.

### Determining anatomic position of umbilicus in Iranian girls, providing quantitative indices and formula to determine neo-umbilicus during abdominoplasty

ESC-ID 489  
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**Background and aim:** Umbilicus plays an important role in the aesthetic appearance of abdomen. So its reconstruction during reconstructive surgeries such as abdominoplasty is a challenge. The aim of this study was to evaluate quantitative indices based on constant skeletal points in the anterior wall of abdomen in order to provide appropriate site of neo-umbilicus during abdominoplasty.

**Material and Methods:** In this descriptive, cross-sectional study we enrolled 65 young adult girls (20-25 years old) Nulliparous, Nulligravid, and without any history of Surgery. Weight, Height (H), Distance from Xiphoid to umbilicus (Xu), Distance from Pubic symphysis to Xiphosternum (Xp), and InterASIS distance of subjects were measured. Data were analyzed by SPSS ver16 using descriptive statistics, multiple regression tests in order to presenting a formula (equation).

**Results:** Mean Age was  $22.74 \pm 1.51$ , Mean Weight =  $54.98 \pm 6.51$ , Mean Height =  $160.91 \pm 4.11$ . BMI calculated as  $21.25 \pm 2.61$ . Mean Xp distance was  $32.26 \pm 2.23$ , and mean Xu distance was  $17.11 \pm 1.64$ . Xu/Xp Ratio (Ratio of UmbilicoXiphoid distance to Pubo-Xiphoid distance) was  $53.06 \pm 3.9\%$ . Data were analyzed using Multiple Regression test and Likelihood ratio and this formula in determining appropriate site of neo-umbilicus during abdominoplasty was suggested:  $Xu = -0.98 + 0.91 Xp - 0.07H$

**Discussion:** By applying these quantitative methods, the natural-looking site of neo-umbilicus could be determined. This could reduce practice errors and increase patients' satisfaction. These findings provide us an evidence to defend against probable legal complaints too. **Key words:** Abdominoplasty, Neo-omphaloplasty, Umbilicus

### The comparison of "HEcBMG" (Human Endochondral Bone Matrix Gelatin) and "DBM" (Demineralized Bone Matrix) in human bone defects healing.

ESC-ID 451  
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**Aim:** Autogeneous bone graft is usual for the prevention of bone loss. Because of the important of the issue and the need of the world health care requirements, this study was conducted in defects from teeth extractions in order to evaluate and compare the effect of "HEcBMG" and "DBM" graft implantation on the repair of bone defect sites. The aim of this study was to compare bone healing induced by Demineralized Bone matrix (DBM) to Human Endochondral Bone Matrix Gelatin (HEcBMG) in teeth extraction's defects.

**Methods and Materials:** After preparation of "HEcBMG" and sterilized, we choose 20 patients, who are systemically health and their age was between 25 to 45. At the next step we put about 2 mg HECBMG in one of the socket and in another socket we put the same amount of "DBM". In continue we did series radiography in period of 0,1,2,4 months with the aim use Kodak E speed film and digital radiography for densitometry analysis of osteoinduction. at the end of we use subtraction software for analytic study.

**Results:** In the bone defects which we had put "HEcBMG" and "DBM" had been observed almost fill of bone within 30 days and bone density was high significantly. Based on observer's report at day 30 55%, 60 days 65% and 120 days 85% of socket became filling up of bone. Bone density of both defects was significant.

**Discussion:** "HEcBMG" has a highly osteoinductive efficacy and stimulator of undifferentiated cells to change to Osteoblast. Jin and Li also reported these results in their investigations. Also Chen and Hue reported relative to other biomaterials, "HEcBMG" had less inflammatory reactions that according our investigations.

### Ethiology, diagnosis and treatment of splenic abscesses

ESC-ID **752**  
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**Introduction:** Splenic abscess is a rare disease, followed by high mortality, with a frequency increasing in the last twenty years.

**Objective:** The aim of the study was to present the thirteen-year experience in the treatment of splenic abscess in the Institute of Digestive System Diseases, Clinical Center of Serbia in Belgrade.

**Material and methods:** 13 patients (five women and eight men, with a mean age  $44,69 \pm 19,62$  years) with the diagnosis of splenic abscess treated at the Institute for digestive system diseases in the period from 1.1.1996. until 16.3.2009. were included in the study.

**Results:** Common symptoms of splenic abscess included fever and weakness (92,31% of patients) and left-upper-quadrant abdominal pain (46,15% of patients). Majority of patients had leukocytosis (53,85%). Seven patients were admitted with a previous diagnosis of endocarditis. Positive microbial cultures were found in 7 patients, with Enterococcus being the most common pathogenic microorganism isolated from the splenic abscess. Ultrasonography and CT proved as the most reliable diagnostic procedures, with CT being superior in the diagnosis of multiple small abscesses. One patient was treated by combined antibiotic therapy alone and one died before splenectomy was performed. The others underwent splenectomy followed by postoperative application of antibiotics in high doses. One patient died of endocarditis after a month; the rest of eleven patients have successfully recovered.

**Conclusion:** Splenic abscess is an uncommon entity and remains a diagnostic challenge because of non-specific symptoms that accompany it. Splenic abscess must be considered in patients with fever, abdominal pain and

leukocytosis. Diagnosis should be established with ultrasonography and abdominal CT. Splenectomy followed by postoperative application of antibiotics remains the standard treatment. Key words: spleen, abscess, splenectomy

### Comparison of treatment methods of osteolytic cysts in children

ESC-ID **981**  
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The classic way of treatment of osteolytic cysts in bones is resection with use of bone grafts. Another method is introduced by Scaglietti in 1974 instillation of Methylprednisolone. New method of osteogenesis stimulated by autogenic growth factor in concentrated platelet fraction of patients blood plasma was introduced in Paediatric Orthopaedics Clinic Medical University in Lublin six years ago. The aim of the study is to compare healing time of benign osteolytic changes in bones with use of Methylprednisolone (Depo-Medrol) and PRP (platelet-rich plasma) The group consists of 62 children with solitary bone cyst of humerus hospitalized in Paediatric Orthopaedics Clinic Medical University in Lublin in years 1992-2006. 50 of them were treated with instillation of Depo-Medrol and 12 with use of platelet-rich plasma. In retrospective analysis of number of applications and time of healing this benign bone lesions in both groups of patients. Average time of remodeling of bone lesion in patients treated with Depo-Medrol was around 36 weeks and in group of patients treated with PRP – 13 weeks. The results of our study shows that use of PRP reduce time of bone remodeling in patients with solitary bone cyst of humerus.

### A mediastinal germ cell tumor of Yolk SAC type - Case report

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**Objective:** We report an extremely rare case of germ-cell tumor localized at the level of anterior mediastinum.

**Clinical presentation:** A 36-year-old man who presented with left subclavial thrombosis was admitted to our hospital for specific cure. Computed tomographic scan of the chest showed a large anterior mediastinal mass. Surgical exam revealed an infiltrative mediastinal tumor involving the branches of the vena from heart basis. The tumor was excised and biopsied for morphological examination. Histologically, the tumor proved to be a mixed carcinoma, composed from papillary and tubular growth patterns. Immunohistochemical stains for alpha-fetoprotein (AFP) was positive in the tumor cells while stains for carci-

noembryonic antigen and placental like alkaline phosphatase (PLAP) were negative. The serum level of alpha-fetoprotein (AFP) of this patient was elevated, as well. This supported the diagnosis of Yolk sac tumor, a rare primary tumor within the mediastinum.

**Conclusion:** Primary mediastinal Yolk sac neoplasm is a rare tumor. The diagnosis should be made not only by morphological studies but also the patient's age and the elevation of serum alpha-fetoprotein. In spite of modern chemotherapy, the prognosis of mediastinal yolk sac tumor remains poor. The single most important prognostic indicator is whether the tumor mass can be completely excised before or after chemotherapy.

### Host-related predictive factors for anastomotic leakage following large bowel resections for colorectal cancer

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**Aim:** To identify the risk, the host-related prognostic factors and their predictive value for anastomotic leakage after colorectal resections following cancer.

**Method:** 993 patients who underwent large bowel resection and primary anastomosis above 12 centimeters from the anal verge, without a temporary or permanent stoma at the Surgical Hospital No.3 (Cluj-Napoca, Romania) were retrospectively reviewed.

**Results:** 32 (3.22 percent) anastomotic leaks were confirmed. Univariate analysis showed that the preoperative variables significantly associated with anastomotic leakage included: weight loss, smoking, cardiovascular disease, lung disease, hypoproteinemia, diabetes, anemia, leukocytosis, presence of two or more underlying diseases. Alcohol use, cerebrovascular disease, bowel preparation, type of anastomosis, tumor location, stage and histology were not significant variables. Hypoproteinemia (total serum protein level = 6 g/dl) and anemia (serum hemoglobin level = 11 g/dl) remained significant in the logistic regression model. The prognostic role of serum hemoglobin and proteins for the anastomotic leak was assessed using ROC curve analysis. For the cut-off value of serum protein level = 5.5 g/dl, a sensitivity of 61.6 percent and a specificity of 84.2 percent were calculated. The area under the curve was 0.703 (p = 0.0024). The area under the curve for serum hemoglobin was 0.616 (p = 0.028). A sensitivity of 64.0 percent and a specificity of 64.7 per cent were obtained for a cut-off value of 9.4 g/dl.

**Conclusion:** A serum protein level lower than 5.5 g/dl and serum hemoglobin lower than 9.4 g/dl could be considered as host-related predictive markers for anastomotic leak in large bowel resections for cancer. Key words Anastomotic leak - risk factors - predictive markers.

### Cell-based therapy for recovery of nasal mucosa in rabbits

ESC-ID **881**  
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**Aim:** Certain surgical procedures carried out on the lachrymal apparatus, nasal chambers and paranasal sinuses include partial or total removal of the mucosal lining. Its recovery has taken – 1.5 - 3 months and usually has complicated by chronic inflammation. In this case common therapeutic approaches are ineffective. We decided to assess the efficiency of the cell-based therapy method for recovery of aseptic nasal mucosal defects in the experiment.

**Materials and methods:** In our study we developed an experimental model of surgical injury of nasal mucosa and assessed the efficiency of transplantation human buccal epithelial cells embedded in collagen gel on proposed model. The experiment was conducted on 14 New Zealand rabbits. To make an experimental defect we excised mucosal flaps (1\*3 mm) on both sides of cartilaginous nasal septum. In the treatment arm we performed transplantation of buccal epithelial cells embedded in collagen gel in the defect region, in the second arm – surgical defect only. We histologically and morphometrically studied the regeneration of nasal mucosa and evaluated the effect of cell-based therapy in 1,2,3,5,7,12,14 days after operation.

**Results:** In the case of cell-based therapy, we have observed decrease in size of fibrinous clots, early completion of the inflammatory infiltration, early initiation and completion of epithelization and more blood vessels compared with the control side of cartilaginous nasal septum (surgical defect without cell-based therapy).

**Conclusion:** We proposed and described the biological model of the surgical defect of nasal mucosa in rabbits. On this model we showed the high efficiency of cell-based therapy – transplantation of buccal epithelial cells in collagen gel in the area of mucosa defect.

### ERCP vs. MRCP

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**Background:** It is controversial whether selective endoscopic sphincterotomy or routine laparoscopic bile duct exploration is the optimal treatment for choledocholithiasis. Magnetic resonance cholangio-pancreatography (MRCP) is a safe and accurate imaging modality; this study evaluated its use in a clinical algorithm for the management of suspected choledocholithiasis.

**Patients and methods:** Consecutive patients presenting with suspected common bile duct (CBD) stones were managed according to an algorithm involving the selective use of MRCP to identify patients who required endoscopic sphincterotomy and bile duct clearance. Following radiological demonstration of a clear CBD, all patients were considered for cholecystectomy.

**Results:** From 157 consecutive patients, 68 proceeded straight to endoscopic sphincterotomy, which was therapeutic in 59. Of 35 who underwent MRCP, choledocholithiasis or tumor obstruction was demonstrated; subsequent endoscopic sphincterotomy was therapeutic. Seventy-four patients subsequently underwent cholecystectomy, with a conversion rate of 9% and a median postoperative stay of 2 day. There were no instances of post-sphincterotomy pancreatitis or haemorrhage requiring transfusion.

**Conclusion:** An algorithm involving selective MRCP with endoscopic sphincterotomy is a safe, effective means of managing suspected choledocholithiasis, particularly where the expertise, equipment or theatre time for laparoscopic bile duct exploration is not routinely available MRCP may become a new additional alternative to ERCP and intraoperative cholangiography.

### **Injury severity score (ISS) and systemic inflammatory response syndrome (SIRS) in predicting mortality and hospitalization length in patients suffering polytrauma admitted to urgent surgery center in Belgrade, Serbia**

ESC-ID **515**  
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**Aim:** Predictive ability of ISS and SIRS in determining the outcome of polytraumatized patients in early stage of treatment (24h of admission).

**Methods and materials:** Trauma is, regardless of age, the third most frequent cause of death in Serbia, compared to the fourth place worldwide. This prospective study emphasizes 42 patients who suffered polytrauma injuries and were admitted to Urgent Surgery Center in Belgrade from 10/01/2008 to 01/31/2009. Injuries are ranked on a scale of 1 to 6, with 1 being minor, 5 severe, and 6 a non-survivable injury. This represents the 'threat to life' associated with an injury and is not meant to represent a comprehensive measure of severity. Each injury is assigned an AIS and is allocated to one of six body regions (head and neck, face, chest, abdomen, extremities (including pelvis), external). Only the highest AIS score in each body region is used. The 3 most severely injured body regions have their score squared and added together to produce the ISS score. Only patients with ISS  $\geq$  16 were considered. Gender, age, injury mechanism, injury type and severity, SIRS parameters, length of stay, complications and mortality were analysed.

**Results:** Out of 42 patients, 10 were female (23.8%) and the rest of 32 male (76.2%). Patients were aged 14-86 (average 46.7). Traffic accidents were found to be the most frequent cause of injuries (57.1%) followed by injuries caused by fall in 7 patients (16.7%), cut/pierced were 5 (11.9%), while the same number of cases - 2 (4.8%) was reported to be a result of blunt trauma and of unknown origin. Firearms and crush syndrome were responsible for the last 2 cases. As a direct result of polytrauma consequences, 18 patients (42.8%) died, 3 of which were female (16.7%), 15 male (83.3%). No statistically significant difference in mortality among genders was found ( $p > 0.05$ ). Comparing groups of survived and deceased

patients who had ISS values less than 25 (severe injury) to the ones who had ISS greater or equal to 25 (critical injury) we found ISS to be statistically highly significant as mortality predictor ( $p < 0.01$ ). SIRS showed similar predictive abilities; difference in SIRS values for survived and deceased patients were also statistically significant ( $p < 0.05$ ). ISS is not in correlation with the length of hospital stay ( $p > 0.05$ ), which directly correlates with SIRS values ( $p < 0.01$ ).

**Conclusion:** Usability of ISS and SIRS in every polytraumatized patient, due to their application simplicity and high complication and mortality prediction abilities, is justified in day-to-day practice.

## *Urology*

### **New method for chemolysis of renal calculi containing uric acid**

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**Introduction:** Urolithiasis is a disease with big social impact, especially in the last decades. The epidemiological tendencies show an increasing number of patients in the working age notably in the industrialized countries. Despite many studies in this field some of the aspects of the ethiopathogenesis of the urolithiasis still remain unclear. The lithiasis is considered as Urate type when the renal calculi are composed of uric acid in keto form. Its treatment usually includes alkalinizing medicaments which bring the pH of urine to 7,0.

**Aim:** The aim of the present study was to evaluate and monitor the effects of a specific chemolytic treatment in patients with urolithiasis in Bulgaria.

**Methods and Materials:** We have synthesised and used for the first time a substance: an ester of fatty acids and polyvalent alcohol (United States patent US 4,816,483). This substance forms a water-soluble complex with uric acid and thus helps its elimination out of the body. The ester is included in a registered product in Bulgaria – "Pharmalyt-neo". We have evaluated retrospectively the effects of therapy with "Pharmalyt-neo". Patients included in the study had either pure urate type or mixed type (renal calculi containing urates and other compounds) of urolithiasis. The effects of the therapy were followed by monitoring the patients' surface tension and pH of urine – laboratory data, as well as the clinical manifestation of renal calculi – imaging data.

**Results:** The treatment with "Pharmalyt-neo" results in a decreased surface tension of the urine in patients with urolithiasis. No statistically significant change in the pH of urine is observed. The substance acts on both pure urate and mixed type of renal calculi. A delay in the formation of kidney stones and therefore a preventive effect is also observed.

**Conclusion:** Urolithiasis treatment based on chemolysis of the renal calculi may be considered as effective both in the cases of prophylaxis and therapy of already manifested stone formation. Our study shows positive results in the

treatment of patients with urate/mixed kidney stones by using a substance which helps the elimination of uric acid.

### The Effects of Acrylamide on sperm parameters and membrane integrity of epididymal spermatozoa in mice

ESC-ID 160  
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*Introduction:* Acrylamide is a chemical reactive substance used in various industries such as polymer industry, cosmetic, adhesives, paper and textile industries, wastewater treatment, and laboratory gels. Recently, the discovery of acrylamide in a variety of human foods like heat-processed starchy foods such as potato chips, French fries, and bread was reported. Acrylamide is known as a carcinogen and cytotoxic material too.

*Purpose:* To determine the effect of acrylamide on membrane integrity and sperm parameters in mice.

*Material and methods:* This experimental study was conducted on thirty male NMRI mice, age 8 to 10 weeks and weight in 25-30 gram which were randomly allotted into three groups, equally. Group I (low dose) and Group II (high dose) were fed on water solutions containing acrylamide 5 and 10 mg/kg/day, respectively, for eight consecutive weeks, while the third group on fresh water only as the control, then sperms analyzed for parameters as well as evaluation of membrane integrity by Hypoosmotic Swelling Test for sperms tail and Eosin Exclusion Test for sperms head. The data analyzed by SPSS v.13 software in ANOVA test. Result: In sperm analysis, the total motility and progressive motility (fast and slow motilities) in both Group I and II was decreased significantly ( $P = 0.00$ ), but no significant change was observed in non-progressive motility ( $P > 0.05$ ). Total motile sperm count decreased significantly only in group II ( $P = 0.01$ ). Sperm morphology was not significantly changed ( $P > 0.05$ ). In membrane integrity evaluation of sperm, functional intact membrane of sperm tail in both Group I and II had significant decrease ( $P = 0.00$ ). However, membrane integrity of sperm head decreased significantly only in Group II ( $P = 0.00$ ).

*Conclusion:* These results indicate that acrylamide through effect on membrane integrity decreased sperm vitality, also causes abnormal sperm parameters in progressive motility and count. Key words: acrylamide, membrane integrity, mouse spermatozoa

### Importance of PSA measurement in serum as screening test and frequency of prostate cancer among male patients for 2005 and 2006 in Canton Tuzla, Bosnia and Herzegovina

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*Introduction:* The prostate is a small gland that, in young adult men, is about the size and shape of a walnut. Prostate is responsible for producing approximately 50 percent of the fluid that combines with sperm to make up ejaculation. The most frequent disease are prostatitis, Benign Prostatic Hyperplasia (BPH) and prostate cancer. Prostate cancer is the second leading cause of cancer death among males.

*Aim:* Aim of this work is to determine the frequency of BPH and prostate carcinoma among tested males in UCC Tuzla during the period of 2005-2006, to determine the frequency of pathohistological changes by age distribution, to determine the prostate specific antigen (PSA) levels and as well Gleason score of prostate cancer.

*Material and Methods:* The study has been conducted by a retrospective analysis, using information from the Department of Pathology UCC Tuzla. All samples were taken using needle biopsy and/or total or partial prostatectomy of patients with prostate problems. All PSA measurements were performed in central laboratory of UCC Tuzla. SPSS 16.0 (Statistical Package for Social Science) computer program is used for processing collected data, and further statistic methods are used: descriptive statistics and Chi<sup>2</sup> test ( $\chi^2$ ).

*Results:* Among 582 patients included in this study (age range 44 to 86 years), prostate cancer was diagnosed in 125 cases (21.47 percent); 78 of these 125 cancers (62.4 percent) had a Gleason score of 7 or higher. PSA was measured to 88 patients with prostate cancer, however this information was missing to 37 cases (29.7 percent). 67.04 percent of all patients with prostate cancer had PSA higher than 10.00 ng/mL, 29.54 percent had PSA between 4.00 ng/mL – 10 ng/mL and 2.4 percent had a PSA lower than 4.00 ng/mL. We have found 437 (75.08 percent) patients with BPH, 125 (21.47 percent) with adenocarcinoma, 14 (2.40 percent) patients with prostatitis and 2 (0.34 percent) with microcarcinoma diagnosed.

*Discussion:* Prostate problems are very common and often for older males. Almost every male over the age of 50 has a small prostate hyperplasia, and a notable portion of them have adenocarcinoma, diagnosed “de novo” or BPH to adenocarcinoma transition. It is important to know that adenocarcinoma treatment is much easier if it is diagnosed at an early stage. Because of that, prostate screening is very important. Every male older than 50 should do at least one digitorectal prostate examination (DrPE) per year. Screening for PSA has led to earlier detection of prostate cancer but elevated serum PSA levels may be present in non-malignant conditions such as benign prostatic hyperplasia (BPH). We believe that the combination of measurement of the serum PSA concentration and rectal examination, with ultrasonography performed in patients with abnormal findings, provides a better method of detecting prostate cancer than rectal examination alone.

*Key words:* PSA, Gleason score, Prostate Cancer

## APPENDIX

### **Morphofunctional changes of the cellular components of the parenchyma and haemomicrocirculatory channel of the adrenal glands on the 14th day of posthypothermical period**

ESC-ID 111  
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*Introduction:* Hypothermia is a serious medical condition caused by an extreme cold. Hypothermia ensues from a long-standing exposure to a cold factor. Among the environmental factors, excessive freezing of the body is of a fairly frequent occurrence. It is regarded as a typical stress promoter which triggers hypothalamo-hypophysis-adrenal system with subsequent release of catecholamines. The impact of the cold factor is also suggested to involve vital morphofunctional changes of the organ. The primary objective of our scientific endeavour was to elucidate these changes by investigating the parenchyma and haemomicrocirculatory channel of the adrenal glands.

*Materials and Methods:* The investigation was performed according to the Animal Testing Legislation of Ukraine and Universal Norms in Bioethics on the 20 mature white breedless male rats with an average weight of about 160-200 g. The animals were divided into two groups: the control (4 rats) and experimental (16 rats). The latter were put into the freeze camera with permanent temperature of about -32°C. Euthanasia was performed due to the inhalation of the ether vapours. Subsequently sampling of the tissue sections was accomplished. These sections of adrenal parenchyma were stained with hematoxylin-eosin and fuxin-picrofuxin to perform a histological investigation. In order to study the adrenal vessels a suspension of azure-blue stain in the ether-chloroform dissolvent was injected through the abdominal aorta. Electron microscopy investigation was performed in a conventional way.

*Results:* On the 14th day of the posthypothermical period significant stabilization features can be observed. The swelling of the cells considerably decreases and the thickness of the glomerular and reticulate zones returns to the norm. The fasciculate zone and adrenal medulla are characterized by hypertrophy, which implies regenerative processes. The cytoplasm of the adrenocortycocytes becomes more imbued with vacuol-like structures, which in our opinion are the analogies of liposomes. Alongside we can observe partially damaged cells surrounded by aggregations of neutrophiles and solitary lymphocytes. Adrenal medulla reflects epenephrocytes with grainy dystrophy features and pieces of hemosiderine. The microcirculatory channel of the adrenal glands contains dilatation of arterial and venous links of the blood channel from the previous term. The diameter of the capillaries of fasciculate zone is about  $7,81 \pm 0,14$  mcm ( $p < 0,05$ ) (in control  $6,97 \pm 0,23$  mcm) and sinusoids of medullary substance  $27,09 \pm 1,52$  mcm ( $p < 0,05$ ) (in control  $21,60 \pm 1,14$  mcm). The level of cortisol in the blood is still high, at the same time the level of epinephrine gradually decreases.

*Conclusions:* Morphofunctional changes of the adrenal glands, mentioned above, prove the activation of intracellular regenerative processes, which is a positive feature for the restoration of the organ's structure. Moreover, received data contributes to pathogenetic therapy of the cold trauma patients by facilitating treatment options.

### **The role of non-invasive ventilation and percutaneous endoscopic gastrostomy and their effect on survival patients with amyotrophic lateral sclerosis (ALS)**

ESC-ID 139  
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*Introduction:* Symptomatic treatment of amyotrophic lateral sclerosis (ALS) primarily based on non-invasive ventilation (NIV) and percutaneous endoscopic gastrostomy (PEG) provides longer survival.

*Aim:* To determine frequency non-invasive ventilation and PEG application in ALS patients and their influence on duration ALS.

*Material and methods:* A total of 229 ALS patient were initially diagnosed between 2003-2007 yrs. we formed a group of 39 ALS patients (23 males and 16 females), mean age of  $58 \pm 9,6$  yrs. 18 with spinal onset and 13 with bulbar onset disease, either with NIV or used NIV and PEG, due to dysphagia and/or respiratory insufficiency. These patients were divided into two groups: 1) only NIV users 2) those with both PEG and NIV. Control group included other ALS patients with definite or probable ALS who did not underwent PEG or NIV. Statistic processing of the given data was done by Kaplan Meier for cumulative survival and Log Rank and Breslow test for valuation statistically significant.

*Results:* In study group 22 patients died (64.70%), and 21 patients (65.45%) in the control group, during the period from 2003- 2007 yrs. The mean survival time from the onset of disease in patients from study group who used NIV was  $5.45 \pm 0.95$  years. When we compare it with controls ( $2.57 \pm 0.24$ ), this difference was statistically significant.

### **Radiographic evaluation and comparison of the effects of Demineralized Bone Matrix (DBM) and autogenous bone graft in reconstruction of bony defects in rats**

ESC-ID 150  
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*Introduction:* Autogenously bone graft is commonly used for the reconstruction of bone defects in routine surgical procedures. The complication of achieving bone grafts and application of them is an important factor that has

been encouraged investigators to detect an alternative and substitute Demineralized Bone Matrix (DBM) which is one of the most famous materials among grafts. Because of the importance of that tissue and the worldwide need of health care societies, for the first time this study was practiced by human bone specialists in order to evaluate and compare the effect of DBM and Auto graft on the reconstruction of bone defects in parietal bone of Rats. The comparison was based on radiographic evaluation by two methods. The aim of the present study was to evaluate the rule of radiographic findings in determining the pattern of osteogenesis in parietal bone of rats.

**Material and methods:** This study was a cross-sectional an experimental research. A total of 12 rats were involved in study. First we produced DBM using shaft of human humerus bone and obtained autograft from the contralateral parietal bone of the rat. In second step the autograft and DBM were placed accidentally in the right and left side of parietal bone of each rat to fill the defects. The rats were sacrificed in 7th, 14th, 24th and 60th day after surgery and placed in four groups according to it. The quality and quantity of bone formation were compared in four groups by two different investigators who were blind of the type of grafts. Investigator used digital and film based methods for investigation and rate the bone formation using a 1-4 rating scale. Then their investigations were compared and analyzed using SPSS.

**Results:** Intra-observer viewer agreement and inter-observer viewer agreement in film based method were 64% and 72.9% respectively. For direct digital method, intra-observer and inter-observer viewer agreement were 80% and 98% respectively. There was no significant difference between two bone substitutes in quantity of bone formation at the end of 60th day.

### Analysis of prostate cancer mortality in Belgrade, 1975-2006

ESC-ID 153  
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**Introduction:** Prostate cancer (PC) is the third most common cancer in men worldwide.

**Aim:** The aim of this study was to analyze mortality from PC in the population of Belgrade, during the period 1975-2006.

**Materials and methods:** Mortality data (official death certificates) for PC have been gathered from published and unpublished material of the Municipal Institute of Statistics in Belgrade. Mortality rates were standardized according to the European standard population. Regression coefficient was determined by Fisher's test.

**Results:** The average standardized mortality rate from PC, in the period 1975-2006 in Belgrade, was 20.14/100,000 (95% confidence interval (CI) 17.30-22.98). During the period observed, the lowest PC mortality rate was 8.96/100,000, and the highest was 39.52/100,000. The risk of fatal outcome from PC increased with age; the highest mortality rate was registered in the oldest age group (70 years and more) – 230.56/100,000 (95% CI 194.42-266.70). The lowest PC mortality rate was obtained for the youngest age group.

### Clinical study of testosterone gel as adjunctive therapy to sildenafil in hypogonadal men with erectile dysfunction who do not respond to sildenafil alone

ESC-ID 228  
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**Objectives:** We have recently shown that, in men with erectile dysfunction (ED), free testosterone (FT) directly correlates with penile arterial inflow. This led us to further investigate the effect(s) of androgen administration on cavernous arteries in patients failing sildenafil treatment.

**Design:** Prospective randomized placebo-controlled pilot study.

**Patients:** Twenty patients with arteriogenic ED as evaluated by dynamic colour duplex ultrasound (D-CDU) studies, normal sexual desire but testosterone (T) and FT in the lower quartile of normal range (low-normal), not responding to sildenafil treatment (100 mg) on six consecutive attempts.

**Measurements:** All patients had D-CDU, hormonal [LH, prostate-specific antigen (PSA), total and free testosterone, sex hormone-binding protein (SHBG), oestradiol], biochemical [haematocrit, low-density lipoprotein (LDL) and HDL cholesterol, triglycerides], and sexual evaluations [International Index of Erectile Function (IIEF)] before and after 1 month of therapy with transdermal testosterone (5 mg/day, n = 10) or placebo along with sildenafil treatment on demand. Measurement of flow parameters by D-CDU on cavernous arteries was the primary endpoint of the study. Improvement of erectile function was assessed using the IIEF questionnaire and the Global Assessment Question (GAQ).

**Results:** One month treatment with transdermal testosterone led to a significant increase in T and FT levels (23.7 ± 3.3 SD vs. 12.8 ± 2.1 nmol/l and 473 ± 40.2 vs. 260 ± 18.1 pmol/l).

### BCG scar size: associations with infant growth, infection, and atopy

ESC-ID 269  
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**Introduction:** A larger scar formed in response to the Bacille Calmette-Guerin (BCG) vaccination may be associated with non-specific beneficial effects on infant immunity and mortality.

**Objectives:** To investigate the relationship of: • nutritional status (birthweight and feeding practices) to BCG scar size • scar size to infant growth, morbidity, and atopy.

**Method:** Follow up of 78 infants from low-income slum communities of Mumbai, India, in a population-based prospective study. The infants received the BCG vaccination at birth and were followed up during infancy. BCG scar measurements and anthropometry (weight, length, skinfolds, circumferences) were performed to a standardised protocol. Z scores-for-age were calculated from the WHO growth standards. Atopic dermatitis and feeding



practices were assessed by questionnaire and morbidity as number of days of fever, cough, and diarrhoea since birth. *Results:* 78 infants were included in the study. 53% were male. Ages ranged from 5.1 to 17.2 months (median 8.3 months). The mean (SD) birthweight and weight-for-age Z-score was 2.71 kg (2.7 kg) and -1.27 (1.05) respectively. The median BCG scar diameter was 3 mm with values ranging from 1 to 9 mm. 39 infants (50%) had experienced either fever, cough, or diarrhoea since birth with a median (IQR) lifetime morbidity of 0.25% (0 to 4.89%). No cases of atopic dermatitis were seen. BCG scar diameter did not relate to birthweight or nutritional status (z-scores) at age of measurement. It was, however, positively correlated to triceps skinfold enlargement during infancy ( $p = 0.03$ ). Smaller scar diameters were associated with exclusive breastfeeding throughout the first month of life.

### **Routine or on demand radiological contrast examination in the diagnosis of anastomotic leakage after esophagectomy**

ESC-ID 277  
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*Background:* To detect anastomotic leakage after esophagectomy in esophageal carcinoma patients, many surgeons apply for a radiological contrast examination routinely. The aim of this retrospective study is to determine the clinical relevance of a routine contrast examination after esophagectomy and to evaluate criteria for a contrast examination on demand.

*Methods:* Data was obtained from 211 patients with cancer of the esophagus or gastro-esophageal junction who underwent an esophagectomy during the period 1991-2004. Retrospectively, we analysed patients regarding anastomosis related characteristics and clinical signs including sepsis, fever  $\geq 39.0^\circ\text{C}$ , leukocytosis  $\geq 20 \times 10^9/\text{ml}$  and pleural effusion.

*Results:* Anastomotic leakage had appeared in 35 of the 211 patients. The clinical signs sepsis (odds ratio (OR) 6.72 (95 per cent confidence interval (CI) 2.57 to 17.56);  $p < 0.000$ ), leukocytosis (OR 2.62 (1.10 to 6.22);  $p < 0.030$ ) and fever (OR 2.34 (1.01 to 5.42);  $p < 0.047$ ) were significantly related to anastomotic leakage. Pleural effusion was not significantly related to anastomotic leakage (OR 2.83 (0.98 to 8.13);  $p = 0.054$ ).

*Conclusion:* Our study suggests that the clinical value for a routinely performed contrast examination is debatable. We recommend performing a contrast examination only when there is clinical suspicion and clinical signs of anastomotic leakage appear such as sepsis, leukocytosis  $\geq 20 \times 10^9/\text{ml}$  and temperature  $\geq 39.0^\circ\text{C}$ .

### **Characterization of pituitary adenylyl cyclase activating polypeptide (PACAP)- and vasoactive intestinal peptide (VIP)-induced pial arteriolar dilation in piglet**

ESC-ID 318  
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*Aim:* PACAP isoforms (PACAP38 and PACAP27) and VIP are structurally related neuropeptides sharing partially common receptors and therefore, a wide spectrum of biological activities. In the central nervous system, these peptides are potent vasodilators; however, little is known on the mechanism of PACAP- and VIP-induced cerebrovascular changes in newborns. Therefore, we sought to characterize the vascular reactivity to PACAP38, PACAP27, and VIP in the piglet model that shows good correlation with the vascular physiology of newborn babies.

*Methods and Materials:* Anesthetized (Na-thiopental 40 mg/kg, ip, followed by  $\gamma$ -chloralose 40 mg/kg, iv), ventilated piglets of either sex (1 day old, 1-2 kg) were equipped with closed cranial window over the left parietal cortex. Pial arteriolar diameters (baseline  $\sim 100 \mu\text{m}$ ) were determined via intravital microscopy using a video microscaler. First, we tested the vasodilator effects of topically applied PACAP38, PACAP27, or VIP at 10<sup>-8</sup> to 10<sup>-6</sup> M concentrations ( $n = 8-8$ ). We also examined, whether COX-derived metabolites play a role in the mechanism of dilation, using the non selective cyclooxygenase (COX)-inhibitor indomethacin (5 mg/kg, iv), the selective COX-1-inhibitor SC-560 (1 mg/kg, iv), and the selective COX 2 inhibitor NS 398 (1 mg/kg, iv,  $n = 8-8$ , respectively). Finally, we determined the effect of the nitric oxide synthase (NOS) inhibition on the PACAP/VIP-dependent pial arteriolar reactivity by intravenously administered N-nitro-L-arginine methyl ester (L NAME, 15 mg/kg,  $n = 8$ ).

*Results:* PACAP38, PACAP27, and VIP evoked significant, dose-dependent, reproducible pial arterial dilations. Percent changes from baseline were  $6 \pm 1-40 \pm 4\%^*$ ,  $5 \pm 1-34 \pm 5\%^*$ , and  $15 \pm 4-68 \pm 4\%^*$  in response to PACAP38, PACAP27, and VIP, respectively.

### **The human circadian rhythm connected to psychosocial state, general health and psychophysiology**

ESC-ID 325  
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*Introduction:* Connection between human circadian rhythm and health is widely debated. Circadian rhythm disruption is suggested as an alternative pathomechanism for several diseases such as sleep disorder, depression, breast cancer etc. Due to these previous findings we wanted to measure the effect of circadian rhythm on health risks and psychosocial state on a healthy sample. Our major question was if we could explore any difference between evening and morning chronotypes concerning

self-reported health, sleep quality and happiness. According to our minor question we examined connections between morning/evening chronotypes and salivary cortisol.

**Material and methods:** A total of 168 healthy women, aged 21-65 years, were recruited, all of them working full-time in Semmelweis University, Budapest. People suffering from serious chronic diseases or taking steroid, hypertensive or anti-inflammatory medication were excluded. Each subject has undergone two 24 hour sessions, one over a working and one over a leisure day. Sessions involved periodic sampling of saliva for the assessment of cortisol. Participants were instructed how to collect saliva samples using Salivettes. Each subject has completed a set of questionnaires including the Hungarian version of Composite Scale of Morningness (Smith), Jenkins Sleep Problems Scale, Patient Health Questionnaire (PHQ-15), WHO Well Being Scale and Beck Depression Inventory. We used spss-13 for statistical analysis, cortisol levels were log-transformed.

**Results:** Patients over 60 years had higher morningness scores than youngsters.

### Experimental investigation of the most suitable osteoblast-differentiation media for human adult stem cells

ESC-ID **351**

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**Introduction:** Stem cell research is a rapidly expanding area of investigation with the ultimate goal to prevent, diagnose and treat various human diseases. By using the most specific culture medium and thus activating certain intercellular signaling pathways, adult stem cells become osteoblasts. This study challenges current protocols by comparing the proliferation effects of different growth factors thought to influence bone genesis.

**Material and Methods:** Adult stem cells isolated from the human bone marrow and osteoblasts isolated from human patella bone are used. Before starting differentiation protocols we identified specific surface markers for stem cells (SSEA-4, CD29, CD105, Oct  $\alpha$ , Nanog and SOX2) and osteoblasts (Osteopontin and Osteonectin). We investigate the proliferation (MTT assay), the intensity of the mineralization process by histology stainings (Alizarin Red S, Alcian Blue and von Kossa) and the presence of alkaline phosphatase in 3 cell culture models: undifferentiated stem cells, predifferentiated stem cells on Matrigel substrate and osteoblastic cells. Cells were cultured in simple osteogenic media and complex medias, supplemented with growth factors: Transforming Growth Factor  $\beta$ , Bone Morphogenic Protein-2, basic Fibroblast Growth Factor, Insulin Growth Factor, Epidermal Growth Factor and commercial serum-free Promocell media for osteoblasts. For the controls we used DMEM/F12 with FCS, L-Glutamine and antibiotics.

**Results:** The MTT assay revealed that stem cell proliferation is most stimulated by IGF and serum-free Promocell media. The most intensive proliferation response was to IGF in all 3 cell culture models, but osteoblasts show the highest sensitivity. Stem cells precultivated on matrigel substrate were more sensitive to FGF, IGF, and EGF. Cells

cultured in medias with IGF and EGF have intense Anti-Alkaline Phosphatase staining.

**Conclusion:** The isolation of human stem cells offers the promise of a remarkable array of novel therapeutics through tissue regeneration and repair, from orthopedics to plastic and reconstructive surgery.

### Clinical features, histology and survival in right- versus left- sided colon cancer

ESC-ID **373**

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**Introduction:** Colorectal cancer is a significant public health problem worldwide. The clinical and pathological differences between right – and left – sided colon cancer are widely debated. These differences are not static and they change over time. A rightward shift in colorectal cancer has been established and also some changes in age distribution. Our objective is to evaluate the differences between right and left colon cancer in patients from the Emergency Hospital of Bucharest.

**Materials and Methods:** A retrospective study that included all patients with colon cancer admitted to the Emergency Hospital of Bucharest in 2003 and 2004 was performed. We tested the differences between right sided (caecum to transverse colon) and left sided (descendent and sigmoid) colon cancer regarding sex, age, tumor localization, stage of diagnosis, the common presenting symptoms and causes of emergency, the histopathological type and grade. In patients that could have been contacted, we followed the emergence of distant metastases or a local recurrence at 1 and 4 years after colorectal surgery and the survival at 4 years. The statistical analysis was performed using SPSS.

**Results:** This study confirms that both women and men develop left colon cancer more often, but right sided colon cancer tends to involve more females than males. There are remarkable differences in age distribution. Right tumors involve the extremes ages. By histology, the right sided colon cancer is more aggressive than the left sided colon cancer.

### Effects of alcohol, Diurnal Rhythm Disturbance and Trolox on the activity of Xanthine Oxidase in rat brain

ESC-ID **458**

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**Background:** Xanthine oxidase (XO) is a rate-limiting enzyme in nuclear acids degeneration through which all purines are channeled for terminal oxidation. These reactions are the major source of superoxide radicals and hydrogen peroxide which then enhance the formation of all oxygen-derived free-radicals. The increased XO activity (XOA) is considered to be important in the pathogene-

sis of the oxidative brain damage. Thus, the elevated XOA is considered to have a negative effect on the brain status. Trolox, the water soluble vitamin E, exhibits some advantages as radical scavenger, compare to alpha- and gamma-tokopherol, but its benefits as an in vivo antioxidant are still under investigation. In this study, the effects of Trolox and alcohol on the XOA in the brain of rat with Diurnal Rhythm Disturbance (DRD), were monitored.

**Materials and methods:** Six groups of 5 rats ( $110 \pm 10$  g) were exposed to different living regimes for a period of 3 weeks. Groups C and CT lived at 12/12 hrs light/dark cycle. Groups D, DT, AD and ADT, were exposed to 24 hrs light to the end of the experiment. Groups AD and ADT were provided with 10% ethanol, while all others received water only, ad libitum. Groups CT, DT and ADT received 200mg/kg Trolox p.o. every day. All animals were fed with standard rodent chow ad libitum. The brains were collected under anesthesia (ether) and stored at  $-81^{\circ}\text{C}$ . After homogenization at  $0^{\circ}\text{C}$ , the samples were centrifuged (4000XG, 10 min,  $+4^{\circ}\text{C}$ ), and the supernatant was analyzed for the activity of XO. XOA was determined by measuring the ability of the sample to transform Xanthine to Uric Acid (Absorbance at 293 nm) for 5 minutes.

**Results:** As result of the stress, the activity of XO increased.

### The impact of Alexithymia on asthma patient management and communication

ESC-ID **493**  
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**Background and Objectives:** Alexithymia is a personality trait associated with difficulty identifying and verbalising feelings [1]. It has been associated with poorly controlled asthma and near-fatal asthma [2]. The primary objectives were to: (1) determine the prevalence of alexithymia in a group of moderate to severe asthmatics who attended an Outpatient Clinic; (2) investigate the relationship between alexithymia and asthma control, management and communication.

**Methods:** Cross sectional study of 25 moderate to severe asthma patients recruited from Royal Adelaide Hospital Outpatients. Participants were either mailed the questionnaire pack or completed it after a clinic appointment. Existing validated questionnaires were used. Statistical analyses were performed using SPSS.

**Results:** In total 11 male (44%) and 14 female (56%) patients with moderate to severe persistent asthma (mean age 44 years  $\pm$  11) participated. Alexithymia scores ranged between 23.0-76.0 ( $= 48.3$ ,  $\text{SD} = 13.2$ ). 12% of participants reported high alexithymia scores, 32% reported borderline alexithymia scores and 56% reported low alexithymia scores. Alexithymia mean scores were not statistically different across sociodemographic variables. A significant correlation was found between alexithymia and asthma control scores.

### Risk factors for hypertension in schooled children

ESC-ID **520**  
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 Institution **Università degli Studi di Palermo**

**Introduction:** High blood pressure, high sodium intake, high body mass index (BMI), low body weight at birth, hypertension in relatives have been reported in children as risk factors for development of hypertension in adulthood. Microalbuminuria (MA) is associated with increased risk of cardiovascular accidents in adults. Few studies have been done relating blood pressure and MA in children.

**Methods:** We registered age, height, weight, systolic (PAS) and diastolic (PAD) blood pressure, birth weight and checked urine by dipstick in 795 schoolchildren in a small town. We registered blood pressure twice and calculated the mean values. We compared our data with values reported by the literature. Familial hypertension (FAM) was diagnosed when a first degree relative was on anti-hypertensive treatment. In 250 children, without urinary alterations, albumin (Al), calcium, sodium, creatinine (Cr) concentration was measured in a first morning urine sample. A multivariate analysis of this data was performed using the SPSS software package.

**Results:** We registered values higher of normal range of PAS in 20% and of PAD in 4%. Blood pressure values higher of 2 standard deviations from the mean, calculated for age and sex of our population, were found in 25 children. Children with hypertension were more often obese (16% vs 6%) and had a familial hypertension (16% vs 8%), in comparison with children without hypertension. Overweight children were 24% (26% Girls, 24% Boys).

### The effect of powder/liquid ratio on flexural strength and shear bond strength to dentin of glass ionomer cements

ESC-ID **529**  
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**Aim:** This study evaluated the effect of variation in powder/liquid (P/L) ratio on shear bond strength to dentin and flexural strength of GICs.

**Method and Materials:** To conduct this study three types of GICs were used (Fuji II, Fuji II LC improved and Fuji IX GP). The cements were mixed using the following P/L ratios: 20% powder less than manufacturer's recommendation ratio, manufacturer's recommended ratio, 20% powder more than recommendation (comprising 9 groups). Shear bond strength (Mpa) evaluated using 90 cylindrical specimens (1.6 x 1mm), 10 in each group, which have been bonded to the superficial conditioned human third molar dentin and loaded under shear stress until fracture and the mode of failure checked under stereomicroscope. To assess the flexural strength (Mpa), 45 specimens (25 \_ 2 \_ 2) were made (5 in each group) and subjected to flexural stress in a universal testing machine (cross-head speed

of 1 mm/min). Mean and standard deviation were calculated and the data subjected to Two-way ANOVA and Tuckey test for analysis.

### Expression of tissue transglutaminase in small intestine biopsy specimens in coeliac disease

ESC-ID **551**  
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Coeliac disease is an autoimmune disorder of the small intestine that occurs in genetically predisposed people of all ages from middle infancy on up. It is caused by a reaction to gliadin, a gluten protein found in wheat. Upon exposure to gliadin, the enzyme tissue transglutaminase modifies the protein, and the immune system cross-reacts with the small-bowel tissue, causing an inflammatory reaction. That leads to flattening of the lining of the small intestine (villous atrophy). Coeliac disease leads to an increased risk of serious complications, which returns to baseline with diet. So, the timely diagnostics is very important. Serological blood tests are the first-line investigation required to make a diagnosis of coeliac disease. Serology for anti-tTG antibodies has a high sensitivity (99%) and specificity (>90%) for identifying coeliac disease. Tissue biopsy is still considered the gold standard in the diagnosis of coeliac disease.

*Aim:* to define tTG expression in duodenal biopsy samples with immunohistochemistry.

*Materials and methods:* 25 duodenal biopsy specimens were analyzed (20 patients with coeliac disease Marsh IIIC and 5 patients with normal mucosa of small intestine). Immunohistochemical staining was performed on formalin fixed tissue. Anti-tTG antibodies (CUB 7402) were used for staining. The quantitative analysis of tTG expression was carried out by image analysis software Aperio ImageScope v. 9.1.19.1571. The expression in superficial epithelium, crypt epithelium and in lamina propria was estimated separately. The total number of weak, moderate and strong positive pixels, and also positivity (total number of positive pixels/total number of pixels) was automatically estimated. Also rates of weak, moderate and strong positivity were defined (number of pixels of certain intensity / total number of pixels).

*Results:* Results of the quantitative analysis of an expression tTG in duodenal biopsy specimens are presented as mean value  $\pm$  standard deviation. Total positivity in superficial epithelium was  $84,0 \pm 15,6$  in 1st group compared to  $47,5 \pm 9,3$  in 2nd group ( $p = 0,0007$ ), in crypt epithelium this rate was  $38,6 \pm 24,3$  in 1st group and  $10,7 \pm 7,3$  in 2nd group ( $p = 0,0074$ ) and  $50,1 \pm 15,1$  in 1st group to  $29,9 \pm 9,1$  in 2nd group ( $p = 0,0043$ ) in lamina propria. Moderate positivity were significantly higher in 1st group in all compartments ( $p < 0,05$ ). The same difference was also between rates of weak positivity in crypt epithelium and in lamina propria. Thus, tTG expression was significantly raised in lamina propria, superficial and crypt epithelium in patients with coeliac disease.

*Conclusions:* immunohistochemical analysis of 25 duodenal biopsy specimens was performed. tTG expression was significantly raised in lamina propria, superficial and

crypt epithelium in patients with coeliac disease. Further researches are needed for estimation of specificity of this phenomenon to coeliac disease.

### The morphological and functional features of pituitary-thyroid axis after central ghrelin administration

ESC-ID **557**  
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*Introduction:* Body weight depends on the balance between energy intake and consumption. An interaction between ghrelin and the thyroid function has been reported almost only in pathophysiological state.

*Aim:* We examined whether intracerebroventricular (ICV) administration of ghrelin affects structure and function of the pituitary-thyroid axis in young adult Wistar male rats.

*Material and methods:* Five doses of ghrelin (1  $\mu$ g rat ghrelin/5  $\mu$ L PBS) or equal volume of PBS were injected every 24h into the lateral cerebral ventricle. Two hours after the last treatment the animals were killed, their pituitaries and thyroids excised and prepared for further immunohistochemical and morphometrical investigation. Serum TSH levels were measured by RIA, while the total T4 and T3 levels were examined by ECLIA.

*Results:* Ghrelin treatment increased pituitary weight.

### Spatial peripheral cues in attraction of attention toward different spatial positions

ESC-ID **572**  
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*Aim:* Visual cues are stimuli that can direct visual attention to or away from a special target in space or time. Several experiments have shown that attention, as measured by simple reaction time to luminance changes, can be shifted in the visual field while the eyes are kept on a fixed position. The shift of attention appears to take place in less than 500ms following a cue indicating the most likely position of the target. The experiment reported here has studied the behavior of spatial peripheral cues in attraction of attention toward different spatial positions.

*Method:* Participants consisted of 10 healthy 20 to 30 year-old volunteers with normal eyesight. Subjects were cued to attend to one of 12 possible stimulus locations which are arranged in any six angles of a virtual hexagon and an other one which is greater than the first and surrounds it. The volunteer should decide, as fast as possible, whether the target, a bar, is presented horizontally or vertically. The reaction times were measured and compared for the stimuli presented in same location, same-hemifield (vertical or horizontal) and same-orientation of the cue.

*Results:* comparison of reaction times to different experimental trials showed that reaction times were shorter in

same-location relative to all other trials, in horizontal trials relative to vertical trials.

### Investigation of peripheral and central blood pressure in young healthy volunteers

ESC-ID 647

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The clinical importance of central blood pressure (BP) has been gradually increasing in recent years, since several studies support that central BP more closely correlates with cardiovascular end-points than peripheral BP does.

**Aims:** We measured the peripheral and the noninvasively determined central blood pressures of healthy, young volunteers, furthermore we examined the correlation of these two parameters with special regard to diurnal variation (8 AM, 12 PM, 5 PM) and gender difference.

**Methods:** The measurements were carried out noninvasively using the SphygmoCor (AtCor Medical, Australia) device.

**Results:** 52 young subjects (23 males and 29 females) were included in the study. The peripheral and central systolic and diastolic BP values did not show any significant diurnal variation either for males, or females. All BP values of the males were higher than that of females ( $p < 0,001$ ). The peripheral systolic BP were higher than the noninvasively measured central systolic pressure during all three times of measurement ( $p < 0,001$ ). The differences for males were 14,33,7 mmHg; 17,43,6 mmHg, and 17,83,1 mmHg ( $p < 0,001$ ), while the amplification of systolic BP for females were 11,53,4 mmHg; 12,73,7 mmHg, and 12,93,5 mmHg ( $p < 0,001$ ), respectively. In case of the diastolic BP we did not find such a similar difference. The pulse pressure (PP) ratio (peripheral PP/ central PP) was significantly increasing for both genders during the day (males:  $p < 0,001$ ; females:  $p = 0,003$ ).

**Conclusion:** We suppose that it is important to determine central blood pressure by non-invasive measurement, especially in young individuals, since the peripheral blood pressure measured at the brachial artery does not reliably represent the actual central pressure conditions.

### Anti-inflammatory Properties of Cydonia oblonga Miller Fruit Aqueous Extract on Rat Paw Edema

ESC-ID 664

Author Zarei M

**Introduction:** Herbal medicines are acceptable alternative to synthetic drugs with the same efficacy and often less side effect. *Cydonia oblonga* Miller (Quince) is administered for treating sore throat, diarrhea, and bowel hemorrhage in traditional medicine. It also appears to have anti-inflammatory effect on the mucous membrane of intestines and stomach. To evaluate its chronic anti-inflammatory properties, we studied the results of intra-peritoneal injections of different doses of aqueous extract of *Cydonia oblonga*

Miller fruit on late phase of formalin induced rat paw edema.

**Materials and Methods:** Forty nine rats (185-240 grams) were randomly divided into six groups. Four groups received *Cydonia oblonga* Miller Fruit extract with different doses of 100, 300, 700, and 1000 mg/kg. Aqueous extract of the fruit was obtained by percolation technique and heating it for 15 minutes. Three other groups were injected with sodium salicylate (300mg/kg) as positive control and normal saline and Distilled water as negative controls groups. The inflammation was induced by injecting formalin (2.5%) once in the first day. The rats paw volumes were measured by a mercury plethysmometer in first day before and after formalin injection, and during the next 9 days, after giving the assigned intervention. Evaluation of anti-inflammatory effects was based on the difference between paws edema on each day and the first day of study. Differences were analyzed with repeated measures ANOVA. Inhibition percentage of each injection was also evaluated as to its efficacy.

**Results:** With the same distribution among groups, the mean of paws volume at the first day before and after inducing inflammation was 1.2  $\pm$  0.14 and 1.48  $\pm$  0.16, respectively. Reduction of the edema in each day compared with the other days was statistically significant,  $p < 0.001$ . The groups which received *Cydonia oblonga* Miller aqueous extract started to show statistically significant reduction of edema from the third day of study compared to the control groups,  $p < 0.05$ . There was no significant difference in reduction of edema between Normal saline and sodium salicylate group ( $p < 0.05$ ), but the reduction of edema in normal saline group was significantly more than distilled water group ( $P > 0.05$ ). The inhibition percentage in the extract injected groups was significantly more than the other groups,  $p < 0.001$ .

**Conclusion:** The results obtained in this study indicate that the fruit aqueous extracts of *Cydonia oblonga* Miller possesses significant anti-inflammatory effects on chronic phase of formalin induced rat paw edema. These effects were comparable with the established anti-inflammatory drug, sodium salicylate ( $p < 0.05$ ). The normal saline began to show anti-inflammatory effects since the 6th day of study which was comparable with Distilled water group, so normal saline has anti-inflammatory effects which could limit its role as a negative control group in anti-inflammatory studies.

### Assessment of right ventricular function in patients with permanent septal pacing versus apical right ventricular pacing

ESC-ID 677

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**Aim:** Permanent pacing is a conventional treatment for incompetent hearts and can prevent the sudden death which is induced by left ventricular (LV) dysfunction. Several studies have focused on the effect of RV septal or apical pacing on LV function but most of them were uncontrolled, poorly matched with short-term follow-up. The assessment of RV function after septal versus apical RV pacing has never been investigated (in Iran). In this

paper we report information about the assessment of RV function in patients with permanent septal versus apical RV pacing.

**Methods and Materials:** We conducted a cohort study of 60 consequences patients who underwent permanent DDDR pacing. The patients were categorised into two groups according to the type of pacing they received. They were followed prospectively by echocardiography evaluation and certain variables were assessed at baseline and one month after pacemaker implantation. we evaluated Right and Left ventricular ejection fraction, cardiac volumes and ejection time and ratio, as our variables to show patients' response to these two types of treatment for this study.

**Results:** The mean age of patients was  $64.6 \pm 7$ , of whom 38.3% were male. The baseline evaluation shows the mean of right ventricular ejection fraction  $48.21 \pm 4.3\%$  in apical group and  $53.61 \pm 3.84\%$  in septal group. The mean left ventricular ejection fraction were  $51.16 \pm 5.93\%$  in apical group and  $56.98 \pm 2.4\%$ . The reduction of RV EF was manifested at apical after one month follow up more significant than septal pacing.

### The interrelation of arsenicosis, malnutrition and arsenic intake from water and food in Nepal

ESC-ID **689**  
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**Background:** Poor nutritional status and chronic arsenic toxicity and have been reported in Nepal. It is estimated that 2.3 million Nepalese are consuming water containing high concentrations of arsenic. Symptoms of arsenicosis include respiratory, nervous, cardiovascular and endocrine toxicity, deleterious birth outcomes, impaired cognitive function and multiple cancers (1).

**Aim:** This study sought to investigate whether the risk of chronic arsenic toxicity increases with compounding factors related to nutritional status.

**Methods and Materials:** 103 male subjects (age range: 25-78, average 47 years) were examined for clinical signs of arsenicosis. Anthropometric measurements were recorded by standard methods. A 7-day quantified Food Frequency Questionnaire was also used to investigate nutritional status. Specific, common foodstuffs were analysed for total arsenic concentration ([As]) by Hydride-Generation Atomic Absorption Spectrophotometry. Drinking water was also analysed for [As] and individual water consumption recorded over a 24-hour time period. Daily Arsenic Intake (DAI) was calculated for each subject per unit body mass (BM). DAI was averaged from water consumption over a 5 year time period, additional intakes of arsenic from common dietary components were also quantified.

**Results:** Water [As] averaged  $181 \mu\text{g/L}$ , with a maximum recording of  $740 \mu\text{g/L}$  (WHO threshold =  $10 \mu\text{g/L}$ ). DAI from water ranged from undetectable levels to  $84.4 \mu\text{g/kgBM/day}$  (average  $11.2 \mu\text{g/kgBM/day}$ ). Tolerable Daily Intake (TDI) is defined by the FAO as  $2.1 \mu\text{g/kgBM/day}$  (2). Although only 22% exhibited clinical signs of toxicity, 69% of subjects consumed arsenic at greater than tolerable levels from drinking water alone. In addition, dietary consumption of arsenic from just four common foodstuffs contributed a mean intake of 2.97

$\mu\text{g/kgBM/day}$  (which increased to  $19.98 \mu\text{g/kgBM/day}$  where contaminated water was used in cooking). Body Mass Index (BMI) ranged from 13.4 to  $32.9 \text{ kg/m}^2$  (average =  $20.5 \text{ kg/m}^2$ ) with 30% of the subjects classified chronic energy deficient. A significant relationship between water arsenic intake and arsenicosis was observed.

### Quality of life after coronary revascularization in patients with chronic stable angina with or without type distal coronary vessels disease

ESC-ID **711**  
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Quality of life in patients after coronary revascularization is the basic treatment direction. Purpose of the study was to describe health status outcomes at 5 years for a cohort patients with distal coronary vessels disease. Patients: 64 patients underwent coronary artery revascularization in 2001-2003. Health-related quality of life (HRQL) was assessed by using Seattle Angina Questionnaire (SAQ). SAQ has well-established psychometric properties, measures broader aspects of the effects of coronary disease than other disease-specific tools. It consists of 19 items grouped in five components: physical functioning (SAQ Phys), angina stability (AS), angina frequency (AF), treatment satisfaction (TS), and QOL perception (SAQ QOL).

**Patients and methods:** All the patients were divided into next groups respectively: Pump-off coronary artery bypass grafted (CABG) (15) and heart-beating CABG (49); aged by years old; patients with distal coronary vessels disease (28); patients with proximal coronary vessels disease (16) according to coronary angiography.

### Apoptosis and extracellular matrix remodeling contribute to left ventricle dysfunction in Monocrotaline-Induced Pulmonary Hypertension

ESC-ID **716**  
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**Background:** Although pulmonary hypertension (PH) selectively overloads the right ventricle (RV), there are several intrinsic alterations in the non-overloaded left ventricle (LV). Our goal was to study the timing of the molecular changes underlying LV dysfunction in monocrotaline-induced PH.

**Methods:** Male Wistar rats (180-200g; n = 48) randomly received a monocrotaline (MCT) injection ( $60 \text{ mg/Kg}$ , sc) or an equal volume vehicle (Ctrl). Hemodynamic studies were carried out and transmural LV myocardial samples were collected for molecular and histological analysis at week 4 (Ctrl-4, n = 6; MCT-4, n = 6), and week 6 after

MCT injection (Ctrl-6, n = 6; MCT-6, n = 6), respectively. Transverse sections (4 $\mu$ m) of LV free wall were stained to evaluate fibrosis (Masson's Trichrome) and apoptosis (TUNEL) and photographed with a digital camera. To quantify the extent of fibrosis, the area percentage of blue staining was measured in 10 fields (400X) randomly selected on each section (ImageJ software). To quantify the apoptosis rate, TUNEL-positive cardiomyocytes were counted in at least 50 optical fields (400X) of each specimen. The apoptotic rate was expressed as a percentage of apoptotic cells of all cardiomyocytes per field. Tenascin-C LV myocardial expression levels were evaluated by real time RT-PCR.

Results were normalized for GAPDH and presented in arbitrary units (AU). The AU was set as the mean obtained for the Ctrl-4 group. Groups were compared with two-way ANOVA.

### Is brain natriuretic peptide level comparable to SCORE Risk Chart in assessing cardiovascular death risk in patients with cardiovascular diseases?

ESC-ID 742  
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**Introduction:** One of the most common causes of death are cardiovascular diseases (CVD). Thus, accurate evaluation of patients with CVD is necessary for the prevention of heart failure and cardiovascular death. SCORE Risk Charts are proved cardiovascular death risk parameter while brain natriuretic peptide (BNP) is a new and independent marker of heart failure and cardiovascular diseases. The aim of the study was to evaluate if BNP serum level correlates with SCORE Risk in patients with cardiovascular diseases.

**Material and methods:** 289 patients aged 62  $\pm$  8 with at least one of the following cardiovascular risk factors: previous myocardial infarction, asymptomatic valvular heart disease, ischemic heart disease, hypertension, diabetes, obesity, hypercholesterolemia, chronic obstructive pulmonary disease and renal failure were investigated. The serum level of BNP using ELISA tests as well as total serum cholesterol level, blood pressure and anamnesis were evaluated. On that basis patients were divided into 3 groups according to the SCORE Risk Chart (group A: 179 patients with automatically high SCORE Risk due to proved CVD, group B: 44 patients with SCORE at least 5% and group C: 66 patients with SCORE below 5%), where group A and B are in the high cardiovascular death risk and group C is in the low risk.

**Results:** The study showed the significant difference in the BNP levels between examined groups. There was statistically higher BNP median in the high SCORE risk groups (A and B) comparing to the group C (p=0.0004). The difference between group A and B was insignificant (p=0.369). The correlation between SCORE Risk Chart and serum BNP levels in the group B and C was assessed with the result of parameter r significantly different from zero (r=0.2541, 95% CI: , p=0.0074).

**Conclusion:** Patients with high cardiovascular death risk have statistically significantly higher BNP serum level than patients with low cardiovascular death risk.

Measuring the BNP level in serum may have additional value in evaluation of the patients with high cardiovascular death risk. In such population deeper analysis of left ventricular function should be performed to detect asymptomatic left ventricular dysfunction. The intensification of the treatment and diminishing of risk factors should be considered in patients with high BNP level.

### Efficiency of application of Loprax and Lorikacin in aged patients with the chronic obstructive lungs disease

ESC-ID 748  
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In research were included 42 patients with the chronic obstructive lungs disease (8 women and 34 men) by age 47 – 72 years, which divided into 2 groups: basic (22 patients) and control (20 persons). The patients of basic group accepted Loprax (cefixim) on a 1 pill (400 mg) orally during 6 days and Lorikacin (amycacin), which was used as inhalations for 500 mg 2 times on days during 6 days, which, preliminary divorcing in 2 ?? of saline, entered with nebulizer. The patients of control group used Cefotaxim in dose 1,0 g 2 times per day intramuscular during 7 days. Under act of the conducted treatment for the patients of basic group more substantial increase of indexes of volume of forced expiration is marked for the first second, namely on the 1th day of treatment the noted index grew on 27,40  $\pm$  2,81, on 3th - on 34,30  $\pm$  3,75 and on 7th – on 46,20  $\pm$  5,21.

### High HDL levels: An additional consideration beyond Low-Density Lipoprotein Cholesterol after acute coronary syndrome (ACS)

ESC-ID 773  
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**Aim:** The purpose of this study was to assess the impact of on-treatment High Density lipoproteins (HDL) on coronary heart disease (CHD) risk after an acute coronary syndrome (ACS). In the previous studies, it has been demonstrated that low-density lipoprotein cholesterol (LDL-C) 70 mg/dl was associated with greater CHD event reduction than LDL-C 100 mg/dl after ACS. However, the impact of high on-treatment HDL on CHD risk beyond LDL-C 70 mg/dl has not been explored.

**Materials and Methods:** We administered atorvastatin 80 mg or pravastatin 40 mg daily on 284 patients. The relationship between on-treatment levels of HDL and LDL-C and the composite end point of death, myocardial infarction (MI), and recurrent ACS were assessed 120 days after initial presentation.

**Results:** High on-treatment HDL (>40 mg/dl) was associated with reduced CHD risk compared with lower HDL in univariate analysis (hazard ratio [HR] 0.73, 95% confidence interval [CI] 0.62 to 0.87; p < 0.001) and in adjust-

ed analysis (HR 0.80, 95% CI 0.66 to 0.97;  $p < 0.025$ ). For each 2-mg/dl increment in on-treatment HDL, the incidence of death, MI, and recurrent ACS was lower by 1.6% or 1.4% after adjustment for LDL-C or non-high-density lipoprotein cholesterol and other covariates ( $p < 0.001$  and  $p < 0.01$ , respectively). Lower CHD risk was also observed with HDL  $>40$  mg/dl and LDL-C 70 mg/dl (HR 0.72, 95% CI 0.54 to 0.94;  $p < 0.017$ ) or high on-treatment HDL, and C-reactive protein (HR 0.59, 95% CI 0.41 to 0.83;  $p < 0.002$ ) compared with higher levels of each variable in adjusted analysis.

**Conclusions:** On-treatment HDL  $>40$  mg/dl was independently associated with a lower risk of recurrent CHD events, lending support to the concept that achieving high HDL may be an additional consideration beyond lowering LDL-C in patients after ACS.

### The method of insulinotherapy in children with diabetes mellitus type 1 and lipid metabolism

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**Objective:** The aim of the study was to find the relationship between the method of treatment in children with diabetes mellitus type 1 and lipid abnormalities.

**Material and Methods:** To the study were included 120 children with diabetes mellitus type 1 aged  $12,8 \pm 3,8$  years and suffering from diabetes for  $4,7 \pm 3,2$  years. 80 patients were treated with many injections of insulin by pens and 40 children treated with continuous subcutaneous insulin infusion (CSII) by personal insulin pump. In all patients HbA1c and plasma concentration of triglycerides, total cholesterol, HDL and LDL-cholesterol and were measured.

**Results:** There were not found significant differences between group treated with pens and group treated with personal insulin pump in concentration of total cholesterol ( $182,4 \pm 43,2$  vs  $192,6 \pm 26,3$  mg/dl), LDL- ( $106,8 \pm 33,8$  vs  $117,8 \pm 25,2$  mg/dl), HDL-cholesterol ( $53 \pm 11,1$  vs  $55,7 \pm 11,6$  mg/dl) and triglycerides ( $105,2$  vs  $96,3$  mg/dl). In both groups was found statistically significant positive correlation between HbA1c level and total cholesterol.

### Virtual oncological liver resection: Enhanced preoperative planning of the residual volume and function in liver surgery – a preliminary report

ESC-ID 841  
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**Introduction:** In the current treatment of liver tumors, the curative approach of partial resection plays the most important role. Although this procedure is considered to be relatively safe, postoperative liver failure by inappro-

priate residual liver volume remains a life-threatening complication, which surgeons strive to avoid. Hence, a tool for the effective planning of postoperative liver volume and functional capacity is necessary to improve patients' outcome. We evaluated a novel system consisting of virtual resection by 3D-computer tomography and the LiMAx-Test for maximal enzymatic liver function capacity for enhanced planning of residual volume and function in a prospective clinical trial.

**Patients and Methods:** 40 patients will be investigated during their perioperative therapy. Till today, eight patients received liver resection and completed the study protocol. Virtual 3D-resection was done by CT analysis (MeVis, Bremen, Germany) using a 4-phase contrast enhanced examination technique (LightSpeed 64®; GE Medical Systems, Milwaukee, IL, USA) before surgery. The proposed cutting plane was either validated or modified by the responsible surgeon. Preoperative enzymatic liver function capacity was measured by the new LiMAx test. A predicted postoperative LiMAx value was calculated using the planned residual liver volume. A second 3D-CT analysis and LiMAx test were performed immediately after surgery.

**Results:** The virtual 3D-resection was successfully implemented into the preoperative phase. This planning tool was positively appraised by the local surgeons. The calculated residual volume was strongly correlated to the actual measured residual volume by a linear coefficient of  $r = 0.962$  ( $p < 0.001$ ). All patients successfully received extended liver resection without postoperative mortality. The median difference between the calculated and the measured LiMAx was  $-36 \mu\text{g}/\text{kg}/\text{h}$  (range  $-81-19 \mu\text{g}/\text{kg}/\text{h}$ ), which might be contributed to additional hepatocyte damage due to intraoperative liver ischemia (Pringle maneuver). The correlation between the calculated and measured liver function was  $r = 0.732$  ( $p = 0.039$ ).

**Conclusion:** The preoperative volume-/function planning is generally feasible and can effectively predict the postoperative liver volume and function. This could substantially improve the security of liver surgery and might decrease postoperative morbidity and mortality.

### Evaluation of effective factors on the survival of the patients with osteosarcoma

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**Introduction:** Osteosarcoma is a primary malignant tumor of the bone that has two types: 1.conventional(75%) 2.variant(25%). Furthermore, osteosarcoma is the most common bone malignancy and has high mortality rate so identifying the effective factors on the survival of these patients will be valuable for therapeutic programs. Considering there have been few researches in this field in Iran, this research was designed to evaluate survival factors in osteosarcoma patients.

**Method and material:** This historical cohort study has performed on all 209 osteosarcoma patients referred to Shafayahyaian hospital in Tehran (referral institution of bone malignancies) during 2001-2007. Patients' characteristics including background variables, symptoms,



tumor location, laboratory data and follow up variables were recorded. Average time of follow up was 24.49(SD = 33.59) months. Data were analyzed by SPSS V.16 software and independent T-test, chi2, Kaplan Meier and Cox regression were used.

**Results:** From all 209 patients, 146(69.9%) were male and 63(30.1%) were female with the mean age of 19.34(SD = 9.29) years. Average period between onset of symptoms and diagnosis of malignancy was 83.41 months (SD = 12.34). During follow up, 44 patients (21.1%) died. And 2-year and 4-year survival rates were 60% and 40% respectively with the mean survival time of 125.41(SE = 16.85) months. Average serum LDH level was significantly higher in patients who died [1147.80(SD = 352.97)U/L VS. 560.52 (SD = 264.01)U/L.

### Evaluation of visul evoked potentials and ERG on patients of SLE

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**Introduction:** Systemic Lupus Erythematosus (SLE) is a multifactorial, chronic, systemic, inflammatory disease, of unknown origin, characterized by the presence of autoantibodies, accumulation of immune complex and polymorphic clinical manifestations. This disease may involve multiple organs and systems. The most common findings are articular, cutaneous, vascular, renal, neurological, cardiac, gastrointestinal, hematological, auditory and ocular abnormalities. Ocular manifestations of lupus are a reflection of a systemic disease. Significant vision loss and associated ocular morbidity are possible in systemic lupus erythematosus, particularly in cases of retinal or central nervous system involvement. Changes in the eyes that occur in SLE are not frequent, but can lead to severe impairment of sight including blindness. Anterior segment findings include keratoconjunctivitis sicca, keratitis and scleritis; retinal manifestations of SLE are cotton wool spots, hemorrhage and vasculitis.

**Aim:** The aim of this research is to prove the possible significant changes in the afferent visual innervation of patients of SLE by using VEP and ERG records.

**Methods:** Within the scope of the research, 8 SLE cases have been focused on, whose VEP-ERG readings had been made in the electrophysiological laboratories of Istanbul University Cerrahpasa Medical Faculty Physiology Department. The standards of the International Committee of Visual Electrophysiology have been extensively provided during the readings. 8 subjects with close intervals of age with the actual patients have been worked on as the control group. Then the results are discussed using the Mann-Whitney U test. Outcomes less than 0,05 are admitted to be significant.

### Juvenile myoclonic epilepsy: Clinical and neurophysiological characteristics

ESC-ID **886**  
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**Introduction:** Juvenile myoclonic epilepsy (JME) is primary, non-progressive, generalized epileptic syndrome associated with myoclonic jerks, usually after waking-up, generalized tonic-clonic seizures, and absence seizures (1,2). There are no abnormalities on neurological examination. Generalized polyspike-and-waves are the most characteristic electroencephalographic pattern. Evoked potentials play a major role in clinical evaluation of myoclonic epileptic syndroms. Neurophysiological studies have reported higher amplitudes of somatosensory-evoked potentials (SEP) (giant SEP) in JME patients (3,4,5). Patophysiological cause of reported abnormalities is probably cortical hyperexcitability.

**Aim:** The aim of this study was to evaluate clinical and neurophysiological characteristics in JME patients, especially SEP.

**Material and methods:** We analyzed medical data of 103 JME patients treated in Clinic of Neurology and Psychiatry of Children and Youth, Medical Faculty, University of Belgrade in period of 2005-2008. Control group consisted of 20 patients with other non-epileptic neurological disorders. SEP examination was performed on apparatus Medelec Sapphire Premiere from median nerve level, using standard International Federation of Clinical Neurophysiology (IFCN) procedure. Data was analyzed by methods of descriptive and analytic statistics.

**Results:** The study was conducted in a group of 103 patients, 43 male (41.7%) and 60 (58.3%) female. Average age was 21.8 years (SD = 5.3). The average age of onset of JME was 13.9 years (SD = 2.8). Onset of disease occurred earlier ( $p < 0.01$ ) in the group of patients with initial absanse seizure. Adequate therapeutic response to valproates (longterm control of epileptic seizures) was gained in all patients (103; 100%). In 73 (70.9%) patients valproates were initial therapy. Others (30; 29.1%) recieved another type of medicament (clobazam, phenobarbiton, lamotrigine, topiramate). Another type of medicament were applied more often ( $p < 0.01$ ) in patients with focal EEG pattern. SEP registration was conducted 33.2 months (SD = 42.5) after JME diagnosis. SEP amplitudes were in physiological range in 62 patients (60.2%), and 41 patients (39.8%) had elevated wave amplitudes (giant SEP). Study shown that amplitudes of following waves: left cortex P25-N35 ( $Z = -3.341$ ,  $p = 0.001$ ), N35-P35 ( $Z = -1.968$ ;  $p = 0.049$ ); right cortex N20-P25 ( $Z = -2.224$ ;  $p = 0.026$ ), P25-N35 ( $Z = -3.376$ ,  $p = 0.001$ ) were higher in examined patients than in control group. Study also shown that lower wave amplitudes correlated with duration of period between beginning of treatment and SEP registration - left cortex N20-P25 ( $q = -0.278$ ,  $p = 0.04$ ), P25-N35 ( $q = 0.241$ ,  $p = 0.000$ ); right cortex P25-N35 ( $q = 0.218$ ,  $p = 0.027$ ).

**Conclusion:** Configuration and amplitude alterations of cortical SEP response are very important parameters in clinical evaluation of myoclonic epilepsies. Our study shows that 39.8% of examined patients had giant SEP amplitudes. In patients with higher SEP amplitudes SEP

was performed earlier than in group with normal findings, indicating SEP normalization with adequate antiepileptic therapy. Our study shown that frequency of pathological findings was 60% when SEP was registered in first month after beginning of valproate therapy. Frequency decreased to 40% in 2-6 months period, and to 20% after two or more years of treatment. This result may suggest early registration of SEP as one of diagnostic procedures in JME.

**Key words:** Juvenile myoclonic epilepsy, somatosensory-evoked potentials

### **Evaluation of periodontal disease in diabetic patients (NIDDM, IDDM) by using CPITN index**

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**Aim:** Among the late complications associated to the diabetes mellitus, periodontal disease has been highlighted, and it can be more severe and refractory to treatment than in healthy subjects. Our objective is to determine the prevalence of gingivitis and periodontitis as well as the Community Periodontal Index of Need of Treatment (CPITN) in diabetic population compared with a control one and analyze the histological characteristics in the gingiva of diabetic patients.

**Methods and Materials:** An analytic case-control study was conducted among 264 IDDM, NIDDM patients attending a diabetes clinic in Tehran, Iran as case group and 246 persons attending Oral Disease Department of Dental Faculty in Tehran, Iran as control group. The study groups included 233 males and 277 females (112males, 134females in control group; 121males, 143females in case group).

**Results:** The severity of periodontal destruction has been shown to be related to the direct and indirect effects of glycemic control, with other factors also being implicated. Periodontitis incidence was 75.5% in NIDDM, 52.9% in IDDM and 26.4% in control group. Also gingivitis incidence (score 1, 2) was 24.5% in NIDDM, 47.1% in IDDM and 66.3% in control group. In comparison to the females, males had more periodontitis but less gingivitis in all groups. This difference was statistically significant.

### **Schwannomas in the cerebellopontine angle – retrospective study of 84 cases for a 3 year period**

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**Objective:** This retrospective study was designed to investigate the incidence, clinical presentations, management

options and outcomes of schwannomas in the cerebello-pontine angle.

**Methods:** A retrospective study was conducted on 84 patients diagnosed with schwannoma in the cerebello-pontine angle and operated between 2005 and 2008 in University Hospital "Sv. Ivan Rilsky" (mean age of 49 years; 52 females and 32 males).

**Results:** In 79% of the cases a retromastoid approach was used while in 15% subtemporal was preferred. The highest percentage (31%) of the patients report onset symptoms dating back 1 to 2 years before diagnosis, 23% - more than 5 years and 16% - 2 to 6 months. Most frequent reported symptoms are hypacusis/anacusis 84%, ataxia - 63%, tinnius - 38%, vertigo and facial palsy - 33%. When divided into two groups - hospitalized for a first operation and hospitalized for a re-operation, we found statistically significant decrease in the tinnitus, ICP like symptoms and bulbar syndrome ( $p < 0,05$ ). The average size of the tumors is 31mm according to MRI and CT imaging. 38% of the masses were removed sub totally, while 35% partially and 28% - grossly total. In 26% of the operated cases endoscopic techniques were used.

**Conclusion:** The results from the analysis of the gathered data correspond widely with the published materials in the literature. We detected some statistically significant differences in the clinical presentations between patients hospitalized for first time and those admitted for reoperation.

### **Correlation between Inducible Nitric Oxide Synthase and Cyclooxygenase-2 Expression in Human Colorectal Adenocarcinoma: A Cross-sectional Study**

ESC-ID **901**  
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**Aims:** Cyclooxygenase (COX) takes action in the prostanoids biosynthesis pathway as the rate limiting enzyme. It is assumed that COX-2 enzyme plays a role in tumor angiogenesis, differentiation, and apoptosis. The inducible isoform of nitric oxide synthetase (iNOS) is expressed in response to inflammatory cytokines and operates through a Ca<sup>2+</sup> independent pathway to produce NO. NO can cause DNA damage and inhibits DNA repair and perhaps contribute to tumour formation. Nonetheless, the expression correlation of Cox-2 and iNOS is not well documented colorectal tumor. In the following study, we have evaluated the expression of iNOS and COX-2 in human colorectal adenocarcinoma.

**Materials and Methods:** Seventeen patients with diagnosed colorectal adenocarcinoma, who underwent surgical resection of tumor, were involved in the study. For each patient, one sample from tumor and one from free surgical margin as formalin fixed paraffin embedded blocks were collected. Both samples were analyzed for iNOS and COX-2 expression by means of immunohistochemistry and western blotting. The results were digitized and semi-quantitatively measured.

**Results:** Immunohistochemistry revealed similar pattern of expression for both iNOS and COX-2, as both were detected in tumoral cells and epithelial cells.

Inflammatory cells were also weakly positive. The mean iNOS and COX-2 levels in western blotting method were significantly higher in tumoral tissue compared to the free surgical margin.

### Prevalence of Polycystic Ovary Syndrome (PCOS) in 15-18 adolescent girls in Tehran

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*Aim:* Polycystic Ovary Syndrome (PCOS) is the most common endocrinopathy in women of reproductive age. The lack of well-defined diagnosis criteria makes identification of this common disease confusing to many clinicians. Considering high prevalence of PCOS, many researches have been made to investigate this disease but unfortunately, few researches in the world have been made in the field of PCOS in teens. Most of studies made on teens had low volumes and short periods. Considering the fact that PCOS is the most prevalent endocrine disease in women and there are also many disagreements regarding etiology and clinical and laboratory signs, execution of a broad and basic research in the field of PCOS among the teens seems quite necessary. The aim of the present study is to determine the prevalence of PCOS in teenage girls of Tehran, the capital city of Iran.

*Material and Methods:* In this cross sectional study efforts have been made to examine and interview with female teens in the age range of 15 to 18. The method of sampling was multi stage random sampling. It has been tried to put those girls having one of the clinical signs of menstruation disorder, hirsutism or obesity, under FSH, LH, FBS, INSULIN and PRL blood tests and then abdominal ultrasound to investigate the prevalence of this disease in the society after approving PCOS diagnosis. The dominant clinical chart of disease may also be described in the society to be studied.

*Results:* This study showed that prevalence of PCOS in this age range is 3/42%.

### Assesment of focal lesions in liver using magnetic resonance imaging - Significance of diffusion weighted imaging

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*Introduction:* The study was design to determine if there is a difference between apparent diffusion coefficient (ADC) values using magnetic resonance imaging (MRI) with different b factors and to compare ADC values of hepatic lesions.

*Patients and methods:* The study included 63 patients with focal hepatic lesions, 52 men (82.5%), age  $62.1 \pm 9.8$  years. Fourteen patients (22.2%) had hepatocellular carcinoma

(HCC) confirmed on surgery, 16 patients (25.4%) were with hepatic metastatic colorectal tumours confirmed on previous surgery, 17 patients (26.9%) with cavernous haemangioma and 16 patients (25.4%) with hepatic cysts based on clinical manifestation, ultrasound/computed tomography/MRI and follow-up results. MRI was performed with 1.5T scanner, with a body coil using EPI sequence with tree b values: 0, 400, 800 s/mm<sup>2</sup>. ADC values were measured in all the lesions, as well as a ratio between the ADC value of the liver and the lesion.

*Results:* ADC values were statistically different among the groups ( $F = 70.7$ ).

### Ischaemic preconditioning decreases stress oxidative and inflammation in a lung auto-transplant animal model

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*Aim:* Ischaemia-reperfusion injury consists of a tissue damage that occurs when blood supply returns to the tissue after a period of ischemia. Damage is caused by oxidative stress and the activation of inflammatory mediators. A particularly destructive aspect of oxidative stress is the production of reactive oxygen species, which are injuring the lipidic cell membrane, in a process called lipidic peroxidation. In the lung, this situation may determine the prolongation of assisted ventilation, the increase of primary graft failure and higher morbidity and mortality. Ischaemic preconditioning has proven to protect several organs from ischaemia-reperfusion injury, but there are no studies regarding to the lung. The aim of this study was to determine the effect of ischaemic preconditioning on oxidative stress and inflammation, and consequently on ischaemia-reperfusion injury, in the lung.

*Methods and materials:* Two groups of 7 large-white pigs (ischaemic preconditioning group and control group) were submitted to a left lung auto-transplant (pneumonectomy, ex-situ superior lobectomy and lower lobe reimplantation). Both groups received the same anaesthetic induction, and same maintenance with propofol. The ischaemic preconditioning group went under two sequences of 5 minutes of ischaemia by clamping the left pulmonary artery, separated by 5 minutes of normal circulation, before the pneumonectomy was performed. In the control group the left pulmonary artery was clamped only before the pneumonectomy. To measure the oxidative stress, the biomarkers gauged were lipidic peroxidation metabolites: malonil-dialdehyde (MDA), lipid hydroperoxides (LPO) and a leucocitary activation marker: myeloperoxidase (MPO); while the cytokines interleukin-1 (IL-1) and tumor necrosis factor (TNF) were used to measure inflammation. Lung samples were collected at three different moments: 1) pre-pneumonectomy (after the ischaemic preconditioning), 2) pre-reperfusion (after the implantation of lower lung lobe) and 3) post-reperfusion. U Mann-Whitney was used as non-parametric test to find statistical meaning.

*Results:* Our results showed, at the post-reperfusion moment, a significant decrease.

## The effects of propranolol on chronic pain in rats and comparing it with morphine and aspirin

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*Introduction:* propranolol competitively blocks response to beta1- and beta2-adrenergic stimulation which results in decreases in heart rate, myocardial contractility, blood pressure, and myocardial oxygen demand. Nowadays it is used for Management of many cardiovascular disorders. Several placebo-controlled studies in adults have found that chronic therapy with propranolol reduces the frequency and severity of migraine in 60 to 80 percent of patients in this investigation an experimental trial study was conducted to evaluate the anti-nociceptive effect of propranolol on formalin induced chronic pain in rats.

*Material and method:* In this study, fifty five male rats randomly divided into 11 groups and administered Normal Saline, Propranolol (20mg/kg), morphine (1,2,4mg/kg), propranolol along with morphine, propranolol pretreated with naloxone (2mg/kg), aspirin (10, 100, 1000mg/kg), aspirin along with Propranolol respectively. Formalin test was conducted to each animal 20 minutes after intra peritoneal injection and pain scores were recorded for 60 minutes.

*Results:* Our results indicated that propranolol decreased pain throughout the Formalin test which was significantly lesser than control group in 2nd phase of formalin induced pain ( $1.52 \pm .060$  vs  $1.93 \pm .039$ ,  $p < .001$ ). the anti-nociceptive effect of propranolol was the same as all three doses of morphine sulfate and was reversed by naloxone. Pretreatment with propranolol before administration of morphine decreased pain sensation but it was not significant to morphine (2mg/kg) group. and also propranolol decreased pain scores in comparison with aspirin 100 and 200mg/kg which it was significant in 6 first time blocks ( $p < .05$ ) and propranolol along with aspirin also decrease hyperalgesia more than aspirin group that it was significant in last 4 time blocks.

*Conclusion:* It can be concluded that administration of propranolol can reduce chronic pain because it decreases hyperalgesia in formalin induced pain model which seems this action has been reversed by naloxone pretreatment is probably due to the activation of opioid receptors by propranolol . and it can develop the anti-inflammatory effect of aspirin.

*Key words:* propranolol, aspirin, Formalin test , inflammation ,chronic pain ,morphine

## Effect of weight loss on metabolic control in obese diabetic patients

ESC-ID **995**  
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*Introduction:* The most important risk factor in development diabetes mellitus type 2 is obesity. It is well known

that obesity has the important role in worsening other risk factors and at the same time is independent factor. Obesity and related diseases has become a significant health problem of the modern world, above the rest diabetes mellitus type 2, hypertension, hyperlipidemia, which all together are a part of metabolic syndrome and significantly contribute increasing cardiovascular risk. Weight loss is basic component in treatment patients with diabetes type 2 and represent an important item in achieving good metabolic control.

*Aim:* The aim of study was to evaluate the effect of weight loss on metabolic control in obese patients (BMI  $\geq 30$ ) with diabetes type 2.

*Material and methods:* The study involves 30 patients with diabetes type 2, age from 40 to 65 years. All patients were submitted by the dietary regimen (1200 kcal/day) in the period of 6 months. Before and at the end of the investigation we measured body weight and height, determined BMI (body mass index), WHR (waist-to-hip ratio), glycemia, plasma insulin levels, HbA1c, total cholesterol, HDL-Ch, LDL-Ch, triglyceride.

*Results:* After 6 months of the study, we found significant decrease of BMI (from  $35,12 \pm 2,12$  to  $32,03 \pm 2,24$ ).

## Autonomic dysfunction in patients with diabetes mellitus and acute myocardial infarction

ESC-ID **998**  
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It is well known fact that patients with diabetes mellitus and autonomic neuropathy have high mortality and worse prognosis due to autonomic dysfunction. The aim: was to establish the grade of autonomic dysfunction in patients with diabetes mellitus and acute myocardial infarction in comparison with patients who did not have diabetes mellitus, but with acute myocardial infarction.

*Methodology:* 1813 patients with acute myocardial infarction are divided into two groups. First group are patients with diabetes mellitus and acute myocardial infarction (512 patients, 27, 8 %); second group are patients with acute myocardial infarction but without diabetes mellitus (1326 patients, 72, 1 %). The function of vagus is examined by application of 3 tests: Valsalva maneuver (VM), Heart rate response to standing test (HRRT), Deep breathing test (DBT). The function of sympathetic is examined by application of 2 tests: Blood pressure response to standing test (BPRT, OH), and Hand grip test (HG).

*Results:* I group of patients, patients with acute myocardial infarction and diabetes mellitus, had positive deep breathing test (DBT) in 56 (83.6%) cases.

### Toxoplasmosis of CNS as opportunistic infection in AIDS (diagnosis and clinic approaches)

ESC-ID **1009**  
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*Introduction:* Toxoplasmosis is the most common cerebral mass lesion encountered in HIV-infected patients, and its incidence has increased markedly since the beginning of the AIDS epidemic. There are occasionally unusual appearances of CNS toxoplasmosis that make diagnosis by standard imaging techniques difficult or impossible.

*Aim:* Researching of clinical, immune and morphologic sings of CNS toxoplasmosis in patients with AIDS.

*Material and Methods:* We investigated 18 patients with HIV stage III-IV, which were referred in ICU of Infectious Clinic of Infectious Disease (Kharkov, Ukraine) in period 2003-2008. Clinical and laboratory investigation performed under clinical guidelines for HIV/AIDS (WHO 2007).

*Results:* Toxoplasmosis was diagnosed in 54% patients with CNS diseases. All patients with CNS toxoplasmosis had gradual diseases onset.

### Treatment of NSCLC – A prognostic index in elderly patients

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Optimal treatment for elderly patients with advanced NSCLC is still debated. Geriatric assessments can contribute to the understanding of this concept, by evaluating independent predictors for treatment tolerance, morbidity and mortality.

*Methods:* Functional status of 148 elderly patients (>65 years) with advanced NSCLC was assessed through a pre-treatment minimal geriatric evaluation questionnaire: 6 items related to Activities of Daily Living (ADL), and 6 to Instrumental Activities of Daily Living (IADL). Study aim was to assign patients to prognostic groups, based on degree of dependence in ADL and IADL (0, 1, 2), and on parameters emerged as significant for survival in a Cox regression model.

*Results:* All cases were pathologically confirmed as NSCLC (squamous 106p, 71.6%; adeno 38p, 25.7%; other 4p, 2.7%) stage IIIA (62p, 41.9%), IIIB (48p, 32.4%), and IV (38p, 25.7%). Significant comorbidities were reported by 90p (60.8%). Hemoglobin (Hb) level cut-off was set at 11 g/dL, and 43p (29.0%) had lower values. Majority of patients received chemo-radiotherapy (sequential 62p, 41.9%; concomitant 37p, 25.0%), while 49p (33.1%) received chemotherapy alone. Mean survival was 11.48 months.

### Sensitivity and specificity of Patient Health Questionnaire-9 in detecting depression among patients with type 2 diabetes

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*Introduction:* Diabetes self-management is essential in the integrated care of diabetic patients. One issue that can affect this important part of diabetes care is depression. Recent studies have shown that the prevalence of depression in diabetic patients is 2-3 times more than general population which is linked with poorer adherence to medications and self-care activities, more reported diabetic symptoms, reduced quality of life, higher mortality and greater healthcare expenditures. So careful management of depression may be necessary to improve quality of life of diabetic patients. However, depression frequently goes undetected and it remains untreated. Although the gold standard for assessment of depression is clinical interview by a psychologist, time and cost factors makes it inapplicable in primary care. Therefore, a brief and rapid instrument for detecting depression in primary care settings may play an important role in the improvement of diabetes care. This study was performed to measure sensitivity and specificity of Patient Health Questionnaire-9 in detecting depression among patients with type 2 diabetes mellitus.

*Method:* This study was conducted in Endocrinology and Metabolism Institute of Iran University of Medical Sciences from January to March 2009. All eligible patients who referred to this institute entered the study after obtaining informed consent. Inclusion criteria included type 2 diabetic patients who could fluently read and speak Persian and had no severe diabetes complications and no diagnosis of active psychosis or dementia. Patients were assessed for depressive symptoms by Patient Health Questionnaire-9 (PHQ-9) and a structured clinical interview based on DSM-IV criteria and the psychologist who interviewed the patients was blinded to results of the questionnaire. All patients who completed the questionnaire took part in the interview and the interviewer was the same for all of them. Baseline characteristics were recorded with a checklist. Sensitivity and specificity of the questionnaire were calculated using a receiver operating characteristic (ROC) analysis.

*Results:* Of 121 participants in this study 73 (60.3%) were female. Mean age, BMI and years since diagnosis of diabetes were 55.12 (SD=9.75), 28.52 (SD=4.99), and 9.84 (SD=8.58). Of the patients 75 (63.6%) were on oral agent and 20 (16.9%) on insulin. Also 21 (17.8%) were using both oral agents and insulin and 2 (1.7%) were not under treatment. ROC analysis showed that PHQ-9 could significantly detect depression in diabetic patients (AUC = 0.84, P < 0.001). Cut point of 11 had the sensitivity = 84%, specificity = 72% and positive likelihood ratio = 3.07.

*Conclusion:* This study shows that cut point of 11 can be use to define likely depression in patients with type 2 diabetes mellitus. Considering high pretest probability of depression in diabetic patients, PHQ-9 may contribute to detection of this disorder in this population.

**Key words:** Diabetes, Depression, Patient health questionnaire-9

### Characteristics of complicated suicides

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**Introduction:** Murder-suicide form a distinct subset of homicide in which the perpetrator kills him or herself after dispatching his or her victims. A variety of categories have been identified that include spousal cases where (a) the action is precipitated by jealousy or ill health, (b) familiar cases where a parent usually kills all of the children and then himself and (c) mixed group consisting of member of religious/political groups who target a large number of victims.

**Aim:** The objective of this work is to carry out the analysis of murder-suicide in Belgrade in 18 years, including the analysis of other characteristics of suicides and homicide such as sex, gender, age, motive, and way of suicide, marital status and profession.

**Material and methods:** During the research the autopsy material of the Forensic Institute, University of Belgrade, was analyzed in 1991 by 2008. There were 60 cases of homicide-suicide. The records of forensic autopsies already carried out were used as well as case-history obtained from suicides' family members, police reports on investigation conducted at the crime scene and possible medical database.

**Results:** There were 60 cases of homicide-suicide over the time period studied. There were 49 male assailants and 3 female assailants ( $x^2 = 40,692$ ).

### A comparison quality of life and sleep in men with benign prostate hyperplasia (BPH) in Poland and Spain

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**Aim:** The lower tract urinary symptoms (LUTS) mainly the symptoms suggestive of BPH are a frequent condition in elderly men. Indeed, the BPH prevalence increases progressive with age. Nocturia is considered as an urinary disorder with own entity more than just a symptom related to a disease. The origin of nocturia may be multifactorial but in many patients with lower tract urinary symptoms suggestive of benign prostatic obstruction constitutes a major cause of nocturia. Indeed, nocturia could be associated to several entities as sleep apnoea, somatic diseases (diabetes, congestive heart failure) or iatrogenic factors as diuretics in treatment of hypertension. To evaluate factors predicting the effect of nocturia in a community-based cohort of adults and quality of sleep using a nocturia-specific quality-of-life (N-QOL) questionnaire, Athen Insomnia Scale (AIS), Pittsburgh Sleep Quality Index (PSQI), Broch Sleep and Insomnia Questionnaire (BSIQ).

**Material and methods:** From January 2005 to May 2007, adults aged 40 years old or older living in Silesia region towns in Poland and in Country Hospital of Albacete in Spain, reporting nocturia of one episode or more per night were interviewed with a 12-item N-QOL questionnaire, consisting of Sleep/Energy and subscales. Univariate analyses were used to analyze the effects of demographic characteristics, frequency and duration of nocturia, and sleeping characteristics on the N-QOL score. Multiple linear regression analysis was used to identify factors predicting the N-QOL score.

**Results:** A total of 216 adults from Poland and 200 from Spain completed this study (mean age 56.7 years). The average N-QOL scores (a lower score indicates worse QOL) were  $93.8 \pm 15.2$ ,  $87.2 \pm 11.8$ ,  $73.6 \pm 15.1$ , and  $63.5 \pm 19.5$  for nocturia episodes of 1, 2, 3, and 4 or more per night, respectively.

We apologise that not all abstracts in this appendix could be published in their complete version. For technical reasons, some abstracts were truncated and the corresponding authors could not be reached in time.

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